RESEARCH ARTICLE

Protozoan Parasites in Group-Living Primates: Testing the Biological Island Hypothesis

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A series of articles by W.J. Freeland published in the 1970s proposed that social organization and behavioral processes were heavily influenced by parasitic infections, which led to a number of intriguing hypotheses concerning how natural selection might act on social factors because of the benefits of avoiding parasite infections. For example, Freeland [1979] showed that all individuals within a given group harbored identical gastrointestinal protozoan faunas, which led him to postulate that social groups were akin to “biological islands” and suggest how this isolation could select specific types of ranging and dispersal patterns. Here, we reexamine the biological island hypothesis by quantifying the protozoan faunas of the same primate species examined by Freeland in the same location; our results do not support this hypothesis. In contrast, we quantified two general changes in protozoan parasite community of primates in the study area of Kibale National Park, Uganda, over the nearly 35 years between sample collections: (1) the colobines found free of parasites in the early 1970s are now infected with numerous intestinal protozoan parasites and (2) groups are no longer biological islands in terms of their protozoan parasites. Whatever the ultimate explanation for these changes, our findings have implications for studies proposing selective forces shaping primate behavior and social organization. Am. J. Primatol. 73:1–8, 2011. © 2011 Wiley-Liss, Inc.

Key words: protozoan infections; biological islands; Kibale National Park; nonequilibrium; climate change

INTRODUCTION

In the 1970s, a series of articles by W.J. Freeland proposed that social organization and social processes were heavily influenced by parasitic infections [Freeland, 1976a, 1977, 1979]. By noninvasive fecal sampling of primates from Kibale National Park, Uganda, Freeland [1979] showed that all individuals within a given social group harbored identical gastrointestinal protozoan faunas, whereas the protozoan faunas between groups differed substantially. This result led him to postulate that social groups were akin to island biotas, because parasite infections suggested that contact among individuals outside the group was extremely limited. He, therefore, dubbed social groups “biological islands” to reflect the intragroup homogeneity in protozoan fauna. Freeland speculated that biological islands result from frequent fecal exposure and, therefore, transmission of protozoans within a group, whereas intergroup differences resulted from group isolation and lack of fecal contamination and parasite transmission among groups. This idea led to a number of intriguing hypotheses concerning social factors upon which natural selection might act because of the benefits of avoiding parasite infections [Chapman et al., 2009; Freeland, 1976b; Loehle, 1995; reviewed in Nunn & Altizer, 2006; Nunn & Dokey, 2006]. For example, Freeland [1979] suggested that avoiding between-group disease transmission could influence dispersal rates, home range sizes, grooming patterns, and daily travel patterns. Loehle [1995]
reinvigorated the debate that social behaviors are influenced by disease risk in a wide variety of animals. To this day, these hypotheses implicate important, but largely untested, selective pressures on the social ecology of group-living animals. Furthermore, the generality of Freeland's initial findings have not been rigorously evaluated; however, a number of studies of group-living mammals have not documented that protozoan species are found either at 0 or 100% prevalence within groups [Ezenwa, 2003; Fernandez-de-Mera et al., 2003; Wenz et al., 2010].

Here, we reexamine the intestinal protozoan faunas of the primate species examined by Freeland, using groups in the same national park (Kibale National Park, Uganda) to test the biological island hypothesis. In addition, we examine the stability of parasite assemblages over time and, if changes were documented, explore factors that drive changes in parasite faunas within the primate community. Our study seeks to assess the generality of Freeland's initial findings while reevaluating the selective pressures on the social ecology of primates.

METHODS

Study Site

Kibale National Park (0°13′–0°41′N and 30°19′–30°32′E) is a 795 km² moist, mid-altitude forest in the western part of Uganda [Chapman & Lambert, 2000; Struhsaker, 1997]. It received 1,701 mm (1990–2009) during two rainy seasons. Kibale receives ~300 mm more rainfall today than it did at the start of the century, has an earlier onset of rains, less frequent droughts, and an average temperature increase of ~4°C over the last 33 years [Chapman et al., 2005].

