

RESEARCH ARTICLE

Evolution of heterophil/lymphocyte ratios in response to ecological and life-history traits: A comparative analysis across the avian tree of life

Piotr Minias 

Department of Biodiversity Studies and Bioeducation, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland

Correspondence

Piotr Minias

Email: pminias@op.pl

Handling Editor: Daniel Ardia

Abstract

1. Lymphocytes and heterophils are the two most abundant leucocyte types, which play a major role in adaptive and innate immune defence, respectively. The ratio of heterophils to lymphocytes (H/L ratio) may reflect a readiness to cope with infection through injury (via heterophils) rather than with a communicable disease (via lymphocytes). Since elevated H/L ratio constitutes a corticosterone-mediated response to external stressors, this trait is generally acknowledged as the robust measure of physiological stress in vertebrates.
2. Here, I hypothesized that baseline H/L ratios of birds (as measured under normal physiological function) could be an important evolutionary trait shaped by species' ecology and life history. I predicted that H/L ratios should be determined by traits related to pathogen exposure (migratoriness, sociality, breeding latitude) and trade-offs between investment in reproduction (clutch size) and self-maintenance (longevity).
3. I compiled published data on leucocyte profiles for nearly 250 bird species representing over half of all extant avian orders and nearly 30% of all extant avian families. Phylogenetically informed comparative methods were used to assess evolutionary associations of H/L ratios with ecological and life-history traits across the avian tree of life.
4. Relatively strong phylogenetic signal and phylogenetic autocorrelation in avian H/L ratios indicated that most diversification in this trait occurred relatively early in bird radiation. Fluctuating selection and drift were identified as the major forces responsible for the evolution of H/L ratios in birds, while low H/L ratios were identified as an ancestral state in birds. Finally, phylogenetically informed Bayesian models showed that H/L ratios correlated negatively with longevity and positively with sociality and breeding latitude.
5. This study was the first to describe the patterns of avian H/L ratio evolution in a broad phylogenetic framework. The results indicate that H/L ratios should not be merely recognized as a proxy for the intraspecific variation in physiological stress, but also as an important evolutionary trait, which probably have adaptive significance visible in a wider phylogenetic perspective.

KEYWORDS

birds, comparative analysis, evolution, H/L ratio, innate and adaptive immunity, life history

1 | INTRODUCTION

The field of comparative immunoeology has rapidly developed over the last two decades, aiming to explain diversity in immune function that is observed among species. It is now well acknowledged that different species have evolutionarily adjusted their immune function to the ecological and environmental context (Hasselquist, 2007). In general, species which are, via ecological constraints, more exposed to higher diversity of pathogens and suffer higher risk of disease should have evolved more robust immune defences (Lindström, Froufopoulos, Pärn, & Wikelski, 2004). In birds, exposure to pathogens can be mediated by ecological factors such as habitat, sociality or migratoriness. For example, high degree of sociality promotes horizontal transmission of pathogens and parasites (Côté & Poulin, 1995), while long-distance migrants are exposed to more diverse pathogen faunas during their annual cycle (Møller & Erritzøe, 1998), so colonial and migratory species are expected to develop immunological mechanisms that would counteract elevated pathogen pressure (Minias, Whittingham, & Dunn, 2017). The level of exposure to diverse pathogens may also vary biogeographically, as there is a negative latitudinal gradient in pathogen richness decreasing from tropics to Arctic (O'Connor, Cornwallis, Hasselquist, Nilsson, & Westerdahl, 2018).

While investment in immune function should be governed by species' ecology, it may also be traded-off against life-history traits. Immune response is energetically costly and can be limited by resource availability, for example by an access to amino acids derived from diet (Bonneaud et al., 2003; Lochmiller & Deerenberg, 2000). Thus, according to the traditional resource allocation-based hypothesis, investment in immune function should limit investment in such life-history components as reproduction and vice versa (Norris & Evans, 2000). In fact, there is robust empirical evidence for trade-offs in resource allocation in immune defence and reproduction (Ardia, 2005; Hanssen, Hasselquist, Folstad, & Erikstad, 2005; Moreno, Sanz, & Arriero, 1999; Saino, Incagli, Martinelli, & Møller, 2002), and a negative effect of reproductive effort on immune responsiveness in birds has been recently supported with a broad-scale meta-analysis (Knowles, Nakagawa, & Sheldon, 2009). On the other hand, immune function can be evolutionarily associated with the pace of life and longevity, since long-lived species that invest more in self-maintenance over reproduction should also develop stronger and better quality immune responses (Tieleman, Williams, Ricklefs, & Klasing, 2005).

Quantification of leucocyte profiles is among the simplest measures of animal immune function (Norris & Evans, 2000). Leucocyte profile is the relative proportion of five white blood cell types: lymphocytes, monocytes, eosinophils, basophils and heterophils (called neutrophils in mammals). Lymphocytes and heterophils are the two

most abundant leucocyte types, together comprising up to 95% of all white blood cells (Davis, Maney, & Maerz, 2008), but they have distinct immune functions. Lymphocytes play a major role in cell-mediated adaptive immunity (T cells) and humoral adaptive immunity (B cells), as they produce immunoglobulins that specifically recognize antigens derived from pathogen processing. In contrast, heterophils are phagocytosing cells of the innate immune system, which form the first line of immune defence against bacterial pathogens in inflammatory lesions. Heterophils recognize evolutionarily conserved molecular motifs (pathogen-associated molecular patterns, PAMPs) of infectious microbes via membrane-spanning Toll-like receptors (TLRs) that represent a decisive determinant of the innate immune response (Kogut et al., 2005). Although innate and adaptive immune systems of vertebrates are not fully independent of each other and there are intricate innate-adaptive connections in the immune response (Fearon & Locksley, 1996; Hoebe, Janssen, & Beutler, 2004), heterophils and lymphocytes are traditionally classified as primary constitutive components of innate and adaptive immune defences, respectively (Lee, 2006; Palacios, Cunnick, Vleck, & Vleck, 2009). Although there is empirical evidence for H/L ratios correlating positively with the strength of innate immune response (MacColl et al., 2017; but see Parejo, Silva, & Avilés, 2007 for lack of correlations) and negatively with humoral adaptive response in wild birds (Kerimov et al., 2018; Krams et al., 2012), it must be acknowledged that H/L ratio does not measure an immune response per se (Davis et al., 2008). However, elevated H/L ratios should be associated with a state of readiness to cope with infection through injury (via heterophils) rather than with a communicable disease (via lymphocytes) (Johnstone, Reina, & Lill, 2012). Thus, H/L ratios are known to change in response to external stressors that can increase the risk of injury, including inclement climatic or weather events, pollution, increased breeding effort, social challenges or parasitic infestation (Minias, Włodarczyk, & Meissner, 2018). Although these changes may proceed rapidly (within tens of minutes) in response to acute stressors (Davis, 2005), baseline H/L ratio levels, as measured under normal physiological function (no acute stress), have been shown to be highly consistent within individuals in a long-term (across-seasonal) perspective (Hörak, Saks, Ots, & Kollist, 2002). Short-term changes in H/L ratios are thought to be mediated by stress hormones (Sapolsky, Romero, & Munck, 2000; but see Müller, Jenni-Eiermann, & Jenni, 2011; for lack of correlations between H/L ratio and corticosterone levels), as glucocorticoids stimulate lymphocytes to undergo transmigration from circulating blood to compartments such as skin, spleen and lymph nodes, where they will be more useful in the event of injury and, at the same time, they induce an influx of heterophils from bone marrow into the blood, which is known as the process of white blood cell trafficking (Dhabhar & McEwen, 1997; Dhabhar, Miller, McEwen, & Spencer, 1996). Thus, H/L ratios

have mostly been used in physioecological research as a simple, but robust measure of physiological stress (Davis et al., 2008).

