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Body size, diet and sociality influence the species richness of parasitic worms in anthropoid primates

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ABSTRACT

Free-ranging animals are exposed to a diverse array of parasitic worms, including nematodes, trematodes, cestodes and acanthocephalans. Across host species, the number and types of parasite species are expected to depend on both host and parasite characteristics. We focused on helminth communities reported from free-living anthropoid primates to investigate factors associated with parasite diversity in a single host clade. We used a comparative data set of 305 host–parasite combinations representing 69 anthropoid primate species and 136 parasite species based on records obtained from the Host–Parasite Database at the Natural History Museum, London. We examined four sets of host characteristics that are predicted to influence parasite diversity in primates: host body size and life history; social contact and population density; diet; and individual ranging behaviour. We controlled for effects of uneven sampling effort on per-host measures of parasite diversity and repeated analyses with and without controlling for host phylogeny. In tests that did not control for host phylogeny, a large number of predictor variables were significantly associated with the diversity of both total helminths and nematode parasites, including body size, life-history variables and day range length. However, multivariate tests revealed that body mass and, to a lesser extent, social group size accounted for most variation in parasite species richness. Analyses that controlled for host phylogeny using independent contrasts showed that diet (estimated as the percentage of leaves in diet) was positively associated with total helminth and nematode parasite diversity in analyses that excluded outliers. Individual ranging behaviour was positively associated with the diversity of parasites with complex life cycles, including cestodes, trematodes and acanthocephalans. Our results demonstrate that several key features of host biology are likely to influence the community diversity of helminths in wild primate populations, including body size, diet, sociality and ranging behaviour.

Keywords: comparative study, helminths, nematodes, parasite species richness, parasitic worms, primates.

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INTRODUCTION

Parasitic worms, commonly termed helminths, are diverse and ubiquitous in wild animal populations, with potentially major impacts on host abundance and evolution (e.g. Dobson and Hudson, 1995; Hudson *et al.*, 1999; Wilson *et al.*, 2002). Wild animals typically are exposed to a variety of helminth species, including nematodes, cestodes, trematodes and acanthocephalans, representing a diverse array of transmission strategies and effects on host fitness (Poulin, 1998b; Morand, 2000; Roberts *et al.*, 2002). A single animal may contain several hundred individual parasites, with host populations harbouring diverse parasite communities (Dobson *et al.*, 1992). For example, feral Soay sheep on the island of St. Kilda harbour 20 different species of helminths alone (Gulland, 1992), and over 43 species of helminths have been reported from four species of zebras in southern Africa (Roberts *et al.*, 2002). Parasites represent an important component of natural communities, and understanding the factors that influence patterns of parasite diversity is vital to identifying ecological principles governing biodiversity and conservation (Cleaveland *et al.*, 2002; Lafferty and Gerber, 2002; Altizer *et al.*, 2003a,b).

Many host traits have been shown to correlate with parasite species richness, but few studies have evaluated a large number of predictor variables simultaneously within a single host clade (Nunn *et al.*, 2003). A broad approach is needed to understand determinants of parasite community diversity because multiple host characteristics may be correlated with one another, and different host characteristics may be important for understanding the species richness of different parasite groups. In this study, we examined the correlates of helminth species richness among anthropoid primate hosts, asking what features of host biology are associated with cross-species variation in parasite diversity. We focused on primates because they represent a diverse and well-studied mammalian order with information available on life history, behaviour, phylogeny and ecology, thus enabling us to test multiple hypotheses for the host traits that influence parasite biodiversity.

We examined four sets of factors that have been predicted to influence the community diversity of parasitic worms in wild hosts: body size and life history; social behaviour; diet; and individual ranging behaviour (Nunn *et al.*, 2003). These variables are predicted to influence host encounter rates with parasites in the wild, and the number of parasite species that can persist in populations or individual animals. A positive association is expected between body mass and parasite diversity because larger-bodied hosts represent larger 'habitats' that provide more niches for colonization (Kuris *et al.*, 1980; Poulin, 1995; Gregory *et al.*, 1996). Body mass also covaries with many life-history and behavioural variables that are predicted to influence parasite species richness. For example, larger-bodied hosts consume more food and therefore are more likely to ingest infectious stages of endoparasites. In mammals, larger-bodied hosts have longer lifespans (Harvey and Clutton-Brock, 1985; Ross and Jones, 1999) and should therefore harbour greater parasite diversity because they are more stable 'islands' for parasites and encounter more parasite species throughout their lifetimes (Pacala and Dobson, 1988). Mathematical models further predict that host life history should interact with key epidemiological processes because high host mortality (and hence short lifespan) is predicted to reduce parasite prevalence and limit the probability of parasite establishment (Anderson and May, 1991; Thrall *et al.*, 1993; De Leo and Dobson, 1996; Altizer and Augustine, 1997). As two measures of host life history we used longevity and age at first reproduction.

