Do Nematode Infections of Red Colobus (*Procolobus rufomitratus*) and Black-and-White Colobus (*Colobus guereza*) on Humanized Forest Edges Differ from Those on Nonhumanized Forest Edges?

Stacey A. M. Hodder · Colin A. Chapman

Abstract Forested edges, especially those that border humanized landscapes, provide opportunities for nonhuman primates and people to interact, and such interactions are predicted to alter disease dynamics. Given the rapid expansion of edge habitats globally, understanding changes occurring on edges is important in evaluating primate behavioral ecology and developing conservation plans. Our research investigates predictions concerning how gastrointestinal parasite and stress levels (cortisol) in red colobus and black-and-white colobus (*Procolobus rufomitratus*; *Colobus guereza*) in Kibale National Park, Uganda, differ between humanized and nonhumanized forest edges. We found *Trichuris* sp., an unidentified strongyle, and *Strongyloides* sp. in the fecal samples. Results did not generally support our expectation that humanized forest edges increase parasite infection and, counter to what we predicted, fecal cortisol did not differ between habitats, suggesting that proximity to edges and/or to humans did not result in increased stress. We conclude that broad habitat classifications, e.g., "humanized," may be too general to identify consistent differences in parasite infection, as other factors, specific to the parasite (e.g., life cycled), host (e.g., immune systems strength), or environment (e.g., moisture level), likely also play important roles.

Keywords Colobus · Cortisol · Edge · Kibale National Park, Uganda · Parasite

S. A. M. Hodder
Department of Anthropology, McGill University, Montreal, Quebec H3A 2T7, Canada

C. A. Chapman
Department of Anthropology and McGill School of Environment, McGill University, Montreal, Quebec H3A 2T7, Canada

C. A. Chapman (✉)
Wildlife Conservation Society, Bronx, NY, USA
e-mail: Colin.Chapman@McGill.ca

Introduction

Anthropogenic disturbance is often speculated to influence the nature of parasite infections in wildlife (Greger 2007), and concern over the potential of zoonotic disease transmission has grown with increasing human encroachment and population size (Chapman *et al*. 2005; Chomel *et al*. 2007; Ferber 2000; Goldberg *et al*. 2007; Wallis and Lee 1999; Wolfe *et al*. 1998). The forested edges of primate habitat, especially those that border humanized landscapes, i.e., areas of intense human use such as villages or crop fields, provide opportunities for humans and nonhuman primates to come into close proximity. Some research indicates that primates inhabiting such areas exhibit differences in their parasite infections compared to primates that do not interact with humans. For example, the prevalence of *Cryptosporidium* sp. in toque macaques (*Macaca sinica*) and purple-faced langurs (*Trachypithecus vetulus*) that ranged into areas used by humans were greater than in monkeys that did not use areas used by humans (Ekanayake *et al*. 2006). Further, a greater prevalence of *Enterobius* sp., *Strongyloides* sp., *Trichuris* sp., strongyle type eggs, *Entamoeba coli*, and *E. hystolytica/dispar* were found in the macaques that ranged in areas used by humans; however, differences in prevalence were not found for other intestinal parasites (Ekanayake *et al*. 2006). Similarly, research was conducted by Chapman *et al*. (2006a) investigated the gastrointestinal parasite infection of colobus monkeys (red colobus [*Procolobus rufomitratus*] and black-and-white colobus [*Colobus guereza*]) inhabiting forest edges and interior forest in Kibale National Park, Uganda. A greater number of colobus monkeys had more than one parasite infection in groups inhabiting forest edges, and the prevalence of an unidentified strongyle was higher in the monkeys that inhabited forest edges. Chapman *et al*. (2006a) speculated contact with humans along the forest edges could explain these findings. However, forest edges far from any human activity were not included in this study, so it is not known whether these results were a consequence of their proximity to edge habitat or a consequence of their proximity to human activity. Thus, it remains unclear what role forest edges play with respect to host–parasite dynamics.

For some parasite species, the microclimate along forest edges is expected to be less conducive to transmission, because forest edges receive increased wind and increased solar radiation and are drier than interior forest environments (Matlack 1993; Murcia 1995). Dry conditions would be expected to desiccate infective stages of some parasite species, decreasing their survival (Appleton and Brain 1995; Gillespie 2001). For example, studies have documented that the number of parasite eggs that are present in the environment that are capable of infecting hosts are lower in the hot, dry months compared to wetter months (Larsen and Roepstorff 1999). However, the response to desiccation is likely species specific, with some parasites, e.g., embryonated *Trichuris* eggs, being much more tolerant to desiccation than infective stage larvae, such as *Strongyloides* and strongyles. However, in general, the results of the colobus parasite study by Chapman *et al*. (2006a, b) are intriguing because they are the opposite pattern of what would be predicted based on the microclimatic differences between edge and interior forest. Thus, other explanations for why the prevalence of certain parasites may be higher along forest edges must be considered. The two most probable explanations are that the primates using the forest edges are stressed by interactions with humans, or that zoonotic transmission is occurring

(Rothman *et al.* 2008). It is very important to point out that without genetic analysis of the parasites, it is not possible to know for certain if human-to-nonhuman primate parasite transmission is occurring (de Gruijter *et al.* 2005). Gasser *et al.* (2009) have recently demonstrated the value of genetic analysis. For example, studies employing only coproscopic analysis have suggested that there was transmission of nodular worm (*Oesophagostomum* sp.) among nonhuman primates and humans and thus suggested that primates poised a health risk to people or vice versa. The eggs and even L3 obtained after coproculture can be identified only to the genus level. In contrast, genetic analysis suggests that *Oesophagostomum bifurcum* in humans is genetically distinct from populations in nonhuman primates and thus demonstrates that these primates do not pose a health risk to people (de Gruijter *et al.* 2004; Gasser *et al.* 2009).