Although H/L ratios are prone to rapid stress-related changes at individual level (Davis, 2005), it has been recently hypothesized that different species might have different baseline levels of heterophils and lymphocytes. This hypothesis was first supported with the comparative analysis of heterophil and lymphocyte numbers in birds, showing their correlated evolution with traits such as body mass, basal metabolic rate and the length of incubation/fledging period (Pap et al., 2015). While the study by Pap et al. (2015) examined absolute heterophil and lymphocyte counts rather than H/L ratios, there is also recent evidence that the relative proportion of heterophils and lymphocytes in peripheral blood could have evolved in response to different ecological and life-history constraints. A comparative analysis of shorebirds Charadrii showed that long-lived species with high annual survival had lower H/L ratios, suggesting that high relative proportion of lymphocytes in circulating blood may be considered as physiological component of slow pace of life (Minias et al., 2018). A possible role of H/L ratio as an evolutionary adaptation was also supported by its intraspecific repeatability (Minias et al., 2018) and relatively high heritability, as shown in poultry (Campo & Davila, 2002) and wild avian species (Wilcoxon, Boughton, Morgan, & Schoech, 2013).

The aim of this study was to reconstruct evolutionary history of H/L ratio and to examine its ecological and life-history correlates in birds. First, I hypothesized that the evolution of H/L ratios in birds should be primarily shaped by either stabilizing or fluctuating selection. To test these two hypotheses, Ornstein–Uhlenbeck (a constant selective optimum) and Brownian motion (a moving selective optimum) models of evolution were fitted to the distribution of H/L ratios across the avian phylogeny. I also tested for time variation in evolutionary rates and for the presence of adaptive radiations in avian H/L ratios. Second, I hypothesized that H/L ratios should be negatively associated with pace of life and longevity (low H/L ratio in long-lived species with long incubation periods), but positively with fecundity (high H/L ratio in species with large clutch size). I also hypothesized that species exposed to more diverse pathogens (migratory and tropical species) and showing higher transmission rates of communicable diseases (social species) should have lower H/L ratios. To test these hypotheses, I compiled published data on leucocyte profiles for nearly 250 bird species and used phylogenetically informed comparative methods to assess evolutionary associations of H/L ratios with ecological and life-history traits across the avian tree of life.

2 | MATERIALS AND METHODS

2.1 | Leucocyte profiles

Data on avian leucocyte profiles were collected from published sources. Only studies on wild-living populations were considered, all data from individuals kept permanently or temporarily (for experimental purposes) in captivity were excluded. I also excluded data

from individuals that were subject to an experimental treatment in the wild (e.g. individuals that were experimentally infected, immunologically challenged or had their plumage manipulated). In such cases, data from experimental control groups were only compiled. Finally, I excluded data from unhealthy individuals, if such information was explicitly specified in the text (e.g. in the case of veterinary screenings). In total, H/L mean estimates were compiled for 239 species from 173 published sources. The data had a wide phylogenetic coverage, representing 56% (20/36) of all extant avian orders and 29% (71/243) of all extant avian families, according to the classification proposed by Winkler, Billerman, and Lovette (2015). Passeriformes order had the largest representation in the data ($n = 115$ species), while non-passerine orders were on average represented by 6.89 ± 2.56 [SE] species per order. Species were much more evenly distributed across families, as each passerine and non-passerine family was represented by an average of 4.28 ± 0.74 [SE] and 2.76 ± 0.41 [SE] species, respectively. Within passerines, four major superfamilies were well represented in the data: Corvoidea ($n = 8$ species), Sylvioidea ($n = 29$ species), Muscicapoidea ($n = 30$ species) and Passeroidea ($n = 38$ species). In total, 415 estimates of H/L ratios were collected, as multiple estimates across different studies were available for some species. The average sampling effort (number of sampled individuals) was 45.7 ± 3.36 [SE] individuals per estimate, and estimates obtained from fewer than three individuals were not included in the dataset.

2.2 | Age, sex and seasonal variation

If possible, separate H/L estimates were collected for males ($n = 53$ estimates) and females ($n = 48$ estimates), but in most cases, sex was not specified. Data on the age of birds were collected according to the following categories: nestlings ($n = 58$ estimates), immatures ($n = 25$ estimates) and adults ($n = 332$ estimates). For adults and immatures, I also collected information on the phase of annual cycle when the measurements were conducted. Following stages of annual cycle were distinguished: breeding season ($n = 243$ estimates), migratory season ($n = 33$ estimates) and non-breeding/non-migratory season ($n = 61$ estimates). Phase of the annual cycle could not be unequivocally determined for twenty estimates (measurements were conducted across different phases).

Since multiple measurements per species were available in the dataset, I used Bayesian phylogenetic mixed models (Hadfield & Nakagawa, 2010) to test for the effects of age, sex and annual stage on H/L ratios in a phylogenetically informed framework. Details of Bayesian modelling are presented below (section 2.5. Comparative analyses). There were significant differences in H/L ratios between age classes, as adults had higher H/L ratios than nestlings (0.059, 95%CI: 0.030 to 0.086; $p < 0.001$). No differences were found between adults and immatures (0.009; 95%CI: -0.044 to 0.071; $p = 0.71$), and thus, these age classes were used in all further analyses. After excluding nestlings from the analyses, 357 estimates ($n = 227$ species) were retained in the dataset and there was significant intraspecific repeatability of mean H/L ratio estimates

obtained from independent studies ($R = 0.73$, 95% CI: 0.65 to 0.80). Also, within adults and immatures, H/L ratios did not differ between sexes (0.013, 95% CI: -0.024 to 0.045, $p = 0.45$) and annual stages (all $p > 0.05$). However, H/L ratio estimates positively correlated with sampling effort (0.013, 95% CI: 0.001 to 0.023; $p = 0.016$), and thus, I controlled for this effect in the analysis of ecological and life-history traits.

2.3 | Ecological and life-history traits

Data on the following ecological and life-history variables were collected for all 239 species: clutch size, incubation period, body mass, sociality, breeding latitude, migratory behaviour and life span. Data on clutch size, incubation period, body mass and sociality were compiled from standard references (del Hoyo, Elliott, & Sargatal, 1992–2011; Snow & Perrins, 1998). Body mass was log-transformed prior to analyses. Sociality was grouped into three categories: (a) solitary species ($n = 155$), (b) semi-social (group-living and semi-colonial) species ($n = 25$), and (c) predominantly colonial species ($n = 59$).

