THE ROYAL SOCIETY

Received 4 July 2002
Accepted 25 October 2002
Published online 9 January 2003

A comparative study of white blood cell counts and disease risk in carnivores

Charles L. Nunn*, John L. Gittleman and Janis Antonovics

Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22904-4328, USA

In primates, baseline levels of white blood cell (WBC) counts are related to mating promiscuity. It was hypothesized that differences in the primate immune system reflect pathogen risks from sexually transmitted diseases (STDs). Here, we test for the generality of this result by examining hypotheses involving behavioural, ecological and life-history factors in carnivores. Again, we find a significant correlation in carnivores between mating promiscuity and elevated levels of WBC counts. In addition, we find relationships with measures of sociality, substrate use and life-history parameters. These comparative results across independent taxonomic orders indicate that the evolution of the immune system, as represented by phylogenetic differences in basal levels of blood cell counts, is closely linked to disease risk involved with promiscuous mating and associated variables. We found only limited support for an association between the percentage of meat in the diet and WBC counts, which is consistent with the behavioural and physiological mechanisms that carnivores use to avoid parasite transmission from their prey. We discuss additional comparative questions related to taxonomic differences in disease risk, modes of parasite transmission and implications for conservation biology.

Keywords: Carnivora; comparative study; immune system; phylogeny; disease risk

1. INTRODUCTION

It is well known that parasites influence the population dynamics and evolution of host species (Grenfell & Dobson 1995). Nevertheless, basic questions about the ecology and evolution of disease have not been addressed (Altizer *et al.* 2003). Are some species more vulnerable to parasites than others? How does the immune system differentially respond to micro- versus macro-parasites? How does the risk of acquiring infectious disease influence individual behaviour? Given recent concerns over emerging diseases in human health and conservation, including catastrophic die-offs in carnivores (Funk *et al.* 2001), there is immediate need to address these fundamental questions. It is unlikely that we will be able to adequately examine such problems for every species, perhaps not even representative species in all higher taxa. Thus, an increasingly valuable approach is to use phylogenetic comparisons to examine broad evolutionary patterns and thereby identify the species and clades that are critical for more detailed investigation (O'Brien *et al.* 2001). Here, we apply such a method to assess the relationship between ecological and behavioural factors that influence the risk of acquiring disease and data on baseline white blood cell (WBC) counts across species of carnivores.

In a recent paper using primate comparative data (Nunn *et al.* 2000; Nunn 2002*a*), we showed that immune system parameters are correlated with mating systems. Baseline WBC counts were higher in more promiscuous species, and this pattern was independent of most other social, ecological and life-history factors. These correlations were confirmed using independent data (M. J. Anderson, J. Hessel and A. F. Dixson, unpublished data). Other factors, such as rainfall, have also been found to account for variation in primate WBC counts (Semple *et al.* 2002). The effect of mating promiscuity was consistent with the hypothesis that sexually transmitted disease (STD), mediated through mating behaviour, has shaped baseline aspects of the primate immune system.

Comparative results should be verified in independent taxa to determine their generality and to assess whether there is evidence that other causal factors are involved. The mammalian order Carnivora is an ideal test case because parasites are well known in this group and there is abundant evidence that they have strong effects on host ecology and evolution. Carnivores are susceptible to a variety of diseases, many of which are often easily transmitted by domestic species (Appel 1987). For example, infectious agents in threatened species such as African wild dogs (*Lycaon pictus*), Ethiopian wolves (*Canis simensis*) and black-footed ferrets (*Mustela nigripes*) reveal a domestic origin. The incidence of lethal diseases such as rabies and canine distemper virus (Funk *et al.* 2001) are well known. Some 'emerging' diseases, such as canine parvovirus, are dramatically increasing worldwide and are found largely in carnivores (Daszak *et al.* 2000). Assessing the importance of infectious disease in the Carnivora is particularly important because many of these species are devastated by range fragmentation and habitat destruction (Murray *et al.* 1999; Funk *et al.* 2001), such that even minor diseases could be detrimental.

Several expected correlates of disease risk are particularly salient for carnivores.

(i) Classical epidemiological theory predicts that disease risk increases with group size and population density as a result of more opportunities for transmission (Anderson & May 1979). In carnivores, viruses such as rabies, canine parvovirus, feline leukaemia and feline viral rhinotracheitis are commonly

*Author and address for correspondence: Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616, USA (cnunn@ucdavis.edu).

© 2003 The Royal Society

transmitted via direct contact (Murray *et al.* 1999). Indeed, disease-induced population declines are likely to be initiated in areas of high population density (Roelke-Parker *et al.* 1996), and sociality is known to influence immune defence (e.g. Møller *et al.* 2001) and patterns of parasite abundance (e.g. Dobson & Meagher 1996; Arneberg *et al.* 1998).

(ii) Carnivores include species that are terrestrial, arboreal and aquatic. Terrestrial as opposed to arboreal animals may experience a risk of acquiring protozoan and helminth parasites from faecal-contaminated soil (Hausfater & Meade 1982), and they may acquire fungal diseases, such as blastomycosis or coccidiosis (Murray *et al.* 1999) from inhalation of dust and soil. Aquatic carnivores (pinnipeds) experience considerable exposure to parasites transmitted in fresh or salt water (Bush *et al.* 1990; Harvell *et al.* 1999; Poulin 1999).

(iii) Disease risk may also increase with the percentage of meat in the diet, because parasites that infect mammalian prey can be directly transmitted to carnivores from their prey (trophic transmission). Pathogens that are transmitted in this way include bacteria (e.g. brucellosis, salmonella, botulism), viruses (e.g. bluetongue, African horse sickness) and protozoa (e.g. toxoplasma, giardia, eimeria; Murray *et al.* 1999).

(iv) STDs are more likely in species that mate with multiple partners (Smith & Dobson 1992; Lockhart *et al.* 1996; Thrall *et al.* 2000), predicting increased immune defence in promiscuous species (Nunn *et al.* 2000; Nunn 2002*a*). Many carnivores, such as African lions and coatis, exhibit extreme polygyny and promiscuity (for a review, see Gompper & Wayne 1996).

(v) Life-history traits may also influence disease risk, because species with slow life histories will tend to come into contact with a greater number of parasites and harbour a more diverse parasite community (Poulin 1995). Conversely, evolution of an extended lifespan itself may be mediated by increased immunological protection to achieve maximum longevity (see also De Leo & Dobson 1996; Møller 1997*b*). As noted below, a slow life history may also favour sexual transmission of parasites (e.g. Thrall *et al.* 1993, 1998).

2. MATERIAL AND METHODS

(a) *Comparative data*

WBC counts for carnivores were collated from the international species information system (ISIS) (Physiological Reference Values CD-ROM, 1999, Minnesota Zoological Garden, Apple Valley, MN, USA). This dataset has been collated primarily for veterinary purposes and contains information on blood cell counts for putatively healthy captive individuals from zoos. Although animals in zoos are exposed to different conditions from their wild counterparts, use of zoo data allows for improved assessment of health status of individual animals and larger numbers of samples per host species. Data on baseline WBC counts from individuals in the wild are unavailable for a sufficient number of species to conduct large-scale comparative tests.