Interpreting the biological significant of indices of parasite infection is difficult. Helminths and protozoans, as well as bacterial and viral infections, can impact host survival and reproduction directly through pathological effects and/or indirectly by the infection reducing the host condition (Coop and Holmes 1996; Hudson 1986). Severe parasitosis can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (Collias and Southwick 1952; Despommier *et al.* 1995). In some cases, animal body condition and reproductive status have been shown to be compromised when parasites inflict substantial energetic costs (Hudson 1986). Less severe infections are more common and may impair nutritional intake; increase energy expenditure; and adversely affect the ability to travel, feed, escape predation, and compete for mates (Coop and Holmes 1996; Hudson *et al.* 1992). Other infections appear to cause little or no effect on host energetics or fitness (Munger and Karasov 1989). In some of these cases, it seems that parasites do not necessarily induce negative effects if hosts have a nutrient surplus concurrent with the infection (Gulland 1992; Munger and Karasov 1989).

One factor that has not received sufficient attention when examining relationships between parasitic infections and use of forest edges is whether the animals on the edges are physiologically stressed. It is well documented that stress can suppress the immune system (Black 1994; Coe and Erickson 1997; Kling *et al.* 1992; Padgett and Glaser 2003). For example, the stress of social separation in capuchin monkeys (*Cebus apella*) causes a decrease in immune function (Kling *et al.* 1992). Primates experiencing increased stress and depressed immune function may be at a higher risk of acquiring more parasites or greater parasite richness than those that experience less stress. This connection has been documented in wild male chimpanzees (*Pan troglodytes*: Muehlenbein 2006). One source of stress for monkeys inhabiting humanized forest edges is the interaction with local residents around the park. Animals in Kibale are known to raid crops, and approximately 80 % of local residents deter animals from raiding their crops, e.g., by guarding, fences, dogs, trapping, poison, shouting, and/or making noise; Naughton-Treves 1997). These interactions are not present along the nonhumanized forest edges and it is possible that these deterrents result in increased stress for animals inhabiting humanized edges, which may lead to suppression of immune systems and elevated parasite infection levels.

We compared nematode infections and levels of fecal cortisol (a measure of stress) in red colobus and black-and-white colobus monkeys inhabiting four habitat types: humanized forest edges (tea and farmland), nonhumanized forest edges, and interior

forest to evaluate whether there are increased levels of parasitism associated with edges as a result of human presence or some other aspect of forest edge environment. We predicted that the colobus monkeys ranging in close proximity to human activity would have higher stress levels and greater nematode prevalence, species richness, and number of eggs per gram of feces than those ranging farther from human activity independent of whether they inhabited forest edges.

The four questions we addressed were:

1. Do colobus monkeys inhabiting forest edges experience greater parasite infections than those inhabiting the interior forest?
2. Do colobus monkeys inhabiting humanized habitats experience greater parasite infections than those inhabiting nonhumanized habitats?
3. Do colobus monkeys inhabiting edge habitats experience greater stress, i.e., higher levels of fecal cortisol, than those inhabiting interior forest?
4. Do colobus monkeys inhabiting humanized habitats experience greater stress than those inhabiting nonhumanized habitats?

Methods

Study Site

Kibale National Park (795 km^2), Uganda is a moist, evergreen forest that receives 1707 mm of rainfall annually (1990–2010, Chapman, *unpubl. data*; Chapman and Lambert 2000). The land adjacent to the park has high levels of human activity. An average farm is *ca*. 1.4 ha, population density is *ca*. 272 individuals/km^2 (Naughton-Treves 1997), and people frequently enter the park for resource extraction, particularly when families are experiencing difficult economic times (MacKenzie *et al*. 2011; Naughton *et al*. 2011). In addition, both large-scale and small-scale tea plantations border Kibale, and plantations span hundreds of hectares to <1 ha (Mulley and Unruh 2004).

We defined humanized forest edges as areas where the forest was adjacent to areas of intense human use. These edges either bordered a tea plantation (hereafter tea edges) or bordered farmland or village area (hereafter agricultural edges). We defined nonhumanized edges as forest edges that had no evidence of human activity and were located in a grassland area in the interior of the park *ca*. 4 km from the park boundary (hereafter grassland edges). Finally, the interior habitat was an area of relatively undisturbed forest adjacent to Makerere University Biological Field Station (known locally as K30).

Fecal Sample Collection and Analysis

We located groups of colobus monkeys by walking along forest edges or along the trail system within the park until a group was encountered. We remained with the group until we collected 15 fecal samples from adults, if the group contained that many individuals, or until the day ended. To endeavor to ensure that fecal samples were independent, i.e., an individual was sampled only once, we attempted to not

collect samples from the same individuals at different times. This was relatively easy for the adult males in the groups, but not for adult females. To minimize the chances that we collected from the same group on different dates, we made sure that the sampling locations were separated by more than the home range of a group (Harris and Chapman 2007). Parasites may not be shedding eggs consistently; thus our estimate of species richness will be an underestimate. We collected fecal samples between April and July 2007 and we stored them in plastic tubes. We recorded the date, time of collection, species, sex, location, and edge type. At the end of each day, 1 g of feces was placed in a 15-ml centrifuge tube along with 2 ml of 10 % formalin. We transported 238 fecal samples to McGill University for analysis.