Social interactions generate a network of contacts through which many parasites spread

within populations (Anderson and May, 1979, 1991), and host behavioural contacts should have overriding importance for parasite community diversity (Morand, 2000; Altizer *et al.*, 2003b). If close proximity or contact among host individuals increases parasite transmission, then greater host sociality or gregariousness should translate to higher parasite prevalence and diversity for directly transmitted species (Freeland, 1976; Loehle, 1995). Increased prevalence and intensity may result directly from social contacts, whereas the size of the parasite community (parasite species richness) may increase because aggregated hosts provide a collectively larger habitat for parasites through effects analogous to island biogeography (Morand, 2000). A large number of epidemiological models, supported by data from several empirical and comparative studies, point to strong links between host density or local group size and the spread and diversity of directly transmitted parasites (Anderson and May, 1979; Packer *et al.*, 1999; Arneberg, 2001, 2002; Altizer *et al.*, 2003b). Among anthropoid primates, we measured social contact using data on average group size, the number of females in the group and local host population density.

Resource use and diet should have a major influence on host exposure to parasitic worms (Guegan and Kennedy, 1993). In primates, invertebrates consumed as prey may serve as intermediate hosts for trophically transmitted parasites (especially trematodes, cestodes and acanthocephalans), predicting increased diversity of complex life-cycle parasites among insectivorous primates relative to those that eat primarily leaves (folivores). We also tested for an effect of folivory, as folivorous primates consume a higher volume of resources and may, therefore, ingest more parasites spread via faecally contaminated food material. Moreover, certain primate species have been reported to ingest leaves with anti-helminthic properties as a form of self-medication (Huffman *et al.*, 1997), although this would lead to predictions of reduced parasite diversity as a function of increased folivory.

Finally, we examined the influence of individual ranging behaviour, based on the prediction that hosts occupying more diverse habitats are likely to encounter a larger number of parasites. Thus, animals that use a larger home range and travel a greater distance per day should encounter more helminth species through contact with the environment and other individuals. As measures of individual ranging patterns, we used information on day range length and home range size.

Because the relative importance of different host characteristics will also depend on the biology of the parasites themselves, we repeated analyses described below for two different functional groups of parasites: those transmitted largely by close or non-close contact, and those characterized by complex life cycles and transmission via intermediate hosts.

The hypotheses discussed above are not mutually exclusive, and independent variables affecting parasite species richness are likely to covary. By focusing on a well-studied host clade and including multiple predictor variables in statistical models, we evaluated the relative importance of different sets of host traits. We also addressed potentially confounding effects of uneven sampling effort and host phylogeny in examining correlates of helminth diversity, and we repeated analyses using phylogenetically independent contrasts.

MATERIALS AND METHODS

Parasite data

Parasite data on anthropoid primates were obtained from the Host–Parasite Database at the Natural History Museum in London, which includes cross-referenced lists of published

records of helminth parasites reported from a diverse range of animal hosts (for a full description, see http://www.nhm.ac.uk/zoology/hp-dat.htm). For each host–parasite record, we entered the Latin binomials for host and parasite. We modified primate host Latin binomials based on Corbet and Hill's (1991) taxonomy and used a phylogeny (Purvis, 1995) based on this taxonomy in comparative analyses described below. Only records from free-ranging populations were used in the final data set, and all but one of the records was published between 1987 and 2002.

Parasite species richness was measured as the log-transformed number of helminth species, including nematodes, cestodes, acanthocephalans and trematodes, reported from each primate host. We examined total helminth diversity and, in separate analyses, we investigated the species richness of nematodes and 'complex life-cycle helminths', with the latter category defined as cestodes, digenean trematodes and acanthocephalans that typically exhibit indirect transmission involving trophic interactions and one or more intermediate hosts. By comparison, most parasitic nematodes in our data set were transmitted by close or non-close contact (e.g. faecal–oral transmission or by contaminated substrates) or biting arthropods (S. Altizer, unpublished data), with fewer than 17% of nematodes transmitted via ingestion of intermediate hosts. Because of their different transmission strategies, the ecological variables affecting these two functional groups of parasites may differ, with complex life-cycle parasites predicted to be more strongly influenced by host ranging behaviour and carnivory (percentage of invertebrates in diet), and nematodes being more strongly influenced by factors affecting host life history, proximity and folivory.

Sampling effort

Size and diversity of the parasite community documented among different hosts may differ due to uneven sampling effort, with host species that are studied to a greater extent having more parasites reported in the published literature. To control for this effect, we followed previous researchers (Gregory, 1990; Poulin, 1995, 1998a; Walther *et al.*, 1995; Morand, 2000) by including measures of sampling effort as covariates in all analyses, as described below. The primary measure of sampling effort that we used was the number of citations from an online database, PrimateLit, which can be accessed at http://primatelit.library.wisc.edu/. This source provides the most complete reference information for journal articles and books on primates, and covers the widest sampling period (1940 to the present). We also repeated all tests using an alternative citation index, the Web of Science (hereafter referred to as WOS; see http://isi0.isiknowledge.com/ for more information), with citation counts from 1975 to the present.

