Note

The Effects of Body Mass on Immune Cell Concentrations of Mammals

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abstract: Theory predicts that body mass should affect the way organisms evolve and use immune defenses. We investigated the relationship between body mass and blood neutrophil and lymphocyte concentrations among more than 250 terrestrial mammalian species. We tested whether existing theories (e.g., protecton theory, immune system complexity, and rate of metabolism) accurately predicted the scaling of immune cell concentrations. We also evaluated the predictive power of body mass for these leukocyte concentrations compared to sociality, diet, life history, and phylogenetic relatedness. Phylogeny explained >70% of variation in both lymphocytes and neutrophils, and body mass appeared more informative than other interspecific trait variation. In the best-fit mass-only model, neutrophils scaled hypermetrically ($b = 0.11$) with body mass, whereas lymphocytes scaled just shallow of isometrically. Extrapolating to total cell numbers, this exponent means that an African elephant circulates 13.3 million times the neutrophils of a house mouse, whereas their masses differ by only 250,000-fold. We hypothesize that such high neutrophil numbers might offset the (i) higher overall parasite exposure that large animals face and/or (ii) the higher relative replication capacities of pathogens to host cells.

Keywords: allometry, comparative, diet, immunology, life history, social system.

Background

Ecological and life-history traits affect variation in immune system architecture among and within species (Nunn 2002; Lee et al. 2008; Brock et al. 2014; Downs et al. 2014). Although rarely considered, these patterns may be manifestations of constraints imposed by body mass because many other host traits important to interactions with parasites also scale with body mass (Downs et al. 2019*b*). Indeed, body mass relates to parasite exposure risk (Dobson and Hudson 1986; Han et al. 2015*a*) as well as some aspects of immunity, such as T cell reactivity (Blount et al. 2003). However, comparatively little research has probed how immune variation scales to body size (Lee 2006; Schoenle et al. 2018; Downs et al. 2019*b*). Body mass has profound effects on many organismal traits (Peters 1983; Calder 1984; Schmidt-Nielsen 1984; Brown et al. 2004). These scaling relationships are typically expressed with the equation $Y = aM^b$, where b represents the scaling exponent between body mass (M) and a focal trait (Y) and a is a constant (Calder 1984; Schmidt-Nielsen 1984). Typically, scaling relationships are investigated from log-log transformations of data, as linear forms of equations ($\log(Y) = \log(a) + b \times \log(M)$) are easier to interpret. For instance, isometric relationships occur when large organisms are found geometrically equivalent to small organisms, that is, $b = 0$ for concentrations. By contrast, allometric relationships occur when the trait of interest is proportionally larger (i.e., hypermetric) or proportionally smaller (i.e., hypometric) in large organisms than in small organisms (Calder 1984; Schmidt-Nielsen 1984).

Although the immune system is multifaceted and we thus might expect different scaling relationships for different types of immune defenses (Lee 2006; Demas et al. 2011; Downs et al. 2019*b*), three theoretical frameworks are useful for making predictions. Two frameworks predict that lymphocyte concentrations, our focal trait in this study, scale isometrically ($b = 0$). First, the protecton theory assumes that organisms require comparable levels of protection, predicting that lymphocyte concentrations should be directly proportional to body mass (Langman and Cohn 1987). Second, scaling based on the complexity of the immune system (henceforth, complexity framework) is derived from the metabolic theory of ecology (Brown et al. 2004; Wiegel and

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Perelson 2004; Perelson et al. 2006). It posits that protection by lymphocytes is dependent on delivery to the site of infection, and, based on assumptions about vasculature and surveillance rates, it too predicts isometric scaling (Wiegel and Perelson 2004; Perelson et al. 2006). This framework was recently extended to predict scaling of innate and adaptive responses to West Nile virus (Banerjee and Moses 2009, 2010; Banerjee et al. 2017). Alternatively, the rate of metabolism theory is derived from the observation that metabolic rates often regulate individual performance (Brown et al. 2004; Careau and Garland 2012). This framework thus predicts a hypometric relationship ($b = -0.25$), largely because basal metabolic rate (BMR) likely drives cellular turnover, differentiation, and trafficking rates, which determine concentrations of cells, including leukocytes (Dingli and Pacheco 2006). For example, the hematopoietic stem cell pool scales similarly with BMR across mammals (Dingli and Pacheco 2006).

Of course, body size is not the only factor apt to affect interspecific variation in leukocyte concentrations or other immune traits. Theory predicts—and many studies reveal—that life-history and ecological traits also explain interspecific variation in immunity (Lee 2006; Schoenle et al. 2018). For example, species that live fast and die young should invest minimally in immunity because of trade-offs with reproduction (Lee 2006). Similarly, trophic level and social system complexity should affect exposure to parasites (Blount et al. 2003; Lee et al. 2008), and as species are not evolutionarily independent units, phylogeny too likely affects immune variation (Harvey and Pagel 1991).