Quantitative variation in host defence mechanisms can be used as an estimate of disease risk in comparisons across species if increasing risk leads to evolutionary increases in host defences (Harvey *et al.* 1991; Møller 1997*a*; Møller *et al.* 1998). In mammals, quantitative measures of immune defence include spleen size (Larson 1985; Nunn 2002*b*) and numbers of circulating leucocytes (Bennett & Hawkey 1988). This approach assumes that cross-species variation in immune system parameters accurately reflects species differences in the ability to ward off infection, thereby offsetting the costs of investment in these defences (Møller *et al.* 1998). The costliness of the immune response is supported by several studies (e.g. Sheldon & Verhulst 1996; Demas *et al.* 1997; Nordling *et al.* 1998; Moret & Schmid-Hempel 2000). Although the costliness of baseline immune system parameters in healthy animals has been evaluated less completely, comparative results demonstrate a relationship between other measures of disease risk (parasite species richness or abundance) and the size of the spleen (John 1995; Morand & Poulin 2000). In addition, the immune response itself, with high levels of leucocytes present only when they are needed, implies that leucocyte production and maintenance are costly.

We focused on overall WBC counts and specific WBC types including neutrophils, lymphocytes, monocytes and eosinophils. Neutrophils and monocytes are part of the innate immune system, while lymphocytes are involved in adaptive immunity and recognition of parasites and pathogens. Eosinophils are thought to fight macroparasites, such as helminths (Roitt *et al.* 1998). Hence, variation in different WBC types may reflect different aspects of disease risk.

All WBC data were absolute counts based on the number of cells per 10^{-9}l of blood. For each carnivore species, an average of 2.1 estimates of overall WBC counts were recorded per animal, and many individual animals (mean = 71.2, range 6–434) were tested per species. We used the unweighted average values provided by ISIS after excluding data with fewer than 10 samples (mean number of samples per species = 157.5, range 10–1257). In assessing the quality of this subset of the ISIS data, we found that one species (*Mustela erminea*) was identified repeatedly as an outlier (e.g. in multivariate analysis of four specific WBC types, jackknifed mahalanobis distance = 6.08). We therefore excluded this species from the dataset, giving a total sample size of 72 species. For 33 of these species, information was available for adult females and males separately, which allowed comparison with the main dataset in which male and female values were combined. We found that male and female values were highly correlated (overall WBC: $r = 0.93$; neutrophils: $r = 0.93$; monocytes: $r = 0.78$; lymphocytes: $r = 0.82$; eosinophils: $r = 0.82$; $n = 33$, $p < 0.0001$ in all tests). For most tests, we therefore used the larger dataset, as this allowed for better control of confounding socio–ecological and life-history variables. In bivariate tests of the effect of mating promiscuity, however, we also provide results using female WBC counts because our measure of promiscuity focused on female behaviour.

Data on group size, population density, life-history variables and substrate use were obtained mainly from published comparative databases (Gittleman 1984, 1985, 1986*a*,*b*, 1991, 1993). We used two life-history variables that provided the largest sample sizes for the comparative tests (gestation length and age at sexual maturity, $n = 65$ and 56 species, respectively). Substrate use was examined as a discrete variable by classifying species as aquatic, fully terrestrial, partly terrestrial or arboreal, with the expectation that disease risk (and thus WBC counts) will decrease progressively from aquatic to arboreal substrates.

Because less is known about disease ecology in marine mammals, the relative disease risks associated with terrestrial and aquatic habitats are difficult to rank. Thus, in a second analysis we compared aquatic with non-aquatic species. For mating promiscuity, each species was classified according to whether females had, per oestrous cycle, a single mate, varied between single and multiple mates, or had many mates (see also van Schaik *et al.* 1999; van Noordwijk & van Schaik 2001). In comparison with analyses in primates (Nunn *et al.* 2000; Nunn 2002*a*,*b*), we lacked information on quantitative measures of promiscuity for carnivores, such as measures of sperm competition based on relative testes mass. The duration of oestrus also fails to quantify promiscuity in carnivores because many species exhibit induced ovulation (e.g. Mead 1989; van Noordwijk & van Schaik 2001), such that mating is required for ovulation and may affect the duration of oestrus that is observed.

(b) *Phylogenetic comparative methods*

We tested the hypotheses by using phylogenetic comparative methods based on independent contrasts (Felsenstein 1985). The tree that we used (Bininda-Emonds *et al.* 1999) is the only complete species-level phylogeny for carnivores, and it provides information on branch lengths needed to standardize contrasts for time since two taxa diverged (Felsenstein 1985; Garland *et al.* 1992). Contrasts were calculated using the computer program CAIC (Purvis & Rambaut 1995), with the BRUNCH algorithm used for bivariate tests that involved analysis of discrete, ranked classifications of mating partner number and substrate use. The discrete variables involved character states that could be ranked from one to three in the case of mating promiscuity and one to four in the case of substrate use, with increasing values corresponding to predictions for increased WBC counts. In tests based on the BRUNCH algorithm, we examined the resulting contrasts because spurious results are possible when more than two character states are used (see Purvis & Rambaut 1995).

CAIC standardizes contrasts using branch length information, and in the process makes several statistical and evolutionary assumptions (Purvis & Rambaut 1995). Analysis of contrasts in relation to branch length and reconstructed nodal values (Garland *et al.* 1992; Purvis & Rambaut 1995; Freckleton 2000; Nunn & Barton 2001) revealed that log-transformed data and equal branch lengths best met the model assumptions for most WBC types and socio–ecological variables. Following transformation of data and branch lengths, the assumptions were generally upheld, but some contrasts were identified as outliers. Outliers may exert undue leverage on the statistical tests and may indicate the presence of unaccounted-for confounding variables or measurement error in the data or phylogeny (Nunn & Barton 2001). We therefore conducted analyses with and without these outliers. Results were generally the same, but removal of outliers is likely to give conservative results and so these results are presented here.

We based our major conclusions on phylogenetic analyses, but we also conducted non-phylogenetic tests for comparison. Results that are found to differ in analyses of contrasts and species values may reflect the existence of confounding variables (Price 1997; Purvis & Webster 1999; Nunn & Barton 2001). Moreover, non-phylogenetic analyses provide more reliable results under an alternative evolutionary model that differs from the Brownian motion assumption of independent contrasts (the 'niche' model; Price 1997; Harvey & Rambaut 2000).

(c) *Statistical analyses*

Many of our variables, including mating promiscuity and substrate use, are available only as discrete classifications. Analyses of discrete and continuous data typically have involved different statistical methods within the framework of independent comparisons (see Purvis & Rambaut 1995; Nunn & Barton 2001). We therefore used multiple statistical approaches to test the hypotheses. First, we performed bivariate tests for all variables, particularly those involving discrete traits analysed with the BRUNCH algorithm (Purvis & Rambaut 1995). This is a standard first step in most comparative studies.