Host traits

We augmented previously compiled comparative databases that included information on life history, sociality, habitat use and diet in primates (e.g. Nunn, 1999; Nunn and van Schaik, 2001). Information on unpublished data is available at http://www.phylodiversity.net/cnunn and is summarized in Table 1. Body size was estimated as mean female body mass (Smith and Jungers, 1997), longevity was measured as maximum recorded longevity in years (Ross and Jones, 1999) and age at first reproduction was measured as age at first birth in years (Ross and Jones, 1999). Using the published literature

Table 1. Data on host characteristics predicted to influence parasite species richness, including the source of data (if published) and number of anthropoid primate host species for which data were available

Variable	Source of data	Number of species
Female mass	Smith and Jungers (1997)	68
Longevity	Ross and Jones (1999)	47
Age at first reproduction	Ross and Jones (1999)	43
Day range length	Unpublished database	55
Population density	Unpublished database	58
No. of females per group	Updated from Nunn and van Schaik (2001)	56
Group size	Updated from Nunn and van Schaik (2001)	68
Home range size	Updated from Nunn and van Schaik (2001)	63
Percent insects in diet	Unpublished database	52
Percent leaves in diet	Unpublished database	55

on primate behaviour and ecology (Nunn and van Schaik, 2001), we obtained estimates of group size (mean number of adult and immature individuals), the number of females per group and population density (based on field studies of local population density, measured as the mean number of animals per km^2). Group size refers to population group size, rather than to smaller foraging units found in some species (Clutton-Brock and Harvey, 1977; Nunn and van Schaik, 2001). We also included measures of day journey length (km) and home range size (ha). Finally, diet was estimated as the percentage of leaves and insects in the diet. Unless otherwise stated, predictor variables were log-transformed before analysis.

Statistical analyses

We used multiple regression analyses to examine the association between parasite species richness and host characteristics. A common problem with such analyses is reduced sample size, because species are excluded from the analysis if they are missing data on any of the predictor variables of interest. For our data, samples sizes varied among predictor variables (Table 1) and overlap among variables was not perfect. We therefore limited the number of variables included in the model by first using focused tests to identify potential independent variables influencing parasite species richness. In each focused test, we included a single host trait and a single measure of sampling effort as independent variables. Variables that were significant or approached significance in focused tests ($P < 0.10$) were then included in iterative, stepwise regression analyses in which variables were entered in forward inclusion, or removed in backward elimination, if the significance probability was less than 0.25. All analyses were repeated for total helminth species richness (using data on all parasite groups collectively), and for nematodes and complex life-cycle helminths tested separately.

When testing specific predictions, we used directed tests rather than one-tailed tests, as these enable the detection of patterns that are opposite to predictions while retaining much of the statistical power of one-tailed tests (Rice and Gaines, 1994). Directed tests allocate a disproportionate probability under the null hypothesis to the tail of the distribution in the predicted direction (γ), while retaining a smaller probability in the opposite tail to detect

unexpected deviations from predictions ($\delta < \gamma$). Directed tests are subject to the constraint that $\delta + \gamma = \alpha$. We followed the guidelines in Rice and Gaines (1994) by setting γ/α to 0.8, giving values of $\gamma = 0.04$ and $\delta = 0.01$.

Phylogenetic comparative methods

Closely related hosts may harbour similar numbers of parasites because of common ancestry rather than similar behavioural or ecological traits, either through co-speciation of hosts and parasites, or because closely related hosts in close geographical proximity share generalist parasites. Methods for incorporating phylogenetic history are now well developed (Harvey and Pagel, 1991; Martins and Hansen, 1996) and have been applied in previous comparative studies of parasite species richness (Poulin, 1995; Gregory *et al.*, 1996; Poulin and Rohde, 1997). To control for the non-independence of species values, we ran analyses using phylogenetically independent contrasts (Felsenstein, 1985). Contrasts were calculated using the computer program CAIC (Purvis and Rambaut, 1995), with phylogenetic information from Purvis (1995). The method of independent contrasts makes several assumptions regarding the evolutionary model, the phylogeny and the quality of the data as representing valid species differences. We tested the assumptions of the method and performed sensitivity tests to determine how violations of these assumptions and different data sets affect the results (Harvey and Pagel, 1991; Garland *et al.*, 1992; Purvis and Rambaut, 1995; Nunn and Barton, 2001). Log-transformed data and branch lengths best met the assumptions of independent contrasts, but our analyses also revealed one or more outliers among the contrasts, which may indicate violation of the assumptions (Purvis and Rambaut, 1995; Harvey and Rambaut, 2000). We therefore conducted analyses with and without outliers, as determined using Mahalanobis distance measures (JMP version 4, Cary, NC). Least-squares regression of phylogenetically independent contrasts were forced through the origin (Felsenstein, 1985).

RESULTS

General patterns

Parasite records from anthropoid primates in the Host–Parasite Database spanned 69 host species and 136 parasite species, encompassed 445 lines of data and included 305 unique host–parasite species combinations. Nematodes were the most diverse group of parasites in our final data set, covering 41 genera, 88 species and 280 unique host–parasite combinations. Fewer records and less species diversity were represented by cestodes, trematodes and acanthocephalan parasite species (Table 2). The average number of helminth species reported per primate host was 4.10 (Table 2), with a maximum of 25 helminths per host reported from *Macaca mulatta*. Nematodes were the most commonly reported helminth type in anthropoid primates and acanthocephalans were the least commonly reported type (Table 2).