Total migration distance was estimated using distribution maps downloaded from BirdLife International website (BirdLife International and Handbook of Birds of the World, 2016) and methodology proposed by Vincze (2016). Shape files were used to calculate geometric centroids of breeding (breeding and resident spatial polygons) and wintering (wintering and resident spatial polygons) ranges with *gCentroid* function in the *rgeos* (Bivand, Rundel, Pebesma, Stuetz, & Hufthammer, 2017) R package. Migration distance was calculated as the geographic distance between centroids of breeding and wintering range using a custom R function developed by Vágási et al. (2016). Geometric centroid of breeding range was used as a measure of breeding latitude in the models.

Data on maximum life span were collected using the AnAge database (de Magalhães & Costa, 2009) integrated within the web portal of the Human Ageing Genomic Resources (Tacutu et al., 2013) or from standard references (del Hoyo et al., 1992–2011). For species which lacked quantitative data on maximum life span (21.3%, $n = 239$), I averaged longevity records available within genus ($n = 29$ species) or family ($n = 22$ species). Although leucocyte profiles were collected exclusively for wild-living populations, longevity records for several ($n = 22$) species originated from captive individuals, and thus, life span estimates needed to be adjusted for the source of data (longevity records are greater in captivity than in the wild; Minias & Podlaskczuk, 2017). Life span estimates also needed to be adjusted for variation in body mass (larger species live longer; Møller, 2007) and sampling effort (probability of recording an extremely old individual increases with sample size, Møller, 2007) prior to analyses. Thus, I extracted residuals of log-transformed life span against log-transformed body mass ($F_{1,234} = 250.0$, $p < 0.001$), sampling effort ($F_{3,234} = 3.45$, $p = 0.017$) and source of data ($F_{2,234} = 8.00$, $p < 0.001$). These residuals, henceforth referred to as residual life span, were used in the comparative analyses.

2.4 | Evolutionary history

The evolutionary history of H/L ratios in birds was analysed using three methodological approaches: fitting macroevolutionary models, estimating phylogenetic signal and phylogenetic autocorrelation, and reconstructing ancestral states.

2.4.1 | Macroevo-lutionary models

Two constant-rate macroevolutionary models were fitted to the data: Brownian motion (BM) and Ornstein-Uhlenbeck (OU). The BM model describes evolutionary dynamics of a trait that changes randomly in direction and distance over any time interval, which is consistent with purely neutral evolution (trait changes only due to genetic drift) or fluctuating selection towards a moving optimum (Hansen & Martins, 1996). In contrast, the OU model describes evolution of traits under stabilizing selection with a constant optimum (Butler & King, 2004; Hansen, 1997). Both models are described with two basic parameters: ancestral state (z_0) and evolutionary rate (σ^2) which is constant through time and across lineages. The OU model is additionally constrained with the third parameter α , which describes the strength of pull towards a central value (selective optimum). Although the BM model is associated with a constant-rate process of evolution, this assumption can be weakened by the phylogenetic scaling parameter λ , which introduces a transformation of the phylogenetic variance-covariance matrix and effectively changes branch lengths of the phylogeny (Pagel, 1999). In general, λ varies between 0 and 1, where 0 indicates phylogenetic independence (producing a single polytomy where all species are equally related), while a value of 1 retains the original tree and indicates that trait evolution corresponds to the standard BM model (Freckleton, Harvey, & Pagel, 2002). With the decreasing of lambda from 1 to 0, internal nodes are pushed towards the root, which is consistent with stronger transformation of phylogenetic variance-covariance matrix and which can reflect temporal variations in the strength of drift or fluctuating selection with a low rate of fluctuation (Revell, Harmon, & Collar, 2008). The second transformation of phylogenetic variance-covariance matrix (δ) was designed to capture variation in the rates of evolution through time (Pagel, 1999). All elements of phylogenetic variance-covariance matrix are raised to the power δ , and the model provides support for exponentially decreasing ($\delta < 1$) or increasing ($\delta > 1$) evolutionary rates. Finally, time-varying rates of evolution can be modelled by incorporation of rate parameter (b) into the BM model. I run two different time-dependent models assuming: (a) linear decrease ($b < 0$) or increase ($b > 0$) in evolutionary rates, and (b) exponential decrease in evolutionary rate ($b < 0$) consistent with early burst model of evolution (adaptive radiation in early evolution) (Blomberg, Garland, & Ives, 2003; Harmon et al., 2010). All macroevolutionary models were fit using the *fitContinuous* function, as implemented in the *GEIGER* package (Harmon, Weir, Brock, Glor, & Challenger, 2008) developed for R statistical environment (R Development Core Team, 2013). Parameters for each model were estimated with 1,000 iterations under the default bounds.

Relative fit of all the models was compared using differences in the Akaike information criterion corrected for small sample sizes (ΔAIC_c) and Akaike weights (ω_i).

2.4.2 | Phylogenetic signal and autocorrelation

Phylogenetic signal and phylogenetic autocorrelation in H/L ratios were assessed using the scaling parameter λ (transformation of phylogenetic variance-covariance matrix under the BM model; Pagel, 1999) and spatial autocorrelation statistic Moran's I (Gittleman & Kot, 1990), respectively. Phylogenetic correlograms of normalized Moran's I (I/I_{\max}) were used to assess the strength of autocorrelation in H/L ratios at different taxonomic levels (genus, family and order). λ was estimated using *phylosig* function in the *PHYTOOLS* (Revell, 2012) package, while Moran's I and correlograms were calculated using the *APE* (Paradis, Claude, & Strimmer, 2004) package in R.

2.4.3 | Ancestral state reconstruction

Ancestral states of H/L ratios were reconstructed using the maximum likelihood approach implemented in *fastAnc* function in *PHYTOOLS* R package. The estimated ancestral states were mapped on the phylogeny using *contMap* function in *PHYTOOLS*, while 95% confidence intervals for point estimates of ancestral states were plotted on the traitgrams using *fancyTree* function from the same package.

2.5 | Comparative analyses

Phylogenetically informed analyses of H/L ratios were conducted using Bayesian phylogenetic mixed models (Hadfield & Nakagawa, 2010), which allowed to incorporate within-species variation in leucocyte profiles and sampling effort by accommodating multiple measurements per species. H/L ratio estimates were log-transformed (using $\log(1 + \text{H/L})$ transformation) to improve normality (skewness: 2.92 ± 0.12 [SE]) and entered as the dependent variable in each model. Residual life span, clutch size, incubation period, migration distance, breeding latitude, body mass and sampling effort were included as covariates, while sociality was included as a fixed factor. To obtain more parsimonious reduced models,

I removed non-significant ($p > 0.10$) predictors from the initial full models and p values were inferred based on z-score (estimate/SE) tests. Phylogeny was reconstructed based on the most recent complete avian time-calibrated phylogeny (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012) with a backbone tree developed by Ericson et al. (2006). To account for phylogenetic uncertainty, each model was run for 100 alternative trees, as downloaded from the BirdTree database (<http://www.birdtree.org>; Jetz et al., 2012). Uninformative priors (variance set to 1 and belief parameter set to 0.002) were used for both fixed and random effects. Two chains with 200,000 iterations were run in each analysis. Burn-in period was set to 150,000, and thinning value was set to 50, yielding a thousand samples per model. The two independent chains converged each time, as assessed with potential scale reduction values < 1.1 (Gelman & Rubin, 1992). All Bayesian phylogenetic mixed models were run in *MCMCGLMM* (Hadfield, 2010) and *MULTREE* (Guillaume & Healy, 2014) packages in R.

