Immune system evolution among anthropoid primates: parasites, injuries and predators

Stuart Semple[*], Guy Cowlishaw and Peter M. Bennett

Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK

In this study we investigate whether present-day variation in a key component of the immune system (baseline leucocyte concentrations) represents evolutionary adaptation to ecological factors. In particular, we test three hypotheses, namely that leucocyte concentrations will be positively related to one of the following: risk of disease transmission between hosts, which is related to host abundance (hypothesis 1), risk of disease infection from the environment due to parasite viability and abundance (hypothesis 2), and risk of injury and subsequent infection, for example following attacks by predators (hypothesis 3). No support was found for hypothesis 1: neither population density nor group size were associated with variation in leucocyte concentrations. Hypothesis 2 was supported: for both sexes, lymphocyte and phagocyte concentrations were positively correlated with annual rainfall, as predicted if interspecific variation in the immune system is related to parasite prevalence (primates suffer higher rates of parasitism in wetter habitats). Support was also provided for hypothesis 3: for both males and females, platelet concentrations were negatively related to body mass, as predicted if injury risk affects immune system evolution, because animals with larger body mass have a relatively lower surface area available to injury. Additional support was provided for hypothesis 3 by the finding that for males, the sex which plays the active role in troop defence and retaliation against predators, concentration of platelets was positively correlated with rate of predation. In conclusion, our analysis suggests that the risk of disease infection from the environment and the risk of injury have played a key role in immune system evolution among anthropoid primates.

Keywords: primates; immune system; leucocyte; parasite; predator; rainfall

1. INTRODUCTION

While theoretical and empirical studies (e.g. Hamilton & Zuk 1982; Sheldon & Verhulst 1996; Norris & Evans 2000) have focused on the role of sexual selection in immune system evolution, markedly less attention has focused on whether variation in the immune system across species reflects evolutionary adaptation to ecological factors. However, many studies have identified social and ecological correlates of parasite abundance and disease prevalence that would be predicted to play a role in shaping the evolution of the immune system.

Two parameters of particular interest in this respect are population density and group size, which may affect parasite transmission rate between hosts. Comparative analysis has indicated that parasite abundance can be strongly correlated with host population density among mammals (Arneberg *et al.* 1998), and meta-analysis has demonstrated that the prevalence and intensity of contagious parasites can be positively correlated with host group size among social animals (Côté & Poulin 1995). More specifically among primates, prevalence of parasitic infection has been found to increase with higher population density (e.g. howler monkeys; Stuart *et al.* 1990), and group size has been found to be positively related to parasitic infection rate (e.g. Amazonian primates; Davies *et al.* 1991).

A further factor which may play a role in affecting patterns of immune system variation among species is habitat humidity and moistness, which can affect disease infection

rate from the environment due to parasite viability and abundance. A number of studies have found a positive correlation between prevalence of ectoparasites and rainfall among mammal species (e.g. Mooring 1995) including anthropoid primates (e.g. baboons and chimpanzees; McGrew *et al.* 1989).

A final ecological parameter which may be important in immune system evolution, but which has received little attention, is injury risk, whether this is due to intraspecific aggression or interactions with predators. As both intraspecific aggressive interactions and attacks by predators can lead to serious injury and a consequent high risk of infection (e.g. Boesch 1991; Drews 1996), it might be expected that these factors could lead to adaptive variation in the immune system.

Here, we investigate whether immune system variation among anthropoid primates reflects evolutionary adaptation to ecological factors. Specifically, we examine concentrations of circulating leucocytes, which are the primary cells involved in immune response (Roitt *et al.* 1998), and test three explicit hypotheses.

(i) *Hypothesis 1: present-day patterns of variation in leucocyte concentrations reflect adaptation to disease transmission rate between hosts due to host abundance.* If this hypothesis is true, it is predicted that leucocyte counts will be greater in species which occur at higher population density, and/or species living in larger groups.

(ii) *Hypothesis 2: present-day patterns of variation in leucocyte concentrations reflect adaptation to disease infection rate from the environment due to parasite viability and abundance.* If this hypothesis is true, it is predicted

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that leucocyte counts will be greater in species inhabiting higher-rainfall areas.

(iii) *Hypothesis 3: present-day patterns of variation in leucocyte concentrations reflect adaptation to risk of injury and subsequent infection.* If this hypothesis is true, it is predicted that leucocyte counts will be lower in species which show larger body mass (because animals with larger body mass have a relatively lower surface area available to injury), and greater in species that experience higher rates of predation.

