

# Integrative Approaches to the Study of Primate Infectious Disease: Implications for Biodiversity Conservation and Global Health

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**ABSTRACT** The close phylogenetic relationship between humans and nonhuman primates, coupled with the exponential expansion of human populations and human activities within primate habitats, has resulted in exceptionally high potential for pathogen exchange. Emerging infectious diseases are a consequence of this process that has the capacity to threaten global health and drive primate population declines. Integration of standardized empirical data collection, state-of-the-art diagnostics, and the comparative approach offers the opportunity to create a baseline for patterns of infection in wild primate populations; to better understand the role of

disease in primate ecology, behavior, and evolution; and to examine how anthropogenic effects alter the zoonotic potential of various pathogenic organisms. We review these technologies and approaches, including noninvasive sampling in field conditions, and we identify ways in which integrative research activities are likely to fuel future discoveries in primate disease ecology. In addition to considering applied aspects of disease research in primate health and conservation, we review how these approaches are shedding light on parasite biodiversity and the drivers of disease risk across primate species. *Yrbk Phys Anthropol* 51:53–69, 2008. © 2008 Wiley-Liss, Inc.

Emerging infectious diseases, fueled by anthropogenic disturbance, pose a serious threat to global health and biodiversity conservation (Daszak et al., 2000; Cleaveland et al., 2001; Pederson et al., 2007; Jones et al., 2008). This is especially true in relation to nonhuman primates, whose close phylogenetic relationship with humans results in high potential for pathogen exchange (Ott-Joslin, 1993; Wolfe et al., 1998; Davies and Pederson, 2008).

Primates have long been the focus of surveillance for potential zoonoses such as yellow fever, malaria, and schistosomiasis (Coatney, 1971; Ghandour et al., 1995; Robertson, 1996). However, interest in primate-associated zoonoses has grown dramatically since the global HIV/AIDS pandemic was traced definitively to zoonotic transmission of SIV-1 from chimpanzees (Gao et al., 1999; Keele et al., 2006). Related retroviruses (i.e., simian foamy viruses) and filoviruses (i.e., ebola virus) continue to pass between wild primates and people with disquieting regularity through the widespread practice of hunting and butchering wild primates (Leroy et al., 2004; Wolfe et al., 2005). Dramatic as they may be, Ebola and HIV/AIDS are only two examples of the multitude of viral, bacterial, fungal, and parasitic pathogens that are readily transmissible from nonhuman primates to humans.

Recent studies have also confirmed transmission of potential pathogens from people and domestic animals to wild primates (Sapolsky and Else, 1987; Nizeyi et al.,

2002; Gillespie and Chapman, 2006, 2008; Chi et al., 2007; Goldberg et al., in press; Rwego et al., in press) and transmission-associated mortality (Köndgen et al., 2008). These confirmations bolster suspicions that epidemics of polio, measles, respiratory diseases, and scabies originated from humans (Goodall, 1983, 1986; Hastings et al., 1991; Hill et al., 2001; Kalema-Zikusoka et al., 2002; Hanamura et al., 2008; Kaur et al., 2008). Wild primates are also threatened by exposure to novel pathogens from wildlife reservoirs. The case of Ebola virus and its impact on chimpanzee (*Pan troglodytes*) and gorilla (*Gorilla gorilla*) populations in Gabon and Congo is perhaps the most dramatic example, with estimates of more than 80% declines in local populations (Leroy et al., 2004; Bermejo et al., 2006). Similarly, *Bacillus*

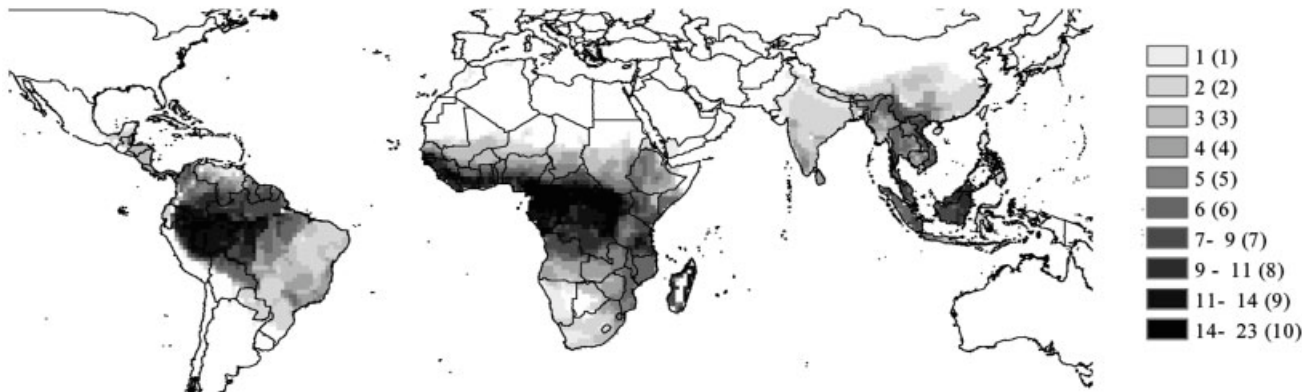
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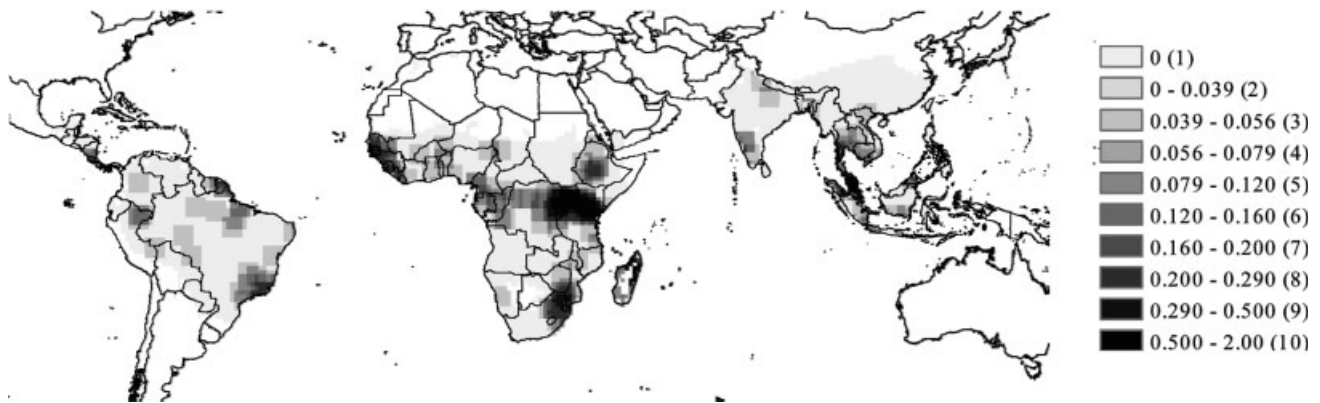
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## Distribution of Primate Species



## Sampling for Parasites



**Fig. 1.** Sampling gaps in our knowledge of primate parasites. The distribution of parasite sampling (bottom) fails to match the distribution of primate richness (top) at a global scale. Information on primate geographical ranges was obtained using geographically rectified range maps for each of the world's primate species, as compiled in a geographical information system (GIS) and used in previous studies of parasite richness in primates (Sechrest et al., 2002; Nunn et al., 2003, 2005). Plot of parasites shows smoothed sampling densities measured as the mean number of sampling localities per square-degree cell, with quantile values in parentheses. Darker shading indicates areas with the highest sampling effort (bottom) and highest primate richness (top). Redrawn using data analyzed by Hopkins and Nunn (2007).

*anthracis* (the causative agent of anthrax), has led to epidemics in chimpanzee populations (Leendertz et al., 2004b, 2006a). It remains to be determined whether such epidemics are part of natural processes or fueled by anthropogenic disturbance at local, regional, and/or global scales.