The park consists of mid-altitude, moist semi-deciduous and evergreen forest (57%), grassland (15%), woodland (4%), lakes and wetlands (2%), and colonizing forest (19%) [Chapman & Lambert, 2000]. Established as a colonial timber reserve in 1932 and designed for the sustainable harvest of hardwoods, Kibale’s management goal shifted to biodiversity conservation when it became a National Park in 1993. The area where this study was conducted (1,500 m elevation), both in the 1974 and in 2008, neighbors Makerere University Biological Field Station, near Kanyawara, and includes two forestry compartments [K14 and K30; see Struhsaker, 1997, for a map of the forestry compartments]. Forestry compartment K-14, a 405 ha forest block, was lightly and selectively harvested from May through December 1969 (averaging 14 m³/ha or 5.1 stems/ha). Twenty-three tree species were harvested, with 9 species accounting for 94% harvest volume. Approximately 25% of all trees in compartment K-14 were destroyed by logging and incidental damage [Chapman & Chapman, 2004; Skorupa, 1988]. K-30 is a 282 ha area that has not been commercially harvested. However, before 1970, a few large stems (0.03–0.04 trees/ha) were removed by pitsawyers. This extremely low level of extraction seems to have had very little impact on the structure and composition of the forest [Chapman & Chapman, 2004; Skorupa, 1988; Struhsaker, 1997]; however, the logging conducted in neighboring forestry compartments may have influenced the demography, social structure, and behavior of animals in the unlogged forest [Bonnell et al., 2010; Chapman et al., 2010c].

Fecal Sample Collection

Our specific goal was to examine the stability of Kibale primate parasite assemblages over time. As a result, we followed the original methods used by Freeland [1979] as closely as possible. We collected fecal samples from adult and juvenile group members, from five to nine groups of each of five species (grey-cheeked mangabeys [Lophocebus albigena], red tail monkeys [Cercopithecus ascanius], blue monkeys [Cercopithecus mitis], black and white colobus [Colobus guereza], and red colobus [Procolobus rufomitratus]) (Table I). Samples were collected from groups inhabiting the same area where Freeland sampled. Freeland did not indicate the number of samples collected per group or group size, and if he had a smaller sample size or groups have become larger it could have created a bias toward more 0 or 100% prevalence estimates in his study. Olive baboons (Papio anubis) were excluded from our comparison, because Freeland [1977, 1979] collected

<table>
<thead>
<tr>
<th>Species</th>
<th># Groups sampled [Freeland, 1977]</th>
<th># Groups sampled (current)</th>
<th>Total number of individuals (current)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procolobus rufomitratus</td>
<td>5</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>Colobus guereza</td>
<td>7</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Lophocebus albigena</td>
<td>11</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Cercopithecus ascanius</td>
<td>3</td>
<td>9</td>
<td>50</td>
</tr>
<tr>
<td>Cercopithecus mitis</td>
<td>3</td>
<td>6</td>
<td>44</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>29</td>
<td>193</td>
</tr>
</tbody>
</table>

Total number of individuals sampled by Freeland [1977] was not disclosed.
nearly all his samples from savannah baboons in the Toro Game Reserve, where hunting decimated this population in the 1980s (CAC and L. Chapman, unpublished data).

All samples were collected immediately after defecation and placed into tubes containing polyvinyl alcohol (PVA). Freeland’s sample collection was conducted between June 1974 and May 1975, whereas our collection occurred between February 2008 and July 2010. Following sample collection, all tubes were sealed and transported to the McGill University for subsequent laboratory analysis.

**Slide Preparation and Protozoan Identification**

Following the protocol outlined by Freeland [1977], we prepared five thin fecal smears for each sample and stained them using trichrome stain [Wheatley, 1951]. We examined slides at 100× magnification using a light microscope (Leica Microscopes, Wetzlar, Germany). In establishing these methods we were acutely aware that the methods we used had to mimic those of Freeland as closely as possible to ensure the results obtained at the two time periods were comparable. The only difference was that we did not fix slides in Schaudin’s solution, because of limitations in obtaining this chemical overseas and our fecal samples were initially placed in PVA solution. Individuals were considered infected by a given protozoan species when their fecal sample harbored one or more distinguishable protozoans of this species.

*Entamoeba histolytica* and *E. dispar* have cysts that are morphologically indistinguishable; it was only recently that *E. dispar* was considered a distinct species [Gattii et al., 2002]. However, *E. histolytica* is pathogenic, whereas *E. dispar* is not. Lacking molecular confirmation, we were unable to differentiate these two species, so we use the name *E. histolytica/dispar*.

To learn to identity protozoans, we found it valuable to use the hematocrit-staining procedure [Bowman, 1999; Garcia, 1999], which is time consuming and expensive relative to other methods. However, to allow comparisons between the infections quantified by Freeland and ourselves, we used only trichrome-stained slides.

It should be noted that neither study could individually recognize each animal in the groups; thus, repeat samples likely occurred, and thus this should be viewed as an index of prevalence. In a quantitative evaluation of this issue, Huffman et al. [1997] contrasted incidences of infection based on the number of fecal samples obtained from chimpanzees (*Pan troglodytes*) vs. that based on the number of known individuals and documented that individual infection rates, the preferred unit of comparison, was statistically higher than rates based on all samples. This is because multiple sampling from the same individual may be required to detect an infection.