Anthropoid primates are the focus of this study because they are a biologically diverse group with well described ecology (e.g. Fleagle 1999), making them an ideal taxon for exploring theories about immune system variation that have previously been tested primarily in birds. Despite their suitability for this type of investigation, to our knowledge only one previous comparative study has examined factors involved in immune system evolution in primates (Nunn *et al.* 2000). This study demonstrated that female promiscuity may have played a key role in determining present-day variation in immune defence, with more promiscuous species showing higher levels of circulating lymphocytes, potentially as an adaptation to the risk of sexually transmitted disease (Nunn *et al.* 2000). Focusing on anthropoid primates has the additional advantage that the elucidation of functional variation in their immune system may have useful practical implications for understanding emerging infectious diseases that pose a risk to both human populations (e.g. Wolfe *et al.* 1998) and endangered wild primates (e.g. Cowlishaw & Dunbar 2000).

Our analysis has two stages. First, we investigate and describe the pattern of variation in leucocyte concentrations across sampling levels (in order to validate the use of the database employed) and taxonomic levels (in order to ascertain the appropriate comparative method to employ). Second, we explore whether patterns of variation in leucocyte concentrations among anthropoid primates can be explained by evolutionary adaptation to ecological factors.

2. MATERIAL AND METHODS

(a) *Data collection*
(i) *Measuring leucocyte concentrations*

In this study, we investigate patterns of immune system variation by examining the concentration of circulating leucocytes in the bloodstream. The vast majority of blood samples used in these analyses were obtained from the routine monitoring of animal health in the Zoological Society of London's collections at Regent's Park and Whipsnade Wild Animal Park between 1965 and 1991. While the obvious differences between the natural and captive environment may mean that data are not representative of wild populations, there are nevertheless considerable advantages to using such captive data. First, all samples were taken from animals held in captivity under similar husbandry conditions; while these conditions varied over time, we believe that this variation will not only be markedly less than that found in the wild but also will not produce a systematic bias across species. Second, these data were collected under standardized conditions from animals judged to be clinically healthy at the time of sampling, so differences between samples should not be

affected by differences in current infections. Finally, consistent methodologies for blood collection and analysis were employed, thus alleviating the problem of differences in methodology between different studies. It is important to point out that leucocyte numbers can be affected by the stressful nature of the sampling procedure (Capitanio *et al.* 1996), and also by other factors such as the time of day when samples are taken (Suzuki *et al.* 1997). However, we do not feel that such factors would have biased our dataset in a systematic way as our data show a high degree of repeatability: most subjects were sampled on multiple occasions and consistent results were obtained for each individual (see below). For further details relating to this dataset, see Hawkey (1975, 1991), Bennett & Hawkey (1988), Hawkey & Bennett (1988) and Bennett *et al.* (1991).

The database comprises haematological counts of 1222 blood samples taken from adult individuals of 33 anthropoid species representing 21 different genera and 6 different families of primates. The abundance of leucocytes in each blood sample was estimated by microscopic count using a haemocytometer and phase contrast microscopy after lysis of red cells with a haemolysing agent (Hawkey 1975, 1991). All leucocyte concentrations were estimated as cells $\times 10^9 \, l^{-1}$. In this study, variation in the median concentrations of three classes of leucocytes is analysed: phagocytes (primarily involved in defence against micro-organism and macro-parasite infection), lymphocytes (primarily involved in immune recognition) and platelets (primarily involved in blood clotting and inflammation) (Roitt *et al.* 1998). While phagocytes and lymphocytes together comprise over 90% of all white blood cells (WBC) across the species in our study, results are also presented for total WBC counts to facilitate comparisons with previous studies. Although platelets are classified as leucocytes (Roitt *et al.* 1998), they are derived from different progenitor cells than other leucocytes and are therefore not included in total WBC counts. Only species for which the number of blood samples was greater than two (for both sexes combined in the initial analysis of taxonomic variation, or for each sex in all other tests) were included in the analyses carried out in this paper. This constraint was imposed in order to reduce the effect of outlying values whilst maintaining statistical power in the analyses. Species values for leucocyte concentrations are shown in table 1.

(ii) *Data on ecological variables*

The database of ecological variables used in this analysis was collated from the literature and is shown in table 1.

(b) *Data analysis*
(i) *Patterns of variation in leucocyte concentrations across sampling levels*

To investigate patterns of variation in concentration of circulating leucocytes at different sampling levels, we carried out a three-level nested analysis of variance (Harvey & Pagel 1991) for each of the three classes of leucocytes. Each sample was scored with respect to sample number, individual identity and species. The percentage of variation that occurred at each level under the nested model was calculated and *F*-ratio statistics were used to estimate whether there was significant independent variation among species (Sokal & Rohlf 1995).