Similar to patterns reported from other recent studies (Raibaut *et al.*, 1998; Nunn *et al.*, 2003), we found that a small number of hosts harboured many parasite species, and most primate species had few parasite records (Fig. 1). Moreover, we found a strong association between the extent to which each host species was studied and the number of parasite species recorded in our database (Gregory, 1990; Poulin, 1995), and this was true for both

Table 2. Diversity of parasitic worms in anthropoid primates as reported by the Host–Parasite Database from the Natural History Museum, London

Parasite group	Number of genera	Number of species	Species per host (mean ± S.E.)	Maximum species per host
Total helminths	66	136	4.10 ± 0.58	25
Nematodes	41	88	2.99 ± 0.45	17
Cestodes	9	15	0.46 ± 0.09	3
Trematodes	15	27	0.58 ± 0.14	6
Acanthocephalans	3	3	0.07 ± 0.03	1

Note: Number of species refers to the total number of worm species reported in the database for each group, and mean species per host indicates the number of parasite species reported per host species.

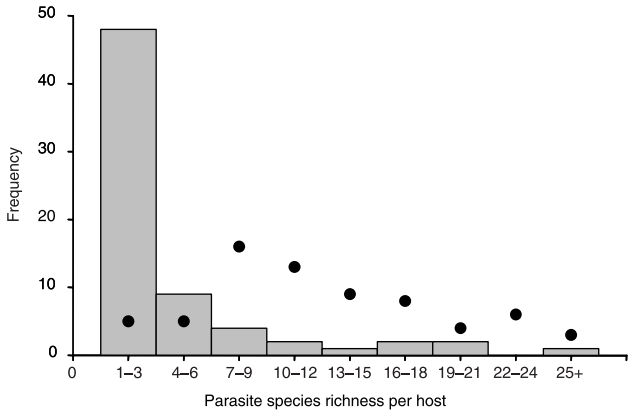

Fig. 1. Distribution of helminth parasite species among anthropoid primate hosts. Grey bars represent parasite species counts per host before controlling for sampling effort. Solid circles represent the distribution of parasite species after controlling for sampling effort (i.e. residuals from a regression of log-transformed parasite species richness against log-transformed PrimateLit citations). Bin ranges for log-transformed values after controlling for sampling effort differed from those shown in the figure and were as follows: Bin 1: −0.44 to −0.33; Bin 2: −0.32 to −0.21; Bin 3: −0.2 to −0.11; Bin 4: −0.1 to 0.0; Bin 5: 0.01 to 0.1; Bin 6: 0.11 to 0.21; Bin 7: 0.22 to 0.32; Bin 8: 0.33 to 0.43; Bin 9: 0.44 to 0.55. The distribution of parasites before controlling for sampling effort was highly aggregated, with most hosts having 6 or fewer parasites and a few host species having 12 or more helminth species. After controlling for sampling effort, parasite diversity exhibited a distribution that was more consistent with normality (see Results).

measures of sampling effort and for all major helminth groups (e.g. total number of helminths: Primate Lit, $t_{68} = 5.79$; WOS, $t_{68} = 5.57$; $P < 0.0001$ in all tests, two-tailed). After controlling for sampling effort by taking residuals, our measures of parasite species richness (PSR) per host more closely resembled a normal distribution (Fig. 1), although statistical tests showed the normality assumption was not always satisfied (e.g. using residuals from a regression of log-transformed total helminth PSR on log-transformed PrimateLit citation counts, the Kolmogorov-Smirnov test statistic was 0.110, d.f. = 69, $P = 0.037$, and the Wilk-Shapiro test statistic was 0.970, d.f. = 69, $P = 0.093$). Residuals from both measures of

sampling effort were highly correlated, indicating that these represented consistent measures of parasite diversity relative to the extent to which each host species had been studied (e.g. the correlation of total helminth PSR residuals based on PrimateLit and WOS: $r = 0.98$, $n = 69$, $P < 0.0001$). The species richness of nematodes was not correlated with the species richness of complex life-cycle helminths (e.g. using residuals from PrimateLit: $r = 0.117$, $n = 69$, $P = 0.339$; similar results were obtained when using the WOS citation counts).

Total helminth species richness

In focused tests of total helminth parasite species richness that did not control for host phylogeny, 7 of the 10 independent variables examined were statistically significant, including body mass, life-history traits, home range size, diet and group size (Table 3). When using both measures of sampling effort, total parasite species richness increased significantly with female body mass, and also increased with longevity and age at first reproduction (Table 3). Total helminth diversity also increased significantly with group size (measured as both the number of females and social group size), but was not significantly associated with population density (Table 3). Total helminth parasite species richness was positively associated with the percentage of leaves in the diet and, for one measure of sampling effort (WOS), the percentage of insects in the diet (Table 3). Finally, home range size was positively associated with helminth parasite species richness for one measure of sampling effort (PrimateLit) and was nearly significant for WOS. The significance of these 'focused' tests was not sensitive to the inclusion of outliers in the data set.