To increase the accuracy of the estimate of parasite infections, we used two parasitological methods to extract parasite eggs from the fecal samples: sodium nitrate flotation (Greiner and McIntosh 2009) and fecal sedimentation (Garcia 1999). Sodium nitrate flotation resulted in one slide per fecal sample, whereas fecal sedimentation resulted in 10–20 slides per fecal sample. We examined a subsample of 25 samples from each edge type for both species using saturated sodium nitrate flotation ($N=200$). We used fecal sedimentation for five red colobus fecal samples from each edge type ($N=20$). For the black-and-white colobus, we examined five samples from the interior and tea using fecal sedimentation and four samples from the agriculture and grassland using this method ($N=18$). We used separate 1-g samples for the sedimentation and floatation. So as not to double the number of statistical tests run when comparing habitats, we combined the counts of these two methods, as was done in the previous study (Chapman *et al*. 2006a, b). It should be cautioned that this produces an index that is internally consistent for this study, but it should not be contrasted to other studies using different methods. We observed larvae in some fecal samples, but did not include them in the analysis because it was very difficult to determine whether they were free-living, i.e., picked up when the fecal material hit vegetation or the ground —this has been shown to occur frequently using genetic analysis (Chapman and Ghai *unpubl. data*)— or whether they had hatched in the gastrointestinal tract of the monkey. Images and egg measurements for these parasites are presented elsewhere (Gillespie *et al*. 2005).

We analyzed a total of 182 fecal samples for cortisol. We collected samples from adults throughout the day. Cortisol levels vary diurnally (Sousa and Ziegler 1998), and we previously documented that for red colobus there is a 1.9 % increase per hour in fecal cortisol levels over the day (Chapman *et al*. 2006b); however, we collected samples from each edge habitat at all times of the day, so a systematic habitat bias is unlikely. We divided samples as follows: red colobus tea ($N=16$), grassland ($N=17$), agriculture ($N=24$), and interior ($N=25$) and black-and-white colobus tea ($N=25$), grassland ($N=25$), agriculture ($N=26$), and interior forest ($N=24$). At the time of collection, we placed fecal samples in 15-ml plastic centrifuge tubes and then in coolers to ensure they remained cold until the end of the day. At the end of the day, we weighed out 1 g of feces and stored the samples in 15-ml centrifuge tubes in a –20 °C freezer. To prepare for cortisol analyses, we thawed samples and weighed out 0.5 g. After thawing, we added a 5.0 pH citrate buffer and 95 % ethanol solution to the 0.5 g of feces and then mixed continuously for 21–27 h using a mechanical shaker. This allowed time for the cortisol to be extracted from the fecal material. At the end of this period, we placed the mixture of feces, citrate buffer, and ethanol in a centrifuge for 30 min. After

centrifuging, we filtered the supernatant containing the cortisol hormone through an Altech maxi-clean cartridge. We placed these cartridges in a freezer until they could be sent to the University of Wisconsin-Madison, where enzyme immunoassays (EIA) were performed and the metabolites of cortisol were reported in nanograms per gram (following Ziegler *et al.* 1995). Detailed information on the cortisol assay, quality controls, and validation with these two colobus can be found in Chapman *et al.* (2006b), Snaith *et al.* (2008).

Analyses

We used three indices to describe infection: prevalence, richness, and an index of intensity (eggs per gram; Bush *et al.* 1997). Prevalence is the number of hosts infected with a particular parasite species divided by the number of hosts examined (in our case a dung sample is considered representative of a single host). Richness is the number of different parasite species observed. We use the number of eggs per gram (EPG) of feces for each parasite (wet weight) as an index of intensity (intensity is formally considered the number of individuals of a particular parasite species in a single infected host, which would typically require a necropsy to determine and this is not possible in this situation, i.e., we are dealing with an endangered species). However, this index should be interpreted with caution because parasite egg production is often highly variable; e.g., female *Trichuris trichiura* in people may produce between 1000 and 46,000 eggs per day (Cheng 1973) and thus EPG may not be indicative of actual infection intensity. However, with *Trichuris* sp. from infected red colobus, we typically obtained <10 eggs/g, but during certain times, individuals in certain groups consistently had much higher loads, i.e., >300 eggs/g for all individuals in a group for an extended time (Chapman *et al. unpubl. data*). This suggests that EPG might be a useful index of parasite infection because high levels are temporally and spatially specific; we recommend that the results concerning load be viewed critically and be considered of interest only if in concordance with other indices of parasite infection.

It should be cautioned that based on the morphology of eggs, larvae, or even adults, it is probably not possible to distinguish between species or important population differences reflecting host specificity (Gasser *et al.* 2009; Ravasi 2009). As a result, without detailed genetic analysis, caution should be used when considering a parasite type to be a single species. However, using "types" provides an initial start to investigate some potentially interesting questions that can be followed up with detailed genetic studies. An excellent example of this can be found when considering *Trichuris* sp. Ooi *et al.* (1993) used scanning electron microscopy to compare infections with *Trichuris* between humans, macaques (*Macaca fuscata*), baboons (*Papio papio*), and swine. Whereas swine whipworm infections (*Trichuris suis*) were identified as distinct, human and nonhuman primate infections were considered identical and thought to be caused by *T. trichuria*. However, the authors noted two morphological variations that differed between nonhuman primate and human infections, i.e., the length of the spicule and the presence of nodular structures at the base of the pericloacal papillae in nonhuman primate whipworms. Whether these differences signal that the *Trichuris* in different primates are different species is unclear. These findings prompted Ravasi (2009) to sequence genetically the *Trichuris* species

of chacma baboons (*Papio hamadryas ursinus*) and compare it with sequences from human *T. trichuria*. Findings demonstrated that baboons harbored a genetically distinct species of *Trichuris*, dubbed *T. papionis*. This was the first study to document a divergent species of *Trichuris* in primates, which was made possible only through genetic analysis.