3 | RESULTS

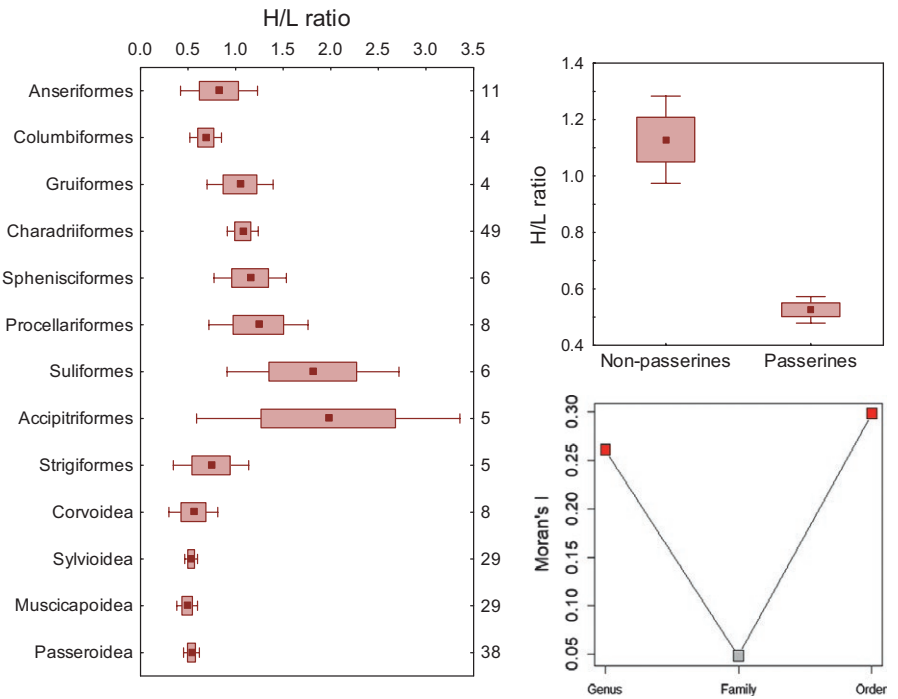
Evolution of avian H/L ratios was best described by the Brownian Motion model adjusted for λ (relative importance across all models: $\omega_i = 1$), which is consistent with the predominant role of fluctuating selection and drift. No support was found for stabilizing selection acting on H/L ratios (Ornstein-Uhlenbeck model), nor for changing rates of H/L ratio evolution (Table 1). There was a moderately strong phylogenetic signal in the data ($\lambda = 0.58$, $p < 0.001$), and relatively high phylogenetic autocorrelation was recorded at the level of genera and orders (Figure 1). In contrast, no significant phylogenetic autocorrelation was recorded at the family level (Figure 1). Ancestral character estimate of H/L ratio at the root node of avian tree was 1.07 (95% CI: -0.88 to 3.01) (Figure 2), while H/L ratio at the basal node of passerines was estimated at 0.68 (95% CI: -0.47 to 1.82) (Figure 2). Ancestral avian lineages (non-passerines) had consistently higher H/L ratios than recently evolved lineages (passerines) (1.13 ± 0.08 vs. 0.53 ± 0.02 ; $p < 0.001$; Figure 1). The highest H/L ratios (> 3.5) were recorded in Psittaciformes, Accipitriformes, Suliformes and Ciconiiformes (Figure 2). In 45.6% of non-passerine species ($n = 114$), heterophils were more abundant than lymphocytes ($\text{H/L} > 1$),

TABLE 1 Relative fit (ΔAIC_c) and parameter estimates of models describing the evolution of H/L ratios in birds

Evolution model	AIC_c	ΔAIC_c	Parameters		
			Evolutionary rate (σ^2)	Ancestral state (z_0)	Model-specific parameters
Brownian motion adjusted for λ	432.0	0.0	0.006	1.05	$\lambda = 0.58$
Ornstein-Uhlenbeck	481.4	49.4	1.85	0.83	$\alpha = 1.95$
Brownian motion adjusted for δ	662.5	230.5	0.021	1.10	$\delta = 2.99^*$
Time-dependent (linear)	687.2	255.2	6E-06	1.10	$b = 99.9^*$
Brownian motion	715.9	283.9	0.058	1.07	—
Time-dependent (early burst)	795.0	363.0	0.15	1.07	$b = 0^*$

Note. Parameters that appeared at default bounds after 1,000 iterations are marked with asterisks.

FIGURE 1 H/L ratios in non-passerine and passerine birds (right upper panel). Order-level and superfamily-level H/L ratios are also shown for non-passerine and passerine birds, respectively (left panel). Sample size (number of species) for each clade is shown at the right vertical axis; data for clades with three or fewer species were not shown. Central point—mean, box—SE, whiskers—95% CI. Phylogenetic correlogram for H/L ratios is shown at the right bottom panel. Moran's I was shown for three taxonomic levels (genus, family and order); significant estimates of phylogenetic correlation were marked in red



while similar situation was recorded in only 3.5% ($n = 113$) passerine species, which made a significant difference ($G = 30.9$, $df = 1$, $p < 0.001$).

Bayesian models identified residual life span, sociality and breeding latitude as valid predictors of H/L ratios in birds (Table 2). I found that species with longer residual life span had lower H/L ratios (Figure 3a) and there was also a positive relationship between avian H/L ratios and breeding latitude (Figure 3b). Social species had higher H/L ratios than solitary species (Figure 4), although there was no significant difference between semi-social and solitary species (Table 2). I found no evidence for the effect of migration distance, clutch size, incubation period and body mass on avian H/L ratios (Table 2).

4 | DISCUSSION

The results of this study provide compelling evidence for the evolution of H/L ratios as an important physiological adaptation in birds. First, I found relatively strong phylogenetic signal and phylogenetic autocorrelation in avian H/L ratios, suggesting that major diversification in this trait occurred relatively early in bird radiation. In fact, there was a significant variation in H/L ratios between old and recently evolved avian lineages, where non-passerines had consistently higher H/L ratios than passerines. Second, I identified fluctuating selection and drift as the major forces responsible for the evolution of H/L ratios in birds. Finally, I found strong support for the correlated evolution of avian H/L ratios with ecological and life-history traits, such as residual life span, sociality and breeding latitude.

The first phylogenetically robust evidence for an evolutionary link between leucocyte profiles and life-history traits of birds came from the study by Pap et al. (2015), who showed that the numbers

of different leucocyte types are developmentally constrained. Both heterophil and lymphocyte numbers correlated positively with the length of incubation period, but negatively with the length fledgling period, indicating that constitutive immune function develops primarily during the embryonic phase (Pap et al., 2015). At the same time, heterophil and lymphocyte counts correlated positively with body mass and negatively with basal metabolic rate, showing that both axes (innate and adaptive) of constitutive immunity might be evolutionarily associated with the pace of life (Pap et al., 2015). Although providing an invaluable insight into the evolution of avian immune function, the study by Pap et al. (2015) had certain methodological limitations, as non-passerines were clearly under-represented in the dataset (ca. 25% out of 105 species) and sample sizes for most non-passerine species were modest (<5 sampled individuals), which could result in inaccurate species-specific mean estimates of leucocyte numbers. Here, I expanded on the study by Pap et al. (2015) by analysing the evolution of avian leucocyte profiles in a broad phylogenetic context (239 species from 71 families). Also, the aim of this study was to examine evolutionary forces shaping the relative proportion of different leucocyte types (heterophils vs. lymphocyte), rather than to examine them as separate measures of immunity.