(ii) *Patterns of variation in leucocyte concentrations across taxonomic levels*

We then estimated the amount of variation in leucocyte concentrations at different taxonomic levels (see Owens & Bennett

Table 1. Concentrations of circulating leucocytes among anthropoid primates, shown as number of cells $\times 10^9 l^{-1}$ of blood (values shown are the mean of the median values for each sex), and ecological variables used in the analysis.

species	total WBC	lymphocytes	phagocytes	platelets	male body mass[a] (kg)	female body mass[a] (kg)	population density[b]	group size[c]	annual rainfall[d] (mm)	predation rate[e]
Aotus trivirgatus	8.70	3.47	5.05	248.0	0.81	0.74	27	4	2739	0.00
Callimico goeldi	10.80	6.82	3.92	496.0	0.50	0.47	—	8	2845	—
Callithrix argentata	6.90	3.61	3.36	283.5	0.33	0.36	6	5	1927	—
Callithrix jacchus	7.40	3.30	3.81	565.5	0.36	0.38	—	4	807	—
Cebuella pygmaea	11.30	5.12	4.21	335.0	0.11	0.12	141	6	2139	—
Cebus albifrons	7.70	2.93	4.77	224.5	3.18	2.29	24	25	2869	—
Cebus capucinus	6.02	2.66	3.65	208.0	3.68	2.54	14	16	3424	—
Cebus olivaceus	6.50	2.95	3.42	221.0	3.29	2.52	25	20	1700	0.03
Cercopithecus diana	6.60	4.30	2.92	415.5	5.20	3.90	18	20	1546	—
Cercopithecus hamlyni	7.95	4.50	3.42	409.0	5.49	3.36	—	10	1827	—
Erythrocebus patas	4.65	2.58	2.29	238.0	12.40	6.50	1	29	1045	0.10
Gorilla gorilla	6.30	2.62	3.47	199.5	169.30	75.70	1	7	2017	—
Hylobates lar	9.25	3.34	6.24	249.0	5.90	5.34	5	3	2662	—
Leontopithecus rosalia	6.60	1.89	3.48	412.0	0.62	0.60	—	5	1093	—
Macaca mulatta	12.20	2.84	7.32	379.0	10.61	7.97	9	33	2758	—
Macaca nemestrina	13.20	2.15	10.58	408.5	9.70	5.84	18	18	1984	—
Macaca nigra	10.80	2.46	6.85	454.5	9.89	5.47	69	20	2662	—
Macaca silenus	6.90	2.82	3.59	406.0	8.72	6.10	4	18	869	—
Macaca sylvanus	8.30	0.94	7.72	230.0	16.00	11.00	41	18	242	—
Mandrillus sphinx	7.30	2.21	4.14	267.0	31.60	12.90	5	95	2017	—
Miopithecus talapoin	6.50	3.36	4.13	249.0	1.52	1.28	92	16	1599	—
Pan troglodytes	10.65	3.46	6.57	205.0	46.00	35.90	3	63	2957	0.03
Papio cynocephalus	5.60	3.53	1.52	208.0	21.68	12.18	10	40	1406	0.11
Papio hamadryas	6.74	1.24	4.98	211.5	17.50	10.28	3	69	250	—
Pithecia pithecia	13.40	8.55	5.60	434.5	1.94	1.58	4	5	2071	—
Pongo pygmaeus	8.80	3.84	4.68	185.5	78.30	35.70	3	1	3904	—
Presbytis entellus	9.50	4.09	5.42	280.5	13.31	10.26	76	19	1407	—
Saguinus fuscicollis	7.25	2.18	5.10	487.0	0.34	0.36	16	7	2139	0.15
Saguinus imperator	12.00	3.48	8.52	550.0	0.47	0.48	9	9	2139	0.15
Saguinus labiatus	9.40	3.96	5.14	537.5	0.49	0.53	11	4	2139	—
Saguinus oedipus	9.50	2.37	6.22	349.0	0.42	0.40	32	6	4313	—
Saimiri sciureus	8.00	3.70	3.90	336.5	0.85	0.68	65	39	1996	—
Theropithecus gelada	7.20	1.62	4.71	222.5	19.00	11.70	69	145	1094	0.00

[a] Body mass shown is for adults; data taken from Smith & Jungers (1997).
[b] Population density is expressed as number of individuals per km². References available from the authors on request.
[c] References available from the authors on request.
[d] Annual rainfall was recorded at weather stations close to the centre of the species' geographic range. References available from the authors on request.
[e] Predation rate is the proportion of animals lost to predators per year. Data taken from Hill & Dunbar (1998).