Second, we performed multiple and iterative stepwise regression analyses (minimum adequate models; Purvis *et al.* 2000) using the following independent variables: body mass, group size, population density, substrate use, percentage of meat in the diet, age at sexual maturity, gestation length and mating promiscuity. We treated the discrete variables as continuous in CAIC. The justification underlying this procedure is that the discrete character states reflect underlying continuous variation in sexual contact frequency (three character states) and substrate use (four character states). The dependent variables were specific WBC types. We used forward and backward stepwise regression to identify variables that explain significant variation in WBC counts (mixed model in JMP v. 4, SAS Institute, Cary, NC, USA). A variable was entered if its significance probability was less than 0.10, or removed if its significance probability was greater than 0.25 (default values in JMP). These analyses were conducted in two ways to maximize the number of variables included in the model, first by entering all variables and performing the stepwise regression, and second by removing all variables. We then re-calculated independent contrasts using the variables that were entered into the stepwise model, repeating the procedure until all of the variables for which contrasts were calculated were entered (although not all variables were significant in these models). We included body mass as a predictor variable in all cases in which life-history traits were included in the iterative stepwise model. If no variables were entered, we used the variables entered at the previous step. We used the results of the iterative stepwise models to construct multiple regression models representing the variables that were most consistently entered into the analyses. Because many of the predictor variables are correlated, including body mass and life history, we also examined variance inflation factors (VIF) as a measure of collinearity (Petraitis *et al.* 1996). In our multiple regression models with all variables entered, the maximum VIF was smaller for independent contrasts analyses (4.34) than for species values (9.86). A VIF of greater than 10 indicates collinearity (Petraitis *et al.* 1996). Thus, our multiple regression analyses of independent contrasts are unlikely to be affected by collinearity.

Third, we used principal components analysis (PCA) to examine how suites of inter-correlated variables are related to WBC counts. We performed PCA on contrasts and species values using the same set of variables that were examined in the multiple regression analysis. We then extracted principal components scores for the variables that accounted for over 70% of the cumulative variance in the data and tested whether these scores correlated with specific WBCs. We followed the methods in Ackerly & Donoghue (1998) for performing PCA on contrasts.

When testing directional predictions for individual variables in the bivariate and final multiple regression models, we used directed tests (see Rice & Gaines 1994). Directed tests enable the detection of patterns that are opposite to predictions while retaining much of the statistical power of one-tailed tests.

Table 1. Bivariate regression analyses of independent contrasts.
('Number of contrasts' refers to the total number of contrasts prior to removing 0 to 6 outliers. Other values are t-statistics. Inclusion of outliers had little effect on the results, with only one minor change for analyses of specific cell types: the significant relationship between group size and neutrophil counts only approached significance when outliers were included ($t_{49} = 1.74$, $p = 0.055$, directed test). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, based on directed tests except for body mass, for which directional predictions were not possible and so two-tailed tests were used. For 'substrate use', positive values represent an increase in WBC counts with increasing use of aquatic and terrestrial substrates, as compared with arboreal substrates. For 'mating partner number', higher values of the three-part ranked classification indicated increased promiscuity, while the dichotomous variable 'monogamy' classified species as monogamous (0) or non-monogamous (1).)

variable	number of contrasts	overall WBC	neutrophils	lymphocytes	monocytes	eosinophils
body mass	67	3.04**	3.51***	−1.17	2.10*	1.13
age at sexual maturity	53	3.84***	4.92***	0.61	2.22*	1.21
gestation	60	2.70**	4.04***	−1.29	0.72	−0.41
group size	50	2.51**	2.36*	0.07	0.85	−0.81
population density	40	1.03	−0.18	3.10**	−0.19	−0.09
substrate use	14	−0.75	−0.06	−0.05	1.86	0.72
percentage of meat in diet	48	1.09	0.32	−0.51	0.80	2.46*
number of mating partners	6	2.49*	2.64*	1.00	0.42	1.58
monogamy	3	2.56	5.50*	1.07	−0.14	1.30

Table 2. Bivariate regression analyses of species values.
('Number of species' refers to total number of data points for each analysis. Other values are t-statistics. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, based on directed tests except for body mass, for which directional predictions were not possible and so two-tailed tests were used. For 'substrate use', positive values represent an increase in WBC counts with increasing use of aquatic and terrestrial substrates. For 'mating partner number', higher values of the three-part ranked classification indicated increased promiscuity, while the dichotomous variable 'monogamy' classified species as monogamous (0) or non-monogamous (1).)

variable	number of species	overall WBC	neutrophils	lymphocytes	monocytes	eosinophils
body mass	72	3.68***	5.46***	−2.67*	4.81***	1.81*
age at sexual maturity	56	3.67***	4.08***	−0.72	2.22*	0.07
gestation	65	6.28***	7.45***	−0.12	3.31**	−0.5
group size	54	1.03	0.96	0.50	1.19	0.04
population density	42	−0.46	−1.33	2.87**	0.05	0.91
substrate use	72	−0.75	0.05	−1.58	1.36	0.34
percentage of meat in diet	51	0.36	0.34	−0.77	0.12	0.18
number of mating partners	55	2.78**	2.58**	1.58	1.81*	−1.17
monogamy	72	1.62	1.12	2.08*	−0.51	−1.46

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 in the opposite direction ($\delta < \gamma$), subject to the constraint that $\delta + \gamma = \alpha$. We followed the guidelines in Rice & Gaines (1994) by setting γ/α to 0.8. In focused tests of particular hypotheses, we did not correct for multiple comparisons among hypotheses, as this is not needed (Rice 1989) and leads to increased type II error rates when investigating broad patterns (see Rothman 1990).

3. RESULTS

(a) *Bivariate analyses*

Results of analyses of independent contrasts are provided in table 1, and those using species values are provided in table 2. In general, both data types were in agreement, although some patterns that were significant using the species data disappeared once phylogeny was taken into account.

Body mass and life-history traits explained significant variation in neutrophil counts (tables 1 and 2). Because neutrophils are the predominant WBC type, this also produced significant results in analyses of overall WBC counts. Body mass and life-history traits also were correlated with the other primary phagocytic cell, monocytes, although analyses of contrasts in gestation length failed to reach significance. A few other results were significant in non-phylogenetic analyses of body mass, involving lymphocytes and eosinophils, but these results were non-significant in analyses based on independent contrasts.

Group size explained significant variation in neutrophil counts in phylogenetic analyses, while population density explained significant variation in lymphocyte counts in analyses of independent contrasts and species values (tables 1 and 2). It was surprising that these two measures of sociality did not provide more congruent results because, among the species in our sample, increased group size is associated with increased population density when using species data ($b = 1.04$, $F_{1,37} = 6.89$, $p = 0.013$

two tailed). However, this pattern is non-significant when using independent contrasts ($b = 0.62$, $F_{1,35} = 2.56$, $p = 0.12$, two-tailed).

Substrate use showed no relationship with WBC counts in any of the analyses (tables 1 and 2). Aquatic carnivores were included with terrestrial species for these analyses, but separate examination of transitions to aquatic habitat revealed a significant increase in overall WBC (three out of three contrasts positive, $t = 3.94$, $p = 0.037$, directed test), neutrophils (three out of three contrasts positive, $t = 3.55$, $p = 0.044$, directed test) and monocytes (three out of three contrasts positive, $t = 6.13$, $p = 0.016$, directed test). Body mass showed a non-significant tendency to increase with increasing use of aquatic environments (three out of three contrasts positive, $t = 2.11$, $p = 0.17$, two-tailed). Although small sample sizes may have reduced the power to detect a significant effect or to control for confounding variables directly, this result indicates that increases in WBC counts among aquatic carnivores are not simply due to increases in body mass.

Percentage of meat in the diet also was unrelated to overall WBC (tables 1 and 2). Particular cell types showed no significant relationships with the percentage of meat in the diet, with the exception of eosinophils, which increased with the percentage of meat consumed in contrasts analysis but not in analysis of species data points (table 2). Animals also may acquire parasites through consumption of insect secondary hosts, but only two species in our dataset were insectivores, ruling out a statistical test of this hypothesis.