Although humans have always shared habitats with nonhuman primates, the dynamics of human-primate interactions have changed dramatically in the recent past. As human population density continues to increase exponentially, speeding the reduction and fragmentation of primate habitat, greater human-primate contact is inevitable and higher rates of pathogen transmission are likely. Our current knowledge regarding wild primate pathogens is minimal and skewed toward regions of long-term primate research (see Fig. 1). Baseline data on patterns of infection in wild primate populations are critical to provide an index of population health and to begin to assess and manage disease risks to nonhuman

primates and humans (Leendertz et al., 2006a,b). In addition, despite the dramatic examples presented earlier, the majority of primate pathogens probably exert chronic, sub-lethal effects on primates in the wild (Nunn and Altizer, 2006). It remains unclear to what extent such endemic pathogens regulate primate populations, impact primate demographics, and alter primate behavior (Chapman et al., 2005). Lastly, considering the evolutionary and ecological links between primates and their pathogens, changes in patterns of infection by naturally occurring pathogens may alert us to potentially imminent threats to primate conservation and human health. Integration of standardized data collection, state-of-the-art diagnostics, and the comparative approach offers the opportunity to create a baseline for patterns of infection in wild primate populations; to better understand the role of disease in primate ecology, behavior, and evolution; and to examine how anthropogenic effects alter the zoonotic potential of various pathogenic organisms.

TABLE 1. Categories of pathogens studied in wild primates and examples of key issues addressed

Category	Example	Species concern	Importance	References
Pathogens causing acute disease, circulating in habitat	Ebola virus	Gorilla, Chimpanzees, Humans	Highest importance, cause major outbreaks and catastrophic decline in some areas with high great ape densities	e.g. Walsh et al., 2003; Huijbregts et al., 2003; Leroy et al., 2004; Bermejo et al., 2006
Pathogens causing acute disease, originating from humans	Respiratory diseases	Great Apes, possibly all primates	High importance in research and tourism projects due to risk of direct or indirect transmission	e.g. Goodall, 1986; Homsy, 1999; Woodford et al., 2002; Nishida et al., 2003; Lonsdorf et al., 2006; Hanamura et al., 2007; Köndgen et al., 2008
Chronic pathogens naturally present in wild primates	Simian immunodeficiency virus (SIV)	Various monkey species, chimpanzees, and gorilla	Important with respect to zoonotic origin of HIV. Importance for wild primates not known	e.g. Gao et al., 1999; Santiago et al., 2003; Bailes et al., 2003; Van Heuverswyn et al., 2006; Keele et al., 2006
Pathogens potentially causing chronic diseases, transmitted from humans	Bacterial enterics (Salmonella, Shigella, <i>E. coli</i> )	Infect all primates	Zoonotic source of dysentery. Importance for wild primates not known	e.g. Goldberg et al., 2007, 2008; Rwego et al., 2008

## EMPIRICAL DATA COLLECTION

Animal populations are predominantly regulated by three factors: availability of quality food, predation, and infectious disease (Lack, 1954; Minchella and Scott, 1991; Dobson, 1995). Although a great deal of research has focused on the effects of food availability and predation on primate abundance (Struhsaker, 1976; Milton, 1982; van Schaik, 1989; Isbell, 1990; Struhsaker and Leakey, 1990; Chapman and Chapman, 1999), the role of infectious disease has remained largely unexplored until recently (Stuart and Strier, 1995; Nunn and Altizer, 2006).

Pathogens play a central role in ecosystems, affecting the ecology and evolution of specific interactions (Esch and Fernandez, 1993), host population growth and regulation (Hudson et al., 1998; Hochachka and Dhondt, 2000), and community biodiversity (Hudson et al., 2002). Pathogens can impact host survival and reproduction directly through pathologic effects and indirectly by reducing host condition (Chandra and Newberne, 1977; Boyce, 1990; Dobson and Hudson, 1992; Hudson et al., 1992; Coop and Holmes, 1996). Severe infection can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (Chandra and Newberne, 1977; Despommier et al., 1995). However, less severe infections are more common and may impair nutrition, travel, feeding, predator escape, and competition for resources or mates, or they may increase energy expenditure (Dobson and Hudson, 1992; Hudson et al., 1992; Coop and Holmes, 1996; Packer et al., 2003). In Table 1, we provide an overview of the general categories of pathogens affecting wild primates and humans with specific examples of impacts on primate conservation and global health.

Understanding pathogen life cycles and modes of infection is essential for understanding how a given pathogen is transmitted and what impact it may have on the host. These details are well known for some pathogens, such as gastrointestinal parasites and enteric bacteria, and remain to be determined for other pathogens, such as many viral pathogens (See Nunn

and Altizer, 2006 for a review). For example, the life cycles and modes of transmission of a diversity of gastrointestinal parasites commonly transmitted among primates involve contact with the free-living stages, ova, or larvae. This may involve contact with contaminated food, water, fecal material, or soil (with vertebrate or invertebrate vectors), or may involve direct contact with infected individuals (see Brown and Neva, 1983 for a review). On the other hand, little information is available with respect to transmission of viruses in primates living in the wild. However, recent findings are revealing transmission patterns of some retroviruses. For example, STLV-1 is transmitted from western red colobus monkeys (*Ptilinopus badius*) to their predator, the western chimpanzees (*Pan troglodytes verus*) resulting in infections with various and in some cases even multiple STLV-1 strains in the chimpanzees (Leendertz et al., 2004c). Noninvasive methods using urine samples for antibody detection revealed significantly that more individuals who participated in meat consumption (9 years or older) were infected compared to younger individuals (Leendertz et al., 2004a). In contrast for Simian Foamy Virus, chimpanzees were infected with SFV strains according to the chimpanzee subspecies investigation (co-evolution between virus and host) (Liu et al., 2008). In addition to infection with the subspecies specific SFV strain, as aforementioned new variants of this virus can be acquired through the predator-prey relationship, resulting in concurrent infections with species-specific and prey-species-specific virus strain in some chimpanzees (Leendertz et al., 2008). Transmission patterns of other viruses continue to be discovered.

Pathogens can be examined in wild primate populations through both invasive and noninvasive sample collection (see below), and details regarding the environment, density, behavior, and health status of primates sampled can provide critical information that may inform infection patterns. For example, habitat attributes can influence infection dynamics in free-ranging primates, with studies demonstrating that infection prevalence is higher in primates ranging in humid compared to more arid habitats (Stuart et al., 1990, 1993).

Evidence of general patterns of seasonal infection in primates is equivocal, with clear seasonal patterns of infection for some primate species (Freeland, 1977; Hausfater and Meade, 1982; Huffman, 1997), and no clear pattern for others (Gillespie et al., 2004, 2005a). Lastly, recent studies demonstrate that patterns of habitat disturbance, such as selective logging and forest fragmentation, can affect primate-pathogen dynamics in dramatic ways (Gillespie and Chapman, 2006, 2008; Gillespie et al., 2005b). Consequently, to improve our understanding of this interplay, researchers should collect data (or cite published data) on climate, habitat, patterns of disturbance, and history of the environment associated with their samples.

Host population density is of central importance to infection rates in directly transmitted pathogens (Poulin, 1998), and population-level studies have demonstrated that host density positively correlates with pathogen prevalence and diversity (Dobson and Meagher, 1996; Packer et al., 1999). However, studies that have concurrently examined primate density and habitat characteristics in relation to primate pathogen prevalence reveal that habitat characteristics may be a better predictor of infection than primate density (Stuart et al., 1993; Gillespie and Chapman, 2006, 2008). Both factors (and others) may play a role in accounting for prevalence of infection, and future research is needed to distinguish more systematically the effects of host density and habitat characteristics on patterns of infections within primate populations (see below for effects of density in interspecific comparisons).