Here, we first asked how body mass scales with lymphocyte and neutrophil concentrations across >250 mammal species spanning a more than 250,000-fold range in body mass. Neutrophils are part of the innate immune system and are important early responders to infections, and lymphocytes include diverse cell types that are functionally involved in adaptive and innate responses, including immunoglobulin production, cytotoxicity, and immune regulation (Murphy et al. 2008; Lanier 2013). Neutrophils and lymphocytes comprise the majority of circulating leukocytes, often >70% (Jain 1993). We then asked how body mass predicted leukocyte concentration variation relative to phylogeny and a combination of life history, diet, and other differences among species.

Methods

Trait Data

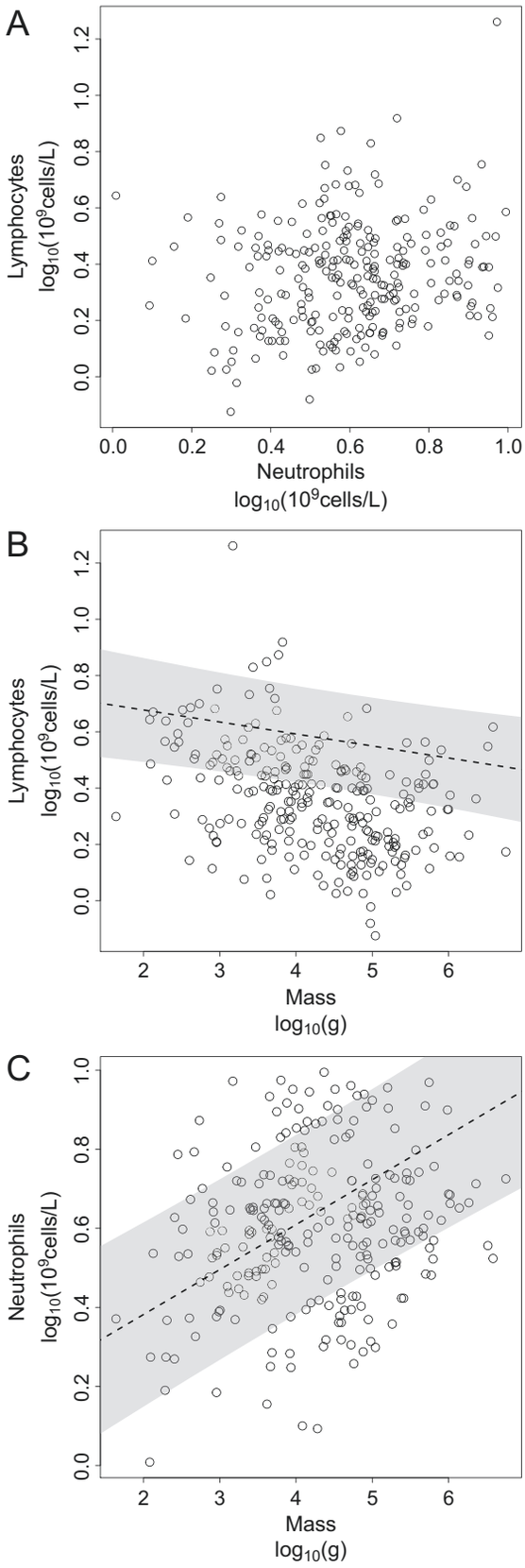
We extracted species means of leukocyte concentrations (cells L^{-1}) for 259 mammalian species from the International Species Information System, now called Species360 (Teare 2013; Gillooly et al. 2017). Species360 data came from healthy captive organisms held at facilities accredited by the Association of Zoos and Aquariums. Although captive animals generally have lower cell counts and can express less variation in immune traits than wild ones (Buehler et al. 2008; Viney et al. 2015; Abolins et al. 2017), using captive animals reduces some confounding effects arising from infections and environmental influences that could occur for wild animals. We extracted mean species data on body mass, trophic level, age at maturation, gestation length, interbirth interval, litter size, maximal life span, and sociality (solitary, pair living, or group living) from PanTHERIA (Jones et al. 2009), AnAge (Tacutu et al. 2012), and other published sources. (Summary data and sources are available in the Dryad Digital Repository; https://doi.org/10.5061/dryad.q4n4884; Downs et al. 2019*a*.) From these data, we calculated maximal reproductive potential for each species as follows:

$$\text{maximal reproductive potential} = \frac{\text{maximal life span} - \text{age of maturation}}{(\text{gestation length} + \text{interbirth interval}) \times \text{litter size}}. \quad (1)$$

Data Analysis

We used an information-theoretic framework to compare hypotheses for scaling relationships between leukocytes and body mass. We fitted three a priori models to $\log_{10}$(lymphocyte) and $\log_{10}$(neutrophil) concentrations: model i, $\log_{10}(\text{leukocyte concentration}) = \log_{10}(a) + 0 \times \log_{10}(\text{mass})$; model ii, $\log_{10}(\text{leukocyte concentration}) = \log_{10}(a) - (1/4) \times \log_{10}(\text{mass})$; and model iii, $\log_{10}(\text{leukocyte concentration}) = \log_{10}(a) + \beta \times \log_{10}(\text{mass})$.