**Ethical Considerations**

The study was entirely noninvasive and observational. Our protocols were approved by the McGill Animal Care Committee, Ugandan Wildlife Authority, and the Uganda Council of Science and Technology. The procedures adhered to the American Society of Primatologists’ Principles for the Ethical Treatment of Nonhuman Primates and Ugandan law.

**RESULTS**

We identified ten protozoans: *Chilomastix mesnili*, *Dientamoeba fragilis*, *Entamoeba coli*, *Endolimax nana*, *Iodamoeba buetschlii*, *Entamoeba hartmanni*, *Entamoeba histolytica/dispar*, *Blastocystis* spp., *Isospora* sp., and *Giardia* duodenalis. Freeland [1979] documented a within-group prevalence of either 0 or 100%, suggesting that an entire primate social group is either uninfected (0) or infected (100%) with a given protozoan parasite (Fig. 1). Contrary to these findings, our results show mixed within-group prevalence, indicating that within a given primate social group, some individuals are infected, whereas others are not (Fig. 1).

Samples collected by Freeland indicated that both colobine species, *P. rufomitratus* and *C. guereza*, were uninfected by protozoans. The absence of protozoan infections was speculated to be the result of the specialized digestive tracts of colobines as a result of their folivorous diets [Freeland, 1979]. Our results, however, indicate that current groups of both *P. rufomitratus* and *C. guereza* have protozoan infections similar to the guenons (*C. ascanius, C. mitis*), and mangabeys (*L. albigena*; Fig. 1) with regard to protozoan species composition and richness.

The overall prevalence of infection within these five primates was higher in our study than in 1979 (Fig. 2). The finding of protozoans in the colobines contributes to this difference. Specifically, the prevalence of *C. mesnili*, *E. nana*, *I. buetschlii*, *E. hartmanni*, *Blastocystis* spp., and *Isospora* was higher in our study than in Freeland’s 1974 and 1975 collections. Notably, Freeland [1979] did not find *Blastocystis* spp. or *Isospora* infections in any primate group, whereas we found these protozoans at an overall prevalence of 36.6 and 14.2% within the primate community, respectively (Fig. 2). Conversely, *Giardia* spp., *E. intestinalis*, and *T. intestinalis* were found at an overall prevalence of 34.5, 34.5, and 3.4%, respectively, by Freeland [1979]. By contrast, we found only one red-tailed guenon group infected with *Giardia* spp.
DISCUSSION

Our results did not support Freeland’s biological island hypothesis [1979] that individuals within a particular social group have identical protozoan faunas, whereas groups differ in their protozoan communities. In contrast, we found mixed prevalences of protozoan infections within groups of all primate species examined. Our test of the biological island hypothesis leads us to reject it for the primates in this area of Kibale in the perspective of presence/absence of parasite species in groups and brings into question the generality of this theory. However, it is possible that while these protozoans are typically present in neighboring groups their populations may differ, because mixing or transmission events are rare. Genetic analysis of the parasites offers a more robust means of quantifying the degree of isolation of parasite population among groups, more directly addressing the ideas of Freeland’s island hypothesis. The value of genetic analysis has recently been demonstrated by Gasser et al. [2009]. 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 that primates posed a health risk to people. The eggs and even L3 obtained after coproculture can only be identified to the genus level. In contrast, genetic analysis suggests that *O. bifurcum* in humans is genetically distinct from populations in nonhuman primates, and thus these primates do not pose a health risk to people [de Gruijter et al., 2004; Gasser et al., 2009].

Our long-term behavioral observations suggest that parasite population mixing will occur fairly frequently among primate groups, and thus these groups are not “biological islands.” For example, in the Kanyawara study area, these five common diurnal primates were in mixed-species associations (i.e., intermingled or within 50 m of another group) on an average of 42.1% of their time [Chapman & Chapman, 2000]; at least five groups of black-and-white colobus used the same small eucalyptus grove of trees [Harris & Chapman, 2007], for more than 4 years 20 animals are thought to have immigrated or emigrated from our main red colobus study group (Chapman, unpublished data), and for more than 8 years, Olupot and Waser [2005] documented 68 animals entering or leaving seven mangabeys groups. All these observations suggest that the...
potential for transmission of protozoan parasites among groups is high.