1995), again using species median values. We used the CAIC program (Purvis & Rambaut 1995) and Purvis's composite phylogeny of the primates (Purvis 1995) to calculate the amount of change that occurred in the concentration of each class of leucocytes at each phylogenetic branching point, or 'node'. These changes are referred to as contrasts. However, the contrasts that are produced by the CAIC program, the 'standardized contrasts', cannot be used to compare the amount of change that occurred at different phylogenetic levels. This is because these contrasts have been 'standardized' to control specifically for inequalities in the variance of change across phylogenetic levels. The absolute values of these standardized contrasts were therefore unstandardized by being multiplied by the square root of the expected variance of the contrast, to yield 'unstandardized contrasts'. Then, for each class of leucocyte, we grouped the unstandardized contrasts according to the phylogenetic level that they represented (see Owens & Bennett 1995). We used three levels of phylogenetic grouping: contrasts between species within genera, contrasts between genera within families, and contrasts between families within the order. We then used analysis of variance to test whether unstandardized contrasts were equally distributed, according to size, among phylogenetic levels. Logarithms were used throughout. For platelets we investigated the residual variation after removing the effect of body size, as platelet concentrations are significantly correlated with body mass (see below).

Table 2. Results of the nested analyses of variance showing patterns of variation across sampling levels in leucocyte concentrations.
(Values show percentage of variation in leucocyte concentration at each level. F-ratios are only significant in analyses of variation at the species level, with $p < 0.001$ for all four classes of leucocytes.)

cell type	among samples/within individuals	among individuals/within species	among species
total WBC	10.9	12.1	77.0
lymphocytes	13.2	16.9	69.9
phagocytes	20.0	10.2	69.8
platelets	4.6	5.2	90.2

(iii) *Examining the relationship between leucocyte concentrations and ecological factors*

The relationship between concentrations of circulating leucocytes and ecological factors was examined for each sex separately using median values for each class of leucocyte. The type of analysis used to examine this relationship depended on the patterns of variation found in leucocyte concentrations across taxonomic levels. If, on the one hand, for a particular class of leucocyte, it was found that the size of unstandardized contrasts was equally distributed among phylogenetic levels, associations between leucocyte concentrations and the ecological factors were examined by the independent contrasts method as implemented by the program CAIC (Purvis & Rambaut 1995), and using Purvis's composite phylogeny of the primates (Purvis 1995).

If, on the other hand, it was found that unstandardized contrasts were significantly greater at one taxonomic level than at the others, then associations between concentrations of that class of leucocytes and the ecological factors examined in this study were tested using non-parametric tests (Spearman correlation) on median values calculated at the taxonomic level in question (see Owens & Bennett 1995). Although analysis of contrasts at the tips alone could also have been used in this case, this approach was not adopted as it would have led to a marked loss of statistical power, due to the reduction in sample size.

Preliminary tests for potential allometric effects of body mass on leucocyte concentrations indicated that one of the classes of leucocytes in this analysis—platelets—was significantly correlated with body mass (see below). Where appropriate, therefore, multiple regression was used to control for the effect of body mass when examining the relationship between platelet concentrations and our ecological variables. All statistical tests are two-tailed.

3. RESULTS

(a) *Patterns of variation in leucocyte concentrations across sampling and taxonomic levels*

Across sampling levels, variation in the leucocyte concentrations among samples from the same individual, or among individuals of the same species, is much lower than variation among species (table 2). This indicates that although there is variation between samples from an individual, and between individuals within a species, nevertheless, individuals of the same species are far more similar than those of different species.

Across taxonomic levels, the size of unstandardized contrasts was not significantly higher at any one taxonomic level compared with the others for concentrations of total WBC, lymphocytes or platelets (total WBC: $F_{2,30} = 0.80$, $p > 0.45$; lymphocytes: $F_{2,30} = 0.59$, $p > 0.55$; platelets: $F_{2,30} = 0.39$, $p > 0.65$). As a result of these findings, com-

parative analysis by independent contrasts was used to examine the relationship between these three classes of leucocytes and the ecological variables examined in this study. In the case of phagocytes, however, unstandardized contrasts were significantly greater at the level of species within genera, as compared to between genera within families, or between families ($F_{2,30} = 6.33$, $p < 0.01$). Consequently, analyses of phagocyte concentrations were carried out using non-parametric tests of species-level data.

(b) *Relationship between ecological factors and concentration of circulating leucocytes in anthropoid primates*

(i) *Hypothesis 1: present-day patterns of variation in leucocyte concentrations reflect adaptation to disease transmission risk between hosts due to host abundance*

Concentrations of circulating leucocytes across anthropoid primate species do not appear to be influenced by our measures of disease transmission risk. Both population density and group size are uncorrelated with total WBC, lymphocytes or phagocytes (population density: $n_{male} = 24$, $n_{female} = 20$, $p > 0.05$ in all cases; group size: $n_{male} = 26$, $n_{female} = 23$, $p > 0.10$). Similar results are obtained for platelet concentrations after the effect of body mass on platelet concentration is taken into account (population density: $n_{male} = 21$, $n_{female} = 16$, $p > 0.45$; group size: $n_{male} = 23$, $n_{female} = 19$, $p > 0.70$).