A wide variety of primate behaviors may influence patterns of infection, including ranging, grooming, inter-individual and inter-group associations, foraging, and interspecies contacts such as preying upon other primates. For example, West African chimpanzees (*Pan troglodytes verus*) have been shown to acquire the retroviruses Simian T-Cell leukemia Virus and Foamy Viruses from their main prey, the red colobus monkey (*Procolobus badius*) (Leendertz et al., 2004c, 2008). Yellow baboons (*Papio cynocephalus*) appear to avoid potential infection by regularly rotating their sleeping sites (Hausfater and Meade, 1982). Likewise, mangabeys (*Lophocebus albigena*) presumably reduce the risk of contact with fecal contamination and consequent infection by traveling further on days of heavy rainfall and avoiding foraging in the same areas on consecutive days (Freeland, 1980). Similarly, Gilbert (1997) suggests that red howlers (*Alouatta seniculus*) reduce contact with parasites by consistently defecating above gaps in the forest vegetation.

Grooming likely affects patterns of infection in complex ways. Although grooming appears to be an important mechanism for removing potentially damaging ectoparasites (Freeland, 1981; Gilbert, 1997), there is no published study on the potential of grooming behavior to alter the risk of infection with other categories of pathogens. Indeed, grooming brings individuals into close contact, increasing the risk of transmission of pathogens with direct life cycles (see Nunn and Altizer, 2006). In addition, ectoparasites often act as intermediate hosts for gastrointestinal parasites (Muller and Baker, 1990; Despommier et al., 1995; Poulin, 1998). Consequently, primates may unintentionally infect themselves with parasites with intermediate hosts by ingesting ectoparasites while grooming (Altizer et al., 2003).

Group size may also impact infection risk (Møller et al., 1993; Altizer et al., 2003). Freeland (1979) found a significant correlation between the size of groups of mangabeys and blue monkeys (*Cercopithecus mitis*) and the number of protozoan infections each group maintained, and Freeland (1980) demonstrated that the prevalence of protozoan infections increased with group size for mangabeys. A meta-analysis of a wide variety of host-parasite relationships showed a positive correlation between intensity of infection by parasites and host group size (Côté and Poulin, 1995). It is expected that for generalist pathogens, the frequency and duration of mixed-species associations affect patterns of infection in similar ways. Thus, by limiting group size and the frequency of multi-species associations, primates may be able to limit their risk of infection (Nunn and Altizer, 2006).

Patterns of primate foraging may also affect patterns of infection. Researchers have systematically documented dietary self-medication in the great apes (Huffman and Wrangham, 1994; Huffman, 1997), and anecdotal evidence supports the potential generality of this behavior among primates and other taxa (Janzen, 1978; Phillips-Conroy, 1986; Glander, 1994; Garber and Kitron, 1997). Consequently, primatologists should be aware of the potential role self-medication may play in the patterns of parasitism they observe.

Although pathogens are a normal component of a functioning ecosystem and low-intensity infections are often asymptomatic (Anderson and May, 1979), anthropogenic change may result in altered transmission rates, pathogen host range, and virulence (Daszak et al., 2000; Patz et al., 2000). Resultant changes in host susceptibility may result in elevated morbidity and mortality, and ultimately, population declines. By evaluating fecal samples for symptoms of illness (e.g., diarrhea and blood) before collection, researchers provide data that can be analyzed in relation to patterns of infection to examine potential relationships between parasitic infections and pathology or disease in free-ranging primates (Leendertz et al., 2006b; Travis et al., 2006).

As in all species, signs of disease are rarely observed in free-ranging primates, as infected individuals often mask weakness to maintain social position and avoid attacks by predators (Alados and Huffman, 2000; Boesch and Boesch-Achermann, 2000; Lonsdorf et al., 2006). Thus, indicators of illness may be subtle, including alteration of daily routine (e.g. leaving a sleeping site late in the morning or preparing for sleep early in the evening), increased time spent resting during the day, decreased food intake rate, and inability to keep up with conspecifics during travel (Huffman and Seifu, 1989). Overt clinical signs should also be observed and recorded, including increased respiratory rate, panting, or inability to sustain physical activity (characteristic of respiratory infections) (Leendertz et al., 2006b). Additional overt symptoms observed in wild primate populations include diarrhea, "runny" noses, paralysis of limbs, vomiting, swelling of body parts and coughing (Goodall, 1970, 1983; Huffman et al., 1996; Boesch and Boesch-Achermann, 2000; Gillespie and Chapman, 2008). Complementary data on social interactions, resource use and ranging patterns of group members can give information on transmission routes and the pattern of spread of an infectious agent (Leendertz et al., 2006b). Timely identification of sick individuals and follow-up monitoring allows for such key observations and rapid necropsy in the event of death.

TABLE 2. Possible analyses and required storage tubes for various collection media used for noninvasive pathogen sampling in primates

Media	Storage tube (ml)	Possible analyses
RNA-later (Ambion)	15–50	Extraction of DNA/RNA, and antibodies
70% Ethanol	15–50	Extraction of DNA/RNA (shorter storage life than RNA-later if not frozen)
Glycerin	15	Bacterial culture
10% Formalin	15–50	Classical parasitology and electron microscopy for viruses and other pathogens
Silica beads	50	Dry feces allowing for extraction of DNA/RNA, antibodies, and hormones

## DIAGNOSTICS

Recent improvements in diagnostic capacity and greater sampling effort are rapidly improving our understanding of the diversity and distribution of pathogens in wild primates (Nunn and Altizer, 2005, 2006). Systematic determination of the presence and prevalence of pathogens in wild primates requires biological material that can be obtained either through invasive sampling (e.g., collection of blood, ectoparasites, or tissue biopsy from anesthetized animals) or through noninvasive collections of naturally excreted materials such as feces or urine. Noninvasive methods are preferred, as they present less risk of injury or death for primates and researchers; however, noninvasive diagnostics are not appropriate for the study of all pathogens, including blood parasites such as *Plasmodium*.

### Noninvasive sample collection

Routine fecal collections to aid in noninvasive surveillance of pathogens in wild primates can be stored at room temperature in several media for weeks to months prior to laboratory-based analyses (Table 2). Freezing or cooling samples can extend viable storage time.

#### Fecal collection protocol.

1. Prepare the media of choice in 15–50 ml collection tubes. Media should account for ca. 30% of the volume of the tube prior to addition of sample.
2. Before collecting feces, examine macroscopically for, and note, consistency, presence of blood, mucus, tapeworm proglottids, and adult or larval nematodes.
3. With gloved hands, use a wooden applicator or spatula to scoop a ca. 2-g sample (15 ml tube) or 6-g sample (50-ml tube) from within the fecal mass into the collection tube. By taking the sample from within the fecal mass, you reduce risks of contamination with environmental sources of viral, bacterial or parasitic contamination.
4. Close tube and label with identification number.
5. Shake tubes vigorously to maximize contact between sample and storage solution (except with silica).
6. Collect replicate sample in similar fashion using other media if supplies allow.
7. Record supplemental data for samples on datasheet (see Fig. 2 for a sample datasheet).
8. If possible, store the tubes in a fridge (about 4°C). If this is not possible, keep as cool as possible in the dark.
9. Shipment: If collaborating with a laboratory outside of the country of sample collection, consult with the national health and wildlife authorities to determine how to obtain appropriate export permits and related documents. Obtain an import permit and related documents from the collaborating laboratory.

It should also be noted that such fecal collections may also allow for paired sampling of stress and reproductive hormones. As technologies for improved field-based hormone extraction and analysis are evolving rapidly, we recommend that field-based researchers consult with specialized hormone assay laboratories well in advance of going to the field.

### Noninvasive diagnostics

Today, methods are available to screen for pathogens, as well as antibodies and antigens, using noninvasive methods. A diversity of enteric bacteria including potentially pathogenic *Salmonella*, *Shigella*, *Camphylobacter*, *Yersinia*, and *Enterococcus* can readily be cultured from fresh primate fecal material using select growth medium in a field setting. Isolated bacterial colonies can then be transported to a laboratory for species confirmation via biochemical and molecular techniques (Rwego et al., in press).

Classical parasitology offers a variety of simple yet effective methods to evaluate primate gastrointestinal parasitic infections based on the collection and analysis of fecal samples, including direct smear, acid-fast staining, fecal floatation, fecal sedimentation, and fecal culturing (Gillespie, 2006).