We ran a stepwise regression analysis of total helminth diversity that included the following independent variables: sampling effort, body mass, longevity, age at first reproduction, number of females per group, group size, home range size and the percentage of leaves in the diet. The analysis revealed that body mass was included in the final model regardless of

Table 3. Results of focused tests of total helminth parasite species richness based on both raw species values and independent contrasts (i.e. controlling for host phylogeny using CAIC)

	Raw species values			Independent contrasts		
Predictor	*N*	*t*	*P*	*N*	*t*	*P*
Female mass	68	3.44	0.001	62	0.91	0.241
Age at first reproduction	43	3.12	0.002	42	0.45	0.408
Longevity	47	2.58	0.008	45	1.33	0.119
Day range length	55	0.76	0.283	50	0.08	0.584
Home range size	63	1.92	0.037	57	0.18	0.538
Females per group	56	2.66	0.008	51	0.93	0.222
Population group size	68	2.18	0.033	62	0.24	0.813
Population density	58	–0.90	0.374	53	–0.02	0.985
Percent leaves in diet	55	2.35	0.014	49	1.21	0.144^{+}
Percent insects in diet	52	–1.68	0.062$^{\text{WOS } P = 0.016}$	48	–0.15	0.549

Note: Only the results of tests conducted with sampling effort based on PrimateLit citations are reported. Significance of results based on another measure of sampling effort (WOS citations) closely matched PrimateLit except where indicated. *N* indicates the number of data points entered into each test. *P*-values are based on directed tests as described in the Methods. $^{+}$Significant result when outliers were removed.

the measure of sampling effort used in the analysis (Fig. 2a). Group size was also entered into the model for both measures of sampling effort, and home range size was entered into the model for one measure of sampling effort (PrimateLit). In the final multiple regression model with body mass, home range size and group size as predictors, body mass was highly significant for both measures of sampling effort (e.g. PrimateLit: $t_{61} = 3.28$, $P = 0.001$; Fig. 2a), and group size showed a positive trend for one measure of sampling effort (PrimateLit: $t_{61} = 1.94$, $P = 0.057$), but home range size did not approach significance when tested using either measure of sampling effort.

In focused tests that controlled for host phylogeny using independent contrasts of total helminth PSR, no host traits approached significance at the 0.05 level for either measure of sampling effort. After excluding outliers, however, the percentage of leaves in the diet was positively and significantly associated with total helminth diversity (PrimateLit: $t_{46} = 2.89$, $P = 0.004$; WOS: $t_{47} = 2.00$, $P = 0.032$). Multiple regression analyses that included sampling effort, body mass and the percentage of leaves in the diet showed that the percentage of leaves was statistically significant for one measure of sampling effort (PrimateLit: $t_{45} = 1.97$, $P = 0.056$) and nearly significant for the other (WOS: $t_{45} = 1.74$, $P = 0.055$), but body mass was not significant in any stepwise tests (Fig. 2b).

Nematode species richness

Results from focused tests of nematode species richness using species values largely paralleled results for total helminth parasite species richness, probably because nematodes comprised the majority of helminths in our data set (Table 2). Thus, body size, age at first reproduction, longevity and number of females in the group were positively associated with nematode parasite species richness for both measures of sampling effort (Table 4). Nematode diversity also increased significantly with the percentage of leaves in the diet, and decreased significantly with the percentage of insects in the diet, and these results held for both measures of sampling effort (Table 4).

In stepwise regression analyses based on those variables that were significant in the focused tests, the final model included body mass, percentage of leaves in diet and the

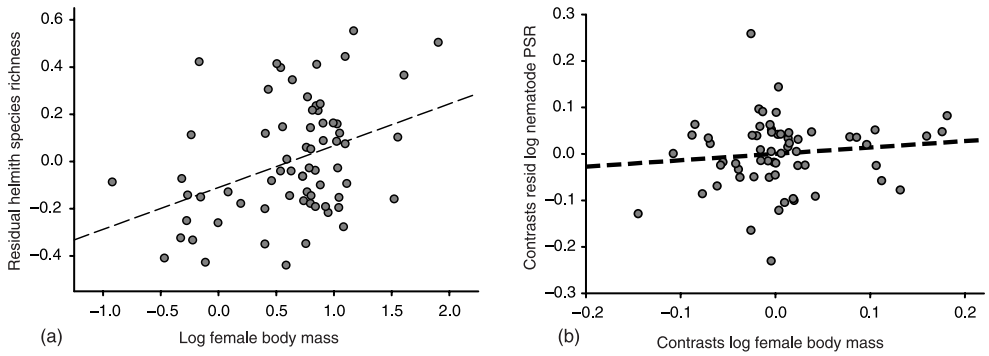

Fig. 2. Relationship between total helminth species richness and mean female body mass based on (a) raw species values and (b) independent contrasts. Sampling effort was controlled by using the residuals from a regression of log-transformed parasite counts against log-transformed PrimateLit citation counts. The results were similar for WOS citation counts.