We fitted data to a log-linear model to determine whether habitat type and parasite infection were related. This analysis reduces the number of comparisons that must be made if differences are nonsignificant and is analogous to using an ANOVA vs. multiple *t*-tests, which also reduces the number of comparisons that must be made. Residuals from the log-linear model allowed us to determine more specifically which habitat(s) had the greatest influence in the χ^2 and Fisher's exact tests (Agresti 1996). A residual of between 2 and 3 indicated that the null hypothesis (lack of association) was not supported (Agresti 1996). Such log-linear models have been used in several studies of parasitism (Chu and Volety 1997; Chourchamp *et al.* 1998; Jokela *et al.* 1999) and are recommended in a number of statistical reviews in ecology (Riggs *et al.* 2010; Trexler and Travis 1993). We addressed prevalence using χ^2 and Fisher's exact tests. We used Mann-Whitney (MW) *U* tests and Kruskall-Wallis (KW) tests to compare parasite EPG and fecal cortisol levels among habitats. We used nonparametric statistics because the data were not normally distributed. Two-tailed tests are reported throughout. We conducted statistical analyses using SPSS 14.0. In this analysis we are assuming that the parasite infections correspond to the habitat that the colobines were found in at the time of fecal sample collection; however, it is possible that ranging behavior of the colobus monkeys may have spanned more than one habitat type.

Results

We found *Trichuris* sp. (superfamily Trichinelloidea), an unidentified strongyle (superfamily Strongyloidea), and *Strongyloides* sp. (superfamily Rhabditoidea) in the fecal samples (Bowman 1999). When interpreting these results it should be noted that it is possible that these types, particularly the unidentified strongyle, represent more than one species and thus have different transmission routes and are affected by environmental factors differently (de Gruijter *et al.* 2005, 2004). Both species of colobus were infected with all three parasite types (Table I).

Parasite Prevalence in Black-and-White Colobus

Prevalence of *Trichuris* sp. in black-and-white colobus differed among habitat types (χ^2=11.926, *P*=0.008). Residuals from the log-linear model indicated that a greater proportion of the monkeys were infected with *Trichuris* sp. in the agricultural than expected if parasite infection and habitat type were not associated (residual=2.275 for agriculture). In contrast, residuals for the tea indicated that fewer monkeys were infected with *Trichuris* sp. than expected (residual=–2.249). We then compared each habitat individually to each of the other habitats and found the agriculture to have more monkeys infected with *Trichuris* sp. than both the grasslands (Fisher's exact test, *P*=0.024) and tea (Fisher's exact test *P*=0.012). Counter to what was expected,

Table I Parasite EPG and prevalence in red colobus and black-and-white colobus inhabiting Kibale National Park, Uganda

Habitat	*Trichuris*	Strongyle	*Strongyloides*	*Trichuris*	Strongyle	*Strongyloides*
	Parasite EPG in red colobus			Parasite EPG in black-and-white colobus		
Agriculture	151	4	7	541	16	3
Tea	113	29	0	217	22	3
Interior forest	140	3	0	683	6	4
Grassland	44	15	0	114	6	64
	Prevalence in red colobus			Prevalence in black-and-white colobus		
Agriculture	0.17	0.04	0.02	0.29	0.04	0.02
Tea	0.17	0.06	0	0.23	0.1	0.02
Interior forest	0.12	0.03	0	0.29	0.03	0.03
Grassland	0.07	0.02	0	0.23	0.06	0.08

Prevalence=how many monkeys are infected with each parasite type.

for the interior habitat there was a tendency for a greater proportion of the monkeys to be infected with *Trichuris* sp. than both grassland (Fisher's exact test, $P=0.092$) and tea (Fisher's exact test, one-tailed $P=0.052$). We found no significant differences for prevalence of *Trichuris* sp. between agriculture and interior or between grassland and tea (Fisher's exact test, $P>0.100$ in each case).

Unlike infection with *Trichuris* sp., infection with the unidentified strongyles was not related to habitat type ($\chi^2=6.012$, $P=0.111$). However, differences between the habitats were found in the prevalence of *Strongyloides* sp. ($\chi^2=7.853$, $P=0.049$). Residuals from the log-linear model indicated that more monkeys were infected with *Strongyloides* sp. in the grassland than would be expected if they lacked association (residual=2.769). When we compared the habitats individually, we found more monkeys to be infected with *Strongyloides* sp. in the grassland than the agriculture ($\chi^2=4.350$, $P=0.038$) and there was a tendency for more to be infected in the tea (Fisher's exact test, $P=0.070$) and interior ($\chi^2=3.007$, $P=0.084$). We found no significant differences between agriculture and interior or between interior and tea (Fisher's exact test, $P>0.100$).

Parasite Prevalence in Red Colobus

Prevalence of *Trichuris* sp. in red colobus also differed according to habitat type ($\chi^2=9.293$, $P=0.026$). Residuals from the log-linear model indicated that fewer monkeys were infected with *Trichuris* sp. in the grassland habitat than would be expected if they lacked association (residual=−2.653). When each habitat was individually compared the samples from the agriculture habitat did not differ from those of the tea habitat ($\chi^2<0.001$, $P=0.50$) for prevalence of *Trichuris* sp. However, both agricultural and tea samples had higher prevalence measures of *Trichuris* sp. than the grassland samples ($\chi^2=6.944$, $P=0.004$; $\chi^2=6.944$, $P=0.004$ respectively). Although these were not significant, both agriculture and tea samples had marginally higher prevalences of *Trichuris* sp. than those from the interior ($\chi^2=1.669$, $P=0.098$; $\chi^2=$

1.669, $P=0.098$ respectively). Samples from the interior had a marginally higher prevalence of *Trichuris* sp. than those from the grassland ($\chi^2=1.926$, $P=0.083$).

