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A COMPARATIVE STUDY OF LEUKOCYTE COUNTS AND DISEASE RISK IN PRIMATES

CHARLES L. NUNN[1]
Biology Department, University of Virginia, Charlottesville, Virginia 22904-4328

Abstract.—Little is known about how the risk of disease varies across species and its consequences for host defenses, including the immune system. I obtained mean values of basal white blood cells (WBC) from 100 species of primates to quantify disease risk, based on the assumption that higher baseline WBC counts will be found in species that experience greater risk of acquiring infectious disease. These data were used to investigate four hypotheses: disease risk is expected to increase with (1) group size and population density; (2) greater contact with soil-borne pathogens during terrestrial locomotion; (3) a slow life history; and (4) increased mating promiscuity. After controlling for phylogeny, WBC counts increased with female mating promiscuity, as reflected in discrete categories of partner number, relative testes mass, and estrous duration. By comparison, the social, ecological, and life-history hypotheses were unsupported in comparative tests. In terms of confounding variables, some WBC types were associated with body mass or activity period, but these variables could not account for the association with mating promiscuity. Several factors may explain why hypotheses involving social, ecological, and life-history factors went unsupported in these tests, including the role of behavioral counterstrategies to disease, restrictions on female choice of mating partners, and the effect of transmission mode on parasite strategies and host defenses.

Key words.—Behavior, comparative study, disease, ecology, independent contrasts, primates.

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Social, ecological, and life-history features are thought to influence the risk of acquiring infectious disease. For example, living in a larger social group is thought to increase the risk of acquiring disease spread through direct contact (Freeland 1976; Møller et al. 1993; Loehle 1995). Studies of intraspecific variation have shown that animals living in larger groups experience increased incidence of certain types of parasites (e.g., helminths infecting feral horses: Rubenstein and Hohmann 1989). Disease risk may therefore be an important factor in social evolution (Alexander 1974; Freeland 1976; Møller et al. 1993; Loehle 1995), but few studies have examined the correlates of disease risk at macroevolutionary scales.

Disease risk is difficult to quantify in comparative studies, thus explaining why so little is known about its correlates across species. With increasing phylogenetic and comparative data, however, three approaches can be used to study macroevolutionary patterns of disease risk. First, patterns of parasite species richness reflect disease risk across species (Morand and Poulin 2000). One weakness with this approach, however, is that sampling effort is one of the strongest correlates of parasite species richness (Gregory 1990) and must be controlled statistically (Gregory 1990; Cotgreave and Clayton 1994; Poulin 1995), which may reduce the power to detect underlying patterns (Harvey et al. 1991).

A second approach for quantifying disease risk uses measures of parasite abundance, including prevalence and intensity of infection, and has provided some insights to the correlates of disease risk across species (John 1995; Arneberg et al. 1998a, b). As with studies of predation (Hill and Dunbar 1998; Janson 1998; Nunn and van Schaik 2000), however, this measure assesses the risk that remains after host counterstrategies, such as the immune system and behavior, have been implemented. Thus, whereas some studies of abundance have provided important insights to host-parasite biology (e.g., John 1995; Arneberg et al. 1998a, b) and should be pursued in future research, studies of abundance may be most powerful for population level analyses (e.g., Rubenstein and Hohmann 1989), including meta-analysis of within-species patterns (Côté and Poulin 1995).

These considerations lead to a third approach, which is the one taken here. Quantitative variation in host defense mechanisms can be used as a surrogate measure of disease risk if increasing risk leads to evolutionary increases in host defenses (Harvey et al. 1991; Møller et al. 1998). In mammals, quantitative measures of immune defense include spleen size (Larson 1985) and numbers of circulating leukocytes (Bennett and 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 defenses (Møller et al. 1998). The costliness of the immune response is supported by several studies (e.g., Sheldon and Verhulst 1996; Demas et al. 1997; Nordling et al. 1998; Moret and Schmid-Hempel 2000). Although the costliness of baseline immune system parameters in healthy animals has been evaluated less completely, comparative research has demonstrated a relationship between other measures of disease risk (parasite species richness or abundance) and the size of the spleen (John 1995; Morand and Poulin 2000). In addition, the immune response itself, with high levels of leukocytes present only when they are needed, implies that leukocyte production and maintenance is costly.

In a previous paper, Nunn et al. (2000) showed that evolutionary transitions to increased mating promiscuity are associated with increases in basal white blood cell (WBC) counts in primates, irrespective of social, ecological, and life-history parameters. These tests used WBC counts from the International Species Information System (ISIS, Minnesota Zoological Garden, Apple Valley, MN), which were obtained from healthy zoo animals for the purpose of constructing

[1] Present address: Section of Evolution and Ecology, University of California, Davis, California 95616; E-mail: cnunn@ucdavis.edu.

physiological references values (i.e., normal ranges) to assess animal health. Nunn et al. (2000) examined only adult female WBC counts to control for the potentially confounding effects of age and sex. Sample sizes can be increased substantially, however, if an average value is taken for all of a species' samples, because sex- and age-specific information is available for only a subset of species listed in the ISIS dataset.

In this paper, I examine the correlates of baseline WBC counts using the larger ISIS database. Use of the larger dataset increases the ability to control for confounding variables other than age and sex. It also allows for improved assessment of the quality of the ISIS data, which is critical for future comparative research on immune system parameters. I used these data to test four a priori hypotheses for the correlates of disease risk in primates: (1) disease risk is expected to increase with group size and population density, via more opportunities for transmission (Møller et al. 1993; Loehle 1995; Dobson and Meagher 1996); (2) risk is expected to increase in species that use the ground for locomotion, because terrestrial primates may experience greater risk of acquiring parasites from fecal-contaminated soil (e.g., Hausfater and Meade 1982); (3) immune system parameters are predicted to show relationships with life-history traits, as species with slow life histories will tend to come into contact with more parasites and may therefore harbor a more diverse parasite community (Poulin 1995); moreover, species with a slow life history may require increased immunological protection to achieve maximum longevity; and (4) sexually transmitted diseases (STDs) are more likely in species that mate with multiple partners (Smith and Dobson 1992; Lockhart et al. 1996; Thrall et al. 1997, 2000), predicting increased immune defense in more promiscuous species.

Materials and Methods

Data on White Blood Cell Counts and Correlations with Phylogeny

I compiled information on the number of institutions reporting WBC counts and, for each WBC type, the mean value, the standard deviation of reported values, and the number of samples (from ISIS Physiological Reference Values CD-ROM 1999). For each species, the mean number of samples was 150 (±234 SD, range = 5–958), with information from an average of 11.4 institutions (±14.0 SD, range = 1–56). Data were available on 99 nonhuman primates, to which I added information on humans (*Homo sapiens*) using Dacie and Lewis (1991) and Borer (2000). I used data on overall WBC counts and specific WBC types, including neutrophils, lymphocytes, monocytes, and platelets. Lymphocytes are involved in adaptive recognition of antigens, neutrophils and monocytes fight off invading pathogens, and platelets are involved in the inflammatory response (Hawkey and Bennett 1988; Roitt et al. 1998). Red blood cells (RBCs) were examined as a possible confounding variable. The effect of terrestrial locomotion was ambiguously supported in the previous study (Nunn et al. 2000). I therefore expanded the analysis by including another WBC type, eosinophils, which are involved in fighting helminths acquired from the soil (Roitt et al. 1998) and therefore serve to test the terrestrial substrate hypothesis.

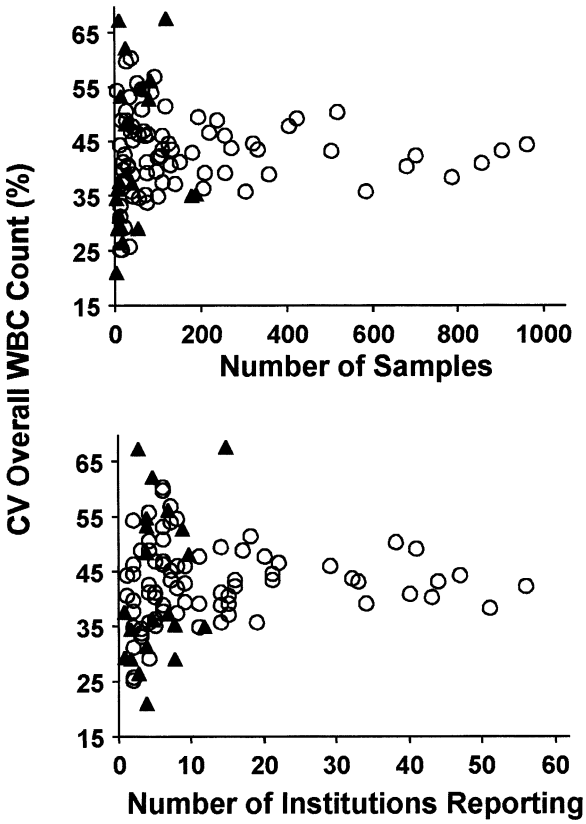

FIG. 1. Variation in overall white blood cell (WBC) count according to sample size. The coefficient of variation (CV) is plotted as a function of number of samples and the number of institutions reporting values. Analysis of the distribution of CV for overall WBC count and each cell type was used to identify outliers. Species that were outliers in at least one of these analyses are indicated by filled triangles and include, with cell type in parentheses (O, overall WBC; N, neutrophil; L, lymphocyte; M, monocyte; E, eosinophil): *Aotus azarae* (N), *Ateles belzebuth* (O), *A. chamek* (M), *Callicebus moloch* (L), *Cebuella pygmaea* (O, L), *Cebus albifrons* (E), *Cercocebus albigena* (M), *Cercopithecus hamlyni* (L), *C. petaurista* (M), *C. pogonias* (N, M, L), *C. wolfi* (E), *Cheirogaleus medius* (N, M), *Macaca nemestrina* (M), *Microcebus coquereli* (M), *Miopithecus talapoin* (E), *Otolemur crassicaudatus* (N), *Presbytis entellus* (E), *P. francoisi* (E), *P. obscurus* (M), *Propithecus tattersalli* (E), and *Theropithecus gelada* (E).