Table 4. Results of focused tests of nematode parasite species richness based on both raw species values and independent contrasts (i.e. controlling for host phylogeny using CAIC)

	Raw species values			Independent contrasts		
Predictor	*N*	*t*	*P*	*N*	*t*	*P*
Female mass	68	3.08	0.002	62	1.27	0.132
Age at first reproduction	43	3.18	0.002	42	1.11	0.172
Longevity	47	1.81	0.048	45	0.98	0.208
Day range length	55	−0.05	0.649	50	−0.51	0.869
Home range size	63	1.22	0.142	57	0.15	0.551
Females per group	56	2.63	0.007	51	1.13	0.165
Population group size	68	1.32	0.190^{+}	62	0.17	0.869
Population density	58	−0.46	0.647	53	0.31	0.755
Percent leaves in diet	55	2.84	0.004	49	2.24	0.019
Percent insects in diet	52	−2.16	0.022	48	−1.09	0.175

Note: Only results of tests conducted with sampling effort based on PrimateLit citations are reported. Significance of results based on another measure of sampling effort (WOS citations) closely matched PrimateLit except where indicated. *N* indicates the number of data points entered into each test. *P*-values are based on directed tests as described in the Methods. $^{+}$Significant result when outliers were removed.

number of females per group. Regression results showed that body mass was significantly associated with nematode parasite species richness for one measure of sampling effort and approached significance for the other (PrimateLit: $t_{53} = 1.91$, $P = 0.039$; WOS: $t_{53} = 1.58$, $P = 0.076$), and the same was true for the number of females in the group (PrimateLit: $t_{46} = 2.29$, $P = 0.017$). The percentage of leaves in the diet was not significantly associated with nematode parasite species richness for either measure of sampling effort in stepwise analyses.

In focused tests of nematode parasite species richness based on phylogenetically independent contrasts, the percentage of leaves in the diet was the only statistically significant variable, and this was significant for both measures of sampling effort (Table 4). When outliers were excluded, this variable became highly significant for both measures of sampling effort (PrimateLit: $t_{46} = 3.75$, $P < 0.0001$; WOS: $t_{47} = 2.88$, $P = 0.004$). Only one other independent variable, the number of females per group, approached significance for phylogenetic tests that excluded outliers, and this was only for one measure of sampling effort (PrimateLit: $t_{47} = 1.75$, $P = 0.055$). Stepwise regression analyses that included sampling effort, body mass and the percentage of leaves in the diet showed that the percentage of leaves was significantly associated with nematode parasite species richness for one measure of sampling effort when outliers were included (WOS: $t_{48} = 1.99$, $P = 0.033$) and for both measures of sampling effort when outliers were excluded (PrimateLit: $t_{46} = 2.40$, $P = 0.013$; WOS: $t_{46} = 2.78$, $P = 0.005$).

Complex life-cycle helminth species richness

Few independent variables were found to be associated with the diversity of complex life-cycle helminths (digenean trematodes, cestodes and acanthocephalans) when examined in focused tests using raw species values. Only day range length was statistically significant for

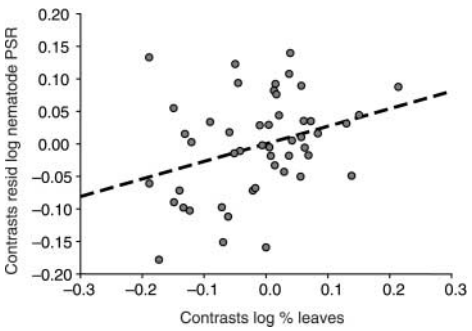

Fig. 3. Relationship between nematode species richness and percent leaves in diet using independent contrasts. Sampling effort was controlled by using residuals from regression of the contrasts of nematode parasites on PrimateLit citation counts. Contrasts were calculated using log-transformed values.

Table 5. Results of focused tests of complex life-cycle parasite species richness based on both raw species values and independent contrasts (i.e. controlling for host phylogeny using CAIC)

	Raw species values			Independent contrasts		
Predictor	*N*	*t*	*P*	N	*t*	*P*
Female mass	68	1.29	0.127	62	−0.34	0.792
Age at first reproduction	43	1.05	0.186	42	−1.04	0.758
Longevity	47	1.60	0.073	45	0.50	0.386
Day range length	55	2.87	0.005	50	1.48	0.092
Home range size	63	1.65	0.066^{+}	57	0.19	0.532
Females per group	56	0.94	0.218	51	−0.36	0.801
Population group size	68	1.81	0.076	62	−0.77	0.443
Population density	58	−1.59	0.132	53	−0.96	0.343
Percent leaves in diet	55	0.07	0.650	49	−0.96	0.212
Percent insects in diet	52	0.24	0.507	48	1.24	0.139

Note: Only results of tests conducted with sampling effort based on PrimateLit citations are reported. Significance of results based on another measure of sampling effort (WOS citations) closely matched PrimateLit except where indicated. *N* indicates the number of data points entered into each test. *P*-values are based on directed tests as described in the Methods. $^{+}$Significant result when outliers were removed.

both measures of sampling effort (Table 5), and home range size approached significance for one measure of sampling effort (PrimateLit). When outliers were excluded, day range length was significant for both sampling effort measures, and home range size became statistically significant when tested together with PrimateLit ($t_{57} = 2.31$, $P = 0.015$). No other independent variables were significantly associated with complex life-cycle helminth parasite species richness, with or without outliers. Because only one independent variable (day range length) was consistently associated with the diversity of complex life-cycle helminths, stepwise regression analyses were not performed.