Like the black-and-white colobus, prevalence of the unidentified strongyle did not differ among habitats for red colobus ($\chi^2=2.667$, $P=0.446$), nor did the prevalence of *Strongyloides* sp. ($\chi^2=6.102$, $P=0.107$).

Parasite EPG in Black-and-White Colobus

Habitat differences were quantified in EPG of *Trichuris* sp. for black-and-white colobus (KW, $P<0.001$). When we compared each habitat type to the other habitat type, black-and white-colobus in agricultural had greater EPG of *Trichuris* sp. than those from both grassland (MW, $P<0.001$) and tea habitats (MW, $P<0.001$). Black-and-white colobus in the interior also had greater EPG of *Trichuris* sp. than those in both the grassland (MW, $P<0.001$) and the tea (MW, $P<0.001$). However, we found no differences between the agricultural and interior (MW, $P=0.612$) or between the grassland and tea (MW, $P=0.358$).

Although the prevalence of strongyle infections did not show differences among habitats, strongyle EPG was marginally differed among habitats (KW, $P=0.094$). We observed significant differences in EPG for *Strongyloides* sp. among habitats (KW, $P=0.045$). Here the samples from grassland had a higher EPG than agriculture (MW, $P=0.038$) and tea (MW, $P=0.034$), and there was a tendency for a similar but nonsignificant trend for the interior forest (MW, $P=0.076$). We found no differences from other habitat comparisons.

Parasite EPG in Red Colobus

We found marginal differences in EPG of *Trichuris* sp. among red colobus in different habitats (KW, $P=0.077$). When we compared samples from each habitat, as predicted samples from the agricultural (MW, $P=0.020$) and tea habitats (MW, $P=0.020$) had higher EPG than those from the grassland. But none of the other comparisons were significantly different (MW, $P>0.10$; Table II). The EPG of the unidentified strongyle did not differ for red colobus among habitat types (KW, $P=0.434$), nor did the EPG of *Strongyloides* sp. (KW, $P=0.109$).

Parasite Richness in Black-and-White and Red Colobus

For black-and-white colobus, we observed no differences in richness among the four habitats. For the red colobus, we found only two parasite types in all three habitat types: *Trichuris* sp. and the unidentified strongyles. We found *Strongyloides* sp. only in fecal samples from the agricultural habitat.

Cortisol Levels in Black-and-White Colobus and Red Colobus

We found no differences among habitats in cortisol levels for black-and-white colobus (KW, $P=0.261$). However, we found differences among cortisol levels for red colobus (KW, $P<0.001$). The cortisol levels in the tea samples were lower than those in the agricultural, grassland, and interior (MW, $P<0.001$ in all cases). We

Table II Summary of the statistical tests used when examining parasite infections of red colobus and black-and-white colobus in Kibale National Park, Uganda

	Black-and-white colobus	
Trichuris	Prevalence	EPG
	Four habitats $\chi^2=11.926$	Four habitats KW
	Two-tailed $P=0.008$	$P<0.001$
Ag vs. Tea	$P=0.006$	$P<0.001$
Ag vs. Int	$P=0.508$	$P=0.306$
Ag vs. Grass	$P=0.012$	$P<0.001$
Int vs. Tea	$P=0.026$	$P<0.001$
Int vs. Grass	$P=0.046$	$P<0.001$
Grass vs. Tea	$P=0.404$	$P=0.179$
Strongyle	Four habitats $\chi^2=6.012$	Four habitats KW
	Two-tailed $P=0.11$	$P=0.094$
Ag vs. Tea		$P=0.038$
Ag vs. Int		$P=0.328$
Ag vs. Grass		$P=0.269$
Int vs. Tea		$P=0.015$
Int vs. Grass		$P=0.145$
Grass vs. Tea		$P=0.087$
Strongyloides	Four habitats $\chi^2=7.853$	Four habitats KW
	Two-tailed $P=0.049$	$P=0.045$
Ag vs. Tea		$P=0.486$
Ag vs. Int		$P=0.342$
Ag vs. Grass		$P=0.019$
Int vs. Tea		$P=0.328$
Int vs. Grass		$P=0.038$
Grass vs. Tea		$P=0.017$
	Red colobus	
Trichuris	Prevalence	EPG
	Four habitats $\chi^2=9.293$	Four habitats KW
	Two-tailed $P=0.026$	$P=0.077$
Ag vs. Tea	$P=0.5$	$P=0.5$
Ag vs. Int	$P=0.098$	$P=0.180$
Ag vs. Grass	$P=0.004$	$P=0.010$
Int vs. Tea	$P=0.098$	$P=0.199$
Int vs. Grass	$P=0.083$	$P=0.081$
Grass vs. Tea	$P=0.004$	$P=0.010$
Strongyle	Four habitats $\chi^2=2.667$	Four habitats KW
	Two-tailed $P=0.446$	$P=0.434$
Strongyloides	Four habitats $\chi^2=6.102$	Four habitats KW
	Two-tailed $P=0.107$	$P=0.109$
Richness		
	Black-and-white colobus: No difference among habitats	Red colobus: *Strongyloides* only found in agriculture habitat

Table II (continued)

Cortisol		
	Black-and-white colobus	Red colobus
	Four habitats KW	Four habitats KW
	$P=0.261$	$P<0.001$
Ag vs. Tea		$P<0.001$
Ag vs. Int		$P=0.187$
Ag vs. Grass		$P=0.331$
Int vs. Tea		$P<0.001$
Int vs. Grass		$P=0.117$
Grass vs. Tea		$P<0.001$

Ag = agricultural edge; Tea = an edge between forest and a tea plantation; Grass = an edge between forest edge grassland with humans present; Int = an edge in the interior of the park between grassland edge and forest where humans were absent. We conducted dyadic tests with MW tests.

found no differences in cortisol for red colobus between agriculture and grassland (MW, $P=0.662$), between agriculture and interior forest (MW, $P=0.374$), or between grassland and interior forest (MW, $P=0.234$).