My study identified residual life span as an important life-history determinant of H/L ratio in birds, as species living longer than expected from the regression of longevity on body mass (higher residual life span) had lower H/L ratio than species having lower residual life span. Similar relationship has been revealed in a comparative analysis of shorebirds, where H/L ratios correlated negatively with life span and annual survival (Minias et al., 2018). This result is consistent with theoretical predictions, as long-lived species are likely to encounter more diverse pathogens throughout their lives (Poulin & Morand, 2000) and they are expected to develop more elaborate

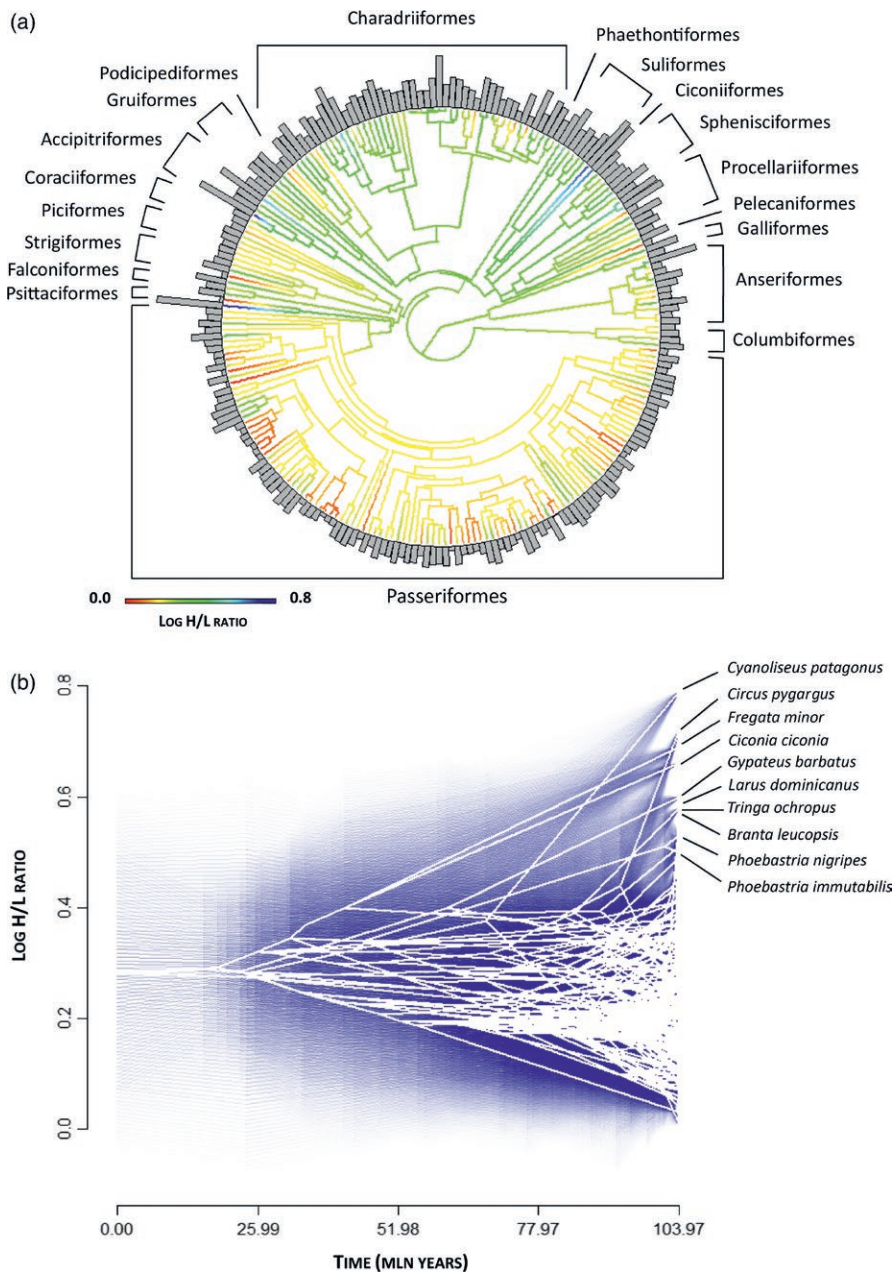


FIGURE 2 Evolution of H/L ratios across the avian tree of life. Bars associated with each terminal branch of avian phylogeny (a) indicate raw values of log-transformed H/L ratio. The vertical position of nodes at the traitgram (b) shows reconstructed ancestral states for H/L ratios, while the horizontal position gives time from the root. Uncertainty is shown via increasing transparency of blue lines plotted along branches and at nodes, which indicate 95% confidence intervals of point estimates

(antibody-mediated) immune defences, whereas short-lived species should rely more heavily on nonspecific and inflammatory immune defences (parasite-exposure hypothesis; Lee, 2006). Since residual life span correlates with developmental time (as measured with incubation period in this study: $r = 0.16$, $n = 239$, $p = 0.013$), it is considered an important component of the pace-of-life syndrome, and the position along the fast-slow continuum has been already shown to be associated with immune function in birds. For example, longer incubation period (slow-living species) correlated with higher natural antibody levels (adaptive immunity), while large clutch size (fast-living species) was associated with higher complement activity (innate immunity) in Neotropical birds (Lee, Wikelski, Robinson, Robinson, & Klasing, 2008). Also, acquired T-cell-mediated immunocompetence (as measured with phytohaemagglutinin assay) varied between life-history axes of birds and it was significantly stronger

in species with slower developmental rate and longer life span (Tella, Scheuerlein, & Ricklefs, 2002). Despite being consistent with theory and previous empirical evidence (Minias et al., 2018), the negative association of avian H/L ratios with residual life span should be treated with caution, as 21% of species included in the analysis lacked quantitative data on longevity, and had their life span estimates extrapolated from closely related taxa.