**Direct smear.** Direct smear involves examining a thin smear of fresh fecal material with normal saline on a microscope slide. Direct smear can demonstrate the presence of helminths and protozoa, but it has limited effectiveness when egg, larvae, or cyst concentrations are not high. In addition, large amounts of detritus in feces can interfere with identifications, and quantitative assessment of egg production is not possible (Gillespie, 2006). Consequently, we recommend this method only as a supplemental procedure for highly motile protozoa.

**Acid-fast stain.** Acid-fast stain facilitates the detection of *Cryptosporidium* oocysts, which are smaller than most other protozoa, but similar in size and shape to yeasts and other debris. *Cryptosporidium* oocysts stain bright red, while yeast and other debris absorb the counterstain. Although acid-fast staining is appropriate for preliminary or clinical confirmation, it is not sensitive enough for monitoring wild primate populations for *Cryptosporidium* sp. Immunofluorescent microscopy, described below, provides a more reliable means of assessing *Cryptosporidium* sp. and several other potentially pathogenic protozoa.

**Fecal flotation.** Fecal flotation is optimal for separating many helminth eggs and protozoan oocysts and cysts from fecal debris in fresh or preserved samples. Occasionally, parasitic mites groomed from the skin will float as well. Solutions of saturated NaCl or sugar can be effective, but NaNO<sub>3</sub> is optimal. ZnSO<sub>4</sub> and MgSO<sub>4</sub> are unsuitable for general analyses because they will not

Field Site:		Community (please use new table for each community):									
Sample Number	Example	1	2	3	4	5	6	7	8	9	10
Sample Type (RNA-later = R, Formalin = F, Silica = S, Glycerine=G)	R,F,S,G										
Name of Individual	Marius										
Date	11.11.2004										
Time of defecation	08:00										
Time of sample collection	08:02										
Place (if possible)	25/64										
Consistence of feces (hard, medium, soft, fluid)	S										
Special content (worms, bones, hair from prey, blood...)	bones and hair from monkey										
Comments	sneezing since 2 days										
Samples at room temperature untill (date)	21.11.2004										
at 4°C untill (date)	10.12.2004										
shippment (date)	10.12.2004										
Collected by (Initials)	FL										
Age and Sex of ape	12 / male										

**Fig. 2.** Sample datasheet providing important supplemental information for fecal samples collected from wild primates. This is the datasheet F. Leendertz and T. Gillespie provide for field-based collaborators involved in the Great Ape Health Monitoring Unit (GAHMU) to ensure standardization among sites.

isolate many of the nematodes commonly infecting wild primates. However,  $ZnSO_4$  is a highly effective solution for this procedure if looking specifically for *Giardia* sp.

**Fecal sedimentation.** Fecal sedimentation allows for the isolation and identification of trematodes (flukes) which, unlike other helminths, are too heavy to rise in flotation solution. Consequently, a combined recovery using fecal flotation and sedimentation techniques is recommended for best results (detailed protocols for these methods are provided in Gillespie, 2006). Fecal centrifugation will enhance sensitivity and allow detection of low numbers of ova. These techniques can be performed sequentially on fresh fecal material or fecal samples preserved in 10% buffered formalin, polyvinyl alcohol, or RNAlater (ambion).

**Fecal culture.** Fecal culture allows for morphological differentiation of nematodes. The similarities in size and appearance of the eggs of different species of gastrointestinal nematodes are such that their differentiation is extremely difficult. Their third-stage larvae, however, are sufficiently different and it is possible to distinguish between different genera, and species in some cases. Fecal culture requires fresh fecal material known to be positive for nematode infection and is suitable for the culture of trichostrongyloid, strongyloid, and rhabditoid larvae (Gillespie, 2006).

**Immunofluorescent microscopy.** Although a number of protozoans can be detected by classical parasitology and light microscopy, a subset of potentially pathogenic protozoa (*Cryptosporidium* sp., *Giardia* sp., and *Cyclospora* sp.) require immunofluorescent microscopy for reliable diagnosis. Pathogen-specific test kits (Meridian Bioscience, Cincinnati, OH) are available for *Cryptosporidium* sp. and *Giardia* sp. that facilitate identification and quantification of infection in association with immunofluorescent microscopy (Salzer et al., 2007). Some pathogens auto-fluoresce, including *Cyclospora* sp., an emerging protozoan pathogen identified in wild African primates (Eberhard et al., 2001). This makes identification and quantification via immunofluorescent microscopy reliable and simple.

**Molecular confirmations.** Molecular confirmations and further examination of epidemiological relationships among pathogens are best done using multilocus sequence typing (MLST), a method for genotyping microbes based on nucleotide sequences of internal fragments of genomically dispersed house-keeping genes (Maiden et al., 1998). Because sequence data are unambiguous, this is the "gold standard." Various PCR-based techniques are available for subtyping gastrointestinal pathogens directly from fecal or environmental samples, and MLST protocols are available for a diversity of organisms that do not require pure culture (Traub et al., 2004; Gatei et al., 2006).

**Antigen and antibody detection.** Antigen and antibody detection from feces or urine is possible for chronically carried pathogens. It has been shown, for example, that antibodies and viral RNA can be detected in feces in primates infected with SIV and Foamy Virus (Santiago et al., 2002, 2003; Ling et al., 2003; Keele et al., 2006; Takehisa et al., 2007; Van Heuverswyn et al., 2007; Liu et al., 2008). Similarly, antibodies against Simian T-cell Leukaemia Virus have been detected in urine (Leendertz et al., 2004c). Other viruses, such as enteroviruses (Kupila et al., 2005), hepatitis B virus (Makuwa et al., 2003), and those causing diarrhea can be detected in feces. Antibody detection requires an immune response in the host that is achieved only after the pathogen is chronically present and when the host maintains high antibody levels. Thus, immediately after or during an acute disease, antibodies can also be found in feces or urine but quickly dissipate to levels that do not allow for detection. Generally, noninvasive antigen and antibody tests have been developed based on individuals known to be infected with a particular pathogen, specifically by comparing results from invasive (blood) tests with outcomes from screening feces or urine.

### Invasive sampling

Invasively collected samples are necessary to study past (acute) infections through detailed antibody screenings; the carrier status of certain pathogens, such as respiratory pathogens, blood parasites, or genital pathogens; and to screen for new pathogens of potential importance for primate health. Once a baseline is established for such infections, further noninvasive tests can be established that allow larger and population oriented screenings. To perform such a screening on primates from the original habitat, samples from wild individuals have to be collected invasively via field immobilization. Anesthesia of wildlife is always connected to a risk for the animals and must be planned with extreme care, using the best available technologies and performed by appropriately trained veterinarians (Kareesh et al., 1998; Leendertz et al., 2006b). The justification should be clear, with the knowledge gained balanced against the risks involved. Once anesthesia is performed, sample collection should be as broad as possible to anticipate possible future questions. This should also be part of any anesthesia conducted in the course of marking or radio collaring project. With respect to wild primates, anesthesia has been performed on species of various sizes, ranging from tamarins to gorillas; field immobilization, examination, and recovery procedures for wild primates are outlined by Glander et al. (1991), Kareesh et al. (1998), and Olupot (2000). Briefly, each animal should receive a complete physical examination including notation of any observed abnormalities, examination of oral cavity, eyes, and ears, auscultation of heart and lungs, and recording body temperature and respiratory rates. External measurements and weight should be recorded, including body length, tail length, girth (at widest point), neck circumference, foreleg length, hind leg length, hind foot length, and forefoot length. Dentition of each animal should be digitally photographed. All persons performing sample collection or getting close to anesthetized primates should wear at a minimum, gloves and surgical mask to avoid pathogen transmission to the primates and vice versa as well as sample contamination. The following samples should be collected:

- Blood: 10–20 ml, not to exceed 1% of body weight, should be collected from the femoral vein after disinfecting the site with alcohol. Appropriate sized needles must be selected to prevent trauma and venipuncture site must be monitored briefly for hematoma formation. Today, collection of EDTA blood is recommended since this allows analyses of whole blood, plasma and “Buffy Coat”. A drop of the blood collected should be used for thin and thick smears, then the collection tube can either be centrifuged to separate plasma from cells or (if no centrifuge is available) EDTA tubes can be stored standing until the plasma has separated from the cell rich fraction. Once separation has occurred, plasma and cells should be stored separately frozen (or if not possible dried).
- Hair: Several pinches of hair pulled from the base of the tail should be collected as a source of genetic material and for detection of integumentary pathogens. A glove or instrument must be used to avoid contamination with human tissue.
- Mouth swabs: Swabs from the inside of the cheeks should be collected for detection of oral pathogens.
- Tracheal swabs: Swabs of the trachea should be collected for detection of respiratory pathogens. This is a challenging procedure for many species and requires some level of expertise. This procedure also presents a small but real risk of animal bite, even in an anesthetized animal, due to jaw reflexes.
- Penile/vaginal swabs: Swabs of the penis or vagina should be collected for detection of sexually transmitted pathogens.
- Rectal/fecal samples: Rectal swabs and fecal samples should be collected for detection of gastrointestinal pathogens.
- Ocular swabs: An eye should be swabbed for detection of ocular pathogens. This should be done with care to avoid corneal trauma from abrasion.
- Nasal swabs: Swabs of the nose should be collected for detection of respiratory pathogens.
- Ectoparasites: The hair and skin of the animals should be examined with fine-toothed combs. Any ectoparasites (e.g. lice, ticks, fleas) thus obtained should be saved for identification in 70% ethanol.

To date several methods are available for sample storage that do not require freezing, such as preservation in RNAlater (Qiagen) or drying (see overview in Leendertz et al., 2006b). Methods for sample conservation should be discussed with specialized laboratories well in advance of going to the field.

Opportunistic necropsies are another mechanism to obtain detailed information about primate pathogens. Because of the risk of disease transmission to scientific researchers, however, care must be taken to ensure that the highest possible safety standards are adopted. Minimal precautions under field conditions should include full body coverage, surgical mask, eye shields, and double gloves. Necropsies should be performed in a defined “contaminated area,” to which only protected personnel have access, and only safely packed and disinfected samples should be transferred outside this area. After the necropsy, all potentially infectious material should be burned or disinfected within the contaminated area. Only suitably trained personnel, such as veterinarians or pathologists, should conduct necropsies (Leendertz et al., 2006b). If suitably trained individuals are unavailable, however, “minimal sampling” procedures can be

performed by trained nonprofessionals. Minimal sampling entails collection of small pieces of muscle, without opening the carcass and thus reducing exposure to blood or other body fluids in the dead animal. These samples can be used to test for some pathogens that can be detected in almost any tissue, such as Ebola virus or *Bacillus anthracis*. However, a complete necropsy, pathological evaluation and sample collection from all organs will significantly enhance the chance of detecting the pathogen(s) responsible for the animal's death, as many pathogens can only be detected from specific organs, especially agents that infect the respiratory or circulatory tracts. Where advanced decomposition has occurred, samples such as muscle, skin or bones should be collected, as analyses may still detect some particularly resilient pathogens or DNA fragments (Leendertz et al., 2006b).

Serum or plasma samples obtained via field immobilization or opportunistic necropsy should be screened for antibodies of common viral and bacterial pathogens. Various immunoassays are available and the procedure should be discussed with collaborating laboratories. Often enzyme immunoassay (EIA) is used, followed by confirmatory western blot assays. Serum samples can also be tested for proteins specific to primate retroviruses using western blotting. In parallel or in addition, samples can be further tested by PCR or reverse-transcription PCR using degenerate primers designed to detect the widest possible range of viruses. Sequence analyses will allow exact characterization of genetic relationship to known viruses. DNA from blood can be used for genetic analysis (microsatellites, nuclear DNA, mitochondrial DNA), to define relatedness among animals, and to test for effects of genetics on disease susceptibility (e.g. Leendertz et al., 2004a).

Thin blood smears should be made immediately after sampling, fixed with 98% methanol, stained with 5% May-Grunwald-Giemsa solution, and examined carefully under light microscopy. This will permit the identification of malaria parasites, trypanosomes (to morphological type) and microfilaria.

Determination of the diversity and categories of pathogens to be assessed should be accomplished through consultation with all stake-holders to ensure that samples will serve the greatest potential. This will often depend on species examined, location, and potential exposure to humans, livestock, and/or introduced species.

### COMPARATIVE STUDIES OF PRIMATE PARASITISM

Field research and diagnostics facilitate investigation of the distribution of parasites among individual primates and the population-level drivers of disease risk. Such detailed species-specific data can be integrated for a diversity of species to investigate variation across species. This comparative approach is critical for understanding patterns of parasitism since many host traits that influence patterns of parasitism vary more among species than within species. For example, terrestrial substrate use (Hausfater and Meade, 1982; Nunn et al., 2000) and body mass (Nunn et al., 2003) may both influence parasitism, and the strongest test of their relative importance will occur across species, rather than within species, since variation in these traits is typically greatest across species. Consider for example, the orders of magnitude variation in body mass from a mouse lemur, at less than 100 g, to a gorilla, at more than 100 kg.

Indeed, for almost all traits of interest to primate parasitology, variation is greater across than within species.

A comparative study investigates variation in traits across species, with the goal of discerning relationships among these traits and how they influence patterns of evolution (Clutton-Brock and Harvey, 1984; Nunn and Barton, 2001). In many cases, these traits are host characters shaped largely by genetic factors, such as brain size or limb length. In other cases, they may be emergent properties of environmental, genetic and social factors, such as patterns of group size or home range area.

Parasitism clearly falls into this latter category, since the parasite community in a population of primates is not inherited like genetically determined traits (Nunn and Altizer, 2006). For example, in a comparative study of parasitism, we might be interested in the correlation between group size and prevalence of infection (Nunn and Heymann, 2005), or the influence of body mass on the number of parasite species found in different primate hosts (Nunn et al., 2003). Important to the comparative studies of parasites, past research has demonstrated that some parasite-related traits correlate with relatedness among primate species. For example, the more closely related are two primate species, the greater their similarity in levels of parasite richness (Nunn et al., 2003) and prevalence (Nunn and Heymann, 2005). Closely related primates are also more likely to share particular parasites (Davies and Pedersen, 2008). These findings suggest that as with other primate traits, parasitism can be examined across species.

Comparative studies of parasites might come across as a rather dry topic. And compiling thousands of records of host-parasite combinations across dozens of primate species (Nunn and Altizer, 2005) can have a soporific effect on even the most passionate admirer of parasites, not to mention those of us with interests that lie more in physical anthropology than parasitology. Take a moment, however, to think about what these records represent and why this endeavor is important. These parasites require primates and their close relatives for reproduction. They are as much a part of the world's biodiversity as the primates they infect. Individuals of any given primate species, such as chimpanzees, exploit a habitat in order to be reproductively successful. The same is true of parasites, and in this context, some primate species will be a more productive habitat for the reproductive success of parasites than other primate species. Parasites also have evolved a fascinating array of adaptations for reproducing through their specific host populations (Price, 1980; Bush et al., 2001). These adaptations include the use of vectors such as mosquitoes, directly passing from host-to-host, or surviving in soil or water for extended periods of time between contacts with a host. This host-parasite relationship requires that hosts and parasites coevolve. Therefore, when a primate goes extinct, its parasites also go extinct—especially its host-specific parasites (Windsor, 1995).

By viewing parasites as biological organisms, a number of fascinating and fundamental questions emerge. What aspects of primate behavior and ecology influence the number of parasites that can infect different primate species? This is essentially a question of parasite species richness and the suitability of different primates for parasites. Do primate behavioral counterstrategies to disease influence parasitism? For example, do hosts that lack a counterstrategic behavior provide a better "habitat" for parasites than hosts that exhibit the behavior?