Model i is an intercept-only model that also corresponds to both the protecton theory and the complexity framework, whereas model ii corresponds to the rate of metabolism framework. Model iii does not correspond to a specific hypothesis but rather estimates the scaling exponent from the data (b is a fixed parameter in models i and ii). All models were fitted as phylogenetic mixed effects models using the MCMCglmm package (Hadfield 2010; Hadfield and Nakagawa 2010). The phylogenetic covariance matrix for this analysis was estimated using a phylogenetic tree constructed with National Center for Biotechnology Information molecular data and phyloT (fig. A1, available online; Letunic 2015). Polytomies were excluded using the randomization process in phyloT. All mixed models were fitted using a weak inverse-gamma prior with shape and scale parameters set to 0.01 for the random effect of phylogenetic variance (app. B; apps. A–C are available online). To fit model ii, a prior was used for the fixed effects portion of the model with regression coefficients set to the a priori



hypothesized values (i.e., −1/4) and heavy prior support placed on that value (app. B). Model outputs confirmed that this approach properly fixed coefficients to the a priori values. Default priors for all other fixed effects were used. Model chains were run for 2.6×10^5 iterations, a 60,000-iteration burn-in, and a 200-iteration thinning interval. Results were robust across alternative priors, and chain length was sufficient to yield negligible autocorrelation. We also estimated unadjusted phylogenetic heritability as a measure of how much of the total observed variation was explained by phylogeny (Housworth et al. 2004) and calculated marginal R^2 as a measure of how much of the total variation was explained by the fixed effects (Nakagawa and Schielzeth 2013). Relative support for each model was determined based on deviance information criterion values and differences among models.

To compare the ability of body mass to explain leukocyte concentrations relative to other biological characteristics, we fitted an additional model (model iv) that included $\log_{10}$(mass), maximum longevity, trophic level, sociality, maximal reproductive potential, and all pairwise interactions between these additional characteristics and $\log_{10}$(mass) as fixed effects. In model iv, we used the scaling exponent from the best supported among models i–iii. Model iv was then compared to models i–iii to determine how much intraspecific immune variation was explained by the biological traits in the model including body mass.

Results

Leukocyte Allometry

Lymphocytes ($\log_{10}$ transformed) and neutrophils ($\log_{10}$ transformed) were positively correlated with each other (Pearson's $r = 0.177, t_{258} = 2.88, P = 0.004$; fig. 1*A*). The top models for both leukocytes included body mass with the scaling exponents determined by the data (table 1). The top models accounted for 71% of variation in lymphocytes and 86% in neutrophils, indicating that these models have high explanatory power. Body mass explained 3% of lymphocyte variation and 9% of neutrophil variation. The scaling exponent for lymphocytes was slightly below zero ($b = -0.04$, 95% credible interval [CI] $= -0.08$ to -0.02), whereas it was above zero ($b = 0.11$, 95% CI $= 0.09$ to 0.14) for neutrophils (fig. 1*B*, 1*C*). Traditionally, allometric studies focused

Figure 1: Relationship between lymphocyte and neutrophil concentrations (*A*; $r = 0.177$). Relationship between host body mass and lymphocyte (*B*) and neutrophil (*C*) concentration from simple allometric models (model iii). Equations: $\log_{10}$(lymphocyte concentration) = 0.76 (0.51 to 0.92) − 0.04 (−0.08 to −0.02) × $\log_{10}$(body mass) + ε; $\log_{10}$(neutrophil concentration) = 0.15 (−0.09 to 0.42) + 0.11 (0.09 to 0.14) × $\log_{10}$(body mass) + ε. The shaded area depicts 95% credible intervals of the slope estimate (β) from the top model.

Table 1: Comparison of models testing the effects of body mass and other factors on lymphocyte and neutrophil concentrations among mammal species

Model	DIC	ΔDIC	λ (95% CI)	Marginal R^2 (95% CI)	Model R^2
Lymphocytes:					
i. β_0	−269.82	−11.25			
ii. $\beta_0 + \beta_1 \times \log_{10}(\text{mass})$	*−274.16*	*−6.92*	*.68 (.45–.82)*	*.03 (0–.08)*	*.71*
iii. $\beta_0 - 1/4 \times \log_{10}(\text{mass})$	−166.65	−114.43			
iv. Full model[a]	**−281.21**	**0**	**.67 (.46–.84)**	**.066 (.03–.14)**	**.74**
Neutrophils:					
i. β_0	−298.80	−40.34			
ii. $\beta_0 + \beta_1 \times \log_{10}(\text{mass})$	*−332.15*	*−6.99*	*.77 (.56–.87)*	*.09 (.05–.22)*	*.86*
iii. $\beta_0 - 1/4 \times \log_{10}(\text{mass})$	−85.74	−253.40			
iv. Full model[a]	**−339.14**	**0**	**.71 (.51–.81)**	**.17 (.09–.28)**	**.85**

Note: For all models, the phylogenetic covariance matrix was estimated using a phylogenetic tree constructed with National Center for Biotechnology Information molecular data and phyloT. Phylogenetic heritabilities (λ) indicate how much of the observed variation in either lymphocytes or neutrophils is explained by phylogeny after controlling for fixed effects. Marginal R^2 is a measure of how much of the total variation was explained by the fixed effects. Model R^2 is an estimate of the overall model fit. CI = credible interval; DIC = deviance information criterion; ΔDIC = differences in deviance information criterion among models. Bold indicates the top model overall, and italics indicate the top mass-only model.