Parasite-exposure hypothesis is also likely to explain the positive latitudinal gradient in avian H/L ratios, as found in this study. Richness and virulence of pathogens and parasites that vertebrate and, specifically, avian hosts are exposed to have been repeatedly shown to increase from high Arctic to the Equator (Bordes, Guégan, & Morand, 2011; Merino et al., 2008; Møller, Arriero, Lobato, & Merino, 2009; Nunn, Altizer, Sechrest, & Cunningham, 2005). There is strong empirical support that this latitudinal gradient in pathogen

TABLE 2 Coefficient estimates and corresponding 95% credibility limits (CL) for ecological and life-history predictors of H/L ratios in birds, as assessed with full and reduced Bayesian phylogenetic mixed models

Predictors	Estimate	Lower 95% CL	Upper 95% CL	p
Full model				
Intercept	0.04	-0.29	0.33	0.79
Residual life span	-0.098	-0.192	-0.013	0.030
Sociality (semi-social vs. solitary)	0.020	-0.026	0.073	0.43
Sociality (social vs. solitary)	0.060	0.000	0.120	0.046
Clutch size	0.003	-0.009	0.014	0.61
Incubation period	0.003	-0.001	0.007	0.22
Migration distance	0.002	-0.004	0.007	0.51
Breeding latitude	0.0008	-0.0001	0.0017	0.082
Body mass	0.016	-0.033	0.059	0.53
Sampling effort	0.012	0.002	0.023	0.018
Reduced model				
Intercept	0.17	-0.07	0.44	0.25
Residual life span	-0.096	-0.189	-0.002	0.020
Sociality (semi-social vs. solitary)	0.022	-0.026	0.069	0.37
Sociality (social vs. solitary)	0.068	0.009	0.134	0.034
Breeding latitude	0.0009	0.0001	0.0018	0.020
Sampling effort	0.012	0.000	0.022	0.026

Note. All values were averaged across 100 different phylogenies. Significant coefficients are marked in bold.

pressure shaped biogeographic variation in the strength of pathogen recognition mechanisms (e.g. via affecting polymorphism levels at the Major Histocompatibility Complex, MHC; O'Connor et al., 2018) and general immune function (Barbosa, Merino, Benzal, Martinez, & García-Fraile, 2007; Martin, Pless, Svoboda, & Wikelski, 2004; Møller, 1998) in birds. Here, a decrease in H/L ratio with decreasing latitude suggests that interspecific variation in avian H/L ratios may be associated with exposure to diverse pathogens and parasites.

Sociality was identified as the last significant predictor of avian H/L ratio, but contrary to my predictions, solitary species had lower H/L ratio than social species. Sociality (coloniality) is often associated with higher infection rate by horizontally transmitted pathogens and parasites, and extensive empirical support exists for positive intraspecific associations between parasitic pressure and group size (Brown & Brown, 2004; Davis & Brown, 1999). On the other hand, evidence for evolutionary association between immune defence and sociality in birds is rather scarce and equivocal (Lee et al., 2008; Møller & Erritzøe, 1996; Møller, Merino, Brown, & Robertson, 2001). It is possible that elevated investment of social species in the front line mechanisms of innate immune defence could reduce the risk of infection taking place (via phagocytic activity of heterophils), simultaneously reducing the need to frequently mount an energetically costly lymphocyte-dependent immune responses. Similar mechanisms have been invoked to explain lower proportion of lymphocytes in scavenging birds that tend to feed in

large groups on patchy resources, which, as in colonial species, facilitate horizontal pathogen transmission (Blount, Houston, Møller, & Wright, 2003). On the other hand, intraspecific agonistic interactions are likely to be much more frequent in social than solitary species, as the incidence of aggressive behaviour is expected to increase as a function of nesting density (Bukacinska & Bukacinski, 1993; Stokes & Boersma, 2000). Since wounding and injuries are recognized as the common cost of aggressive interactions in colonially breeding birds (Pierotti & Annett, 1994), it seems possible that these species may need effective inflammatory responses (via heterophils), resulting in high H/L ratios.

The results of this study provided support for relatively strong phylogenetic signal and phylogenetic autocorrelation in H/L ratio across the avian tree of life, indicating that most diversification in this trait occurred early in bird radiation. Evolution of avian H/L ratios was best described by the model of Brownian motion adjusted for λ , consistent with the role of drift and fluctuating selection towards a moving optimum. While phylogenetically informed Bayesian modelling showed that avian H/L ratios were evolutionarily determined by certain ecological and life-history trait, at the same time I found evidence for specific phylogenetic patterns in the evolutionary history of this trait. Most notably, old evolutionary lineages (non-passerines) had consistently higher H/L ratios than more recent lineages (passerines), indicating that this may be an ancestral character in birds. This is consistent

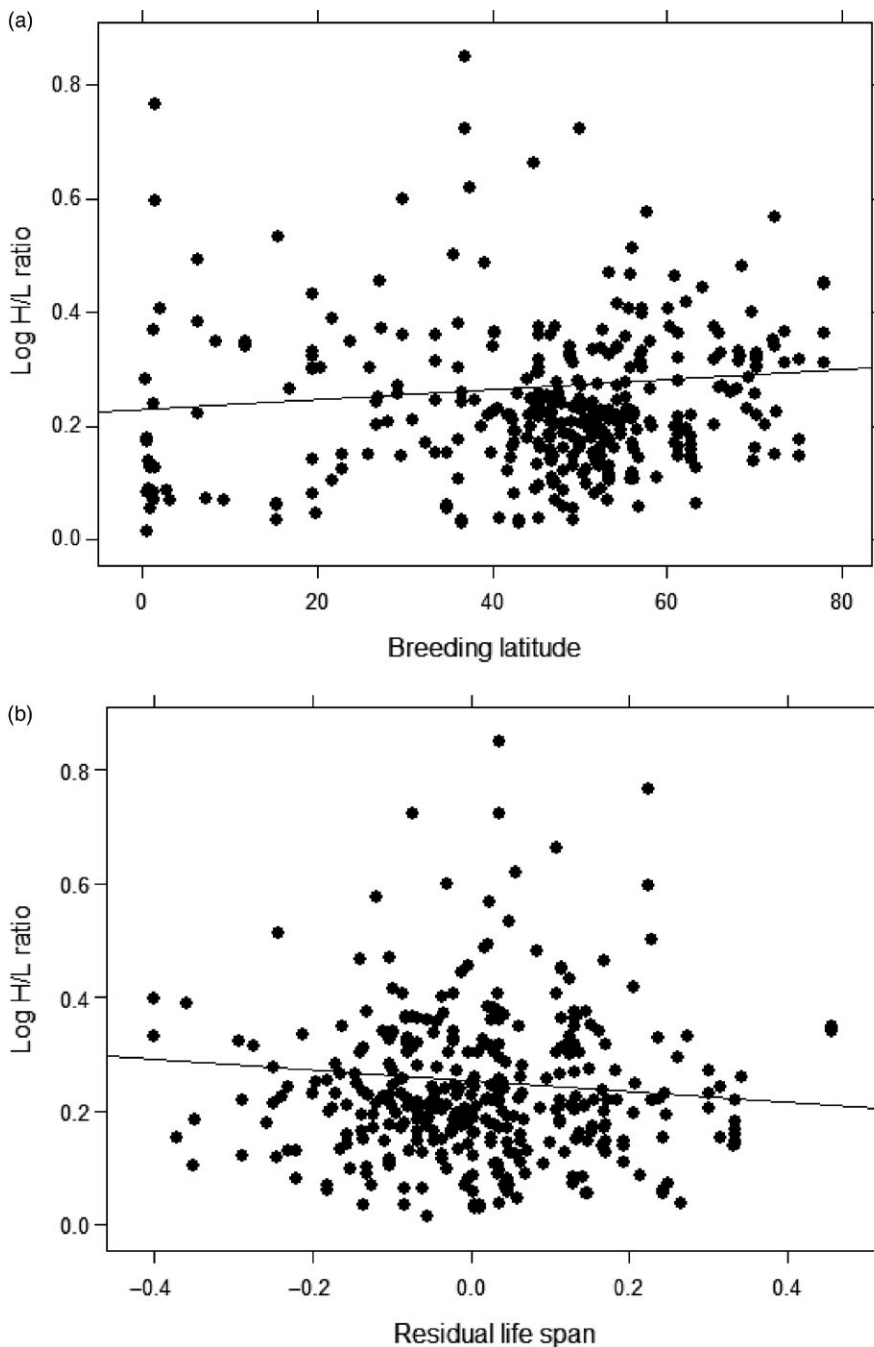


FIGURE 3 Correlations of avian H/L ratios with breeding latitude (a) and residual life span (b), as assessed with Bayesian phylogenetic mixed models