I assessed the quality of the ISIS dataset in several ways. If methods of obtaining WBC counts differ among the institutions reporting values, then species values gathered from more institutions should show greater variation in mean reported WBC counts. I tested this possibility using the coefficient of variation (CV = 100[standard deviation/mean]) but found no significant effect of the number of institutions reporting values (Fig. 1, Table 1). Moreover, sample size was not significantly correlated with CV (Table 1). Nonetheless, small samples or few reporting institutions characterized all outliers among CV scores, and there was a trend for samples gathered from a greater number of institutions to have higher mean CVs. To deal with these issues, I therefore generated two restricted datasets to determine if inclusion of the least certain values affected the comparative results. The first restricted dataset excluded humans and those species with particularly low or high CVs among any WBC type, as deter-

TABLE 1. Effects of sample size on white blood cell (WBC) counts in multiple regression. Based on independent contrasts with equal branch lengths, unlogged data, and exclusion of species with a single sample and humans. Elimination of outliers did not change the results substantially but did reduce the significance of analyses involving lymphocytes ($F_{1,81} = 2.04$, $P = 0.16$). Nonphylogenetic tests provided similar results.

	Number of contrasts	Number reporting institutions			Number of samples		
		$b_{NumInst}$	$F_{NumInst}$	P-value[1]	b_{Sample}	$F_{SampleSize}$	P-value[1]
Overall WBC	93	0.079	0.21	0.65	−0.0021	0.04	0.85
Neutrophils	92	0.17	0.38	0.54	−0.0098	0.28	0.60
Lymphocytes	92	0.39	3.55	0.06	−0.020	1.98	0.16
Monocytes	92	0.30	0.49	0.49	−0.0012	0.001	0.97
Eosinophils	92	0.72	1.35	0.25	−0.047	0.54	0.46
Platelets	75	−0.18	0.81	0.37	0.029	0.58	0.45

[1] Two-tailed tests.

mined in the outlier analysis ($n = 78$ species; see Fig. 1 legend). The second restricted dataset removed species from restricted dataset 1 that had small samples (<10), further reducing the sample size by one species for overall WBC, neutrophils, and lymphocytes; three species for monocytes; and ten species for eosinophils. For overall WBC counts, these two steps reduced variation in CV scores (two-tailed test of equal variance: $F_{98,76} = 1.55$, $P = 0.048$; Sokal and Rohlf 1995).

To further assess the ISIS data, I tested whether WBC counts were correlated with phylogeny by using phylogenetic autocorrelation (Moran's *I*; Gittleman and Kot 1990) and the test for serial independence (TFSI; Abouheif 1999). These tests investigate phylogenetic signal in the data, rather than assessing data quality directly, but a finding of low phylogenetic signal may indicate that WBC counts are measured with great error within species, making them unsuitable for cross-species analysis. I did not use nested ANOVA to assess phylogenetic correlations of WBC counts (Bennett and Hawkey 1988; S. Semple, G. Cowlishaw, and P. M. Bennett, unpubl. ms.) because of unequal sample sizes in the nested classes (Sokal and Rohlf 1995) and because this approach offers no clear statistical criteria for deciding whether a trait is significantly correlated with phylogeny.

Moran's *I* is a measure of the similarity of species values at successive taxonomic levels (Gittleman and Kot 1990). I used the computer program Phylogenetic Autocorrelation (Luh et al. 1995) to calculate statistics, with evolutionary relationships represented as a taxonomy (Fleagle 1988) that is generally congruent with existing phylogenetic information (e.g., Purvis 1995). Four taxonomic rankings were used, representing suborder, infraorder, family-subfamily, and genus. As shown in Figure 2, this analysis revealed a strong association between WBC counts and phylogeny.

The TFSI measures the degree of nonrandomness in a series of continuous values, such as those along the tips of a phylogeny. A randomization procedure is used to deal with the arbitrariness of the species' order in a binary tree (Abouheif 1999). To implement TFSI, I used the program Phylogenetic Independence (Reeve and Abouheif 1999), with explicit phylogenetic information based on Purvis (1995). Statistical significance was tested using simulations to generate a null distribution ($n = 1000$ simulations), as described in Abouheif (1999). The computer program requires a fully bifurcating phylogeny, and so five polytomies were randomly resolved in MacClade (Maddison and Maddison 1992) prior to running the test. These polytomies were near the tips of the tree and within genera, so that random resolution had little effect on the statistics, which was confirmed by using a different random arrangement of the polytomous nodes. As with Moran's *I*, TFSI demonstrated a high correlation between phylogeny and overall WBC counts ($P = 0.001$) and specific WBC types ($P < 0.003$ in all cases). These tests therefore make a strong case for the quality of the WBC counts in the ISIS dataset and the applicability of phylogenetic comparative methods for analyzing these data.

Testing for Correlated Evolution

To test for correlated evolution of two or more traits, I used methods based on independent contrasts (Felsenstein 1985; Harvey and Pagel 1991), as implemented by the computer program CAIC (Purvis and Rambaut 1995). For statistical analysis of data involving a discrete independent variable, I used the BRUNCH algorithm (Purvis and Rambaut 1995). This method calculates contrasts with the direction of subtraction such that the discrete variable is positive, retaining the direction of subtraction for contrasts in the continuous variable. The prediction to test is whether contrasts in the continuous dependent variable show consistent directionality and magnitude over evolutionary increases in the discrete independent variable, which I tested using a *t*-test (Purvis and Rambaut 1995).

The method of independent contrasts makes a number of assumptions regarding the evolutionary model, the phylogeny, and the quality of the data as representing valid species differences. I tested the assumptions of independent contrasts and performed sensitivity tests to determine how violations of these assumptions and different datasets affect the results. These tests are critical for interpreting results from independent contrasts (Garland et al. 1992; Purvis and Rambaut 1995) and therefore are described in detail in what follows. To streamline the text, however, only those sensitivity tests that provided different results are reported.

To ensure that the contrasts were properly standardized using branch length information, I tested for an association between the absolute value of the contrasts and their standard deviations (Garland et al. 1992; Purvis and Rambaut 1995). I also examined the association between contrasts and estimates of nodal values (Purvis and Rambaut 1995; Freckleton 2000). These tests revealed that log-transformed data and branch lengths best met the assumptions for the majority of

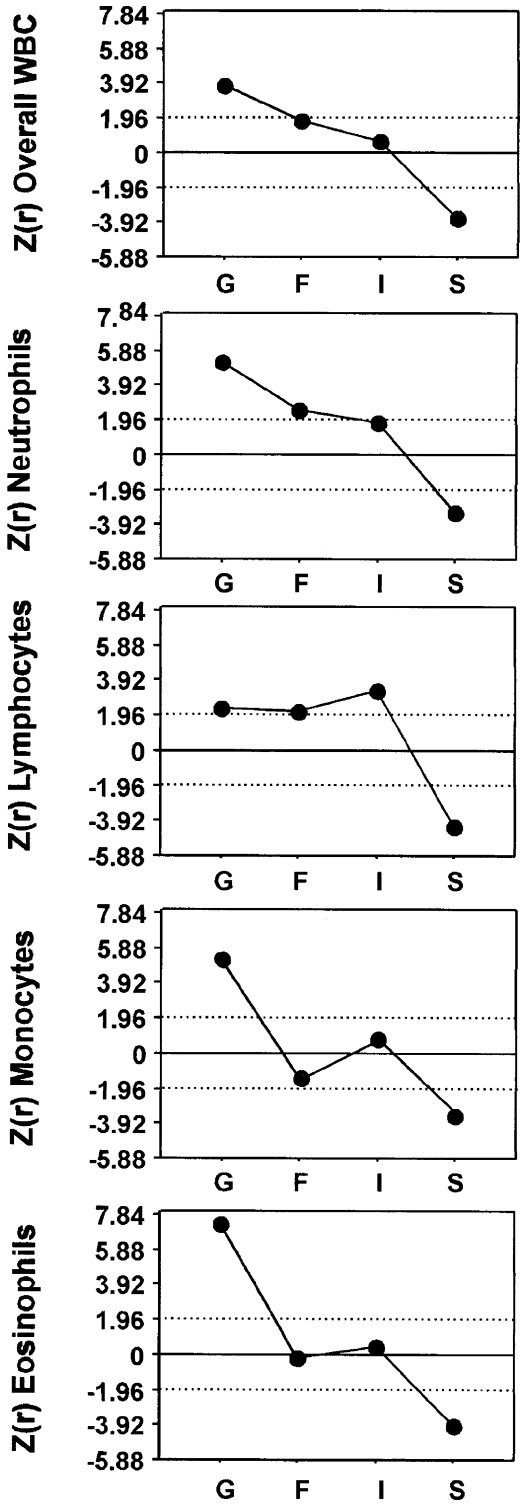

FIG. 2. Phylogenetic autocorrelation and Moran's *I*. Plots show *Z*(*r*) statistics from phylogenetic autocorrelation analysis relative to taxonomic level (G, genus; F, family; I, infraorder; S, suborder). Results are shown for overall white blood cell (WBC) counts and particular WBC types. Significance levels for the *Z*(*r*) scores are indicated with lines at 1.96 and −1.96. These results are based on restricted dataset 1. The plots show that trait values at the genus level were more similar than expected by chance, values generally decreased with increasing taxonomic distance, and values for suborders were significantly negative.

socioecological variables, but unstandardized contrasts best met the assumptions for overall and specific WBC types. (Unstandardized contrasts are equivalent to assuming equal branch lengths, with no adjustment of internal branches for contrasts involving reconstructed nodes; see Felsenstein 1985; Purvis and Rambaut 1995). Comparative tests of the hypotheses were repeated using standardized contrasts for WBC parameters, and these provided generally consistent results. Confounding variables shared through common descent and violations of the assumptions of independent contrasts may produce outliers in contrasts analysis (Price 1997; Purvis and Webster 1999; Harvey and Rambaut 2000; Nunn and Barton 2000). I therefore conducted analyses with and without outlying contrasts, as determined using Mahalanobis distance measures in bivariate and multivariate tests. Removal of outliers is likely to give conservative results. Such results were therefore given precedence in this paper.

Another assumption is that the phylogeny used in the tests accurately reflects the evolutionary history of the species being compared. The Purvis (1995) tree is the only composite phylogeny available for all living primates with information on branch lengths. I based my conclusions on the phylogenetic analyses, but for three reasons I also provide nonphylogenetic results for comparison. First, patterns that hold in nonphylogenetic tests are unlikely to be overturned in phylogenetic analyses when alternative trees become available. Second, comparison of phylogenetic and nonphylogenetic results can often reveal the presence of confounding variables (Price 1997; Purvis and Webster 1999; Nunn and Barton 2000, 2001), which is critical for understanding the suite of parameters that explain variation in a dependent variable and the interactions among these parameters. Finally, nonphylogenetic tests provide more reliable results under an alternative model of evolution (Price 1997; Harvey and Rambaut 2000). In cases of discrete data, nonphylogenetic statistical results may have greatly inflated degrees of freedom (Garland et al. 1993; Nunn and Smith 1998), as compared to continuous data with $n - 1$ contrasts for n species, and should therefore be interpreted with caution.