Evolutionary increases in mating promiscuity were associated with increased WBC counts (figure 1; tables 1 and 2). With regard to specific WBC types, neutrophils were statistically significant in analyses of independent contrasts, while the relationships with other WBC types were positive but not significant. Body mass and life-history traits also increased with increasing promiscuity, with results approaching significance (body mass: $t_5 = 2.15$, $p = 0.08$; age at sexual maturity: $t_4 = 2.71$, $p = 0.054$; gestation: $t_4 = 2.07$, $p = 0.11$; all two-tailed). In analyses using species values, total WBC, neutrophils and monocytes were positively related to mating promiscuity (table 2). Three contrasts were available for the more conservative analyses of monogamous versus non-monogamous species. Significant results were obtained for overall WBC and neutrophils in contrasts analyses (table 1) and lymphocytes in tests that used species values (table 2). Finally, results were upheld for neutrophils using the smaller dataset on adult female WBC counts (four out of four contrasts were positive, $t_3 = 3.44$, $p = 0.026$, directed test), although other cell types were not significant (lymphocytes: $t_3 = 0.00$, $p = 0.62$; monocytes: $t_3 = 2.01$, $p = 0.086$; eosinophils: $t_3 = -0.15$, $p = 0.69$).

(b) *Multiple regression analyses*

We performed multiple regression and iterative stepwise regression analyses using specific WBC types (table 3). No variables were statistically significant in the multiple regression analyses that included all socio–ecological variables, possibly because sample sizes were too small after excluding species that lacked the necessary information on all variables.

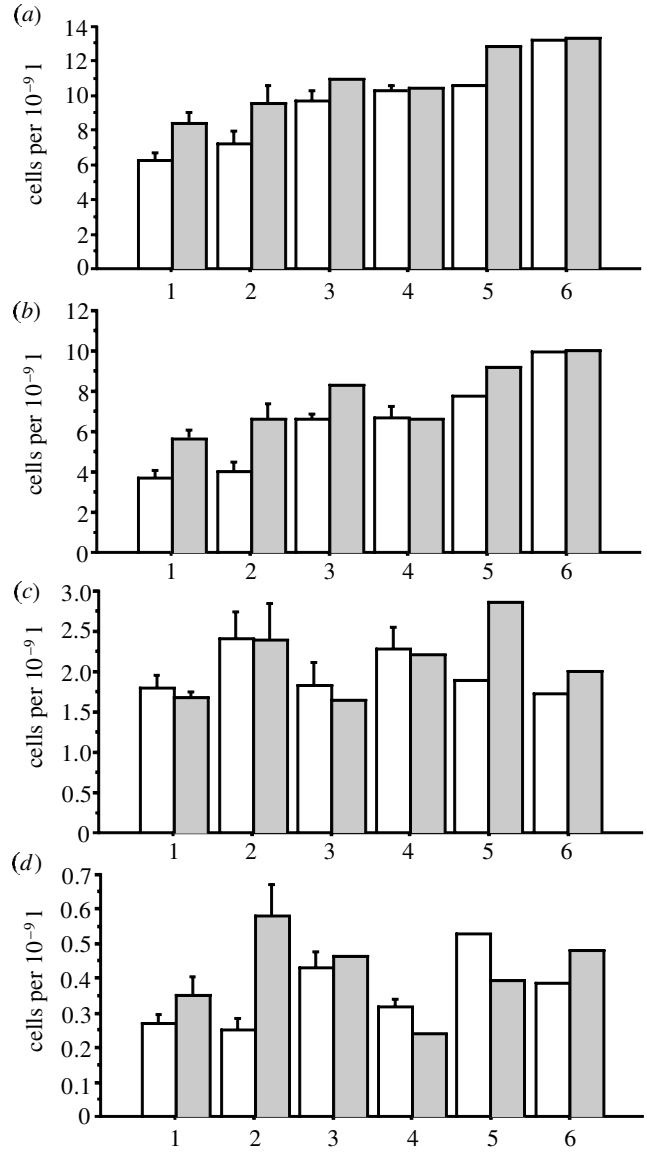

Figure 1. Comparison of (*a*) overall WBCs, (*b*) neutrophils, (*c*) lymphocytes and (*d*) monocytes. Bars represent mean blood cell counts for comparisons of less promiscuous taxa (open bars) with those that are relatively more promiscuous (grey bars) for six pairs of taxa. Standard errors are provided for bars representing averaged values of two species (i.e. a contrast involving a higher node). Contrasts used in the analyses were differences in bar height corrected for branch length. Taxa used in the comparisons were: 1, *Alopex lagopus*, *Otocyon megalotis*, *Urocyon cinereoargenteus*, *Vulpes velox*, *V. vulpes*, *V. zerda* versus *Tremarctos ornatus*, *Ursus americanus*, *U. arctos*, *U. maritimus*; 2, *Amblonyx cinereus*, *Aonyx capensis*, *Enhydra lutris*, *Lontra canadensis*, *Martes pennanti*, *Mephitis mephitis*, *Mustela erminea*, *M. nigripes*, *Nasua narica*, *Potos flavus*, *Procyon lotor*, *Spilogale putorius* versus *Halichoerus grypus*, *Odobenus rosmarus*, *Phoca vitulina*, *Zalophus californianus*; 3, *Canis latrans*, *C. lupus*, *C. mesomelas*, *C. rufus* versus *Lycaon pictus*; 4, *Leopardus pardalis*, *L. wiedii* versus *Oncifelis geoffroyi*; 5, *Hyanea hyaena* versus *Crucuta crocuta*; 6, *Panthera pardus* versus *P. leo* (less promiscuous species listed first).

By sequentially adding and removing particular variables, the iterative stepwise regression analysis identified a smaller subset of variables that explained significant variation in specific WBC types. We found that neutrophil

Table 3. Stepwise regression analyses of independent contrasts.
(* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, based on directed tests in the final multiple regression model except for body mass, for which directional predictions were not possible and so two-tailed tests were used. Values are *t*-statistics. For 'substrate use', positive values represent an increase in WBC counts with increasing use of aquatic and terrestrial substrates. For 'mating partner number', higher values of the three-part ranked classification indicated increased promiscuity.)

	neutrophils		lymphocytes		monocytes		eosinophils	
	multiple regression	iterative stepwise	multiple regression	iterative stepwise	multiple regression	iterative stepwise	multiple regression	iterative stepwise
$n_{contrasts}$	28	46	28	32	28	31	28	37
body mass	1.63	2.08*	−1.04		0.38	0.52	1.29	2.14*
age at sexual maturity	−0.98		0.46		−0.71		−1.96	−2.12
gestation	1.55	3.17**	0.48		0.94	−0.21	0.22	
group size	0.21		−0.30		0.66	0.38	−1.00	−1.95
population density	0.39		1.88	3.53***	0.33		−0.25	
substrate use	−0.79		1.38		0.30		−1.28	−1.25
percentage of meat in diet	0.96	1.73	−0.27		1.34	1.30	0.99	
number of mating partners	0.74		1.26	1.50	0.50	0.10	0.98	2.34*

counts were predicted by body mass and gestation length. The percentage of meat in the diet was retained in the stepwise model but was not significant in the final analysis. Nearly identical results were obtained in analyses of species values: body mass and gestation were the only variables entered in the final model and both were highly significant ($t_{62} = 3.17$ and 5.08, respectively, $p < 0.01$).