The biological island hypothesis is potentially important, as evidenced by its popularity [Freeland, 1979, has been cited 88 times—Web of Science Search July 10, 2011] and the diversity of hypotheses it has led to [Loehle, 1995; Nunn & Altizer, 2006]. However, our results suggest that the hypothesis may not be generally applicable. Both our study and Freeland’s were cross-sectional surveys. Although we made every possible effort to replicate Freeland’s methods [1977, 1979], the possibility still exists that documented differences are a result of differences in the methods used or the skills of the researchers. Even if the differences we documented are authentic, a great many ecological factors differ between the 1970s and today in Kibale National Park. As a result, it is very difficult to attribute differences in parasitism over nearly 35 years to any single factor or set of factors. An increase in home range overlap or dispersal overtime might account for the observed change, but we view this as unlikely. Logging had occurred in areas adjacent to the study area in 1969 [Struhsaker, 1997], which would have likely increased overlap and dispersal as groups adjusted to reduction in food supply and not decreased it, which would be required if this mechanism was to account for the quantified changes we documented. Here, we discuss two hypotheses that could, in principle, account for the differences we observed—environmental change and nonequilibrium states.

We documented two general changes in protozoan parasite community of primates in the study area of Kibale over the nearly 35 years between sample collections: (1) the colobines found free of parasites in the early 1970s are now infected with numerous intestinal protozoan parasites and (2) groups are no longer biological islands in terms of their protozoan parasites. It is possible that differences in parasite infections over the last 35 years in Kibale correspond to environmental changes between the two collection periods. Overall, Kibale receives ~300 mm more rainfall today than it did at the start of the century (Fig. 3), an earlier onset of the rains, less frequent droughts, and an average temperature increase of ~4°C over the last 33 years [Chapman et al., 2005; Stampone et al., 2011]. Any of these conditions, alone or in combination, could have affected the suitability of the local landscape for the transmission of intestinal parasites infections [Chapman et al., 2010b; McGrew et al., 1989; Stoner, 1996; Stuart & Strier, 1995; Stuart et al., 1998]. For example, high rainfall may allow parasite eggs and infective stage larvae to persist in the environment longer, and thus increase infection risk [Gillespie, 2001; Grove, 1989]. Although parasite–host dynamics may respond to increasing moisture in different ways, increasing moisture is associated with increased macroparasite persistence, development, and transmission [Dobson et al., 1992; Nunn, 2009; Nunn & Altizer, 2006; Nunn et al., 2005; Stromberg, 1997]. Depending on the specifics of the parasite life cycle, higher temperature can, up to a threshold, reduce development time of some intestinal parasites [Claxton et al., 1999; Kutz et al., 2005; Poulin, 2006; Smith, 1990; van Dijk et al., 2010], as well as alter parasite prevalence and host–parasite dynamics [Kutz et al., 2009]. These environmental changes suggest that transmission of protozoans would have increased over the last 35 years. If this were to be the case, this is in the direction of change that would be expected to account for the colobines found free of parasites in the early 1970s now becoming infected. However, it is not clear how environmental changes related to climate would lead to particular social groups no longer having identical protozoan faunas as Freeland [1979] documented. The wetter and warmer conditions might have promoted transmission, which would presumably lead to members of the same group all having the same protozoan infections, not having mixed prevalence of protozoan infections as Freeland [1979] documented. The wetter and warmer conditions might have promoted transmission, which would presumably lead to members of the same group all having the same protozoan infections, not having mixed prevalence of protozoan infections as Freeland [1979] documented.

The differences we documented in parasite infections between now and 35 years ago could result from forest and primate communities existing in nonequilibrium states [Chapman et al., 2010a,c]. Using 18 years of data on forest change, we previously concluded that what was considered mature forest in Kibale was actually disturbed in the recent past, possibly 500–1,000 years ago [Chapman et al., 2010a]. Similarly, using nearly 40 years of census data that employed identical census
routes and methods, we demonstrated that abundance of some primate species are declining, whereas others are either increasing or stable and that these changes can be independent of changes in their food resources [Chapman et al., 2000c; Struhsaker, 1997, 2010]. Such changes have led to local changes in primate density, which is a factor known to influence the transmission of many parasite species [Altizer et al., 2003; Anderson & May, 1992; Bonnell et al., 2010]. However, we do not see any consistent response in protozoan infections that seem to be owing to these documented density changes.

In conclusion, our results suggest that the biological island hypothesis is not applicable to primate groups in Kibale National Park, Uganda, today. Whatever the ultimate explanation, this finding has implications for studies examining proposing selective pressures that may have shaped primate behavior and social organization. The comparison we attempted between data collected so far apart in time also draws attention to the importance of continuous, long-term prospective studies of primate parasitism.

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