To assess how data quality affects the results, I performed sensitivity analyses with all primate values from the ISIS dataset and the two restricted datasets mentioned above.

Comparative Data on Primate Socioecological Variables

For group size, population density, and the percentage of time terrestrial, I used information from an unpublished comparative database (the unpublished socioecological data used in this study are available via http://faculty.virginia.edu/charlienunn). In addition to the percentage of time terrestrial, substrate use was examined as a discrete variable, with arboreal species scored as 1, terrestrial species in a wooded environment as 2, and terrestrial species in an open (savanna) environment as 3 (Nunn and van Schaik 2001). To increase the statistical power of analyses of discrete substrate categories in BRUNCH tests (Purvis and Rambaut 1995), I used only those species that provided unambiguous contrasts in substrate use. Information on activity period (diurnal vs. nocturnal) was taken from Nunn and van Schaik (2001). Data on age at first reproduction, longevity, and the interbirth in-

terval were taken mainly from Ross and Jones (1999), and body masses for males and females were obtained from Smith and Jungers (1997). Tests involving body mass used female values (except when calculating testes residuals, where I used male mass from Harcourt et al. 1995; see below), but sensitivity tests using male body mass provided similar results.

For analysis of mating partner number, species were classified as in van Schaik et al. (1999), with females having a single mate per estrous cycle (1 mate), variation between single and multiple mates per cycle (1+ mates), and many mates. A few species were added to van Schaik et al. (1999) or reclassified based on new information and to ensure that unambiguous contrasts in mating activity were used in BRUNCH analyses. In particular, it was sometimes difficult to classify species to the 1+ mates versus many mates categories. Thus, *Alouatta caraya* was given the same value as *Ateles* (=many mates), as were *Cebus* and *Saimiri*, for comparison to the more monogamous *Pithecia pithecia* and *Callicebus moloch*, respectively. All callitrichids were assigned 1+ mates (Goldizen 1988) except for *Callimico goeldii*, which is polygynous (=1 mate per estrous cycle). *Hapalemur griseus* was added (=1 mate) to contrast with *Lemur catta* (=many mates). *Pan paniscus* was assigned a value higher than *Pan troglodytes* (Wrangham 1993). *Homo sapiens* was included in some analyses (classified as 1 mate), although I eliminated humans in analyses of the restricted datasets (see above). Nocturnal species were removed from this analysis to avoid the potentially confounding effects of activity period and, in the case of *Aotus*, extremely high eosinophil counts (see Results).

For exploratory analyses of potentially confounding variables, specific predictions were not possible. I therefore used two-tailed tests with $\alpha = 0.05$. For testing the hypotheses, however, specific directional predictions were possible and one-tailed tests were used. In addition to being justified in the case of specific predictions, an important advantage to using one-tailed tests is that they increase statistical power, which may be important for comparative tests with a limited number of evolutionary transitions. I followed Rothman (1990) in testing each hypothesis without adjusting for experimentwise error rate. Instead, I thoroughly assessed potentially confounding socioecological variables, and I explored the a priori predictions in multiple ways, retaining those that were robust to methodology and dataset.

RESULTS

General Patterns and Confounding Variables

Data on overall WBC counts and specific WBC cell types were available for species representing all the major primate evolutionary radiations (Lorisoidea, Lemuroidea, Ceboidea, Ceropithecoidea, Hominoidea). I found consistent differences across radiations in some WBC types, with relatively high neutrophil counts in New World monkeys and the apes and high lymphocyte counts in lorisid prosimians (Fig. 3). As demonstrated previously (Hawkey 1977), night monkeys (*Aotus* spp.) had remarkably high eosinophil counts (Fig. 3).

First, I examined whether there were consistent correlations among the different WBC types. Most WBC types were associated positively in contrasts analysis (Table 2), whereas

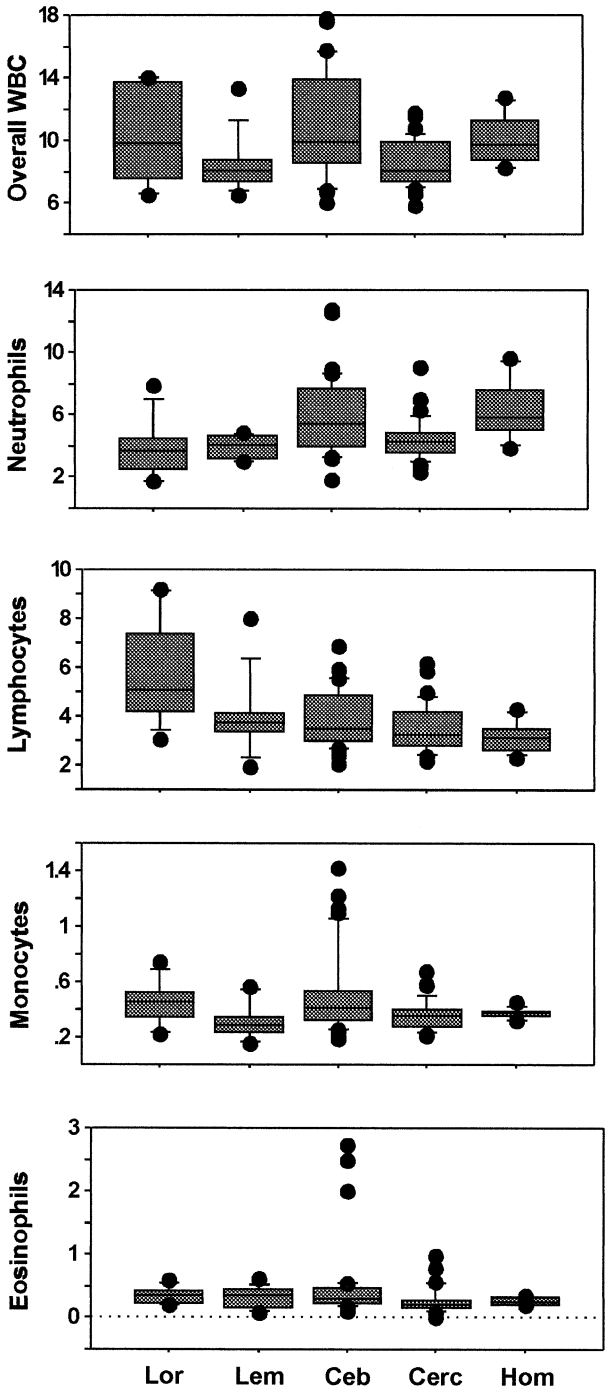

FIG. 3. Broad patterns in white blood cell (WBC) counts across primate radiations. Panels show box plots for overall WBC and specific types of WBC. Boxes indicate upper and lower quartiles (the interquartile range), divided at the median, with vertical lines representing the smallest and largest values within 1.5 interquartile ranges. Values beyond these ranges are plotted individually. Lor, Lorisoidea; Lem, Lemuroidea; Ceb, Ceboidea; Cer, Cercopithecoidea; Hom, Hominoidea. Units are cells $\times 10^9$ per L. The prominent outliers in the eosinophil plot of New World monkeys (Ceboidea) are *Aotus* monkeys (see also Hawkey 1977).

TABLE 2. Associations among white blood cell types.

Comparison	Number of contrasts[1]	Slope[2]	*F*-statistic[3]
Neutrophils–lymphocytes	88	−0.096	1.11
Neutrophils–monocytes	90	0.27	6.59**
Neutrophils–eosinophils	90	0.16	0.99
Lymphocytes–monocytes	89	0.51	22.5***
Lymphocytes–eosinophils	88	0.78	16.7***
Monocytes–eosinophils	86	0.13	0.67

[1] Sample sizes differ because of exclusion of different numbers of outliers among contrasts (see Materials and Methods).

[2] Slope is the second variable of the pair regressed on the first. Regression rather than correlation was used because the method of independent contrasts assumes no intercept (Garland et al. 1992), which is more easily implemented for regression than correlation in existing statistical packages (e.g., Barton and Harvey 2000).

[3] * $P < 0.05$, ** $P < 0.025$; *** $P < 0.01$, two-tailed tests. The degrees of freedom for significance tests were 1 and (number of contrasts − 1) because the line is forced through the origin in statistical analysis of contrasts (Garland et al. 1992).

overall WBC counts were unrelated to platelets ($b_{\text{platelets}} = -0.04$, $F_{1,82} = 0.16$, $P = 0.69$) or RBCs ($b_{\text{rbc}} = -0.017$, $F_{1,85} = 0.21$, $P = 0.65$; see also Bennett and Hawkey 1988). Ecological and social correlates of RBC and platelet counts, about which little are known (Bennett and Hawkey 1988; Hawkey et al. 1991; Semple et al., unpubl. ms.), are therefore unlikely to confound testing of the hypotheses involving WBC.

All lorisids are nocturnal and have high lymphocyte counts (Fig. 3), suggesting that lymphocytes are associated with activity period. Among four contrasts available in activity period, lymphocytes were significantly higher in nocturnal species ($t = 3.21$, df = 3, $P < 0.05$), but this significant result disappeared when using standardized contrasts ($t = 2.41$, df = 3, $P = 0.09$). Use of other WBC types produced nonsignificant results ($P = 0.17$–0.21). In the restricted datasets, only three contrasts in activity period were available, but the increase in lymphocytes among nocturnal species still approached significance (e.g., restricted dataset 1, unstandardized contrasts: $t = 3.58$, df = 2, $P = 0.07$).

Diet is an important socioecological variable in primate comparative studies (Milton and May 1976; Clutton-Brock and Harvey 1977; Janson and Goldsmith 1995; Nunn and Barton 2000). Thus, diet (folivory vs. frugivory) may influence WBC counts or their correlates, but clear predictions are difficult to formulate. One possibility is that folivorous primates adjust immune defenses, either positively or negatively, to maintain symbiotic bacteria needed to digest leaves (Fleagle 1988). Another is that folivores are more energetically constrained (e.g., Milton 1984; Janson and Goldsmith 1995), such that they cannot bear the costs of higher levels of baseline immune defense. Perhaps reflecting these contradictory, but not mutually exclusive, predictions, analysis of contrasts revealed no significant effect of percentage of leaves in the diet, with a nearly even mix of positive and negative slopes for the different cell types and *P*-values ranging from 0.37 to 0.95 ($n > 67$ in all tests). Use of discrete categories of diet (frugivores vs. folivores) also failed to show any relationship between WBC and diet (seven contrasts, $P = 0.39$–0.91). Transitions to predominantly gum or insect eating (from folivory or frugivory), however, revealed that lymphocytes increased significantly ($t = 2.72$, df = 5, $P = 0.04$), with an increase in eosinophils approaching significance ($t = 2.48$, df = 5, $P = 0.06$), but no other cell types were significant. Many nocturnal species specialize on gums and insects (Bearder 1987), and this may explain the association between nocturnality and high lymphocyte counts (or vice versa).