Discussion

Our results are complex because we studied two primate species, three parasite species with different life history strategies, and three indices of infection. However, despite this complexity general patterns were revealed. First, our results suggest that broad habitat classifications such as "edge" or "interior" are too general and that parasite infections are likely affected by more specific conditions, such as type of edge and interactions between specific edge condition and the life history of particular parasite species. Second, we predicted both humanized habitats (agriculture and tea) to have greater parasite infections than the nonhumanized habitats (interior forest and grassland); however, in general, we found that parasite infections were not greater in colobus found in humanized habitat. In fact, black-and-white colobus groups using the two humanized habitats did not have more elevated parasite infections compared to groups in the two nonhumanized habitats. For red colobus the results were mixed. Thus, differences in the nature of the infections lacked clear associations with specific habitat classifications such as "edge" or "humanized." Third, fecal cortisol did not differ between habitats for the black-and-white colobus, suggesting that proximity to edges and/or humans did not result in increased stress for these monkeys. We found differences in cortisol levels between habitats for red colobus, but the differences were not related to the specific habitat types as predicted. Fecal samples from the agriculture (humanized edge), grassland (nonhumanized edge), and interior forest (nonhumanized, nonedge) exhibited significantly higher cortisol levels than the fecal samples from the tea (humanized edge).

Neither edge habitat nor humanized edge habitat was clearly associated with higher levels of parasite infection or stress; thus classifications such as "humanized" may be inadequate. We suggest that the lack of association found here relates to a

poor understanding of the conditions that create richer parasite infections with higher prevalence levels and EPG and changes in stress. The predictions we made here about forest and humanized edges were logical extensions of previous research. For example, a 6-year study by Chapman *et al.* (2006b) found that red colobus monkeys in forest fragments had cortisol levels that were *ca*. 3.5 times those of groups in the intact forest and this difference was likely not related to differences in group size (Snaith *et al.* 2008). Fragments contain a large edge component. This elevated cortisol level corresponded to higher prevalence of nematodes, and through a path analysis it was demonstrated that food availability had a strong direct effect on population size, but it also had an indirect effect via parasite infection. We based our predictions on these findings and the general relationships that have been documented between cortisol, immune systems depression, and parasite infections (Muehlenbein 2006). However, even if these factors influence parasite infections as we envision, the infections could be similarly affected by other, unmeasured, factors. For example, low levels of canopy opening increased the light levels to individual trees, which resulted in higher protein concentrations in sun-exposed leaves, while fiber content remained constant (Ganzhorn 1995). Colobus biomass has been documented to be positively related to the protein-to-fiber ratio of available leaves (Chapman *et al.* 2004; *cf.* Chapman *et al.* 2010). Thus, it is conceivable that the high light associated with trees being on the forest edges means that the food supply for colobines is improved in this habitat, which could lower infections (Gulland 1992; Milton 1996). Alternatively, food resources associated with crop raiding or human settlements may provide food resources and influence parasite infections (Weyher 2009; Wenz *et al.* 2010).

The results obtained here also do not support the notion that the microclimatic differences that characterize edges affect infections in a consistent manner. Forest edges receive increased wind and increased solar radiation, and thus are drier than interior forest environments (Matlack 1993; Murcia 1995). Based on these dry conditions one would expect a consistent decrease in parasite infection for the parasite types we examined, with the possible exception of *Trichuris*. The observed patterns are not consistent with this expectation.

The results of our study indicate that broad habitat classifications, e.g., "humanized," may be too general to identify consistent differences in parasite infection. We suggest that the way forward is to focus on one species of parasite at a time and then add the knowledge of each of these individual species up to form a picture of how the parasite community is responding and thus to speculate on how this will influence the primates. By examining only one species at a time, it will be possible to perform more detailed analysis of transmission and in some cases evaluate the density of infective stages in different types of habitats. However, the evaluation will be complex, as it will also need to include the primate's response to the habitat. For example, are the monkeys physiologically stressed in particular types of edges, or do some habitats offer better nutrition, offsetting potential stress or greater infection risk? Following this approach and investigating the effect of such infections on the hosts' behavior and fitness will provide a significant step forward in understanding the consequences of edges, and fragmentation in general, on the conservation of primates.

References

Agresti, A. (1996). *An introduction to categorcial data analysis*. New York: John Wiley & Sons.

Appleton, C. C., & Brain, C. (1995). Gastrointestinal parasites of *Papio cynocephalus ursinus* living in the Central Namib Desert, Namiba. *African Journal of Ecology, 33*, 257–268.

Black, P. H. (1994). Central nervous system-immune system interactions – psychoneuroendocrinology of stress and its immune consequences. *Antimicrobial Agents and Chemotherapy, 38*, 1–6.

Bowman, D. D. (1999). *Georgis' parasitology for veterinarians*. St. Louis: Elsevier.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology, 83*, 575–583.

Chapman, C. A., Chapman, L. J., Naughton-Treves, L., Lawes, M. J., & McDowell, L. R. (2004). Predicting folivorous primate abundance: validation of a nutritional model. *American Journal of Primatology, 62*, 55–69.

Chapman, C. A., Gillespie, T. R., & Goldberg, T. L. (2005). Primates and the ecology of their infectious diseases: how will anthropogenic change affect host-parasite interactions? *Evolutionary Anthropology, 14*, 134–144.