[a] $\beta_0 + \beta_1 \times \log_{10}(\text{mass}) + \beta_2 \times \text{max reproduction} + \beta_3 \times \text{max longevity} + \beta_4 \times \text{trophic level} + \beta_5 \times \text{sociality} + \beta_6 \times \log_{10}(\text{mass}) \times \text{max reproduction} + \beta_7 \times \log_{10}(\text{mass}) \times \text{max longevity} + \beta_8 \times \log_{10}(\text{mass}) \times \text{trophic level} + \beta_9 \times \log_{10}(\text{mass}) \times \text{sociality}$.

on equations examine only the relationship between body mass and the trait of interest (i.e., no other fixed effects are included). Thus, the scaling exponents from these models are most comparable to the vast literature reporting scaling exponents, and the best test of the predictions in the literature include body mass as the main/only predictor of leucocyte concentrations. Phylogeny accounted for 68% of variation in lymphocytes and 77% in neutrophils.

Best-Fit Models for Leukocyte Variation

The scaling exponent for body mass in model iv was fitted to the data rather than being set a priori. For both leukocytes, the full models (model iv) had more support than the mass-only models (model iii), indicating that ecological and life-history characteristics explained some interspecific variation. The full model accounted for 74% of variation in lymphocytes and 85% in neutrophils, indicating that these models have high explanatory power (table 1). However, life-history and ecological traits increased the explanatory power of fixed effects to only 6.6% for lymphocytes and 17% for neutrophils. Phylogeny accounted for 71% of variation in lymphocytes and 67% in neutrophils (fig. 2). The scaling exponent for lymphocytes was slightly below zero ($b = -0.09$, 95% CI $= -0.19$ to 0.00), whereas it was above zero ($b = 0.16$, 95% CI $= -0.07$ to 0.38) for neutrophils.

Discussion

Among the more than 250 mammal species spanning five orders of magnitude in body mass, we found little support for available theoretical frameworks for the scaling of immune defenses. The mass-only models for lymphocytes did not support any of the a priori predictions, and body mass explained only ~3% of variation in this cell type. No theory predicted the hypermetric scaling exponent ($b = 0.11$) supported for neutrophils, and as with lymphocytes, body mass explained only a small portion (~9%) of the variation in neutrophils. This outcome changed, however, when controlling for phylogeny (~23%). Adding ecological and life-history traits increased the explanatory power of the nonphylogenetic effects to 17% of total variation in neutrophils and 6.6% in lymphocytes. Altogether, phylogeny explained the most variation in neutrophil and lymphocyte concentrations, and our full models accounted for 74% of variation in lymphocytes and 85% in neutrophils.

Allometric Scaling of Leukocyte Concentrations

Our a priori hypotheses for scaling exponents were not supported (for the mass-only models). In the mass-only and full models, lymphocyte concentrations had a shallow hypometric slope with body mass, which is consistent with prior observations in primates and carnivores (Nunn et al. 2000, 2003*b*) but not Neotropical bats, which showed hypermetric scaling, or rodents, which exhibited isometric scaling (Schneeberger et al. 2013; Tian et al. 2015). These inconsistencies could arise, in part, because circulating lymphocytes are a heterogeneous class of cells with different functions and states of activation (Westermann and Pabst 1990). Alternatively or additionally, the proportion of lymphocyte types might differ among species. For example, 15.4% of lymphocytes in human