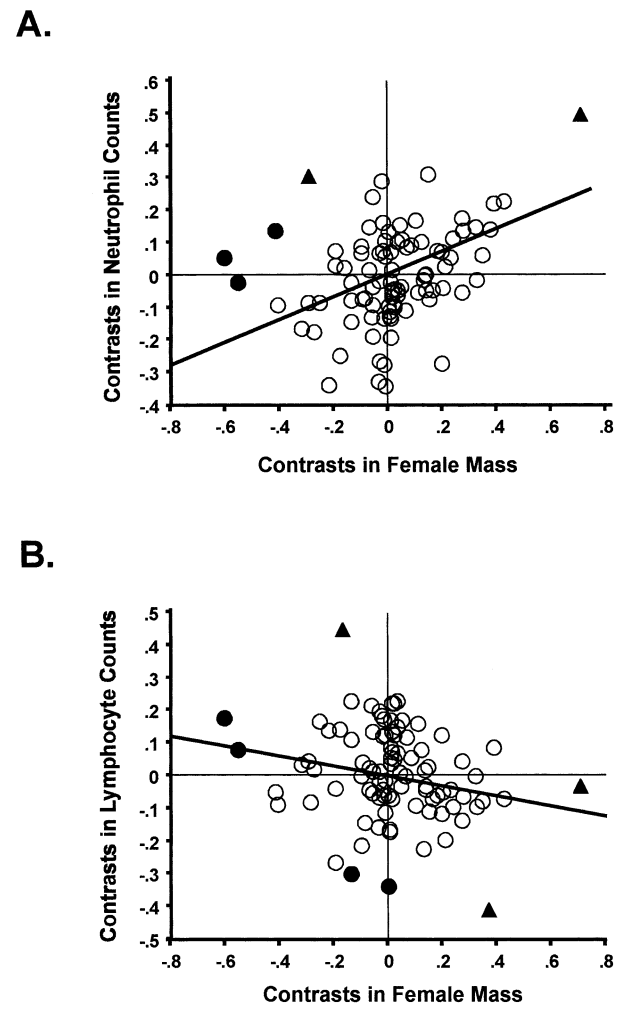

FIG. 4. Allometric patterns in neutrophil and lymphocyte counts. Plots show allometric relationships involving (A) neutrophils and (B) lymphocytes. Lines are slopes from least-squares regression after removal of outliers and with the intercept forced through the origin. Outliers involving at least one nocturnal species are shown as filled triangles, and other outliers are indicated with filled circles. The *x* variable is not positive in this contrasts plot (Garland et al. 1992; Purvis and Rambaut 1995) because multiple variables were entered in the CAIC computer program, with a column of random numbers provided as the independent variable. Thus, the direction of subtraction for calculating contrasts is arbitrary for the plotted values (see Garland et al. 1992).

Finally, I tested for relationships among immune system parameters and body mass (Bennett and Hawkey 1988). Overall WBC counts increased with female body mass, approaching significance in a two-tailed test ($b = 0.11$, $F_{1,85} = 3.51$, $P = 0.06$). This pattern was the consequence of a highly significant relationship involving neutrophils (Fig. 4; $b =$

TABLE 3. Sociality and white blood cell counts.

Test	Group size			Population density		
	LS Slope	*F*-statistic[1]	Number of contrasts	LS Slope	*F*-statistic[1]	Number of contrasts
Overall WBC	−0.024	0.19	83	−0.039	2.24	62
Neutrophils	0.024	0.11	84	−0.074	2.79	62
Lymphocytes	−0.11	2.58	83	0.065	3.57*	62
Monocytes	−0.055	0.58	82	−0.050	1.91	60
Eosinophils	−0.047	0.21	81	0.052	0.74	57
Platelets	−0.10	2.90	75	0.012	0.18	55

[1] * $P < 0.05$ in one-tailed tests based on a priori predictions. The degrees of freedom for significance testing are 1 and (number of contrasts − 1) because the line is forced through the origin in statistical analysis of contrasts (Garland et al. 1992).

0.32, $F_{1,86} = 14.9$, $P = 0.0002$; nonphylogenetic: $b = 0.08$, $F_{1,95} = 8.07$, $P = 0.006$), which are the most common WBC type and therefore a major component of overall WBC. Lymphocytes declined significantly with body mass ($b = -0.15$, $F_{1,91} = 4.53$, $P = 0.04$), but this relationship disappeared after exclusion of outliers ($b = -0.084$, $F_{1,84} = 1.26$, $P = 0.26$). Three of these outliers involved at least one nocturnal species (Fig. 4), and when the analysis was restricted to diurnal species, results were not significant (with outliers included: $b = -0.079$, $F_{1,78} = 1.07$, $P = 0.30$). Other WBC types showed no significant relationship with body mass in contrasts analysis (e.g., eosinophils: $b = 0.052$, $F_{1,85} = 0.16$, $P = 0.69$). In nonphylogenetic tests, the negative relationship between body mass and lymphocytes was highly significant ($b = -0.10$, $F_{1,97} = 22.6$, $P < 0.0001$), eosinophils showed a significantly negative relationship ($b = -0.097$, $F_{1,97} = 4.34$, $P = 0.04$), and monocytes approached significance ($b = -0.054$, $F_{1,97} = 3.48$, $P = 0.07$). These results in nonphylogenetic analyses likely reflect the presence of other confounding variables involving activity period or diet (feeding on insects or gums), which are correlated with body mass across species (Clutton-Brock and Harvey 1977) and shared through common descent.

In sum, these tests suggest that shifts in activity pattern (or correlated shifts in diet) may affect variation in lymphocytes. Moreover, body mass may be a confounding variable in analyses of neutrophils and overall WBC. These variables were therefore taken into account in the tests that follow.

Hypotheses for Disease Risk

Group size and population density

For overall WBC and most WBC types, I found no significant effect of group size or population density on WBC counts with one exception, lymphocytes, which were positively associated with population density (Table 3). Sensitivity tests using the restricted datasets and an alternative compilation of population density (Wrangham et al. 1993) provided no significant results in this test. For other cell types, many of the tests produced slopes that were opposite to predictions (Table 3). Nonphylogenetic analyses further showed that lymphocytes and eosinophils decline with group size ($b = -0.087$, $F_{1,91} = 9.08$, $P = 0.0033$, and $b = -0.13$, $F_{1,91} = 4.27$, $P = 0.042$ respectively). Hence, these tests provided no support for the predicted positive relationship between measures of sociality and WBC counts.

Terrestrial substrate use

Higher WBC counts were predicted in more terrestrial primates, but I found no support for this prediction when using the percentage of time terrestrial (Table 4). I also tested the hypothesis using discrete classifications of arboreal, semiterrestrial, and terrestrial species. Only one significant result was found: in all of eight contrasts, neutrophil counts increased over transitions to increased terrestriality ($t = 4.76$, df = 7, $P = 0.001$). Similar results were found using the restricted datasets, but in nonphylogenetic tests significance disappeared. No consistent trends were found for platelets to increase with increasing terrestriality.

The significant result involving neutrophils may reflect a confounding association between this WBC type and body mass (Fig. 4), as more terrestrial primates have greater mass (Clutton-Brock and Harvey 1977). Consistent with this explanation, body mass increased significantly over transitions in discrete substrate use categories ($t = 3.41$, df = 7, $P = 0.011$; see also Nunn and Barton 2001). The percentage of time terrestrial, however, was not significant in bivariate tests (Table 4) or in multiple regression analyses that included female mass. Nonetheless, multivariate results involving substrate categories and neutrophils were sensitive to the dataset used and the methods of analysis, making it difficult to rule out an independent effect of terrestrial substrate use on neutrophils.

Another approach to disentangling the roles of substrate use and body mass is to use a blood cell type that is uncorrelated with body mass but involved in combating diseases that are likely to be acquired through proximity to soil. I therefore examined patterns in eosinophil counts, because

TABLE 4. White blood cell counts and the percentage of time terrestrial.

Immune system variable	Number of contrasts	LS Slope	*F*-statistic[1]
Overall WBC	39	−0.0014	3.24
Neutrophils	39	−0.00026	0.07
Lymphocytes	39	−0.0025	7.09
Monocytes	39	0.0013	1.08
Eosinophils	39	−0.0049	6.97
Platelets	35	−0.00030	0.14

[1] No results were in the predicted direction and significant. The degrees of freedom for significance tests were 1 and (number of contrasts − 1) because the line is forced through the origin in statistical analysis of contrasts (Garland et al. 1992).

this blood cell combats macroparasites (Roitt et al. 1998), such as helminths, which are commonly present in the soil. Contrary to predictions, however, eosinophils declined with increasing terrestriality (Table 4) and over discrete transitions in substrate use ($t = -0.53$, df = 7, $P = 0.69$).

Life-history traits

To examine how WBC counts covary with life-history traits involving longevity, age at first reproduction, and the interbirth interval, I first regressed contrasts in these life-history traits on contrasts in body mass (significant in all cases) to calculate residuals that are independent of body mass (Harvey and Pagel 1991). Residual longevity and age at first reproduction were not significantly correlated with any WBC type in one-tailed tests. Lymphocytes increased with residual interbirth interval ($b = 0.33$, $F_{1,53} = 5.87$, $P = 0.01$), but no other cell types were significantly positive. The significant result with lymphocytes approached significance in sensitivity tests ($P < 0.10$). I also examined values of life-history parameters without controlling for body mass, but the results from these analyses were significant only in some analyses involving neutrophils, which may reflect an underlying association between this cell type and body mass (Fig. 3).

Mating promiscuity

In comparison to the other predictions, I found a strong association between WBC counts and female mating promiscuity. For overall WBC and all specific cell types, trends were in the predicted direction (Fig. 5). Overall WBC increased significantly (11 of 12 contrasts positive, $t = 3.17$, df = 11, $P = 0.005$), and this appears to be driven mainly by an increase in neutrophils (nine of 12 positive, $t = 2.74$, df = 11, $P = 0.01$) and a nearly significant increase in monocytes (eight of 12 positive, $t = 1.69$, df = 11, $P = 0.06$; other WBC types: lymphocytes, $t = 0.06$, df = 11, $P = 0.47$; eosinophils, $t = 1.23$, df = 11, $P = 0.12$). Eight of 12 contrasts in platelet counts, however, were negative ($t = -2.09$, df = 11, $P = 0.061$). In the restricted datasets, results remained significant for overall WBC and neutrophils, with monocytes significant in some tests. Nonphylogenetic analyses revealed a similar pattern but no significant results, perhaps due to confounding effects of combining data from the different evolutionary radiations (see Figs. 2, 3).