Chapman, C. A., & Lambert, J. E. (2000). Habitat alteration and the conservation of African primates: case study of Kibale National Park, Uganda. *American Journal of Primatology, 50*, 169–185.

Chapman, C. A., Speirs, M. L., Gillespie, T. R., Holland, T., & Austad, K. M. (2006a). Life on the edge: gastrointestinal parasites from the forest edge and interior primate groups. *American Journal of Primatology, 68*, 397–409.

Chapman, C. A., Wasserman, M. D., Gillespie, T. R., Speirs, M. L., Lawes, M. J., Saj, T. L., & Ziegler, T. E. (2006b). Do nutrition, parasitism, and stress have synergistic effects on red colobus populations living in forest fragments? *American Journal of Physical Anthropology, 131*, 525–534.

Chapman, C. A., Struhsaker, T. T., Skorupa, J. P., Snaith, T. V., & Rothman, J. M. (2010). Understanding long-term primate community dynamics: implications of forest change. *Ecological Applications, 20*, 179–191.

Cheng, T. C. (1973). *General parasitology*. New York: Academic Press.

Chomel, B. B., Belotto, A., & Meslin, F. X. (2007). Wildlife, exotic pets and emerging zoonoses. *Emerging Infectious Diseases, 13*, 6–11.

Chourchamp, F., Yoccoz, N. G., Artois, M., & Pontier, D. (1998). At-risk individuals in feline immuno-deficiency virus epidemiology; evidence from a multivariate approach in a natural population of domestic cats (*Felis catus*). *Epidemiology and Infection, 121*, 227–236.

Chu, F. L. E., & Volety, A. K. (1997). Disease processes of the parasite *Perkinsus marinus* in eastern oyster *Crassostrea virginica*: minimum does of infection initiation, and interaction of temperature, salinity and infective cell does. *Disease of Aquatic Organisms, 28*, 61–68.

Coe, C. L., & Erickson, C. M. (1997). Stress decreases lymphocyte cytolytic activity in the young monkey even after blockade of steroid and opiate hormone receptors. *Developmental Psychobiology, 30*, 1–10.

Collias, N., & Southwick, C. (1952). A field study of population density and social organization in howling monkeys. *Proceedings of the National Academy of Sciencesof the USA, 96*, 143–156.

Coop, R. L., & Holmes, P. H. (1996). Nutrition and parasite interaction. *International Journal of Parasitology, 26*, 951–962.

de Gruijter, J. M., Gasser, R. B., Polderman, A. M., Asigri, V., & Dijkshoorn, L. (2005). High resolution DNA fingerprinting by AFLP to study the genetic variation among *Oesophagostomum bifurcum* (Nematoda) from human and non-human primates from Ghana. *Parasitology, 130*, 229–237.

de Gruijter, J. M., Ziem, J., Verweij, J. J., Polderman, A. M., & Gasser, R. B. (2004). Genetic substructuring within *Oesophagostomum bifurcum* (Nematoda) from human and non-human primates from Ghana based on random amplified polymorphic DNA analysis. *American Journal of Tropical Medicine and Hygiene, 71*, 227–233.

Despommier, D. D., Gwazda, R. W., & Hotez, P. J. (1995). *Parasitic diseases*. New York: Springer-Verlag.

Ekanayake, D. K., Arulkanthan, A., Horadagoda, N. U., Sanjeevani, G. K. M., Kieft, R., Gunatilake, S., & Dittus, W. P. J. (2006). Prevalence of *Cryptosporidium* and other enteric parasites among wild non-human primates in Polonnaruwa, Sri Lanka. *American Journal of Tropical Medicine and Hygiene, 74*, 322–329.

Ferber, D. (2000). Human diseases threatens great apes. *Science, 289*, 1277–1278.

Ganzhorn, J. U. (1995). Low-level forest disturbance effects on primary production, leaf chemistry, and lemur populations. *Ecology, 76*, 2048–2096.

Garcia, L. S. (1999). *Practical guide to diagnostic parasitology*. Washington, DC: ASM Press.

Gasser, R. B., de Gruijter, J. M., & Polderman, A. M. (2009). The utility of molecular methods for elucidating primate-pathogen relationships the *Oesophagostomum bifurcum* example. In M. A. Huffman & C. A. Chapman (Eds.), *Primate parasite ecology: the dynamics and study of host-parasite relationships* (pp. 47–62). Cambridge: Cambridge University Press.

Gillespie, S. H. (2001). Intestinal nematodes. In S. H. Gillespie & R. D. Pearson (Eds.), *Principles and practice of clinical parasitology* (pp. 561–583). New York: John Wiley & Sons.

Gillespie, T. R., Greiner, E. C., & Chapman, C. A. (2005). Gastrointestinal parasites of the colobus monkeys of Uganda. *Journal of Parasitology, 91*, 569–573.

Goldberg, T. L., Gillespie, T. R., Rwego, I. B., Wheeler, E., Estoff, E. L., & Chapman, C. A. (2007). Patterns of gastrointestinal bacterial exchange between chimpanzees and humans involved in research and tourism in western Uganda. *Biological Conservation, 135*, 511–517.

Greger, M. (2007). The human/animal interface: emergence and resurgence of zoonotic infectious diseases. *Critical Reviews in Microbiology, 33*, 243–299.

Greiner, E. C., & McIntosh, A. (2009). Collection methods and diagnostic procedures for primate parasitology. In M. A. Huffman & C. A. Chapman (Eds.), *Primate parasite ecology: The dynamics and study of host-parasite relationships* (pp. 3–28). Cambridge: Cambridge University Press.