(ii) *Hypothesis 2: present-day patterns of variation in leucocyte concentrations reflect adaptation to disease infection risk from the environment due to parasite viability and abundance*

There is evidence that concentrations of circulating leucocytes are influenced by disease infection risk from the environment: lymphocyte counts are positively correlated with rainfall among males ($b = 0.42$, $n = 26$, $p < 0.01$; figure 1a); a similar near-significant relationship is found for lymphocyte concentrations among females ($b = 0.37$, $n = 23$, $p = 0.068$; figure 1b). A positive correlation with rainfall is also found for phagocyte concentrations in both sexes (males: $r = 0.54$, $n = 26$, $p < 0.01$; females: $r = 0.53$, $n = 23$, $p < 0.01$; figure 1c,d). By contrast, in neither sex does rainfall correlate with platelets, after the effect of body mass on platelet concentration is taken into account ($n_{male} = 23$, $n_{female} = 19$, $p > 0.05$).

(iii) *Hypothesis 3: present-day patterns of variation in leucocyte concentrations reflect adaptation to risk of injury and subsequent infection*

There is evidence that concentrations of circulating leucocytes may be affected by injury risk. For both sexes,
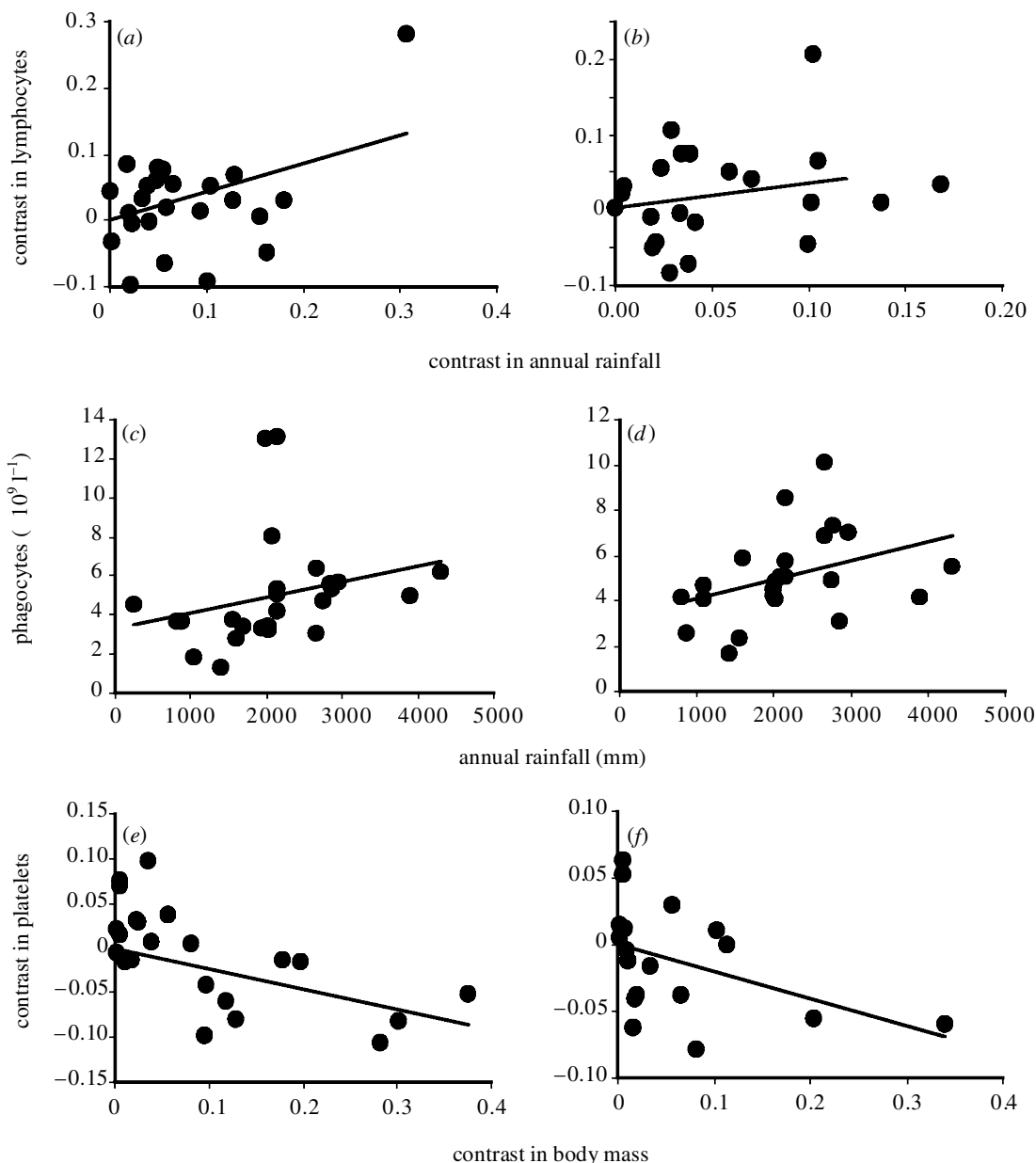
Figure 1. Relationship between ecological variables and WBC counts among male (left-hand side of figure) and female (right-hand side) anthropoid primates. (*a*,*b*) Relationship between median concentration of circulating lymphocytes and the annual rainfall close to the centre of the geographic range, based on independent contrasts generated using CAIC (Purvis & Rambaut 1995). The line is a best fit least-squares linear regression through the origin. (*c*,*d*) Relationship between median concentration of circulating phagocytes and the annual rainfall close to the centre of the geographic range, based on raw species data. The line is a best fit least-squares linear regression. (*e*,*f*) Relationship between median concentration of circulating platelets and adult body mass, based on independent contrasts. The line is a best fit least-squares linear regression through the origin.