None of the independent variables were significantly associated with complex life-cycle helminth parasite species richness in focused tests using independent contrasts, and this was

true for both measures of sampling effort and with and without outliers included in the statistical tests. We therefore did not pursue stepwise or multiple regression analyses of independent contrasts for this dependent variable.

DISCUSSION

Our results demonstrate that four key features of host biology were associated with the diversity of helminth parasites in wild primate populations. First, larger-bodied primates harboured a greater diversity of both total helminths and parasitic nematodes in non-phylogenetic analyses. Second, resource use influenced patterns of parasite species richness, with leaf-eating primates exhibiting increased species richness of nematodes and, to a lesser extent, total helminths when tested using independent contrasts. Third, sociality played a role in accounting for parasite diversity, with group size and the number of females per group explaining significant variation in the diversity of all helminth parasites and of nematodes, respectively, in non-phylogenetic analyses. Finally, individual ranging patterns, including home range size and day range length, accounted for the diversity of total helminths and complex life-cycle helminths, again in non-phylogenetic analyses. Thus, the strength of the effects differed according to the method of analysis and the types of helminths examined.

We followed previous researchers by implementing two methodological advances to study parasite species richness. First, we controlled for effects of uneven sampling effort on per-host measures of parasite diversity by repeating analyses with two different citation counts as estimates of the extent to which each host species has been studied (Gregory, 1990; Poulin, 1995; Walther *et al.*, 1995; Poulin and Rohde, 1997; Morand and Harvey, 2000; Arneberg, 2002). Measures of sampling effort were positively associated with estimates of helminth parasite species richness in all analyses, both with and without controlling for host phylogeny. Although the total number of individuals sampled for each host species might also provide another means to control for sampling effort (Walther *et al.*, 1995; Nunn *et al.*, 2003), this information was not available in the present study. Using a different data set, however, Nunn *et al.* (2003) showed that residual parasite diversity estimates based on citation indices and the number of animals sampled were significantly positively correlated, indicating that these two measures of sampling effort provide similar assessment of the extent to which host taxa have been studied for parasites.

Second, we controlled for phylogeny in our comparative tests by using independent contrasts (Felsenstein, 1985; Purvis, 1995). We found that this greatly affected the conclusions of the statistical tests, with some variables, such as body mass, being highly significant in non-phylogenetic tests, but non-significant once phylogeny was taken into account. In fact, several previous studies of parasite species richness have reported that the significance of independent variables depended on whether tests controlled for host phylogeny. In birds, for example, Poulin (1995) found an association between body mass and helminth species richness when using species values, but this pattern disappeared after correcting for phylogeny. In an analysis of terrestrial mammals, Morand and Poulin (1998) found that body mass became non-significant after taking phylogeny into account, and the effect of population density actually switched signs, becoming positive when controlling for phylogenetic relationships. Finally, Nunn *et al.* (2003) found similar effects in micro- and macroparasites reported from primates using an independently derived data set, with body mass becoming largely non-significant once phylogeny was taken into account.

An emerging pattern from several recent comparative studies of parasite species richness is that host body mass often is significant in non-phylogenetic analyses, but becomes non-significant once phylogeny is taken into account. Few previous studies have attempted to address the causes that underlie this pattern (Nunn *et al.*, 2003). One possibility is that body mass may be linked with many different processes affecting parasite transmission and persistence, including niches available for parasite colonization, ingestion of parasites through increased metabolic needs, host density and habitat use, and the correlated effects of body mass with host life-history traits (see above and Gregory *et al.*, 1996). For example, large-bodied hosts of some mammalian orders, such as primates, tend to be terrestrial (Clutton-Brock and Harvey, 1977; Harvey and Clutton-Brock, 1985; Nunn and Barton, 2001) and most have 'slow' life histories (e.g. increased longevity, delayed age at first reproduction). Whereas some associations between body mass and other host traits are expected to be positive, other correlations are negative (e.g. population density declines with increasing body mass). Thus, a high degree of collinearity among predictor variables, and a combination of positive and negative associations with parasite species richness, may lead to unstable statistical models. More complete data for a large number of host characteristics may provide more consistent results in phylogenetic and non-phylogenetic tests, and should also pinpoint better the processes by which body size influences host–parasite interactions.