Results for lymphocytes in multivariate tests mirrored those from bivariate analyses. Population density was the only significant variable in the iterative stepwise regression model, with mating promiscuity retained in all models but not significant in the final test (table 3). As with the bivariate analyses, the results using species values differed when analysing variation in lymphocytes, with a positive relationship found between lymphocyte counts and mating promiscuity ($t_{52} = 2.83$, $p = 0.004$, directed test) and a negative relationship with body mass ($t_{52} = -3.24$, $p = 0.002$, two-tailed).

For monocytes and eosinophils, few results were significant in the stepwise regression analyses, although in both cases multiple variables were retained in successive iterations of analysis (table 3). The only significant result from the final multiple regression model involves mating promiscuity: more promiscuous species of carnivores had higher eosinophil counts when controlling for five other socio–ecological variables. Non-phylogenetic analyses were more striking and consistent. Body mass and population density explained significant variation in both monocytes (body mass: $t_{51} = 4.08$, $p = 0.0002$, two-tailed; population density: $t_{51} = 2.20$, $p = 0.02$, directed test) and eosinophils (body mass: $t_{32} = 3.68$, $p = 0.001$, two-tailed; population density: $t_{32} = 2.92$, $p = 0.004$, directed test).

(c) *Principal components analysis*

PCA revealed that body mass, age at sexual maturity and female mating promiscuity represent a suite of correlated characters. The first three principal components explained a combined 78.6% of the variance. The first principal component accounted for 38.9% of the variance in the independent variables in table 3. Body mass, age at sexual maturity and mating promiscuity had positive weightings, all with eigenvectors greater than 0.25 (table 4). Other variables also showed strong effects on the first principal component (PC-1), including group size, population density (negative) and substrate use. PC-1 was a strong predictor of neutrophils and monocytes, but not of lymphocytes or eosinophils (table 5). The primary factors that accounted for PC-2 included gestation, population density, substrate use (negative) and percentage of meat in the diet (negative), while gestation, group size (negative) and mating promiscuity (negative) were the top three factors accounting for PC-3. However, both PC-2 and PC-3 failed to explain significant variance in any of the WBC counts (table 5). Analyses of species data revealed a similar pattern, with the first three principal components explaining 77.8% of the variation in the independent variables, and PC-1 accounting for significant variation in neutrophil counts ($t_{27} = 3.97$, $p = 0.0005$, two-tailed). The analysis involving monocytes and PC-1, however, did not reach significance in tests that used species values ($t_{27} = 1.67$, $p = 0.11$, two-tailed).

Bivariate analyses revealed inter-relationships among body mass, life history and mating promiscuity. These variables are expected to relate to the risk of acquiring STDs as follows. Sexual transmission of parasites is favoured in larger-bodied hosts, in which population density is low (Thrall *et al.* 1998). Large-bodied hosts have slower life histories, which is further expected to increase the benefits of sexual versus non-sexual transmission (Thrall *et al.* 1993). Finally, in mammalian species with slower life histories, including longer inter-birth intervals, promiscuous mating by females may be used to counter the effects of increased male–male competition (Mitani *et al.* 1996), particularly infanticide. In lions, for example, females mate with multiple males to reduce the risk of infanticide (Packer & Pusey 1983; see also van Noordwijk & van Schaik (2001)).

Thus, in a final test, we performed PCA on contrasts in body mass, gestation, age at sexual maturity and the three-part measures of mating promiscuity. Using inde-

Table 4. Eigenvectors from PCA of contrasts.

variable	PC-1 (38.9%)	PC-2 (25.9%)	PC-3 (13.8%)
body mass	0.356	−0.001	0.202
group size	0.207	0.189	−0.453
age at sexual maturity	0.306	0.183	0.178
gestation	0.135	0.359	0.276
mating promiscuity	0.282	0.173	−0.268
population density	−0.230	0.293	−0.223
substrate use	0.231	−0.293	−0.126
percentage of meat in diet	0.179	−0.317	−0.090

Table 5. Analyses of leucocyte counts in relation to principal components.
(Values are t-statistics, with $n = 28$ contrasts. $^{*}p < 0.05$, $^{**}p < 0.01$, based on two-tailed tests.)

independent variable	neutrophils	lymphocytes	monocytes	eosinophils
PC-1	2.85**	−0.51	2.39*	−0.01
PC-2	1.57	1.97	−0.10	−0.87
PC-3	0.58	−1.86	−0.75	0.64

pendent contrasts, PC-1 explained over 53% of the variation in these character states, with all variables showing positive eigenvectors greater than 0.38. PC-2 accounted for an additional 20.7% of the variation, with a large positive weighting for mating promiscuity and negative ones for gestation and mass. PC-3 accounted for 16.6% of the variation and reflected variation mainly in gestation, age at sexual maturity (negative) and body mass (negative). Among these variables, PC-1 was a significant predictor of neutrophils ($t_{41} = 4.03$, $p = 0.0002$, two-tailed) and monocytes ($t_{41} = 2.97$, $p = 0.005$, two-tailed). All other analyses were non-significant. Analyses of species data yielded similar results. PC-1 explained 63.2% of the variance and had loadings of greater than 0.40 for each of the four variables. This variable explained significant variation in neutrophils ($t_{44} = 6.03$, $p < 0.0001$, two-tailed) and monocytes ($t_{44} = 2.66$, $p = 0.011$, two-tailed), but not for other WBC types.

4. DISCUSSION

Our results show highly significant correlations between WBC counts and various ecological and behavioural characteristics of carnivores. Because our analyses used independent contrasts, these correlations indicate that evolutionary changes in WBC counts have accompanied other evolutionary changes related to the ecology of these animals. It is much harder to establish clear cause-and-effect relationships. In one or more tests, the comparative patterns support body size, sociality, life history and mating promiscuity as correlates of WBC counts. In bivariate tests, we found support for the effect of mating promiscuity on overall and specific WBCs, which is congruent with results found in primates (Nunn *et al.* 2000). The other highly significant relationship observed in primates, between body mass and neutrophil counts, is also found in carnivores.

One surprising result was the weak support for an effect of diet in carnivores. We hypothesized that meat eaters should have elevated blood cell counts to withstand contact from diseased prey. No consistent support was found for this hypothesis despite great variability in meat eating among the Carnivora, and while some bivariate tests involving eosinophils and neutrophils showed an effect of diet, there was no evidence for their having an effect in the multivariate analyses. Surprisingly, although the carnivore disease literature is replete with examples of protozoan, bacterial and viral infections acquired from prey (see Murray *et al.* 1999), little is known about the frequency with which carnivore populations contract these diseases, individual variability of showing symptoms once contaminated prey are eaten and whether some prey species pass on pathogens more than others. Moreover, to our knowledge, no study has evaluated the relative transmission frequency of food-borne versus socially transmitted parasites in carnivores. Therefore, current information on food-borne diseases in carnivores is inadequate to assess the importance of this route of infection.