Gulland, F. M. D. (1992). The role of nematode parasites in Soay sheep (*Ovis aries* L.) mortality during a population crash. *Parasitology Research, 105*, 493–503.

Harris, T. R., & Chapman, C. A. (2007). Variation in the diet and ranging behavior of black-and-white colobus monkeys: implications for theory and conservation. *Primates, 28*, 208–221.

Hudson, P. J. (1986). The effect of a parasitic nematode on the breeding population of red grouse. *Journal of Animal Ecology, 55*, 85–92.

Hudson, P. J., Dobson, A. P., & Newborn, D. (1992). Do parasites make prey vulnerable to predation: red grouse and parasites. *Journal of Animal Ecology, 61*, 681–692.

Jokela, J., Dybdahl, M. F., & Lively, C. M. (1999). Habitat-specific variation in life-history traits, clonal population structure and parasitism in a freshwater snail (*Potamopyrgus antipodarum*). *Journal of Evolutionary Biology, 12*, 350–360.

Kling, A., Lloyd, R., & Tachiki, K. (1992). Stress of social separation on immune function and brain neurotransmitters in cebus monkey (*Cebus apella*). *Ontogenetic and Phylogenetic Mechanisms of Neuroimmunomodulation, 650*, 257–261.

Larsen, M. N., & Roepstorff, A. (1999). Seasonal variation in development and survival of *Ascaris suum* and *Trichuris suis* eggs on pastures. *Parasitology, 1999*, 209–220.

Matlack, G. R. (1993). Microenvironment variation within and among forest edge sites in the eastern United States. *Biological Conservation, 66*, 185–194.

MacKenzie, C., Chapman, C. A., & Sengupta, R. (2011). Spatial patterns of illegal resource extraction in Kibale National Park, Uganda. *Environmental Conservation, 39*, 8–50.

Milton, K. (1996). Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler (*Alouatta palliata*) population in Panama. *Journal of Zoological Society of London, 239*, 39–63.

Muehlenbein, M. P. (2006). Intestinal parasite infections and fecal steroid levels in wild chimpanzees. *American Journal of Physical Anthropology, 130*, 546–550.

Mulley, B. G., & Unruh, J. D. (2004). The role of off-farm employment in tropical forest conservation: labor, migration, and small holder attitudes towards land in Western Uganda. *Journal of Environmental Management, 71*, 193–205.

Munger, J. C., & Karasov, W. H. (1989). Subleathal parasites and host energy budgets: tapeworm infection in white-footed mice. *Ecology, 70*, 904–921.

Murcia, C. (1995). Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution, 10*, 58–62.

Naughton-Treves, L. (1997). Farming the forest edge: vulnerable places and people around Kibale National Park, Uganda. *Geographical Review, 87*, 27–49.

Naughton, L., Alix-Garcia, J., & Chapman, C. A. (2011). A decade of forest loss and economic growth around Kibale National Park, Uganda: lessons for poverty reduction and biodiversity conservation. *Proceedings of the National Academy of Sciences of the USA, 108*, 13919–13924.

Ooi, H. K., Tenora, F., Itoh, K., & Kamiya, M. (1993). Comparative study of *Trichuris trichiura* from nonhuman primates and from man, and their differences with *T. suis*. *Journal of Veterinary Medical Science, 55*, 363–366.

Padgett, D. A., & Glaser, R. (2003). How stress influences the immune response. *Trends in Immunology, 24*, 444–448.

Ravasi, D. F. C. (2009). *Gastrointestinal parasite infections in chacma baboons* (Papio h. ursinus) *of the Cape Penninsula, South Africa: The influence of individual*, *troop, and anthropogenic factors*. D. Phil. thesis, University of Capetown.

Riggs, M. R., Haroldson, K. J., & Hanson, M. A. (2010). Analysis of covariance models for data from observational field studies. *The Journal of Wildlife Management, 72*, 34–43.

Rothman, J. M., Pell, A. N., & Bowman, D. D. (2008). Host-parasite ecology of the helminths in mountain gorillas. *Journal of Parasitology, 94*, 834–840.

Snaith, T. V., Chapman, C. A., Rothman, J. M., & Wasserman, M. D. (2008). Bigger groups have fewer parasites and similar cortisol levels: a multi-group analysis in red colobus monkeys. *American Journal of Primatology, 70*, 1–9.

Sousa, M. B. C., & Ziegler, T. E. (1998). Diurnal variation on the excretion patterns of fecal steroids in common marmoset (*Callithrix jacchus*) females. *American Journal of Primatology, 46*, 105–117.

Trexler, J. C., & Travis, J. (1993). Nontraditional regression analyses. *Ecology, 74*, 1629–1637.

Wallis, J., & Lee, D. R. (1999). Primate conservation: the prevention of disease transmission. *International Journal of Primatology, 20*, 803–826.

Wenz, A., Heyman, E. W., Petney, T. N., & Taraschewski, H. F. (2010). The influence of human settlements on the parasite community in two species of Peruvian tamarin. *Parasitology, 137*, 675–684.

Weyher, A. H. (2009). Crop raiding: The influence of behavioral and nutritional changes on primate-parasite relationships. In M. A. Huffman & C. A. Chapman (Eds.), *Primate parasite ecology: The dynamics and study of host-parasite relationships* (pp. 403–422). Cambridge, UK: Cambridge University Press.

Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A., & Lal, A. A. (1998). Wild primate populations in emerging infectious disease research: the missing link? *Emerging Infectious Diseases, 4*, 149–158.

Ziegler, T. E., Scheffler, G., & Snowdon, C. T. (1995). The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, *Saguinus oedipus*. *Hormones and Behavior, 29*, 407–424.