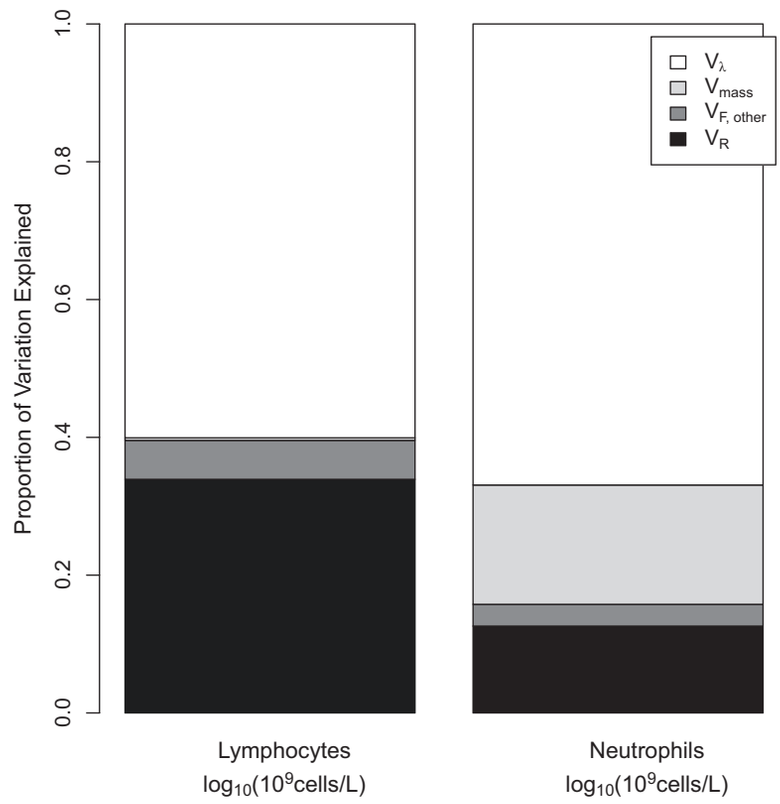


Figure 2: Estimated proportion of variance of lymphocyte and neutrophil concentrations explained by allometric models including additional ecological and life-history parameters (model iv). V_λ = variance explained by the random effects (i.e., phylogeny); V_{mass} = variance explained by body mass; $V_{F, other}$ = variance explained by all other fixed effects; V_R = residual variance.

females are T helper cells and 6% are B cells, whereas in female Wistar rats 59.0% are T helper cells and 24.5% are B cells (Franch et al. 1993; Evans et al. 1999).

Our most intriguing result was the hypermetric relationship detected for neutrophil concentrations in the mass-only models, particularly as all a priori hypotheses predicted isometry or hypometry. Our finding is consistent with observations in bats (Schneeberger et al. 2013) and rodents (Tian et al. 2015). When life-history and ecological variables were included (model iv), the mean estimate for the scaling exponent was also hypermetric, but the 95% CIs overlapped zero. The addition of maximal reproductive effort seemed to mitigate some of the relationship between body mass and neutrophil concentrations.

Although we found that body mass explained a very small proportion of the interspecific variation in neutrophil concentrations, the biological effect could be profound. Because blood volume scales isometrically (Prothero 2015), hypermetric scaling for neutrophil concentrations translates to hypermetric scaling of whole-animal neutrophil counts. To illustrate, we extrapolated from our equation for the best mass-only model (model iii, $b = 0.11$) to total neutrophils by using published body masses, blood volumes, and blood densities (Riches et al. 1973; Prothero 1980; Teare 2013). We used the mass-only model to be consistent with published allometric studies, which have traditionally used models that account for only body mass. We found that a 15-g mouse is expected to circulate 2.74×10^{15} neutrophils, whereas a 3,800-kg African elephant would circulate 3.65×10^{22} neutrophils (Riches et al. 1973; Prothero 1980). This represents a difference of seven orders of magnitude in neutrophil number over a difference of five orders of magnitude in body mass.

Why large animals require so many more neutrophils is currently unclear, but the performance-safety trade-off hypothesis predicts that large animals should overinvest in safety (e.g., immunity; Harrison 2017). The mechanism whereby neutrophils protect hosts may also provide some insight. Neutrophils are a major component of mobile, constitutive innate immunity, and they respond to diverse parasites by moving quickly to infected areas, consuming microbes, and/or dying (Nathan 2006; Hidalgo et al. 2019).

Hosts must produce or mobilize new neutrophils rapidly or maintain high enough constitutive numbers to preempt proliferating parasites (Nathan 2006; Hidalgo et al. 2019). If host cell turnover rates scale hypometrically, as predicted (Brown et al. 2004; Dingli and Pacheco 2006), hosts would have a comparatively weaker capacity to produce new defensive cells, providing a reproductive (and evolutionary) advantage to microbes. Relatedly, large animals are disproportionately more likely to be exposed to new parasites than are small ones, putting them at a further disadvantage (Dobson and Hudson 1986). Although a given gram of tissue in a large host is less exposed to parasites because of a low ratio of surface area to volume, absolute exposure to parasites is probably much higher (Dobson and Hudson 1986; Downs et al. 2019*b*). Given the broad protective effects of neutrophils, disproportionately high concentrations in large mammals might act as a generic first line of defense against diverse and often novel threats.

Influence of Ecological Traits and Life-History Traits on Leukocyte Concentrations

Our modeling approach allowed us to assess whether sociality, trophic level, maximal longevity, and maximal reproductive potential was more informative about interspecific variation in leukocyte concentrations than body mass. The addition of other variables improved model fit for both leukocyte types, and one pattern we detected is consistent with the literature. Specifically, both leucocyte concentrations decreased with reproductive potential (table C1, available online); species with reproductively conservative life histories had comparatively higher concentrations than did productive species. We caution overinterpreting these results, however, because the joint explanatory value of life-history variables was small and because our analytical approach was not designed to determine which ecological and life-history traits were most important in explaining interspecific variation.