platelet concentrations are negatively related to body mass (males: $b = -0.23$, $n = 23$, $p < 0.01$; females: $b = -0.20$, $n = 19$, $p < 0.05$; figure 1*e*,*f*). In neither sex are total WBC, lymphocyte or phagocyte concentrations related to body mass ($n_{male} = 26$, $n_{female} = 23$, $p > 0.05$ in all cases). Among males, the rate of predation is also positively correlated with platelet concentration after the effect of body mass is controlled for by multiple regression ($\beta = 9.41$, $n = 7$, $p < 0.01$; figure 2). None of the other measures of leucocyte concentrations for males are significantly associated with predation rate (total WBC, lymphocytes and phagocytes: $n = 7$, $p > 0.60$ in each case). Predation rate is also uncorrelated with total WBC, lymphocytes, phago-

cytes ($n = 6$, $p > 0.30$ in each case) or platelets ($n = 5$, $p > 0.55$) among females.

4. DISCUSSION

Investigation into the evolutionary history and ecological correlates of immune system variation has been seriously hampered by a lack of sufficient data for a wide number of species. Our study of anthropoid primate species assessed the concentration of circulating leucocytes, a parameter which provides a useful index of immune function (e.g. Zuk *et al.* 1995; Møller 1998; Norris & Evans 2000). Our analyses indicate that immune system vari-
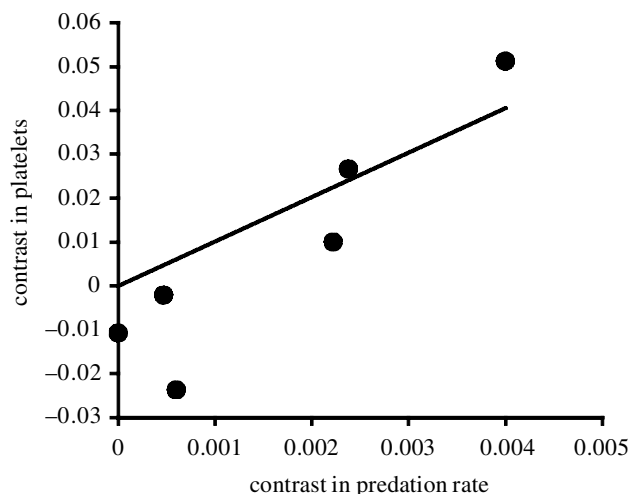
Figure 2. Relationship between the median concentration of circulating platelets and predation rate among males, based on independent contrasts. The line is a best fit least-squares linear regression through the origin. This relationship remains significant after the effect of body mass is taken into account.

ation across anthropoid primates reflects evolutionary adaptation to ecological factors, and further indicates that the type of ecological parameter involved may be critical.

The results of this study suggest, first, that disease transmission risk between hosts (indexed by group size or population density) is a less important determinant of interspecific variation in the immune system than the potential disease infection rate from the environment (indexed by annual rainfall and thus habitat moistness). Nunn *et al.* (2000) similarly found no relationship between a variety of leucocyte counts and either group size or population density across primates, using a different database from ours comprising a different combination of species (these authors did not investigate the effects of rainfall). The effect of habitat moistness on infection rate from the environment is reflected in our finding that species living in wetter habitats show increased circulating concentrations of both phagocytes, which are primarily involved in micro-organism and macro-parasite infection, and lymphocytes, which are responsible for the specific immune recognition of pathogens.