Various aspects of carnivore digestion and feeding ecology may reduce the chances of infection via prey. Carnivores have digestive systems that rapidly expel or pass foods (Davis 1964; Van Soest 1994), with simple stomachs and relatively short intestines (roughly half the length of a herbivore) that increase food passage rate and therefore minimize opportunities for parasite transmission. Also, prey contaminated with transmissible parasites may give off olfactory and visual cues that would lead a predator to avoid these individuals. Regurgitation is known for most carnivore species, a behavioural response that effectively eliminates the chance of a pathogen entering the system. This may select for more rapid infection by parasites in the gut, but such behavioural counterstrategies should reduce the risk of parasite uptake, and may prevent parasites from reaching later portions of the gut where, for some parasites, infection often takes place. Finally, although carnivores are well known for eating meat, most species in the order supplement their diets with other foodstuffs. This means that, on a relative basis, even a meat eater may have opportunities to avoid contaminated prey.

Similar to primates, carnivore species with promiscuous mating systems have relatively high WBC counts. Spotted hyaenas (*Crocuta crocuta*) provide an example of how pathogens may become prevalent as a result of the mating and social system. Spotted hyaenas have group sizes that are among the largest reported for a carnivore (up to 80 individuals), comprising a strict hierarchy of females that are dominant over males, with all females in a group reproducing and maintaining massive maternal creches of up to 30 young of different ages and among up to 20 litters (Mills 1990; Frank *et al.* 1991; Hofer & East 1996). Behavioural interactions within large clans are intense, with much physical and behavioural contact amongst individuals. Although spotted hyaenas have been found with a broad array of viruses and other pathogens (for a review, see Mills & Hofer 1998), these have not been systematically analysed with regard to sociality or frequency of inter-individual contact.

Canids are the primary clade showing an association between monogamy and comparatively low WBC counts. The grey wolf (*Canis lupus*), red fox (*Vulpes vulpes*) and maned wolf (*Chrysocyon brachyurus*) all have low WBC counts and are classic examples of mating systems based around a monogamous breeding pair, offspring of that year and some offspring from previous seasons (Macdonald 1981; Moehlman 1989). Reports of promiscuity in these species are extremely rare (Mech & Nelson 1989). If the immune system is closely tied to levels of promiscuity, then monogamous species may be especially vulnerable to new diseases. This has not been systematically investigated in the Canidae, although a large number of diseases have been catalogued in this group (Laurenson *et al.* 1998; Murray *et al.* 1999; Woodroffe 1999).

One promiscuous species that does not fit the promiscuity–immunity association is the California sea lion (*Zalopus californianus*), which has comparatively low WBC counts. Although it is typically classified as promiscuous, the California sea lion is an example of a species that falls into other categories in some circumstances. For example, mating in sea lions can be variable depending on type of habitat, temperature and foraging conditions (Stirling 1983). Females may form temporary groups, moving around temporary rookeries selecting mates; in other areas, females may form groups for thermoregulation but mate with the nearest male; or, females do not form any type of group but select mates during solitary encounters. It would be interesting to assess whether carnivores with flexible mating systems, particularly those in the intermediate promiscuity category, adjust their baseline immune systems facultatively in response to varying levels of promiscuity. An alternative mechanism is that the number of lifetime partners is the important variable and has selected for an intermediate cell count regardless of individual behaviour.

Our multivariate statistical analyses did not rule out alternative explanations for the association between mating promiscuity and leucocyte counts. In primates, control of confounding variables proved more straightforward, as some of our measures of promiscuity, such as relative testes mass, were quantitative and calculated so as to be independent of body mass. Correlations among the predictor variables make this issue more problematic for carnivores, and from our PCA analyses all that can be concluded is that a suite of characters involving socio–ecology, life history and mating system covary with leucocyte counts.

It is also likely that a suite of characters involving increased sociality and slower life history leads to conditions favouring greater promiscuity. For example, promiscuous mating increases with group size, as noted above for the spotted hyena. Similarly, the association between life history and leucocytes may reflect covariation with mating promiscuity because life history and promiscuity are intertwined: long-lived hosts may be more likely to have multiple mating partners over their lifetimes, and STDs should have a greater impact on animals with slow than fast life histories (Thrall *et al.* 1993; Loehle 1995; De Leo & Dobson 1996). Moreover, once established, STDs tend to be immuno-evasive and are likely to last throughout the lifetime of the host (Lockhart *et al.* 1996), which makes them more of a problem for long-lived host species. Until more quantitative data are available on carnivore mating promiscuity, the relative roles of these variables are likely to remain elusive in multivariate tests.

Information is also needed on the mechanism by which an increase in WBC counts reduces risk of acquiring STDs. In other mammals, neutrophils and monocytes engulf sperm and seminal fluids in the female reproductive tract immediately following copulation (Austin 1975; Phillips & Mahler 1977; Pandya & Cohen 1985; Barratt *et al.* 1990) and the same is likely to be true in carnivores. Phagocytosis of sperm may be involved in other aspects of mammalian reproduction, however, including cryptic female choice (Eberhard 1996).

More terrestrial species of primates were found to have higher neutrophil counts and are also larger in body mass (Nunn *et al.* 2000; Nunn 2002*a*). Compared with primates (Clutton-Brock & Harvey 1977; Nunn & Barton 2001), substrate use in carnivores is generally unrelated to body mass (Gittleman 1985). Aquatic carnivores showed elevated baseline levels of neutrophils and monocytes. It is noteworthy that the number of eosinophils, which are reported to be important in fighting macroparasite infections (Roitt *et al.* 1998), such as those acquired from the soil, showed no relationship to terrestrial substrate use in either mammalian order (for primates, see Nunn (2002*a*)).

In conclusion, phylogenetic comparisons across primates and carnivores reveal consistent patterns between WBC counts and mating promiscuity, although in carnivores additional factors are involved. The consistency of our results within two independent mammalian orders therefore provides support for both this general pattern and for the importance of examining baseline immunological parameters in a broad evolutionary context. Ideally, the hypothesis that mating promiscuity influences the immune system could be tested using information on the diversity and prevalence of STDs. Such information, however, is not widely available and is generally known only for humans and species of economic importance, such as livestock (Lockhart *et al.* 1996). Comparisons of immunity and disease risk across taxa are also important in a conservation context. Most work to date has involved piecing together the transmission and virulence of pathogens after the fact, then working to solve or prevent the spread of disease. By establishing the role of baseline levels of immunity and response, it may be possible to better anticipate which species are at greater risk from endemic

(autochthonous) infections as well as diseases that are transmitted from domestic animals.

We thank Sonia Altizer, Kate Jones, Andrew Read, Carel van Schaik and two anonymous reviewers for helpful discussion or comments on the manuscript. We also acknowledge Cathy Williams for calling our attention to the ISIS dataset, and ISIS for making these important data readily available. This research was supported by a National Science Foundation postdoctoral research fellowship in Biological Informatics to C.L.N., by NIH grant GM60766-01 to J.A., and by the National Centre for Ecological Analysis and Synthesis (NCEAS) in Santa Barbara, CA.

REFERENCES

Ackerly, D. D. & Donoghue, M. J. 1998 Leaf size, sapling allometry and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *Am. Nat.* **152**, 767–798.

Altizer, S. M. (and 11 others) 2003 Social organization and disease risk in mammals: integrating theory and empirical studies. *A. Rev. Ecol. Syst.* (In the press.)

Anderson, R. M. & May, R. M. 1979 Population biology of infectious diseases: part 1. *Nature* **280**, 361–367.