with the prevailing view on the evolution of vertebrate immune system, where mechanisms of acquired (adaptive) immunity are thought to be of more recent evolutionary origin and may have, to some extent, replaced the innate immune defence (Råberg et al., 2002). Although it is generally agreed that innate and adaptive axes of immune system became tightly coadapted in the modern vertebrates, interacting and complementing each other (Fearon & Locksley, 1996; Hoebe, Janssen, & Beutler, 2004), it is also acknowledged that these two branches of immunity are partially interchangeable over evolutionary times (Råberg et al., 2002). This view was supported by the finding of negative genetic and phenotypic correlations between innate and adaptive branches

of immune system in poultry and wild-living birds (Blount et al., 2003; Cheng, Rothschild, & Lamont, 1991). High relative importance of innate (vs. adaptive) immune defence for non-passerine birds has also been suggested by the molecular studies of avian MHC genes (pathogen recognition receptors of the adaptive immune system), showing that the number of MHC loci in non-passerines is evolutionarily constrained and consistently lower than in passerines (Bollmer, Dunn, Whittingham, & Wimpee, 2010; Minias, Pikus, Dunn, & Whittingham, 2019). This may suggest that pathogen recognition in non-passerines could be primarily governed by innate immune genes, such as TLRs, which are primarily expressed at heterophils/neutrophils and macrophages.

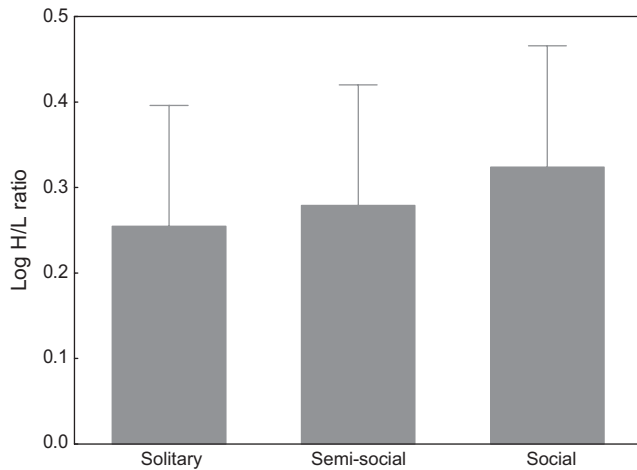


FIGURE 4 Mean (\pm SE) H/L ratio in solitary, semi-social and social species of birds. Estimates were obtained with Bayesian phylogenetic mixed models

5 | CONCLUSIONS

This study was the first to describe the patterns of avian H/L ratio evolution in a broad phylogenetic framework. The results of phylogenetically informed Bayesian modelling provided evidence for the effects of ecological (sociality and breeding latitude) and life-history (longevity) traits on the evolution of H/L ratio in birds. At the same time, low H/L ratios were identified as an ancestral state in birds. In conclusion, the study indicates that H/L ratios should not be merely recognized as a proxy for the intraspecific variation in physiological stress, but also as an important evolutionary trait. Although it remains to be determined whether H/L ratio is an adaptive trait itself, the results of this study strongly suggest that the H/L is indicative for an interplay between immunity, physiology and ecology, which probably have adaptive significance visible in a wider phylogenetic perspective.

ACKNOWLEDGEMENTS

I thank two anonymous reviewers for constructive comments on the earlier draft of the manuscript.

DATA ACCESSIBILITY

Raw data have been deposited in the Dryad Digital Repository: <https://doi.org/10.5061/dryad.s3cc172> (Minias, 2019).

ORCID

Piotr Minias  <https://orcid.org/0000-0002-7742-6750>

REFERENCES

Ardia, D. R. (2005). Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows.

- Journal of Animal Ecology*, 74, 517–524. <https://doi.org/10.1111/j.1365-2656.2005.00950.x>
- Barbosa, A., Merino, S., Benzal, J., Martinez, J., & García-Fraile, S. (2007). Geographic variation in the immunoglobulin levels in pygoscelid penguins. *Polar Biology*, 30, 219–225.
- BirdLife International and Handbook of Birds of the World (2016). *Bird species distribution maps of the world*, ver. 6.0. Retrieved from <http://datazone.birdlife.org/species/requestdis>.
- Bivand, R., Rundel, C., Pebesma, E., Stuetz, R., & Hufthammer, K. O. (2017). *Rgeos: Interface to Geometry Engine – Open Source (GEOS)*. R package ver. 0.3-23. Retrieved from <https://CRAN.R-project.org/package=rgeos>
- Blomberg, S. P., Garland, T., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution*, 57, 717–745. <https://doi.org/10.1111/j.0014-3820.2003.tb00285.x>
- Blount, J. D., Houston, D. C., Møller, A. P., & Wright, J. (2003). Do individual branches of immune defence correlate? A comparative case study of scavenging and non-scavenging birds. *Oikos*, 102, 340–350. <https://doi.org/10.1034/j.1600-0706.2003.12413.x>
- Bollmer, J. L., Dunn, P. O., Whittingham, L. A., & Wimpee, C. (2010). Extensive MHC class II B gene duplication in a passerine, the common yellowthroat (*Geothlypis trichas*). *Journal of Heredity*, 101, 448–460. <https://doi.org/10.1093/jhered/esq018>
- Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B., & Sorci, G. (2003). Assessing the cost of mounting an immune response. *The American Naturalist*, 161, 367–379. <https://doi.org/10.1086/346134>
- Bordes, B., Guégan, J. F., & Morand, S. (2011). Microparasite species richness in rodents is higher at lower latitudes and is associated with reduced litter size. *Oikos*, 120, 1889–1896. <https://doi.org/10.1111/j.1600-0706.2011.19314.x>
- Brown, C. R., & Brown, M. B. (2004). Empirical measurement of parasite transmission between groups in a colonial bird. *Ecology*, 85, 1619–1626. <https://doi.org/10.1890/03-0206>
- Bukacińska, M., & Bukaciński, D. (1993). The effect of habitat structure and density of nests on territory size and territorial behaviour in the Black-headed Gull (*Larus ridibundus* L.). *Ethology*, 94, 306–316.
- Butler, M. A., & King, A. A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *The American Naturalist*, 164, 683–695. <https://doi.org/10.1086/426002>
- Campo, J. L., & Davila, S. G. (2002). Estimation of heritability for heterophil: Lymphocyte ratio in chickens by restricted maximum likelihood. Effects of age, sex, and crossing. *Poultry Science*, 81, 1448–1453. <https://doi.org/10.1093/ps/81.10.1448>
- Cheng, S., Rothschild, M. F., & Lamont, S. J. (1991). Estimates of quantitative genetic parameters of immunological traits in the chicken. *Poultry Science*, 70, 2023–2027. <https://doi.org/10.3382/ps.0702023>
- Côté, I. M., & Poulin, R. (1995). Parasitism and group size in social animals: A meta-analysis. *Behavioral Ecology*, 6, 159–165.
- Davis, A. K. (2005). Effects of handling time and repeated sampling on avian white blood cell counts. *Journal of Field Ornithology*, 76, 334–338. <https://doi.org/10.1648/0273-8570-76.4.334>
- Davis, J. A., & Brown, C. R. (1999). Costs of coloniality and the effect of colony size on reproductive success in purple martins. *Condor*, 101, 737–745. <https://doi.org/10.2307/1370060>
- Davis, A. K., Maney, D. L., & Maerz, J. C. (2008). The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. *Functional Ecology*, 22, 760–772. <https://doi.org/10.1111/j.1365-2435.2008.01467.x>
- de Magalhães, J. P., & Costa, J. (2009). A database of vertebrate longevity records and their relation to other life-history traits. *Journal of Evolutionary Biology*, 22, 1770–1774. <https://doi.org/10.1111/j.1420-9101.2009.01783.x>
- del Hoyo, J., Elliott, A., & Sargatal, J. (1992–2011). *Handbook of the birds of the world*. Vol. 1–16. Barcelona, Spain: Lynx Edicions.