The relationship between mating partner number and neutrophils could be confounded with body mass if mass increases with partner number. Consistent with this possibility, body mass increased significantly over contrasts in mating partner number (10 of 12 contrasts positive, $t = 2.73$, df = 11, $P = 0.02$, two-tailed). The following tests deal with confounding variables and small samples in BRUNCH analyses by using surrogate measures for mating promiscuity that are continuous rather than discrete, including testes mass and the duration of estrus (Nunn et al. 2000). After controlling for body mass, testes mass reflects sperm competition and thus serves to quantify the number of partners a female has during estrus (Harcourt et al. 1981, 1995). I found a significant association between body mass and testes mass (Harcourt et al. 1995) among species with corresponding information on

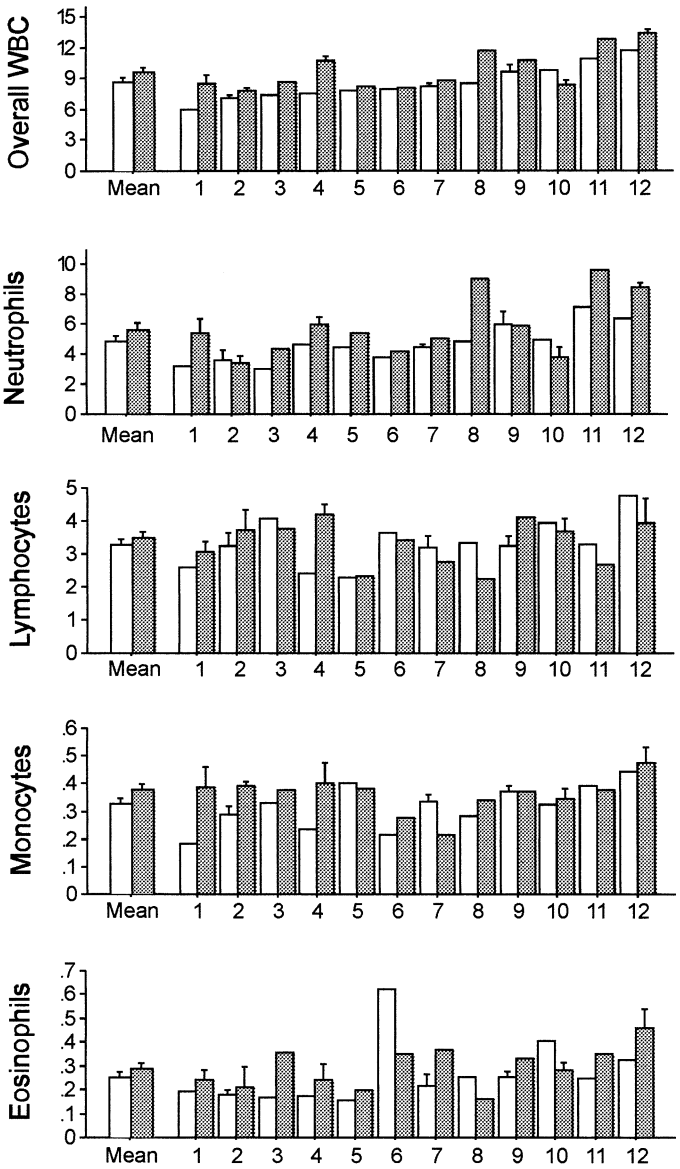

FIG. 5. White blood cell (WBC) counts and the number of mating partners. Bars represent mean blood cell counts for comparisons of less promiscuous taxa (open bars) to those that are relatively more promiscuous (closed bars) for 12 pairs of taxa. Columns labeled mean refer to the average of the 12 contrasts for these comparisons. 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 these comparisons are: (1) *Callimico goeldii* vs. *Callithrix jacchus* + *Saginus* spp. + *Leontopithecus rosalia* + *Cebuella pygmaea*; (2) *Cercopithecus* spp. + *Erythrocebus patas* vs. *Cercocebus* spp.; (3) *Hapalemur griseus* vs. *Lemur catta*; (4) *Macaca silenus* vs. *Macaca* spp.; (5) *Homo sapiens* vs. *Gorilla gorilla*; (6) *Eulemur rubriventer* vs. *Varecia variegata*, *Presbytis* non-*entellus* spp. vs. *Presbytis entellus*; (8) *Theropithecus gelada* vs. *Papio* sp.; (9) *Hylobates* spp. vs. *Pongo pygmaeus*; (10) *Callicebus moloch* vs. *Cebus* spp. + *Saimiri* spp.; (11) *Pan troglodytes* vs. *Pan paniscus*; (12) *Pithecia pithecia* vs. *Alouatta caraya* + *Ateles paniscus* + *Lagothrix lagotricha*.

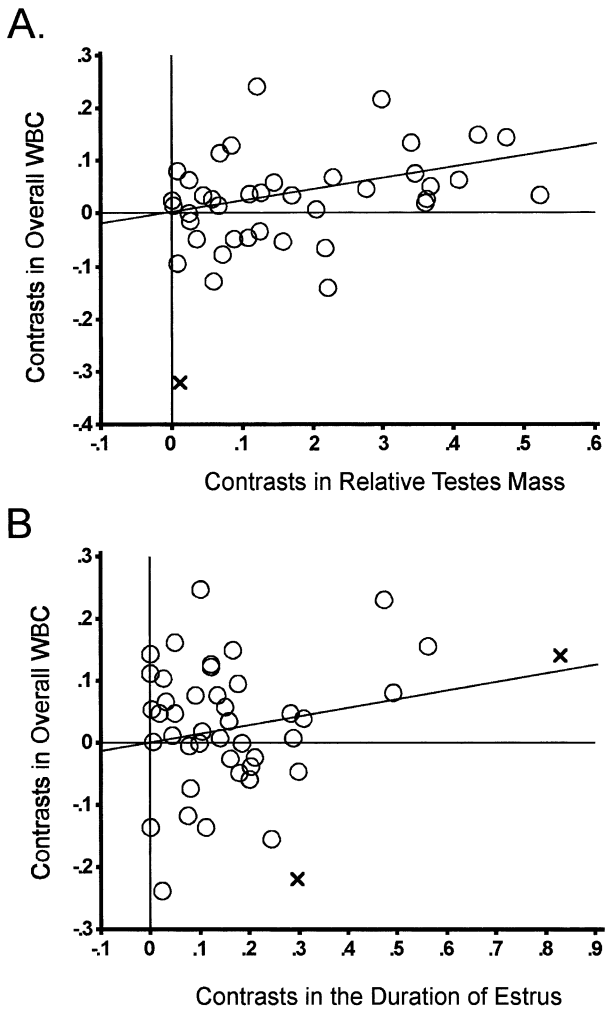

FIG. 6. Quantitative measures of female mating promiscuity. Plots show the relationship between (A) overall white blood cell (WBC) count and relative testes mass and (B) the duration of estrus. Datapoints are independent contrasts, with outliers indicated by Xs. The text provides results with outliers excluded, but results remained significant if outliers were included in the analysis (testes residuals: $F_{1,39} = 7.92$, $P = 0.004$, duration of estrus: $F_{1,43} = 4.35$, $P = 0.022$).

WBC counts ($b = 0.65$, $F_{1,38} = 26.0$, $P < 0.0001$). This slope estimate was therefore used to calculate residuals that are independent of body mass (Harvey and Pagel 1991). A longer duration of estrus enables females to mate with more males and is therefore associated with increased female mating promiscuity (van Schaik et al. 1999). The duration of estrus was not associated with body mass ($b = 0.099$, $F_{1,41} = 0.58$, $P = 0.45$, two-tailed).

Evolutionary increases in relative testes mass were associated with increasing overall WBC counts (Fig. 6; $b = 0.19$, $F_{1,38} = 11.3$, $P = 0.001$) and, of the specific WBC types, neutrophils ($b = 0.30$, $F_{1,37} = 9.19$, $P = 0.002$). Other cell types, including lymphocytes, monocytes, and eosinophils, were in the predicted direction but not statistically significant ($P = 0.09$–0.29). The second measure of promiscuity provided congruent results: overall WBC counts increased with estrous duration (see Fig. 6; $b = 0.18$, $F_{1,41} = 5.09$, $P = 0.015$), and sensitivity tests revealed that increases in neutrophils ($b = 0.23$, $F_{1,33} = 5.32$, $P = 0.014$) and eosinophils ($b = 0.35$, $F_{1,43} = 7.06$, $P = 0.01$, without exclusion of outliers) explain the significant overall result. Nonphylogenetic tests gave consistent results, with overall WBC increasing with residual testes mass ($b = 0.077$, $F_{1,39} = 3.24$, $P = 0.04$) and the duration of estrus ($b = 0.073$, $F_{1,43} = 3.59$, $P = 0.03$).

Previous work in birds has shown that another immune system parameter, the spleen (Møller 1997), is related to measures of sexual selection. I therefore tested whether sexual dimorphism in body mass, a correlate of male intrasexual competition in primates (Clutton-Brock et al. 1977; Mitani et al. 1996), was positively associated with WBC counts. Contrary to this prediction, overall WBC counts showed no association with body size dimorphism ($b = -0.002$, $F_{1,89} = 0.0009$, $P = 0.51$). Other WBC types also were not significant (e.g., neutrophils: $b = 0.095$, $F_{1,87} = 0.05$, $P = 0.59$).

Finally, I tested the hypotheses using multivariate methods and found strongest support for mating promiscuity as a predictor of variation in WBC counts. In a stepwise regression model with overall WBC as the dependent variable and group size, percentage of time terrestrial, female body mass, and relative testes mass as independent variables, only relative testes mass was entered and statistically significant ($b = 0.25$, $F_{1,18} = 7.75$, $P = 0.006$). Similar results were obtained in nonphylogenetic tests, when using neutrophils as the dependent variable, with the duration of estrus substituted for relative testes mass, and when treating the number of mating partners as a continuous variable.