Nevertheless, it is somewhat surprising that ecological and life-history variables explained so little variation in our study because they have been important for explaining variation in immune defenses in other interspecific ecoimmunology studies (e.g., Tieleman et al. 2005; Lee 2006; Martin et al. 2006, 2007). This apparent discrepancy might arise because different taxonomic groups exhibit different scaling relationships. Also, immune defenses, including leukocyte concentrations, are highly dynamic (Buehler et al. 2012; Hegemann et al. 2012, 2013), and oftentimes investment in immune defenses depends on current or past infection, host stress, age, season, and nutritional condition (Downs et al. 2014; Wilcoxen et al. 2015; MacColl et al. 2017). Intraspecific variation likely accounts for some of the unexplained variation in our data, and not accounting for it prevented us from investigating scaling relationships within species (Downs and Dochtermann 2014). In the end, as immune defenses relate differently to life-history variation, depending on their relative costs and benefits (Schmid-Hempel and Ebert 2003; Martin et al. 2007; Schoenle et al. 2018), immune defenses are apt to scale differently among and within species, depending on the types of protection they provide.

Phylogeny

Phylogenetic relatedness explained the majority of variation in leukocyte concentrations in our models. Given our analysis, the most we can conclude is that leukocyte concentrations have evolved slowly enough that the signature of phylogeny is observable. Similar phylogenetic conservation has been observed for heterophil-lymphocyte ratios in birds (Minias 2019), bactericidal capacity among some Carnivora (Heinrich et al. 2016), and body mass–parasite diversity relationships in primates (Nunn et al. 2003*a*). In contrast, genus did not predict reservoir status among rodent species (Han et al. 2015*b*), and phylogeny was not predictive of outcomes of epidemiological dynamics (Han et al. 2015*a*). We therefore encourage additional large studies involving broad taxonomic coverage to provide insight into how phylogeny shapes various aspects of host competence (Downs et al. 2019*b*).

Conclusion

The coarse nature of leukocyte concentrations and our reliance on zoo-housed animals might obscure some of the eco-evolutionary relevance of our results. Still, our study found an unexpected pattern in the scaling of neutrophils that was not predicted by previously developed theoretical frameworks. Although we do not yet know the mechanism driving these patterns, body size does appear to impose a modest but detectable constraint on mammalian immunity (Bennett 1987; Brown et al. 2004). The cross-species patterns we found for the scaling of immune cells by using a comparative approach indicate a need for the development of theory about how the architecture of the immune system changes across species and suggest that such an approach can be fruitful. Further, broad comparative patterns can be integrated into models to improve our understanding and management of disease dynamics (De Leo and Dobson 1996; Banerjee et al. 2017; Downs et al. 2019*b*).

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Data and Code Availability

Data used in this analysis are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.q4n4884; Downs et al. 2019*a*).

Literature Cited

Abolins, S., E. C. King, L. Lazarou, L. Weldon, L. Hughes, P. Drescher, J. G. Raynes, J. C. Hafalla, M. E. Viney, and E. M. Riley. 2017. The comparative immunology of wild and laboratory mice, *Mus musculus domesticus*. Nature Communications 8:1–14.

Banerjee, S., and M. Moses. 2009. A hybrid agent based and differential equation model of body size effects on pathogen replication and immune system response. Pages 14–18 *in* P. S. Andrews, J. Timmis, N. D. L. Owens, U. Aickelin, E. Hart, and A. Hone, eds. Artificial immune systems. Springer, Berlin.

———. 2010. Scale invariance of immune system response rates and times: perspectives on immune system architecture and implications for artificial immune systems. Swarm Intelligence 4:301–318.

Banerjee, S., A. S. Perelson, and M. Moses. 2017. Modelling the effects of phylogeny and body size on within-host pathogen replication and immune response. Journal of the Royal Society Interface 14:20170479. https://doi.org/10.1098/rsif.2017.0479.

Bennett, A. F. 1987. The accomplishments of ecological physiology. Page 364 *in* M. E. Feder, A. F. Bennett, W. W. Burggren, and R. B. Huey, eds. New directions in ecological physiology. Cambridge University Press, Cambridge.

Blount, J. D., D. C. Houston, A. P. Møller, and J. Wright. 2003. Do individual branches of immune defence correlate? a comparative case study of scavenging and non-scavenging birds. Oikos 102:340–350.

Brock, P. M., C. C. Murdock, and L. B. Martin. 2014. The history of ecoimmunology and its integration with disease ecology. Integrative and Comparative Biology 54:353–362.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85:1771–1789.

Buehler, D., B. Pierce, and B. Tieleman. 2008. Captive and free-living red knots *Calidris canutus* exhibit differences in non-induced immunity that suggest different immune strategies in different environments. Journal of Avian Biology 39:560–566.

Buehler, D., F. Vézina, W. Goymann, I. Schwabl, M. Versteegh, B. Tieleman, and T. Piersma. 2012. Independence among physiological traits suggests flexibility in the face of ecological demands on phenotypes. Journal of Evolutionary Biology 25:1600–1613.