The relationships found in this study between annual rainfall and both phagocyte and lymphocyte concentrations are consistent with previous studies of intraspecific variation in primate parasite loads that have described higher infection rates during wet seasons within populations (e.g. howler monkeys (Milton 1996), chimpanzees (Huffman *et al.* 1997) and gorillas (Watts 1998)), and across primate populations in wetter habitats (e.g. baboons and chimpanzees (McGrew *et al.* 1989) and howler monkeys (Stuart *et al.* 1990)). This finding is also consistent with the observation that previous studies examining the effect of population density and group size on disease prevalence are often confounded by habitat differences, and where it is possible to disentangle these effects the role of habitat is predominant (reviewed in Stuart & Strier 1995). Stuart *et al.* (1993), for example, found that across woolly spider monkey populations, parasite infection rates were lowest in the population with the larg-

est and most cohesive group, and highest where the population density was lowest but the habitat was wettest.

A second ecological factor associated with interspecific variation in leucocyte counts is injury risk. As we might expect, the effects of injury risk are expressed through the concentration of circulating platelets (the leucocyte that plays the primary role in blood clotting and thus wound closure). Platelet counts correlate negatively with body mass in both sexes, and they are the only leucocyte to exhibit such an allometric relationship. This negative correlation might reflect the decrease in surface area/volume ratio associated with increasing body mass; this would lead to a relatively smaller area of skin being exposed to potential injury in larger animals, and thus selection for lower platelet concentrations. While the risk of injury from predation also appears to be strongly associated with the concentration of circulating platelets, it is notable that this result is only observed in males. This finding is consistent with the fact that among anthropoid primates it is the males who play the active role in group defence and retaliation against predators (e.g. capuchins (van Schaik & van Noorwijk 1989), baboons (Cowlishaw 1994) and red colobus (Stanford 1995)).

Whilst, overall, our findings indicate that evolutionary adaptation to ecological factors may explain a significant proportion of interspecific variation in the baseline concentrations of circulating lymphocytes, phagocytes and platelets, we found no such correlates of total WBC counts. This indicates that if immune system evolution is to be studied through measures of leucocyte concentrations, simply using total WBC counts may result in the failure to detect significant relationships involving separate classes of leucocytes.

While a lot of research has focused on the evolutionary and ecological causes and consequences of parasitism and disease among primates (e.g. Freeland 1980; Stuart & Strier 1995; Sorci *et al.* 1997), there has been very little possibility to examine the role of such factors in shaping the evolution of the immune system in this order, primarily due to the paucity of data available. This study, together with that of Nunn *et al.* (2000), thus represents one of the first attempts to conduct a comprehensive investigation of interspecific immune system variation in anthropoid primates. A crucial next step will be to test the results presented in this paper using a database of leucocyte concentrations collected from a larger number of primate species in the wild. Perhaps the most important question to explore in future is the nature of the relationship between components of the immune system among primates, such as leucocyte concentrations, and immunocompetence—the ability to minimize the fitness costs incurred as a result of infection (Owens & Wilson 1999).

REFERENCES

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.)

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

Bennett, P. M., Gascoyne, S. C., Hart, M. G., Kirkwood, J. K. & Hawkey, C. M. 1991 Development of Lynx: a computer application for disease diagnosis and health monitoring in wild mammals, birds and reptiles. *Vet. Rec.* **128**, 496–499.

Boesch, C. 1991 The effects of leopard predation on grouping patterns in forest chimpanzees. *Behaviour* **117**, 220–242.

Capitanio, J. P., Mendoza, S. P. & McChesney, M. 1996 Influences of blood sampling procedures on basal hypothalamic–pituitary–adrenal hormone levels and leukocyte values in rhesus macaques (*Macaca mulatta*). *J. Med. Primatol.* **25**, 26–33.

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

Cowlishaw, G. 1994 Vulnerability to predation in baboon populations. *Behaviour* **131**, 293–304.

Cowlishaw, G. & Dunbar, R. I. M. 2000 *Primate conservation biology*. Chicago University Press.

Davies, C. R., Ayres, J. M., Dye, C. & Deane, L. M. 1991 Malaria infection-rate of Amazonian primates increases with body-weight and group-size. *Funct. Ecol.* **5**, 655–662.

Drews, C. 1996 Contexts and patterns of injuries in free-ranging male baboons (*Papio cynocephalus*). *Behaviour* **133**, 443–474.

Fleagle, J. G. 1999 *Primate adaptation and evolution*, 2nd edn. New York: Academic.

Freeland, W. J. 1980 Primate social groups as biological islands. *Ecology* **60**, 719–728.