Appel, M. J. (ed.) 1987 *Virus infections of carnivores*. New York: Elsevier.

Arneberg, P., Skorping, A., Grenfell, B. & Read, A. F. 1998 Host densities as determinants of abundance in parasite communities. *Proc. R. Soc. Lond.* B **265**, 1283–1289. (DOI 10.1098/rspb.1998.0431.)

Austin, C. R. 1975 Sperm fertility, viability and persistence in the female tract. *J. Reprod. Fertil.* **22**(Suppl.), 75–89.

Barratt, C. L. R., Bolton, A. E. & Cooke, I. D. 1990 Functional significance of white blood cells in the male and female reproductive tract. *Hum. Reprod.* **5**, 639–648.

Bennett, P. M. & Hawkey, C. M. 1988 Comparative haematology: phylogenetic and ecological aspects in mammals and birds. In *Animal clinical biochemistry* (ed. D. J. Blackmore), pp. 33–48. Cambridge University Press.

Bininda-Emonds, O. R. P., Gittleman, J. L. & Purvis, A. 1999 Building large trees by combining phylogenetic information: a complete phylogeny of the extant Carnivora (Mammalia). *Biol. Rev. Camb. Phil. Soc.* **74**, 143–175.

Bush, A. O., Aho, J. M. & Kennedy, C. R. 1990 Ecological versus phylogenetic determinants of helminth parasite community richness. *Evol. Ecol.* **4**, 1–20.

Clutton-Brock, T. H. & Harvey, P. H. 1977 Primate ecology and social organization. *J. Zool. Lond.* **183**, 1–39.

Daszak, P., Cunningham, A. A. & Hyatt, A. D. 2000 Emerging infectious diseases of wildlife. Threats to biodiversity and human health. *Science Wash.* **287**, 443–449.

Davis, D. D. 1964 The giant panda: a morphological study of evolutionary mechanisms. *Fieldiana Zool. Mem.* **3**, 1–339.

De Leo, G. A. & Dobson, A. P. 1996 Allometry and simple epidemic models for microparasites. *Nature* **379**, 720–722.

Demas, G. E., Chefer, V., Talan, M. I. & Nelson, R. J. 1997 Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* **42**, R1631–R1637.

Dobson, A. P. & Meagher, M. 1996 The population dynamics of brucellosis in the Yellowstone National Park. *Ecology* **77**, 1026–1036.

Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton University Press.

Felsenstein, J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15.

Frank, L. G., Glickman, S. E. & Licht, P. 1991 Fetal sibling aggression, precocial development, and androgens in neonatal spotted hyenas. *Science* **252**, 702–705.

Freckleton, R. P. 2000 Phylogenetic tests of ecological and evolutionary hypotheses: checking for phylogenetic independence. *Funct. Ecol.* **14**, 129–134.

Funk, S. M., Firello, C. V., Cleaveland, S. & Gompper, M. E. 2001 The role of disease in carnivore ecology and conservation. In *Carnivore conservation* (ed. J. L. Gittleman, S. Funk, D. Macdonald & R. K. Wayne), pp. 443–466. Cambridge University Press.

Garland, T. J., Harvey, P. H. & Ives, A. R. 1992 Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **4**, 18–32.

Gittleman, J. L. 1984 The behavioural ecology of carnivores. PhD thesis, University of Sussex, UK.

Gittleman, J. L. 1985 Carnivore body size: ecological and taxonomic correlates. *Oecologia* **67**, 540–554.

Gittleman, J. L. 1986*a* Carnivore brain size, behavioral ecology, and phylogeny. *J. Mammal* **67**, 23–36.

Gittleman, J. L. 1986*b* Carnivore life-history patterns: allometric, phylogenetic, and ecological associations. *Am. Nat* **127**, 744–771.

Gittleman, J. L. 1991 Carnivore olfactory bulb size: allometry, phylogeny and ecology. *J. Zool. Lond.* **225**, 253–272.

Gittleman, J. L. 1993 Carnivore life histories: a re-analysis in the light of new models. In *Symp. Zool. Soc. Lond. vol. 65: mammals as predators* (ed. N. Dunstone & M. L. Gorman), pp. 65–86. Oxford University Press.

Gompper, M. E. & Wayne, R. K. 1996 Genetic relatedness among individuals within carnivore societies. In *Carnivore behavior, ecology, and evolution*, vol. 2 (ed. J. L. Gittleman), pp. 429–452. Ithaca, NY: Cornell University Press.

Grenfell, B. T. & Dobson, A. P. (eds) 1995 *Ecology of infectious diseases in natural populations*. Cambridge University Press.

Harvell, C. D. (and 12 others) 1999 Marine ecology: emerging marine diseases, climate links and anthropogenic factors. *Science Wash.* **285**, 1505–1510.

Harvey, P. H. & Rambaut, A. 2000 Comparative analyses for adaptive radiations. *Phil. Trans. R. Soc. Lond.* B **355**, 1599–1605. (DOI 10.1098/rstb.2000.0721.)

Harvey, P. H., Read, A. F., John, J. L., Gregory, R. D. & Keymer, A. E. 1991 An evolutionary perspective: using the comparative method. In *Parasite–host associations* (ed. C. A. Toft, A. Aeschlimann & L. Bolis), pp. 344–355. Oxford University Press.

Hausfater, G. & Meade, B. J. 1982 Alternation of sleeping groves by yellow baboons (*Papio cynocephalus*) as a strategy for parasite avoidance. *Primates* **23**, 287–297.

Hofer, H. & East, M. L. 1996 The components of parental care and their fitness consequences: a life-history perspective. *Vehandlungen der Deutschen Gessell schraft fur Zoologie* **89**, 149–164.

John, J. L. 1995 Parasites and the avian spleen. *Biol. J. Linn. Soc.* **54**, 87–106.

Larson, S. G. 1985 Organ weight scaling in primates. In *Size and scaling in primate biology* (ed. W. L. Jungers), pp. 91–113. New York: Plenum.

Laurenson, K., Sillero-Zubiri, C., Thompson, H., Shiferaw, F., Thirgood, S. & Malcolm, J. 1998 Disease as a threat to endangered species: Ethiopian wolves, domestic dogs and canine pathogens. *Anim. Conserv.* **1**, 273–280.

Lockhart, A. B., Thrall, P. H. & Antonovics, J. 1996 Sexually transmitted diseases in animals: ecological and evolutionary implications. *Biol. Rev.* **71**, 415–471.

Loehle, C. 1995 Social barriers to pathogen transmission in wild animal populations. *Ecology* **76**, 326–335.

Macdonald, D. W. 1981 Resource dispersion and the social organization of the red fox, *Vulpes vulpes*. In *Proc. World Furbearer Conf* (ed. J. A. Chapman & D. Ursley), pp. 918–949. Falls Church, VA: R. H. Donnelly and sons.

Mead, R. A. 1989 Reproduction in mustelids. In *Conservation biology and the black-footed ferret* (ed. U. S. Seal, E. T. Thorne, M. A. Bogan & S. H. Anderson), pp. 124–137. Yale University Press.

Mech, L. D. & Nelson, M. E. 1989 Polygyny in a wild wolf pack. *J. Mammal* **70**, 675–676.

Mills, G. & Hofer, H. 1998 *Hyaenas*. Cambridge: IUCN Publications.

Mills, M. G. L. 1990 *Kalahari hyaenas*. London: Unwin Hyman.