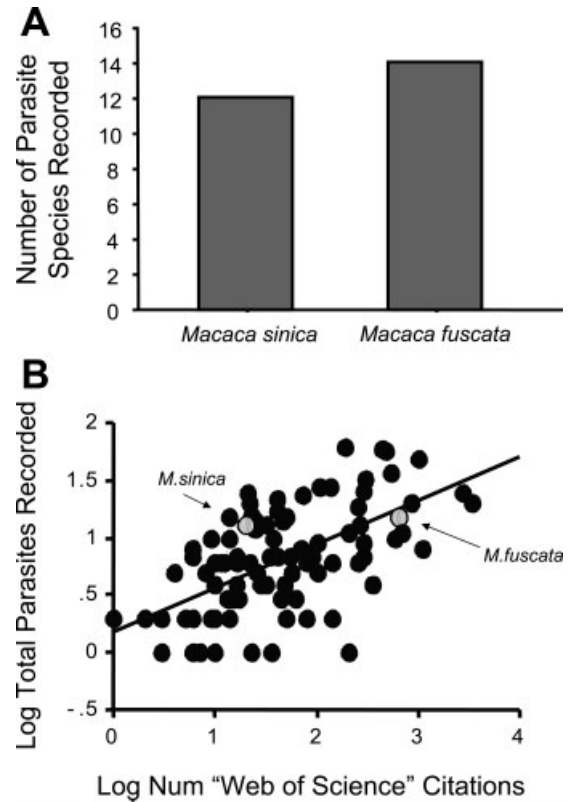
Has host speciation and extinction influenced parasite diversification? For example, given that primate hosts are undergoing an extinction crisis, are their parasites also experiencing higher rates of extinction? These questions relate to key issues in primate behavior and evolution, and have implications for understanding human evolution at global and evolutionary scales (Low, 1990; Guernier et al., 2004; Nunn and Altizer, 2006).

The most detailed comparative focus in primates involves the measure of parasite species richness, or PSR (Nunn et al., 2003, 2004, 2005; Nunn and Altizer, 2006; Nunn and Dokey, 2006; Vitone et al., 2004). PSR is simply measured as the number of parasites per host species. It is important to be as complete in sampling for parasites as possible. Omission of a group of hosts may lead to a gross underestimate of the number of parasites and may greatly affect the results of a comparative study. To this end, Charles Nunn and Sonia Altizer produced the Global Mammal Parasite Database (Nunn and Altizer, 2005). This database represents all known host-parasite records for primates and other major mammalian groups. The primate data are updated as new data becomes available, and the entire database is available for use via the Internet (<http://www.mammalparasites.org/>).

Even with a concerted effort to record all the records of parasites in primates, sampling effort will be biased. For example, groups that live in easily accessible habitats, such as terrestrial species like baboons, tend to be better studied than inaccessible species such as monkeys that live high in the trees or in swamp forests. Hopkins and Nunn (2007) found a striking mismatch between primate host density and sampling for parasites—the richest primate communities are under-sampled relative to less rich primate communities (see Fig. 1).

These sampling biases make it necessary to control for sampling effort using statistical approaches. Two major methods have been taken. One uses data compiled on the number of host individuals sampled per study, on the basis that having more animals included in a study will result in more records of parasites (Gregory, 1990; Walther et al., 1995). One problem with this approach is that some species are studied intensively for only one or a few parasites, particularly for zoonotic pathogens such as the virus that causes yellow fever or the protozoa that cause malaria. Thus, the sampling effort may be high in terms of animals sampled, yet not reflect the breadth of sampling, which is necessary for assessing patterns of parasite richness.

Another approach for controlling for sampling effort is to simply investigate how well different species have been studied, which can easily be accomplished using citation counts from online bibliographic databases. In comparative studies of primate parasite richness, the number of citations is often an excellent predictor of parasite number (see Fig. 3), outperforming even ecological variables in multivariate tests (e.g., Nunn et al., 2005). PrimateLit (<http://primatelit.library.wisc.edu/>) provides an excellent resource for tallying citation counts for primates. This bibliographic database includes peer-reviewed journal publications, which are not included in standard bibliographic search engines, as well as books. It is important to keep in mind that most attempts to control for sampling effort have ignored the importance of seasonal effects. In principle, one could restrict studies to those that have sampled throughout the year, but this would substantially reduce the number of species and is not currently feasible with the data at hand.



**Fig. 3.** Sampling effort and parasite counts. (A) Comparative tests of parasite richness require that the investigator control for how well different host species have been studied. For example, when we compared the number of parasites recorded in two species of macaques using the first version of the Nunn and Altizer (2005) database, we found that *Macaca fuscata* had 14 parasite species, while *M. sinica* had 12 parasite species. This difference could reflect that *M. fuscata* is better studied than *M. sinica*. (B) After controlling for sampling effort using ISI's Web of Science, *M. sinica* has more parasites than *M. fuscata*, with positive and negative residuals for these species, respectively. The association between citation counts and the number of parasites recorded in different anthropoid primate species is highly significant ( $t_{99} = 7.96$ ,  $P < 0.001$ , see Nunn et al., 2003).

After controlling for sampling effort, other factors influence parasite richness in primates. The major ecological and social factors that have been investigated are summarized in Table 3 and discussed in what follows (see also Nunn and Altizer, 2006).

One major predictor of parasite number is primate population density (Nunn et al., 2003). Population density was investigated based on the hypothesis that as host density increases, the conditions for parasite establishment will be met for more parasite species (Anderson and May, 1982). Consistent with this hypothesis, primate population density predicts parasite number for all parasites combined, and the diversity of the three major groups of parasites reported in primates: helminths, protozoa, and viruses (where "parasite" includes macroparasites such as helminths and protozoa, and microparasites such as fungi, viruses, bacteria, and protozoa).

In addition to host population density, geographic range is a significant predictor of both viral and protozoan species richness (Nunn et al., 2003, 2005). Geographic range is a measure of the total area covered by a species. It is commonly included in tests of parasite

TABLE 3. Summary of ecological and social variables that have been the focus of comparative studies of primate parasites

Variable	Effect
Population density	Significant in comparative tests across species, but less so within species, where habitat differences may play a bigger role.
Geographic range size	Significant predictor of viruses and protozoa in comparative tests across species. A larger geographic range could indicate greater habitat variability, overlap with more other species, or a larger host population size.
Body mass	Not significant in comparative tests that control for phylogenetic relatedness.
Latitude	Higher diversity of protozoa at lower latitudes.
Terrestrial substrate use	Not a significant predictor of parasite richness or immune defenses across species.
Group size	Not a significant predictor of parasite richness or immune defenses across species in most tests, although covaries with prevalence of malaria in New World primates.

richness because a host species with a larger geographic range typically will be exposed to a wider diversity of parasites (Dritschilo et al., 1975; Price and Clancy, 1983; Gregory, 1990). It is also possible that the size and density of geographic range are related by causal mechanisms, as the multiplication of these two variables represents an estimate of global population size. Given that a larger host population is thought to support more parasite species (e.g., Bagge et al., 2004), it could be that the critical variable in comparative tests of parasite richness is really this estimate of global population size (Nunn et al., 2003, 2005).

Body mass is also predicted to affect the number of parasites harbored by different host species, due to several mechanisms. For example, a host with a larger body mass will tend to require more resources, and therefore may incidentally ingest more infectious stages of parasites (Nunn and Altizer, 2006). In addition, according to island biogeography theory applied to parasites, a larger host might represent a larger island for parasites, resulting in more niches for parasites to colonize (Kuris et al., 1980). In most comparative studies, however, body mass has not emerged as a significant predictor of parasite richness or prevalence once phylogeny is taken into account (Nunn et al., 2003; Vitone et al., 2004; Nunn and Heymann, 2005). Thus, associations with body mass in nonphylogenetic tests (e.g., Davies et al., 1991) are likely to be artifacts of phylogenetic nonindependence. This provides strong evidence in favor of conducting phylogeny-based tests of parasite traits in primates (Nunn and Barton, 2001).