Hamilton, W. D. & Zuk, M. 1982 Heritable true fitness and bright birds: a role for parasites. *Science* **218**, 384–387.

Harvey, P. H. & Pagel, M. D. 1991 *The comparative method in evolutionary biology*. Oxford University Press.

Hawkey, C. M. 1975 *Comparative mammalian haematology*. London: Heinemann.

Hawkey, C. M. 1991 The value of comparative haematological studies. *Comp. Haem. Int.* **1**, 1–9.

Hawkey, C. M. & Bennett, P. M. 1988 Comparative haematology: clinical aspects in mammals and birds. In *Animal clinical biochemistry: the future* (ed. D. Blackmore), pp. 49–62. Cambridge University Press.

Hill, R. A. & Dunbar, R. I. M. 1998 An evaluation of the roles of predation rate and predation risk as selective pressures on primate grouping behaviour. *Behaviour* **135**, 411 430.

Huffman, M. A., Gotoh, S., Turner, L. A., Hamai, M. & Yoshida, K. 1997 Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains, Tanzania. *Primates* **38**, 111–125.

McGrew, W. C., Tutin, C. E. G., Collins, D. A. & File, S. K. 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. 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.

Møller, A. P. 1998 Evidence of larger impact of parasites on hosts in the tropics: investment in immune function within and outside the tropics. *Oikos* **82**, 265–270.

Mooring, M. S. 1995 The effect of tick challenge on grooming rate by impala. *Anim. Behav.* **50**, 377–392.

Norris, K. & Evans, M. R. 2000 Ecological immunology: life history trade-offs and immune defence in birds. *Behav. Ecol.* **11**, 19–26.

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

Owens, I. P. F. & Bennett, P. M. 1995 Ancient ecological diversification explains life-history variation among living birds. *Proc. R. Soc. Lond.* B **261**, 227–232.

Owens, I. P. F. & Wilson, K. 1999 Immunocompetence: a neglected life-history trait or conspicuous red herring? *Trends Ecol. Evol.* **14**, 170–172.

Purvis, A. 1995 Composite estimate of primate phylogeny. *Phil. Trans R. Soc. Lond.* B **348**, 405–421.

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

Roitt, I., Brostoff, J. & Male, D. (eds) 1998 *Immunology*, 5th edn. London: Mosby.

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

Smith, R. J. & Jungers, W. L. 1997 Body mass in comparative primatology. *J. Hum. Evol.* **32**, 523–559.

Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*, 3rd edn. New York: Freeman.

Sorci, G., Morand, S. & Hugot, J. P. 1997 Host–parasite coevolution: comparative evidence for covariation of life-history traits in primates and oxyurid parasites. *Proc. R. Soc. Lond.* B **264**, 285–289. (DOI 10.1098/rspb.1997.0040.)

Stanford, C. B. 1995 The influence of chimpanzee predation on group-size and antipredator behavior in red colobus monkeys. *Anim. Behav.* **49**, 577–587.

Stuart, M. D. & Strier, K. B. 1995 Primates and parasites: a case for a multidisciplinary approach. *Int. J. Primatol.* **16**, 577–593.

Stuart, M. D., Greenspan, L. L., Glander, K. E. & Clarke, M. R. 1990 A coprological survey of parasites of wild mantled howling monkeys, *Alouatta palliata palliata*. *J. Wildl. Dis.* **26**, 547–549.

Stuart, M. D., Strier, K. B. & Pierberg, S. M. 1993 A coprological survey of parasites of wild muriquis, *Brachyteles arachnoides*, and brown howling monkeys, *Alouatta fusca*. *J. Helminth. Soc. Wash.* **60**, 111–115.

Suzuki, S. (and 10 others) 1997 Circadian rhythm of leucocytes and lymphocyte subsets and its possible correlation with the function of the autonomic nervous system. *Clin. Exp. Immunol.* **110**, 500–508.

van Schaik, C. P. & van Noorwijk, M. A. 1989 The special role of male *Cebus* monkeys in predation avoidance and its effects on group composition. *Behav. Ecol. Sociobiol.* **24**, 265–276.

Watts, D. P. 1998 Seasonality in the ecology and life histories of mountain gorillas (*Gorilla gorilla beringei*). *Int. J. Primatol.* **19**, 929–948.

Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A. & Lal, A. A. 1998 Wild primate populations in emerging infectious disease research: the missing link? *Emerging Infect. Dis.* **4**, 149–158.

Zuk, M., Johnsen, T. S. & Maclarty, T. 1995 Endocrine–immune interactions, ornaments and mate choice in red jungle fowl. *Proc. R. Soc. Lond.* B **260**, 205–210.