Calder, W. A. 1984. Size, function, and life history. Harvard University Press, Cambridge, MA.

Careau, V., and T. Garland Jr. 2012. Performance, personality, and energetics: correlation, causation, and mechanism. Physiological and Biochemical Zoology 85:543–571.

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

Demas, G. E., D. A. Zysling, B. R. Beechler, M. P. Muehlenbein, and S. S. French. 2011. Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts. Journal of Animal Ecology 80:710–730.

Dingli, D., and J. M. Pacheco. 2006. Allometric scaling of the active hematopoietic stem cell pool across mammals. PLoS ONE 1:e2.

Dobson, A., and P. Hudson. 1986. Parasites, disease and the structure of ecological communities. Trends in Ecology and Evolution 1:11–15.

Downs, C. J., J. S. Adelman, and G. E. Demas. 2014. Mechanisms and methods in ecoimmunology: integrating within-organism and between-organism processes. Integrative and Comparative Biology 54:340–352.

Downs, C. J., and N. A. Dochtermann. 2014. Testing hypotheses in ecoimmunology using mixed models: disentangling hierarchical correlations. Integrative and Comparative Biology 54:407–418.

Downs, C. J., N. A. Dochtermann, R. Ball, K. C. Klasing, and L. B. Martin. 2019*a*. Data from: The effects of body mass on immune cell concentrations of mammals. American Naturalist, Dryad Digital Repository, https://doi.org/10.5061/dryad.q4n4884.

Downs, C. J., L. A. Schoenle, B. A. Han, J. F. Harrison, and L. B. Martin. 2019*b*. Scaling of host competence. Trends in Parasitology 35:182–192.

Evans, D. M., I. H. Frazer, and N. G. Martin. 1999. Genetic and environmental causes of variation in basal levels of blood cells. Twin Research 2:250–257.

Franch, A., C. Castellote, C. Pelegrí, E. Tolosa, and M. Castell. 1993. Blood B, T, $CD4^+$ and $CD8^+$ lymphocytes in female Wistar rats. Annals of Hematology 67:115–118.

Gillooly, J. F., J. P. Gomez, and E. V. Mavrodiev. 2017. A broad-scale comparison of aerobic activity levels in vertebrates: endotherms versus ectotherms. Proceedings of the Royal Society B 284: 20162328.

Hadfield, J. D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software 33:1–22.

Hadfield, J. D., and S. Nakagawa. 2010. General quantitative genetic methods for comparative biology: phylogenies, taxonomies and multi-trait models for continuous and categorical characters. Journal of Evolutionary Biology 23:494–508.

Han, B. A., A. W. Park, A. E. Jolles, and S. Altizer. 2015*a*. Infectious disease transmission and behavioural allometry in wild mammals. Journal of Animal Ecology 84:637–646.

Han, B. A., J. P. Schmidt, S. E. Bowden, and J. M. Drake. 2015*b*. Rodent reservoirs of future zoonotic diseases. Proceedings of the National Academy of Sciences of the USA 112:7039–7044.

Harrison, J. F. 2017. Do performance-safety trade-offs cause hypometric metabolic scaling in animals? Trends in Ecology and Evolution 32:653–664.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford University Press, New York.

Hegemann, A., K. D. Matson, C. Both, and B. I. Tieleman. 2012. Immune function in a free-living bird varies over the annual cycle, but seasonal patterns differ between years. Oecologia 170:605–618.

Hegemann, A., K. D. Matson, M. A. Versteegh, A. Villegas, and B. I. Tieleman. 2013. Immune response to an endotoxin challenge involves multiple immune parameters and is consistent among the annual-cycle stages of a free-living temperate zone bird. Journal of Experimental Biology 216:2573–2580.

Heinrich, S. K., B. Wachter, O. H. Aschenborn, S. Thalwitzer, J. Melzheimer, H. Hofer, and G. A. Czirjak. 2016. Feliform carnivores have a distinguished constitutive innate immune response. Biology Open 5:550–555.

Hidalgo, A., E. R. Chilvers, C. Summers, and L. Koenderman. 2019. The neutrophil life cycle. Trends in Immunology 40:584–597.

Housworth, E. A., E. P. Martins, and M. Lynch. 2004. The phylogenetic mixed model. American Naturalist 163:84–96.

Jain, N. C. 1993. Essentials of veterinary hematology. Blackwell, Philadelphia.

Jones, K. E., J. Bielby, M. Cardillo, S. A. Fritz, J. O'Dell, C. D. L. Orme, K. Safi, W. Sechrest, E. H. Boakes, and C. Carbone. 2009. PanTHERIA: a species-level database of life history, ecology, and geography of extant and recently extinct mammals. Ecology 90:2648.

Langman, R. E., and M. Cohn. 1987. The ET (elephant-tadpole) paradox necessitates the concept of a unit of B-cell function: the protection. Molecular Immunology 24:675–697.