DISCUSSION

These results suggest that risk of STD has shaped macroevolutionary patterns of immune defense parameters in primates (Nunn et al. 2000). Compared to diseases with other transmission modes, little is know about STDs in the wild, but several STDs have been identified in nonhuman primates, including herpesvirus and simian immunodeficiency virus (SIV; Lockhart et al. 1996). There was little consistent support for other factors that are putatively linked to disease risk, including large group size, high population density, terrestrial locomotion, and a slow life history. In addition to the specific hypotheses, I found comparative patterns involving body mass, activity period, and some aspects of diet. These relationships, however, are unlikely to provide alternative explanations for higher WBC counts among promiscuous primates. In terms of assessing data quality, which is important for future comparative tests using the ISIS dataset, I found no significant effect of sample size or number of reporting institutions on variation in mean WBC counts, a strong association between mean WBC counts and phylogeny, and generally consistent results in sensitivity tests of the data and with alternative evolutionary assumptions.

The investigation of confounding variables suggests that nonsexual factors may also influence WBC counts. Thus, Semple et al. (unpubl. ms.) used a different dataset to show that WBC counts are higher in primates that typically inhabit moist environments, consistent with greater parasite risk in such locations (e.g., McGrew et al. 1989; Stuart et al. 1990;

Milton 1996). In the ISIS dataset, some less promiscuous species have relatively high WBC counts and therefore raise questions about potentially confounding variables. For example, the siamang (*Hylobates syndactylus*) is a generally monogamous gibbon but has higher WBC counts than other gibbons (12.38 vs. 8.93 $\times$ 10^9 cells per L averaged for four other gibbon species). It is tempting to attribute this difference to extrapair copulations that have been observed in the siamang (Palombit 1994). Because extrapair copulations are not limited to this gibbon species (Reichard 1995), however, a more likely explanation involves the large body mass of the siamang relative to other gibbons (Smith and Jungers 1997). Another exception is the white-faced saki (*Pithecia pithecia*; overall WBC = 11.66 $\times$ 10^9 cells per L). Sakis are seed predators (Robinson et al. 1987), suggesting that risk of infection from cracked teeth or cut gums might explain their higher WBC counts. When looking across species at other seed predators (Rowe 1996), however, there was no clear effect of seed eating on overall WBC counts ($n = 7$, $t = 0.74$, df = 6, $P = 0.49$, two-tailed). More fine-grained tests, using information on the percentage of seeds in the diet and the hardness of those seeds, may reveal an effect of seed eating on neutrophil counts, because this cell type shows a trend to increase with discrete shifts to seed eating ($n = 7$, $t = 1.88$, df = 6, $P = 0.11$, two-tailed).

I also examined the effect of terrestrial substrate use in greater detail, because this hypothesis was ambiguously supported in an earlier analysis that used a smaller sample of species (Nunn et al. 2000). In particular, it was impossible to tease apart the effects of substrate use and body mass on neutrophil counts in the smaller dataset because terrestrial locomotion increases with body mass in primates (Clutton-Brock and Harvey 1977; Nunn and Barton 2001). Two lines of evidence from above suggest that body mass is the primary variable explaining differences in neutrophil counts. First, the percentage of time terrestrial was not associated with neutrophils (see Table 4), yet as expected, the percentage of time terrestrial increased with body mass in this dataset ($b_{\text{mass}} = 38.2$, $F_{1,37} = 6.16$, $P = 0.018$, two-tailed). Second, eosinophils did not increase with terrestrial substrate use despite their role in combating macroparasites acquired from the soil (Roitt et al. 1998). Analysis of other taxonomic groups may provide additional insights to the role of terrestrial locomotion in disease risk. In carnivores, for example, body mass and substrate use are uncorrelated (Gittleman 1985), providing a means to assess their relative contributions to variance in neutrophil counts.

Another important difference emerged between these tests and the study of Nunn et al. (2000). Here, I found that neutrophils were the primary WBC type correlated with mating promiscuity, whereas Nunn et al. (2000) showed that all WBC types increased with mating promiscuity (eosinophils were not examined). This difference may be related to the ages and sexes of the animals included in the two studies. In the present analyses, I increased sample sizes for the comparative tests by combining male and female values without regard to age of the animals, but Nunn et al. (2000) treated the sexes separately, focusing on adult females. To assess whether the age of the animals was responsible for the different results with particular cell types, I reanalyzed the patterns using only adult or immature animals ($n = 43$, male and female values combined). Statistically significant results emerged for each WBC type when using the adult dataset, but not when using values from immature animals (Table 5). The stronger result in sexually mature animals supports the interpretation that mating behavior influences WBC counts, and neutrophils, monocytes and eosinophils are (nonsignificantly) higher, on average, in adult than immature animals.

TABLE 5. Transitions in mating promiscuity and immature versus adult white blood cell counts.

	Immature WBC		Adult WBC	
	Contrasts in predicted direction	*t*-statistic[1]	Contrasts in predicted direction	*t*-statistic[1]
Overall WBC	6/7	1.91	7/7	3.87***
Neutrophils	5/7	1.01	6/7	3.22***
Lymphocytes	5/7	2.07*	7/7	3.50***
Monocytes	4/7	1.11	5/7	2.14*
Eosinophils	4/7	1.03	5/7	1.13
Platelets	3/7	−0.74	2/7	−1.47

[1] * $P < 0.05$, ** $P < 0.025$, *** $P < 0.01$, one-tailed tests.

Why Are the Other Hypotheses Not Supported?

The hypotheses for disease risk are not mutually exclusive. Thus, it was surprising that only the mating promiscuity hypothesis was supported in these comparative tests. One possibility is that the tests have low statistical power and that the nonsignificant results for the nonmating hypotheses are Type II errors (Cohen 1988; Thomas and Juanes 1996). Although use of one-tailed tests increased the statistical power to detect patterns, I investigated the power of these tests based on the smallest sample size in tests of the key hypotheses (39 contrasts, testing the percentage of time terrestrial). With a significance criterion of 0.05 and a medium effect size (=0.25; see Cohen 1988; Erdfelder et al. 1996), the statistical power of a one-tailed correlation test was reasonably high (=0.61, calculated using G*Power, Ver. 2.0, see Erdfelder et al. 1996). Moreover, patterns in three of the four key WBC types in this test, excluding platelets, were in a direction opposite to predictions.

Thus, low statistical power is unlikely to explain the nonsignificant findings, raising the question of what factors account for the differential support of mating promiscuity over the other nonmating hypotheses. At least three factors may explain why the other hypotheses went unsupported.

Use of data from captive animals

It is possible that by using data on immune system parameters from zoo animals, this study is less likely to find an effect of group size and substrate use. This assumes that current housing affects WBC counts in addition to any genetic influences, which may be true, and it suggests that data on wild animals, which are presently unavailable for a sufficient number of species, might resolve the hypotheses differently. There are several reasons, however, to prefer samples from zoo animals to those from the wild. First, use of zoo data allows more accurate assessment of the health of individual animals, which is necessary for estimating basal

WBC counts. Second, wild animals may experience greater individual variation in the factors that influence WBC counts, given that they often experience greater energetic demands, less balanced diets, more variation in food intake, and exposure to a potentially wider array of parasites and pathogens. Third, use of data from captive animals provides larger samples, which will tend to improve estimates of mean cell counts for a species. Finally, obtaining blood samples from wild animals involves darting or trapping, which may initiate immune and inflammatory responses and thus bias WBC counts (Roitt et al. 1998). Similar stress responses may occur in captive animals, but the magnitude of these effects is likely to be greater in unhabituated animals exposed to greater environmental stresses in the wild.

Fewer behavioral counterstrategies to sexually transmitted diseases

The absence of behavioral counterstrategies may make immune compensation more important for STDs than for parasites transmitted through the soil or by social contact. Behavioral counterstrategies operate in conjunction with the immune system to defend the body from parasites and pathogens (Hart 1990; Møller et al. 1993; Loehle 1995). These behaviors therefore modulate disease risk and reduce the need for immunological protection. For example, experiments have demonstrated that female rodents can detect infections in conspecifics, including potential mates, through olfactory cues, and they take appropriate action to evade infectious disease (e.g., Kavaliers et al. 1997; Penn et al. 1998; Klein et al. 1999). Similarly, primates are thought to avoid fecal contamination of the soil (Hausfater and Meade 1982), and they expend considerable amounts of energy avoiding biting insects that serve as disease vectors (Dudley and Milton 1990), including anointment with chemical defenses (Valderrama et al. 2000)

Compared to parasites with other transmission modes, less is known about behavioral counterstrategies to STDs (e.g., Loehle 1995). Plausible behavioral mechanisms to avoid STDs may occur before or after copulation. In terms of precopulatory strategies, one possibility is to identify infected individuals and avoid mating with them. Thus far, there have been no experimental studies to determine if olfactory cues could be used to discriminate individuals with STDs, as compared to disease with other transmission modes in the abovementioned experiments on rodents (Kavaliers et al. 1997; Penn et al. 1998; Klein et al. 1999). Genital inspections are common prior to mating in many species of primates (Dixson 1998), but tend to be by males inspecting females (C. Nunn, unpubl. comparative dataset) and so probably function to assess fertility rather than STD infection. Visual identification of infected partners is also likely to be difficult because many STDs are not directly observable. Thus, whereas some human STDs exhibit visible symptoms in some infected individuals, such as syphilis and genital herpes, others tend to exhibit few readily observable symptoms, including chlamydia and HIV (Holmes et al. 1994). Several factors may lead to low detectability of STDs. If choices are made based on infection cues, this is likely to reduce the virulence of the STD and reduce the importance of detection, as examined in a recent theoretical model (Knell 1999). Moreover, infected hosts and parasites will have congruent interests in hiding the infection status of the host: Individuals identified as infected would have low reproductive success, as would the pathogen infecting them. Finally, avoidance of STDs through female choice will be difficult in one-male groups because alternative mating partners are unavailable to females, or under male sexual coercion in multimale groups (Smuts and Smuts 1993; Clutton-Brock and Parker 1995), but I found no association between WBC counts and measures of sexual dimorphism.

Postcopulatory mechanisms to avoid STDs may also exist. For example, Hart et al. (1987) showed that genital grooming reduces sexual transmission of bacteria in male rats. In some prosimian primates, males orally groom their genitals following mating (e.g., ringtailed lemurs, *Lemur catta*; Jolly 1966). Genital grooming rates should therefore increase over evolutionary transitions in mating promiscuity, but few data are available for testing this prediction. This also leaves unexplained the behaviors used by anthropoid primates, including humans (Donovan 2000a, b), to avoid STDs. Monkeys and apes have been reported to inspect their own genitals following mating (C. Nunn, unpubl. comparative database), but it is unclear how this might reduce the risk of STDs. Postcopulatory urination is a common strategy to avoid STDs in humans, and it may kill pathogens in the male's urethra (Donovan 2000b), but in nonhuman primates little is known about this behavior in relation to copulation.