Diet has been shown to be an important predictor of helminths in birds and fish, with omnivory being associated with greater parasite diversity (Bell and Burt, 1991; Guegan and Kennedy, 1993; Galaktionov, 1996). Multivariate tests that also controlled for host phylogeny showed that, among primates, the percentage of leaves in hosts' diets explained significant variation in the diversity of nematode parasites. Primates that consume more leaves tend to be larger in body size and, consequently, may consume more total biomass, thus leading to greater probabilities of ingesting infectious stages of gastrointestinal nematode parasites. Moreover, folivory may be associated with a higher probability of consuming faecal-contaminated material (relative to frugivory or insectivory), leading to greater opportunities for transmission of macroparasites with direct life cycles. For example, Freeland (1980) found that mangabeys (*Cercocebus albigena*) commonly defecate on vegetation, and predicted that mangabeys should move longer distances during dry weather to minimize the risk of parasite infection.

The results for parasitic nematodes were generally congruent with those for total helminth diversity, with body size and sociality significant in non-phylogenetic tests, and diet (percentage of leaves) significant in tests based on independent contrasts (see Table 4). By comparison, virtually no variables tested were significant in analyses of complex life-cycle helminths. Individual ranging behaviour (day range length) was statistically significant in one test, perhaps because animals that use a larger home range and travel a greater distance per day encounter more parasite species. It is surprising that insectivory was not positively associated with the diversity of complex life-cycle helminths, as invertebrates consumed as prey may serve as intermediate hosts for trematodes, cestodes and acanthocephalans. One possible explanation may be that other invertebrate groups, including molluscs or non-insect arthropods, are more important than insects as intermediate hosts for trophically transmitted parasites. This result may also be due to our focus on anthropoid primates, few of which are exclusive insectivores. Expanding the taxonomic scope to include prosimian primates may produce significant results for analyses of insectivory. Finally, by relying on multiple host species for transmission, the diversity of complex life-cycle helminths may be 'decoupled' from the traits of any single host.

Another possible cause for the lack of significant results involving complex life-cycle parasites concerns the relative abundance of different groups of helminths that infect anthropoid primates. In the Host–Parasite Database used in this study, nematodes represented the greatest number of species and the highest parasite diversity on a per-host species basis (Table 2). This was also found using an independent compilation of parasites from the published literature (Nunn *et al.*, 2003). Such a pattern may be caused by reporting biases or the ease of sampling hosts for nematodes, but could also be caused by limitations on parasite community diversity for those species spread through complicated transmission cycles requiring multiple host species. For example, complex life cycles may be more difficult to maintain, leading to the extinction of host–parasite relationships, and such complex multi-host associations are difficult to form, reducing the diversification of parasites that specialize on such multi-host associations. Our failure to identify any strong independent variables associated with the group that includes cestodes, trematodes and acanthocephalans may result from their relative rarity among primate parasites (e.g. far fewer species and lower taxonomic diversity relative to nematodes; Table 2).

One major difference between this study and the results of an earlier analysis of primate parasites (Nunn *et al.*, 2003) is that population density was not significant in any tests based on records from the Host–Parasite Database, whereas Nunn *et al.* (2003) reported that parasite diversity increased significantly with population density in tests that controlled for host phylogeny. Previous studies have also found that population density can produce inconsistent results (Morand and Poulin, 1998), possibly due to error in measuring this variable, or because patterns of helminth diversity are more finely tuned to overall population size (total number of animals in a population) rather than density – that is, the number of animals per unit area (Nunn *et al.*, 2003). In fact, although Nunn *et al.* (2003) found that population density was an important predictor of protozoan and viral parasite diversity, the results for population density as a predictor of helminth species richness were weak relative to other parasite types, with results affected by inclusion of outliers. Moreover, the host species represented in our study did not overlap perfectly with the species analysed by Nunn *et al.* (2003), with only 61 species in common among the 69 species that we analysed.

Anthropoid primates are hosts to an incredible diversity of helminth parasites, with the records in the current data set alone encompassing 136 parasite species. Each primate species harboured an average of more than 4 different helminth species, with up to 25 parasite species in a single host (*Macaca mulatta*). These numbers probably far underestimate the actual diversity of helminths infecting wild primates, with records limited by sampling of wild hosts, biases towards parasites of greatest concern to human health, and taxonomic resolution of helminths recovered from wild populations. In considering the role of parasites and infectious diseases in primate conservation, it is important to assess the relative impacts of primate extinctions on the biodiversity represented by their parasites. For example, many parasites specific to endangered or threatened mammals may themselves become extinct with their specific hosts (Gompper and Williams, 1998), and hosts that lose their parasites during population declines or in captive breeding programmes may also lose their ability to respond to future parasite threats (Cunningham, 1996; Altizer *et al.*, 2003a).

To summarize, our analyses of helminth diversity in a well-studied host group demonstrated that broad patterns of parasite species richness were explained by a relatively small number of host characteristics, especially host body size, social group size, diet and, to a lesser extent, individual ranging behaviour. Thus, processes driven by host biology are likely

to both facilitate and limit the diversity of host–parasite interactions observed in natural populations. A better understanding of these processes should provide insights into the types of parasites that threaten rare or endangered species. Moreover, incorporating characteristics of the parasites themselves, including specificity and transmission strategy, will allow us to test process-oriented hypotheses about host–parasite combinations that occur in wild populations.

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