It is widely believed that parasite risk increases close to the equator in humans. A recent study confirmed this expectation (Guernier et al., 2004). Is the same true of nonhuman primates? Nunn et al. (2005) found support for this prediction in the case of protozoa (see Fig. 4) and vector-borne protozoa. The relationship shown in Figure 3 is statistically significant in a bivariate test ( $t_{107} = -3.14, P < 0.01$ ) and in multivariate models that include additional predictor variables, such as global population

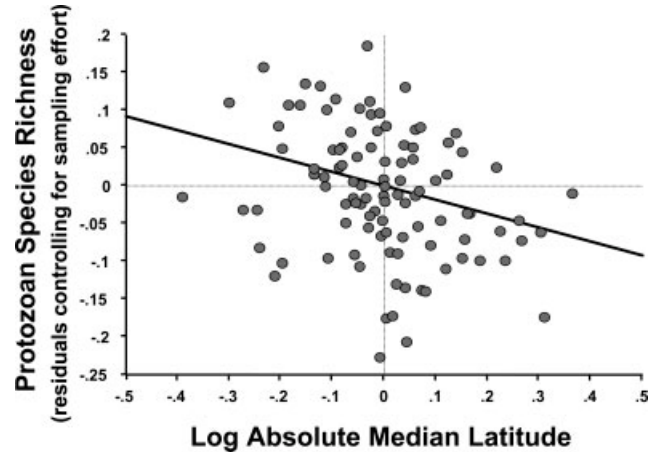


Fig. 4. Latitudinal gradient in protozoan species richness. Evolutionary increases in latitude for different lineages of primates are associated with declines in protozoan parasite richness, a finding that also holds in nonphylogenetic tests using species values (Nunn et al., 2005). In other words, species further from the equator harbor fewer protozoan parasites. Redrawn using data analyzed by Nunn et al. (2005).

size (Nunn et al., 2005). The unexplained variation in the bivariate plot reflects the influence of other factors on parasite richness, such as host population size, climatic variation, and error associated with counting parasites. Given that many vector borne diseases, such as malaria, are highly virulent and can be transmitted to a broad range of primate hosts, these results are important in the context of human health and conservation of biodiversity. As the climate warms, the distribution of vectors is likely to expand, resulting in increased disease risk to both humans and wild primates.

The nonsignificant predictor variables are equally important to understand as the significant factors, especially when there is good reason to predict that a particular variable will influence parasite richness. In addition to body mass, one such variable is substrate use. It seems plausible that an organism that commonly uses the ground will be exposed to more parasites than an organism in the trees, specifically through terrestrial contact with infectious stages of parasites in the soil and water (Nunn and Altizer, 2006). Yet this seems not to be the case in primates (Nunn et al., 2003). Terrestrial substrate use also is not a significant positive predictor of investment in immunity after controlling for body mass, e.g. in terms of white blood cell counts (Nunn, 2002a; Nunn et al., 2000) or spleen size (Nunn, 2002b). Thus, while terrestrial species might be exposed to different types of parasites, such as schistosomes that are spread through contact with water (e.g., Muller-Graf et al., 1997), the comparative data indicate that the number of parasites does not differ between terrestrial and arboreal species.

As another example, it is widely believed that living in a larger group increases the risk of infection for individual hosts (e.g., Altizer et al., 2003; Møller et al., 1993), resulting in a predicted positive correlation between group size and parasite richness. Again, a variable that would seem to be a “sure thing” in comparative studies of parasitism failed to deliver across multiple types of studies in primates, including tests of parasite richness (Nunn et al., 2003) and immune defenses (Nunn et al.,

2000; Nunn, 2002a,b; Semple et al., 2002). Moreover, the analyses that revealed robust effects of group size linked sociality to malaria (Davies et al., 1991; Nunn and Heymann, 2005). Counter to standard predictions, this involves vector-borne transmission rather than close contact among social hosts (see below).

Some recent work on insect immune defenses (and an accompanying model) provides insights as to why group size might not be an important variable in comparative studies of primate parasitism (Wilson et al., 2003). In this study, investment in immune defense declined with increasing group size, a direction that was contrary to standard predictions. The authors noted when a population is subdivided into groups; it acts to quarantine parasites in those groups, which could reduce the ability of parasites to become established in the population (and thus produces weaker selection on immune defenses). Applying this principle to primates, rates of between-group movement (transfer) could be more relevant for predicting parasite success (i.e. richness or prevalence) than is group size (see also Watve and Jog, 1997; Nunn and Altizer, 2006).

It is also possible to investigate variation in parasite prevalence across species, where prevalence is calculated as the proportion of hosts in a group or population that are infected (and averaged to produce a value for each species). Such an approach has been taken in studying malaria prevalence. In a pathbreaking study, Davies et al. (1991) first investigated this question in New World primates. They found animals with greater body mass or living in a larger group exhibited higher prevalence of malaria. In a later study, Nunn and Heymann (2005) investigated these patterns using phylogeny-based methods, which help account for the nonindependence of species values by investigating evolutionary change in two more traits (Harvey and Pagel, 1991; Nunn and Barton, 2001). They found that evolutionary changes in group size covaried with evolutionary changes in malaria prevalence, but that body mass was no longer significant after taking phylogeny into account (suggesting that body mass is not the primary evolutionary driver of differences in prevalence across species). Both sets of authors interpreted their group size results in terms of vector behavior, with a larger group of primates emitting more of the chemical attractants that draw in vectors, resulting in a higher rate of infection. Nunn and Heymann (2005) further proposed that animals sleeping in "closed" sleep sites experience lower levels of malaria infection, probably by limiting the diffusion of chemical attractants used by the mosquito vectors (see also Heymann, 1995, 2001).

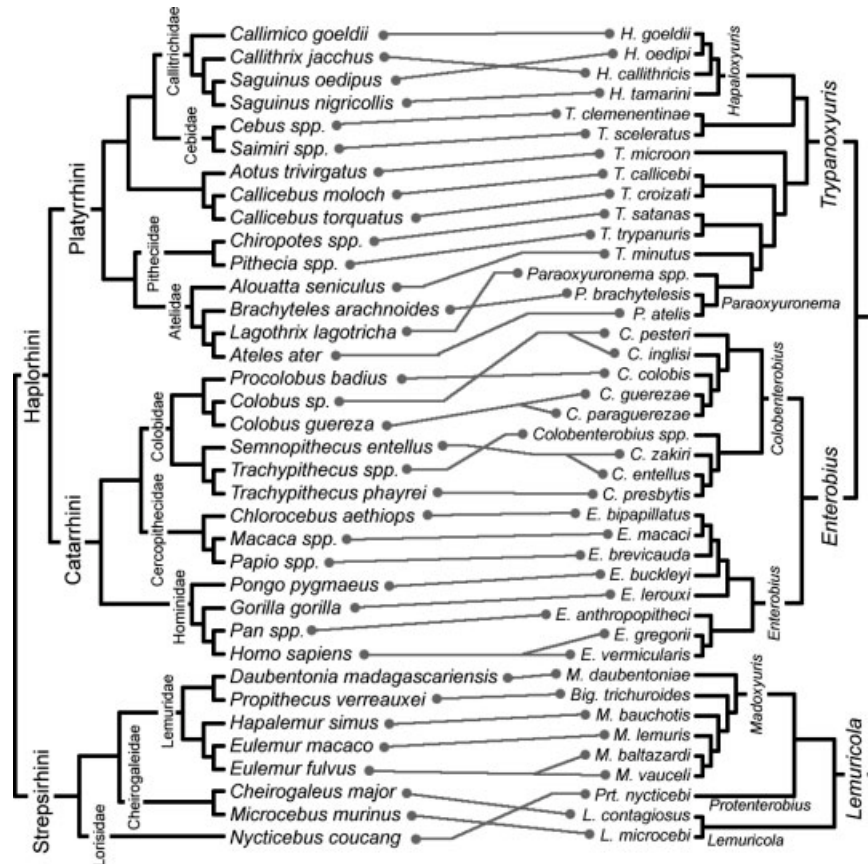
Another type of comparative study considers whether hosts and parasite phylogenies exhibit congruence. A number of studies of primates and their parasites have been conducted, and some of these are considered classics in parasitology, such as the study of primate pinworms (Brooks and Glen, 1982). A very simple (but important) question is the following: do parasite lineages tend to cospeciate with primate hosts, so that when a lineage of hosts splits, the parasites also show a corresponding split at the same point in evolutionary history, with each parasite lineage following one of the host lineages? This is exactly what is found in the case of primate pinworms (Hugot, 1999). Although the associations are not perfect (see Fig. 5), there is general congruence between the host and parasite phylogenies; statistical tests confirmed this in reconstructed coevolutionary scenarios for prosimians and New World monkeys. Cases of

incongruence can be due to a variety of factors that can be quantified using phylogenetic techniques, including parasite extinctions, speciation events, and transfers to new hosts (Page and Charleston, 1998).