Mitani, J., Gros-Louis, J. & Richards, A. F. 1996 Sexual dimorphism, the operational sex ratio, and the intensity of male competition in polygynous primates. *Am. Nat.* **147**, 966–980.

Moehlman, P. D. 1989 Intraspecific variation in canid social systems. In *Carnivore behavior, ecology and evolution* (ed. J. L. Gittleman), pp. 143–163. Ithaca, NY: Cornell University Press.

Møller, A. P. 1997*a* Immune defence, extra-pair paternity, and sexual selection in birds. *Proc. R. Soc. Lond.* B **264**, 561–566. (DOI 10.1098/rspb.1997.0080.)

Møller, A. P. 1997*b* Parasitism and the evolution of host life history. In *Host–parasite evolution* (ed. D. H. Clayton & J. Moore), pp. 105–127. Oxford University Press.

Møller, A. P., Dufva, R. & Erritzoe, J. 1998 Host immune function and sexual selection in birds. *J. Evol. Biol.* **11**, 703–719.

Møller, A. P., Merino, S., Brown, C. R. & Robertson, R. J. 2001 Immune defense and host sociality: a comparative study of swallows and martins. *Am. Nat.* **158**, 136–145.

Morand, S. & Poulin, R. 2000 Nematode parasite species richness and the evolution of spleen size in birds. *Can. J. Zool.* **78**, 1356–1360.

Moret, Y. & Schmid-Hempel, P. 2000 Survival for immunity: the price of immune system activation for bumble-bee workers. *Science* **290**, 1166–1168.

Murray, D. L., Kapke, C. A., Evermann, J. F. & Fuller, T. K. 1999 Infectious disease and the conservation of free-ranging large carnivores. *Anim. Conserv* **2**, 241–254.

Nordling, D., Andersson, M., Zohari, S. & Gustafsson, L. 1998 Reproductive effort reduces specific immune response and parasite resistance. *Proc. R. Soc. Lond.* B **265**, 1291–1298. (DOI 10.1098/rspb.1998.0432.)

Nunn, C. L. 2002*a* A comparative study of leukocytes counts and disease risk in primates. *Evolution* **56**, 177–190.

Nunn, C. L. 2002*b* Spleen size, disease risk and sexual selection: a comparative study in primates. *Evol. Ecol. Res* **4**, 91–107.

Nunn, C. L. & Barton, R. A. 2001 Comparative methods for studying primate adaptation and allometry. *Evol. Anthropol.* **10**, 81–98.

Nunn, C. L., Gittleman, J. L. & Antonovics, J. 2000 Promiscuity and the primate immune system. *Science Wash.* **290**, 1168–1170.

O'Brien, S. J., Eizirik, E. & Murphy, W. J. 2001 On choosing mammalian genomes for sequencing. *Science Wash.* **292**, 2264–2265.

Packer, C. & Pusey, A. E. 1983 Adaptations of female lions to infanticide by incoming males. *Am. Nat.* **121**, 716–728.

Pandya, I. J. & Cohen, J. 1985 The leukocytic reaction of the human uterine cervix to spermatozoa. *Fertil. Steril.* **43**, 417–421.

Petraitis, P. S., Dunham, A. E. & Niewiarowski, P. H. 1996 Inferring multiple causality: the limitations of path analysis. *Funct. Ecol.* **10**, 421–431.

Phillips, D. M. & Mahler, S. 1977 Leukocyte emigration and migration in the vagina following mating in the rabbit. *Anat. Rec.* **189**, 45–60.

Poulin, R. 1995 Phylogeny, ecology, and the richness of parasite communities in vertebrates. *Ecol. Monogr.* **65**, 283–302.

Poulin, R. 1999 Speciation and diversification of parasite lineages: an analysis of congeneric parasite species in vertebrates. *Evol. Ecol.* **13**, 455–467.

Price, T. 1997 Correlated evolution and independent contrasts. *Phil. Trans. R. Soc. Lond.* B **352**, 519–529. (DOI 10.1098/rstb.1997.0036.)

Purvis, A. & Rambaut, A. 1995 Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Comput. Appl. Biosci.* **11**, 247–251.

Purvis, A. & Webster, A. J. 1999 Phylogenetically independent contrasts and primate phylogeny. In *Comparative primate socioecology* (ed. P. Lee), pp. 44–68. Cambridge University Press.

Purvis, A., Gittleman, J. L., Cowlishaw, G. & Mace, G. M. 2000 Predicting extinction risk in declining species. *Proc. R. Soc. Lond.* B **267**, 1947–1952. (DOI 10.1098/rspb.2000.1234.)

Rice, W. R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Rice, W. R. & Gaines, S. D. 1994 Heads I win, tails you lose: testing directional alternative hypotheses in ecological and evolutionary research. *Trends Ecol. Evol.* **9**, 235–237.

Roelke-Parker, M. E. (and 14 others) 1996 A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* **379**, 441–445.

Roitt, I. M., Brostoff, J. & Male, D. K. 1998 *Immunology*. London: Gower Medical Publishing.

Rothman, K. J. 1990 No adjustments are needed for multiple comparisons. *Epidemiology* **1**, 43–46.

Semple, S., Cowlishaw, G. & Bennett, P. M. 2002 Immune system evolution among anthropoid primates: parasites, injuries and predators. *Proc. R. Soc. Lond.* B **269**, 1031–1037. (DOI 10.1098/rspb.2001.1950.)

Sheldon, B. C. & Verhulst, S. 1996 Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends Ecol. Evol.* **11**, 317–321.

Smith, G. & Dobson, A. P. 1992 Sexually transmitted diseases in animals. *Parasitol. Today* **8**, 159–166.

Stirling, I. 1983 The evolution of mating systems in pinnipeds. In *Advances in the study of mammalian behavior* (ed. J. F. Eisenberg & D. G. Kleiman), pp. 489–527. Special Publication no. 7, American Society of Mammalogists, Shippensberg, PA.

Thrall, P. H., Biere, A. & Antonovics, J. 1993 Plant life history and disease susceptibility: the occurrence of *Ustilago–Violacea* on different species within the Caryophyllaceae. *J. Ecol.* **81**, 489–498.

Thrall, P. H., Antonovics, J. & Wilson, W. G. 1998 Allocation to sexual versus nonsexual disease transmission. *Am. Nat.* **151**, 29–45.

Thrall, P. H., Antonovics, J. & Dobson, A. P. 2000 Sexually transmitted diseases in polygynous mating systems: prevalence and impact on reproductive success. *Proc. R. Soc. Lond.* B **267**, 1555–1563. (DOI 10.1098/rspb.2000.1178.)

van Noordwijk, M. A. & van Schaik, C. P. 2001 Reproductive patterns in eutherian mammals: adaptations against infanticide? In *Infanticide by males and its implications* (ed. C. P. van Schaik & C. H. Janson), pp. 322–360. Cambridge University Press.

van Schaik, C. P., van Noordwijk, M. A. & Nunn, C. L. 1999 Sex and social evolution in primates. In *Comparative primate socioecology* (ed. P. C. Lee), pp. 204–240. Cambridge University Press.

Van Soest, P. J. 1994 *Nutritional ecology of the ruminant*. Ithaca, NY: Cornell University Press.

Woodroffe, R. 1999 Managing disease threats to wild mammals. *Anim. Conserv* **2**, 185–193.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.