Thus, it is too early to know whether behavioral counterstrategies are less effective against STDs, as compared to diseases with other transmission modes. But if correct, then the absence of effective behavioral counterstrategies to STDs could explain the need for increased immunological protection, leading to less support for the nonsexual hypotheses in comparative tests.

Transmission mode and host-parasite interactions

STDs tend to be persistent and immunoevasive (Quinn 1992; Lockhart et al. 1996). By comparison, many socially transmitted diseases have a hit-and-run strategy, with infection and transmission occurring before the inducible immune system can be activated (Antia et al. 1994; Roitt et al. 1998). Given that mucosal defenses can prevent establishment of STDs (Quinn 1992; Cohen et al. 1994), it may be more important to have higher circulating leukocytes when the risk of acquiring STDs increases. Important questions remain, however, regarding the relationship between circulating leukocytes and mucosal immunity in the genitourinary tract (Hogarth 1982; Quinn 1992; Cohen et al. 1994).

In this regard, neutrophils play an active role in phagocytosis of sperm in the female reproductive tract in mammals (humans: Pandya and Cohen 1985; Barratt et al. 1990; rabbits: Phillips and Mahler 1977) and may be critical for preventing STD infection. Destruction of sperm may function to reduce disease transmission, because seminal fluids are known to carry sexually transmitted pathogens (Lockhart et al. 1996). We have therefore interpreted the association between WBC and promiscuity in the context of STDs (the STD hypothesis; Nunn et al. 2000), but phagocytosis of

sperm raises an alternative hypothesis with congruent predictions (the sperm hypothesis). In particular, it is possible that higher basal neutrophil counts function in eliminating sperm from the female reproductive tract regardless of sexually transmitted pathogens. Females may benefit in several ways from removal of unneeded sperm. For example, sperm antigens can cause an immune response and can lead to infertility (Schwimmer et al. 1967; Hogarth 1982; Marshburn and Kutteh 1994). More speculatively, the selective destruction of a particular male's sperm may be an important factor in cryptic female choice (Eberhard 1996). These hypotheses are difficult to disentangle without additional information on the interacting effects of number of mating partners, the diversity of sperm in relation to the immune response, and the overall quantity of sperm to be removed in each estrous cycle. It is entirely plausible, however, that timely removal of sperm involves both elimination of STDs and basic maintenance of the female reproductive tract. Thus, genital secretions also are rich in mucous and immunoglobulins, particularly IgA (Cohen et al. 1994), both of which are involved in preventing the entrance of bacteria and viruses into the body (Roitt et al. 1998).

Conclusions

These comparative results support the hypothesis that female mating promiscuity explains variation in immune defense parameters in primates to the exclusion of other variables involving social, ecological, and life-history factors (Nunn et al. 2000). The interpretation of these results in the context of STDs assumes that higher basal WBC cell counts reflect greater ''immuno-preparedness'' (Møller 1997; Møller et al. 1998). This assumption is reasonable given current knowledge, but additional information is needed on the mechanisms of immune defense against STDs. A complete understanding of the macroevolutionary correlates of risk requires comparative tests using the other surrogate measures of risk mentioned above as well as other approaches, including experiments. Little is known about STDs in nature (Loehle 1995; Lockhart et al. 1996), but these results underscore the need for such information. Finally, similar studies in other groups of organisms are likely to reveal important insights to the correlates of disease risk. Such insights have potentially important implications for our understanding of evolution, animal conservation, and human health.

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Literature Cited

Abouheif, E. 1999. A method for testing the assumption of phylogenetic independence in comparative data. Evol. Ecol. Res. 1: 895–909.

Alexander, R. D. 1974. The evolution of social behavior. Annu. Rev. Ecol. Syst. 5:325–383.

Antia, R., B. R. Levin, and R. M. May. 1994. Within-host population dynamics and the evolution of maintenance of microparasite virulence. Am. Nat. 144:457–472.

Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998a. Host densities as determinants of abundance in parasite communities. Proc. R. Soc. Lond. B 265:1283–1289.

Arneberg, P., A. Skorping, and A. F. Read. 1998b. Parasite abundance, body size, life histories, and the energetic equivalence rule. Am. Nat. 151:497–513.

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

Barton, R. A., and P. H. Harvey. 2000. Mosaic evolution of brain structure in mammals. Nature 405:1055–1058.

Bearder, S. K. 1987. Lorises, bushbabies, and tarsiers: diverse societies in solitary foragers. Pp. 11–24 *in* B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham and T. T. Struhsaker, eds. Primate societies. Univ. of Chicago Press, Chicago, IL.

Bennett, P. M., and C. M. Hawkey. 1988. Comparative haematology: phylogenetic and ecological aspects in mammals and birds. Pp. 33–48 *in* D. J. Blackmore, ed. Animal clinical biochemistry. Cambridge Univ. Press, Cambridge, U.K.

Borer, W. Z. 2000. Reference intervals for the interpretation of laboratory tests. Pp. 1205–1213 *in* R. E. Rakel, ed. Conn's current therapy. W. B. Saunders, Philadelphia, PA.

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

Clutton-Brock, T. H., and G. A. Parker. 1995. Sexual coercion in animal societies. Anim. Behav. 49:1345–1365.

Clutton-Brock, T. H., P. H. Harvey, and B. Rudder. 1977. Sexual dimorphism, socionomic sex ratio and body weight in primates. Nature 269:797–800.

Cohen, J. 1988. Statistical power analysis for the behavioral sciences. Erlbaum Associates, Hillsdale, NJ.

Cohen, M. S., R. D. Weber, and P. A. Mardh. 1994. Genitourinary mucosal defenses. Pp. 117–127 *in* K. K. Holmes, P. F. Sparling, P. A. Mardh, S. M. Lemon, W. E. Stamm, P. Piot and J. N. Wasserheit, eds. Sexually transmitted diseases. McGraw-Hill, New York.

Côté, I. M., and R. Poulin. 1995. Parasitism and group size in social animals: a meta-analysis. Behav. Ecol. 6:159–165.

Cotgreave, P., and D. H. Clayton. 1994. Comparative analysis of time spent grooming by birds in relation to parasite load. Behaviour 131:171–187.

Dacie, J. V., and S. M. Lewis. 1991. Practical haematology. Churchill Livingstone, Edinburgh.

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

Dixson, A. F. 1998. Primate sexuality. Oxford Univ. Press, Oxford, U.K.

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

Donovan, B. 2000a. The repertoire of human efforts to avoid sexually transmissible diseases: past and present. Part 1. Strategies used before or instead of sex. Sex. Transm. Infect. 76:7–12.

———. 2000b. The repertoire of human efforts to avoid sexually transmissible diseases: past and present. Part 2. Strategies used during or after sex. Sex. Transm. Infect. 76:88–93.

Dudley, R., and K. Milton. 1990. Parasite deterrence and the energetic costs of slapping in howler monkeys, *Alouatta palliata*. J. Mammal. 71:463–465.

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

Erdfelder, E., F. Faul, and A. Buchner. 1996. GPOWER: a general power analysis program. Behav. Res. Meth. Instr. Comp. 28: 1–11.