Another type of coevolutionary question asks whether aspects of primate diversification have influenced patterns of parasitism or, to reverse causality and pose a more provocative question, have patterns of parasitism influenced primate host diversification? Nunn et al. (2004) investigated this pair of questions in a comparative study of parasite richness and host diversification. If one looks at primate phylogeny, it becomes apparent that some lineages have radiated more rapidly than others (Purvis et al., 1995), with increased diversification the result of increased speciation and/or decreased extinction. Nunn et al. (2004) found a positive correlation between the rate of diversification and parasite richness in primates. One explanation for this pattern is that having higher numbers of parasites might increase the rate of diversification, for example if parasites play a role in sexual selection (Lande, 1981; Barraclough et al., 1995; Turelli et al., 2001). Using data on measures that may be subject to sexual selection, such as genital coloration and sexual dimorphism, Nunn et al. (2004) found no support for this hypothesis. Alternatively, it could be that higher rates of diversification have produced conditions that make diversifying lineages more attractive habitats for parasites. Consistent with this possibility, measures of geographic range overlap covaried positively with parasite richness of protozoa and especially viruses; however, rates of diversification were unrelated to measures of geographic range overlap. Hence, this intriguing pattern has yet to receive a satisfying explanation.

A coevolutionary perspective also raises important questions concerning the conservation implications of parasites. Although many studies have demonstrated the potential negative consequences of parasites for primate conservation (see above), fewer have considered the benefits of parasites, or how extinction of parasites might be driven by population declines of primates. In recent comparative research, Altizer et al. (2007) detected hints that declines in parasite biodiversity are linked to declines in primate hosts. By comparing parasite richness in threatened and nonthreatened primates, they found that threatened species have fewer parasites (threatened corresponded to "vulnerable" or above on the IUCN Red List, Hilton-Taylor, 2002). This effect was documented for all major groups of parasites (protozoa, helminthes, and viruses), and for both generalist and specialist parasites. A plausible interpretation is that as the primate host populations become smaller and more fragmented, they support fewer species of parasites. If correct, this indicates that parasites are going extinct before their hosts, causing a cascading loss of biodiversity. In terms of conservation, biologists typically attempt to preserve intact ecosystems, i.e. the predators, prey and all other organisms in the community. As parasites are lost, host populations could become unstable, and thus conservation of parasites may be as important as conserving predators in threatened ecosystems (Hudson et al., 2006). And more generally, parasites are part of biodiversity and thus deserve conservation action as much as their primate hosts (Windsor, 1995, 1998).

Our understanding of primate disease biology is in its infancy, and the future holds many exciting challenges for comparative studies of primate parasites. First, the



**Fig. 5.** Cospeciation of primates and their pinworms. Primate phylogeny is shown on the left, pinworm phylogeny on the right. The two phylogenies show general congruence, indicating that when host lineages split, so do parasite lineages. Redrawn from Hugot (1999).

uneven geographic sampling of parasites suggests that many important gaps must be filled (Hopkins and Nunn, 2007), and methods of controlling for geographic sampling gaps are needed. We should be cautious in our interpretations from existing databases, given that more complete sampling could change some of the conclusions. Although there are no indications that we should be concerned about possible biases in studies of how host traits covary with parasitism, the geographical biases could have negative effects on our understanding of how environmental factors influence parasites. It is the field primatologists and parasitologists who make the comparative studies possible, and their research should be supported so that future comparative research will also be possible, especially when field work occurs in an area or host species identified as a “sampling gap” (Hopkins and Nunn, 2007).

Second, a number of future directions in comparative methodology and informatics are likely to provide new insights to comparative parasitology in the future. These include methods for controlling for phylogenetic uncertainty in comparative tests (Huelsenbeck et al., 2000; Lutzoni et al., 2001), as well as more sophisticated approaches for investigating evolutionary history using phylogenies (Pagel 1997, 1999). Computer programs to implement these and other advances have become readily available in the past 2 years (e.g., Pagel and Meade, 2007), and they could be applied to investigate parasite

richness or prevalence. From an informatics perspective, as more data become available online, it will be easier to incorporate taxonomic revisions on both primate hosts and their parasites—an essential undertaking, as this can greatly impact the number of parasites recorded.

Third, most of the comparative research on primate parasites has focused on parasite richness, leaving studies of parasite prevalence behind. It should also be possible to create new measures that integrate data on prevalence and richness—a parasite that occurs at low prevalence should be counted as fewer “units” in tallying parasite counts than a parasite that occurs more widely. Such a method has been used at least once (Preston et al., unpublished results), and offers much promise for broader application in the future. In a similar fashion, we desperately need information on the effects of parasites on individual hosts, because measures of “virulence” form an essential element of epidemiological models and should influence the ability of parasites to establish in host populations (and should thus influence parasite richness, see Anderson and May, 1982). More specifically, a parasite that causes fewer negative effects on its hosts (i.e., lower mortality) will be more likely to persist, and yet we presently lack tabulations of parasite virulence to use in comparative studies of primate parasitism.

Lastly, it is important to note that most of the comparative studies of primate parasites have been conducted

on counts of parasites without regard to the transmission modes of those strategies. Thus, we might expect that the predictors of helminth richness, for example, will be different than the predictors of fecally-transmitted parasites. Given that most major groups of parasites exhibit diverse transmission modes, this is likely to be a weakness of most previous research, and analyses of parasite counts broken down by transmission strategy could produce new insights or stronger support for transmission-focused hypotheses (e.g. Nunn et al., 2005; Nunn and Dokey, 2006).

## CONCLUSIONS

Integration of standardized empirical data collection, state-of-the-art diagnostics, and the comparative approach has the potential to rapidly improve our knowledge of parasites and the factors that influence parasitism within and across primate species. As our knowledge of parasites in wild populations accumulates, our understanding of disease dynamics in space and time will improve. Similarly, with more background data on patterns of infection, it will become possible to better identify pathogens responsible for wildlife declines (Leendertz et al., 2006a,b), and to target other infectious diseases that pose the greatest risks to human health.

In this article, we also integrate results from within populations and those obtained across species. In some cases, results that were significant across species (Nunn et al., 2003) were less consistently significant within species (Gillespie et al., 2004). Indeed, results from different levels of analysis will be needed for different questions. In the case of anthropogenic drivers of parasitism, for example, it might be easier to examine variation within populations, where data on stump density in the home ranges of different groups can be used to proxy human effects; similar measures would be more challenging to devise at the species level. For some other traits, however, more variation exists among different species than within them, for example in the case of body mass, substrate use and population density, or the trait only exists at the species level, such as geographic range size. Thus, the focused and broad-scale perspectives complement one another and will be needed to provide a richer understanding of the role of parasites in primate behavior and ecology. To this end, we hope this article will stimulate collaborations among field primatologists, ecologists, evolutionary biologists, and members of the medical and public health community, and we look forward to seeing what future research reveals about primate disease ecology.

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