Lanier, L. L. 2013. Shades of grey—the blurring view of innate and adaptive immunity. Nature Reviews Immunology 13:73.

Lee, K. A. 2006. Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology 46:1000–1015.

Lee, K. A., M. Wikelski, W. D. Robinson, T. R. Robinson, and K. C. Klasing. 2008. Constitutive immune defences correlate with life-history variables in tropical birds. Journal of Animal Ecology 77:356–363.

Letunic, I. 2015. phyloT: phylogenetic tree generator. http://phylot.biobyte.de.

MacColl, E., K. Vanesky, J. Buck, B. M. Dudek, C. A. Eagles-Smith, J. A. Heath, G. Herring, C. Vennum, and C. J. Downs. 2017. Correlates of immune defenses in golden eagles. Journal of Experimental Zoology A 327:243–253.

Martin, L. B., D. Hasselquist, and M. Wikelski. 2006. Investment in immune defense is linked to pace of life in house sparrows. Oecologia 147:565–575.

Martin, L. B., Z. M. Weil, and R. J. Nelson. 2007. Immune defense and reproductive pace of life in *Peromyscus* mice. Ecology 88:2516–2528.

Minias, P. 2019. Evolution of heterophil/lymphocyte ratios in response to ecological and life-history traits: a comparative analysis across the avian tree of life. Journal of Animal Ecology 88:554–565.

Murphy, K., P. Travers, and M. Walport. 2008. Janeway's immunobiology. Garland Science, New York.

Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. Methods in Ecology and Evolution 4:133–142.

Nathan, C. 2006. Neutrophils and immunity: challenges and opportunities. Nature Reviews Immunology 6:173–182.

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

Nunn, C. L., S. Altizer, K. E. Jones, and W. Sechrest. 2003*a*. Comparative tests of parasite species richness in primates. American Naturalist 162:597–614.

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

———. 2003*b*. A comparative study of white blood cell counts and disease risk in carnivores. Proceedings of the Royal Society B 270:347–356.

Perelson, A. S., J. G. Bragg, and F. W. Wiegel. 2006. The complexity of the immune system: scaling laws. Pages 451–459 *in* T. S. Deisboeck and J. Yasha Kresh, eds. Complex systems science in biomedicine. Springer, New York.

Peters, R. 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

Prothero, J. W. 1980. Scaling of blood parameters in mammals. Comparative Biochemistry and Physiology A 67:649–657.

———. 2015. The design of mammals: a scaling approach. Cambridge University Press, Cambridge.

Riches, A., J. Sharp, D. B. Thomas, and S. V. Smith. 1973. Blood volume determination in the mouse. Journal of Physiology 228:279–284.

Schmid-Hempel, P., and D. Ebert. 2003. On the evolutionary ecology of specific immune defence. Trends in Ecology and Evolution 18:27–32.

Schmidt-Nielsen, K. 1984. Scaling: why is animal size so important? Cambridge University Press, Cambridge.

Schneeberger, K., G. Á. Czirják, and C. C. Voigt. 2013. Measures of the constitutive immune system are linked to diet and roosting habits of Neotropical bats. PLoS ONE 8:e54023.

Schoenle, L. A., C. J. Downs, and L. B. Martin. 2018. An introduction to ecoimmunology. Pages 901–931 *in* E. L. Cooper, ed. Advances in comparative immunology. Springer, Cham.

Tacutu, R., T. Craig, A. Budovsky, D. Wuttke, G. Lehmann, D. Taranukha, J. Costa, V. E. Fraifeld, and J. P. De Magalhães. 2012. Human ageing genomic resources: integrated databases and tools for the biology and genetics of ageing. Nucleic Acids Research 41:D1027–D1033.

Teare, J. A., ed. 2013. ISIS physiological reference intervals for captive wildlife: a CD-ROM. International Species Information System, Bloomington, MN.

Tian, J., A. Courtiol, K. Schneeberger, A. D. Greenwood, and G. A. Czirjak. 2015. Circulating white blood cell counts in captive and wild rodents are influenced by body mass rather than testes mass, a correlate of mating promiscuity. Functional Ecology 29:823–829.

Tieleman, I. B., J. B. Williams, R. E. Ricklefs, and K. C. Klasing. 2005. Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. Proceedings of the Royal Society B 272:1715–1720.

Viney, M., L. Lazarou, and S. Abolins. 2015. The laboratory mouse and wild immunology. Parasite Immunology 37:267–273.

Westermann, J., and R. Pabst. 1990. Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system? Immunology Today 11:406–410.

Wiegel, F. W., and A. S. Perelson. 2004. Some scaling principles for the immune system. Immunology and Cell Biology 82:127–131.

Wilcoxen, T. E., D. J. Horn, B. M. Hogan, C. N. Hubble, S. J. Huber, J. Flamm, M. Knott, L. Lundstrom, F. Salik, and S. J. Wassenhove. 2015. Effects of bird-feeding activities on the health of wild birds. Conservation Physiology 3:cov058.

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