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

Fleagle, J. G. 1988. Primate adaptation and evolution. Academic Press, New York.
Freckleton, R. P. 2000. Phylogenetic tests of ecological and evolutionary hypotheses: checking for phylogenetic independence. Funct. Ecol. 14:129–134.
Freeland, W. J. 1976. Pathogens and the evolution of primate sociality. Biotropica 8:12–24.
Garland, T. J., P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Syst. Biol. 4:18–32.
Garland, T., Jr., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. Syst. Biol. 42:265–292.
Gittleman, J. L. 1985. Carnivore body size: ecological and taxonomic correlates. Oecologia (Heidelb.) 67:540–554.
Gittleman, J. L., and M. Kot. 1990. Adaptation: statistics and a null model for estimating phylogenetic effects. Syst. Zool. 39: 227–241.
Goldizen, A. W. 1988. Tamarin and marmoset mating systems: unusual flexibility. Trends Ecol. Evol. 3:36–40.
Gregory, R. D. 1990. Parasites and host geographic range as illustrated by waterfowl. Funct. Ecol. 4:645–654.
Harcourt, A. H., P. H. Harvey, S. G. Larson, and R. V. Short. 1981. Testis weight, body weight and breeding system in primates. Nature 293:55–57.
Harcourt, A. H., A. Purvis, and L. Liles. 1995. Sperm competition: mating system, not breeding system, affects testes size of primates. Funct. Ecol. 9:468–476.
Hart, B. L. 1990. Behavioral adaptations to pathogens and parasites: five strategies. Neurosci. Biobehav. Rev. 14:273–294.
Hart, B. J., E. Korinek, and P. Brennan. 1987. Postcopulatory genital grooming in male rats: prevention of sexually transmitted infections. Physiol. Behav. 41:321–325.
Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, Oxford, U.K.
Harvey, P. H., and A. Rambaut. 2000. Comparative analyses for adaptive radiations. Proc. R. Soc. Lond. B. Biol. Sci. 355:1–7.
Harvey, P. H., A. F. Read, J. L. John, R. D. Gregory, and A. E. Keymer. 1991. An evolutionary perspective: using the comparative method. Pp. 344–355 *in* C. A. Toft, A. Aeschlimann, and L. Bolis, eds. Parasite-host associations. Oxford Univ. Press, Oxford, U.K.
Hausfater, G., and B. J. Meade. 1982. Alternation of sleeping groves by yellow baboons (*Papio cynocephalus*) as a strategy for parasite avoidance. Primates 23:287–297.
Hawkey, C. M. 1977. The haematology of exotic mammals. Pp. 103–160 *in* R. K. Archer, L. B. Jeffcott and H. Lehmann, eds. Comparative clinical haematology. Blackwell Scientific, Oxford, U.K.
Hawkey, C. M., and P. M. Bennett. 1988. Comparative haematology: clinical aspects in mammals and birds. Pp. 49–62 *in* D. J. Blackmore, ed. Animal clinical biochemistry. Cambridge Univ. Press, Cambridge, U.K.
Hawkey, C. M., P. M. Bennett, S. C. Gascoyne, M. G. Hart, and J. K. Kirkwood. 1991. Erythrocyte size, number and hemoglobin content in vertebrates. Br. J. Haematol. 77:392–397.
Hill, R. A., and R. I. M. Dunbar. 1998. An evaluation of the roles of predation rate and predation risk as selective pressures on primate grouping behaviour. Behaviour 135:411–430.
Hogarth, P. J. 1982. Immunological aspects of mammalian reproduction. Praeger Scientific, Glasgow, Scotland.
Holmes, K. K., P. F. Sparling, P. A. Mardh, S. M. Lemon, W. E. Stamm, P. Piot, and J. N. Wasserheit. 1994. Sexually transmitted diseases. McGraw-Hill, New York.
Janson, C. H. 1998. Testing the predation hypothesis for vertebrate sociality: prospects and pitfalls. Behaviour 135:389–410.
Janson, C. H., and M. Goldsmith. 1995. Predicting group size in primates: foraging costs and predation risk. Behav. Ecol. 6: 326–336.
John, J. L. 1995. Parasites and the avian spleen. Biol. J. Linn. Soc. 54:87–106.
Jolly, A. 1966. Lemur behavior. Univ. Chicago Press, Chicago, IL.
Kavaliers, M., D. D. Colwell, K. P. Ossenkopp, and T. S. Perrot-Sinal. 1997. Altered responses to female odors in parasitized male mice: neuromodulatory mechanisms and relations to female choice. Behav. Ecol. Sociobiol. 40:373–384.
Klein, S. L., H. R. Gamble, and R. J. Nelson. 1999. *Trichinella spiralis* infection in voles alters female odor preference but not partner preference. Behav. Ecol. Sociobiol. 45:323–329.
Knell, R. J. 1999. Sexually transmitted disease and parasite-mediated sexual selection. Evolution 53:957–961.
Larson, S. G. 1985. Organ weight scaling in primates. Pp. 91–113 *in* W. L. Jungers, ed. Size and scaling in primate biology. Plenum, New York.
Lloyd, S. 1995. Environmental influences on host immunity. Pp. 327–361 *in* B. T. Grenfell and A. P. Dobson, eds. Ecology of infectious diseases in natural populations. Cambridge Univ. Press, Cambridge, U.K.
Lockhart, A. B., P. H. Thrall, and J. Antonovics. 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.
Luh, H.-K., J. L. Gittleman, and M. Kot. 1995. Phylogenetic autocorrelation. Ver. 1.1. Available via ftp://ftp.math.utk.edu/pub/luh/PA/hqx.
Maddison, W. P., and D. R. Maddison. 1992. MacClade. Sinauer Associates, Sunderland, MA.
Marshburn, P. B., and W. H. Kutteh. 1994. The role of antisperm antibodies in infertility. Fertil. Steril. 61:799–811.
McGrew, W. C., C. E. G. Tutin, D. A. Collins, and S. K. File. 1989. Intestinal parasites of sympatric *Pan troglodytes* and *Papio* spp. at two sites: Gombe (Tanzania) and Mt. Assirik (Senegal). Am. J. Primatol. 17:147–155.
Milton, K. 1984. Habitat, diet, and activity patterns of free-ranging woolly spider monkeys (*Brachyteles arachnoids* E. Geoffroy 1806). Int. J. Primatol. 5:491–514.
———. 1996. Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata*) population in Panama. J. Zool. (Lond.) 239:39–63.
Milton, K., and M. L. May. 1976. Body weight, diet and home range area in primates. Nature 259:459–462.
Mitani, J., J. Gros-Louis, and A. F. Richards. 1996. Sexual dimorphism, the operational sex ratio, and the intensity of male competition in polygynous primates. Am. Nat. 147:966–980.
Møller, A. P. 1997. Immune defence, extra-pair paternity, and sexual selection in birds. Proc. R. Soc. Lond. B 264:561–566.
Møller, A. P., R. Dufva, and K. Allander. 1993. Parasites and the evolution of host social behavior. Adv. Study Behav. 22:65–102.
Møller, A. P., R. Dufva, and J. Erritzoe. 1998. Host immune function and sexual selection in birds. J. Evol. Biol. 11:703–719.
Morand, S., and R. Poulin. 2000. Nematode parasite species richness and the evolution of spleen size in birds. Can. J. Zool. 78: 1356–1360.
Moret, Y., and P. Schmid-Hempel. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. Science 290:1166–1168.
Nordling, D., M. Andersson, S. Zohari, and L. Gustafsson. 1998. Reproductive effort reduces specific immune response and parasite resistance. Proc. R. Soc. Lond. B 265:1291–1298.
Nunn, C. L., and R. A. Barton. 2000. Allometric slopes and independent contrasts: a comparative test of Kleiber's law in primate ranging patterns. Am. Nat. 156:519–533.
———. 2001. Comparative methods for studying primate adaptation and allometry. Evol. Anthropol. 10:81–98.
Nunn, C. L., and K. K. Smith. 1998. Statistical analyses of developmental sequences: the craniofacial region in marsupial and placental mammals. Am. Nat. 152:82–101.
Nunn, C. L., and C. P. van Schaik. 2000. Intersexual conflict and ecological factors in primate social evolution. Pp. 388–419 *in* C. P. van Schaik and C. Janson, eds. Infanticide by males and its implications. Cambridge Univ. Press, Cambridge, U.K.
———. 2001. Reconstructing the behavioral ecology of extinct primates. Pp. 159–216 *in* J. M. Plavcan, R. F. Kay, W. L. Jungers, and C. P. van Schaik, eds. Reconstructing behavior in the fossil record. Kluwer Academic/Plenum, New York.

Nunn, C. L., J. L. Gittleman, and J. Antonovics. 2000. Promiscuity and the primate immune system. Science 290:1168–1170.
Palombit, R. A. 1994. Extra-pair copulations in a monogamous ape. Anim. Behav. 47:721–723.
Pandya, I. J., and J. Cohen. 1985. The leukocytic reaction of the human uterine cervix to spermatozoa. Fertil. Steril. 43:417–421.
Penn, D., G. Schneider, K. White, P. Slev, and W. Potts. 1998. Influenza infection neutralizes the attractiveness of male odour to female mice (*Mus musculus*). Ethology 104:685–694.
Phillips, D. M., and S. Mahler. 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.
Price, T. 1997. Correlated evolution and independent contrasts. Philos. Trans. R. Soc. Lond. B 352:519–529.
Purvis, A. 1995. A composite estimate of primate phylogeny. Philos. Trans. R. Soc. Lond. B 348:405–421.
Purvis, A., and A. Rambaut. 1995. Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. Comput. Applic. Biosci. 11: 247–251. Available via http://www.bio.ic.ac.uk/evolve/software/caic/index.html.
Purvis, A., and A. J. Webster. 1999. Phylogenetically independent contrasts and primate phylogeny. Pp. 44–68 *in* P. Lee, ed. Comparative primate socioecology. Cambridge Univ. Press, Cambridge, U.K.
Quinn, T. C. 1992. Sexually transmitted diseases. Advances in host defense mechanisms. Vol. 8. J. I. Gallin and A. S. Fauci, series Eds. Raven Press, New York.
Reichard, U. 1995. Extra-pair copulation in a monogamous gibbon (*Hylobates lar*). Ethology 100:99–112.
Reeve, J., and E. Abouheif. 1999. Phylogenetic independence. Ver. 1.1. Available via http://life.bio.sunysb.edu/ee/ehab.
Robinson, J. G., P. C. Wright, and W. G. Kinzey. 1987. Monogamous cebids and their relatives: intergroup calls and spacing. Pp. 47–53 in B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham, and T. T. Stuhsaker, eds. Primate societies. Univ. Chicago Press, Chicago, IL.
Roitt, I. M., J. Brostoff, and D. K. Male. 1998. Immunology. Gower Medical Publishing, London.
Ross, C., and K. E. Jones. 1999. Socioecology and the evolution of primate reproductive rates. Pp. 73–110 *in* P. C. Lee, ed. Comparative primate socioecology. Cambridge Univ. Press, Cambridge, U.K.
Rothman, K. J. 1990. No adjustments are needed for multiple comparisons. Epidemiology 1:43–46.
Rowe, N. 1996. The pictorial guide to the living primates. Pogonias Press, East Hampton, NY.
Rubenstein, D. I., and M. E. Hohmann. 1989. Parasites and social behavior of island feral horses. Oikos 55:312–320.
Schwimmer, W. B., K. A. Ustay, and S. J. Behrman. 1967. An evaluation of immunologic factors of infertility. Fertil. Steril. 18:167–180.
Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol. Evol. 11:317–321.
Smith, G., and A. P. Dobson. 1992. Sexually transmitted diseases in animals. Parasitol. Today 8:159–166.
Smith, R. J., and W. L. Jungers. 1997. Body mass in comparative primatology. J. Hum. Evol. 32:523–559.
Smuts, B. B., and R. W. Smuts. 1993. Male aggression and sexual coercion of females in nonhuman primates and other mammals: evidence and theoretical implications. Adv. Study Behav. 22: 1–63.
Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3rd ed. W. H. Freeman and Co., New York.
Stuart, M. D., L. L. Greenspan, K. E. Glander, and M. R. Clarke. 1990. A coprological survey of parasites of wild mantled howling monkeys, *Alouatta palliata palliata*. J. Wildl. Dis. 26: 547–549.
Thomas, L., and F. Juanes. 1996. The importance of statistical power analysis: an example from *Animal Behaviour*. Anim. Behav. 52:856–859.
Thrall, P. H., J. Antonovics, and J. D. Bever. 1997. Sexual transmission of disease and host mating systems: within-season reproductive success. Am. Nat. 149:485–506.
Thrall, P. H., J. Antonovics, and A. P. Dobson. 2000. Sexually transmitted diseases in polygynous mating systems: prevalence and impact on reproductive success. Proc. R. Soc. Lond. B 267: 1555–1563.
Valderrama, X., J. G. Robinson, A. B. Attygalle, and T. Eisner. 2000. Seasonal anointment with millipedes in a wild primate: A chemical defense against insects? J. Chem. Ecol. 26:2781–2790.
van Schaik, C. P., M. A. van Noordwijk, and C. L. Nunn. 1999. Sex and social evolution in primates. Pp. 204–240 *in* P. C. Lee, ed. Comparative primate socioecology. Cambridge Univ. Press, Cambridge, U.K.
Wrangham, R. W. 1993. The evolution of sexuality in chimpanzees and bonobos. Hum. Nature 4:47–79.
Wrangham, R. W., J. L. Gittleman, and C. A. Chapman. 1993. Constraints on group size in primates and carnivores: population density and day-range as assays of exploitation competition. Behav. Ecol. Sociobiol. 32:199–209.

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