- Dhabhar, F. S., & McEwen, B. (1997). Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: A potential role for leukocyte trafficking. *Brain, Behaviour and Immunity*, 11, 286–306. <https://doi.org/10.1006/brbi.1997.0508>
- Dhabhar, F. S., Miller, A. H., McEwen, B. S., & Spencer, R. L. (1996). Stress-induced changes in blood leukocyte distribution – role of adrenal steroid hormones. *Journal of Immunology*, 157, 1638–1644.
- Ericson, P. G., Anderson, C. L., Britton, T., Elzanowski, A., Johansson, U. S., Källersjö, M., ... Mayr, G. (2006). Diversification of Neoaves: Integration of molecular sequence data and fossil. *Biology Letters*, 2, 543–547. <https://doi.org/10.1098/rsbl.2006.0523>
- Fearon, D. T., & Locksley, R. M. (1996). The instructive role of innate immunity in the acquired immune response. *Science*, 272, 50–54. <https://doi.org/10.1126/science.272.5258.50>
- Freckleton, R. P., Harvey, P. H., & Pagel, M. (2002). Phylogenetic analysis and comparative data: A test and review of evidence. *The American Naturalist*, 160, 712–716. <https://doi.org/10.1086/343873>
- Gelman, A., & Rubin, D. B. (1992). Inference from iterative simulation using multiple sequences. *Statistical Science*, 7, 457–511. <https://doi.org/10.1214/ss/1177011136>
- Gittleman, J. L., & Kot, M. (1990). Adaptations: Statistics and null model for estimating phylogenetic effects. *Systematic Zoology*, 39, 227–241. <https://doi.org/10.2307/2992183>
- Guillerme, T., & Healy, K. (2014). mulTree: A package for running MCMCglmm analysis on multiple trees. *Zenodo*. <https://doi.org/10.5281/zenodo.12902>
- 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., & Nakagawa, S. (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. <https://doi.org/10.1111/j.1420-9101.2009.01915.x>
- Hansen, T. F. (1997). Stabilizing selection and the comparative analysis of adaptation. *Evolution*, 51, 1341–1351. <https://doi.org/10.1111/j.1558-5646.1997.tb01457.x>
- Hansen, T. F., & Martins, E. P. (1996). Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution*, 50, 1404–1417.
- Hanssen, S. A., Hasselquist, D., Folstad, I., & Erikstad, K. E. (2005). Cost of reproduction in a long-lived bird: Incubation effort reduces immune function and future reproduction. *Proceedings of the Royal Society of London B*, 272, 1039–1046. <https://doi.org/10.1098/rspb.2005.3057>
- Harmon, L. J., Losos, J. B., Davies, T. J., Gillespie, R. G., Gittleman, J. L., Bryan Jennings, W., ... Purvis, A. (2010). Early bursts of body size and shape evolution are rare in comparative data. *Evolution*, 64, 2385–2396.
- Harmon, L. J., Weir, J., Brock, C., Glor, R. E., & Challenger, W. (2008). GEIGER: Investigating evolutionary radiations. *Bioinformatics*, 24, 129–131. <https://doi.org/10.1093/bioinformatics/btm538>
- Hasselquist, D. (2007). Comparative immunoeology in birds: Hypotheses and tests. *Journal of Ornithology*, 148, 571–582. <https://doi.org/10.1007/s10336-007-0201-x>
- Hoebe, K., Janssen, E., & Beutler, B. (2004). The interface between innate and adaptive immunity. *Nature Immunology*, 5, 971–974.
- Hörak, P., Saks, L., Ots, I., & Kollist, H. (2002). Repeatability of condition indices in captive greenfinches (*Carduelis chloris*). *Canadian Journal of Zoology*, 80, 636–643. <https://doi.org/10.1139/z02-038>
- Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K., & Mooers, A. O. (2012). The global diversity of birds in space and time. *Nature*, 491, 444–448. <https://doi.org/10.1038/nature11631>
- Johnstone, C. P., Reina, R. D., & Lill, A. (2012). Interpreting indices of physiological stress in free-living vertebrates. *Journal of Comparative Physiology B*, 182, 861–879. <https://doi.org/10.1007/s00360-012-0656-9>
- Kerimov, A. B., Ilyina, T. A., Ivankina, E. V., Bushuev, A. V., Sokolova, O. V., & Rogovin, K. A. (2018). Melanin-based coloration and immunity in polymorphic population of pied flycatcher, *Ficedula hypoleuca*. *Evolutionary Ecology*, 32, 89–111. <https://doi.org/10.1007/s10682-017-9926-z>
- Knowles, S. C., Nakagawa, S., & Sheldon, B. C. (2009). Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: A meta-regression approach. *Functional Ecology*, 23, 405–415. <https://doi.org/10.1111/j.1365-2435.2008.01507.x>
- Kogut, M. H., Iqbal, M., He, H., Philbin, V., Kaiser, P., & Smith, A. (2005). Expression and function of Toll-like receptors in chicken heterophils. *Developmental and Comparative Immunology*, 29, 791–807.
- Krams, I., Vrublevska, J., Cirule, D., Kivleniece, I., Krama, T., Rantala, M. J., ... Hörak, P. (2012). Heterophil/lymphocyte ratios predict the magnitude of humoral immune response to a novel antigen in great tits (*Parus major*). *Comparative Biochemistry and Physiology Part A*, 161, 422–428. <https://doi.org/10.1016/j.cbpa.2011.12.018>
- 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., Wikelski, M., Robinson, W. D., Robinson, T. R., & Klasing, K. C. (2008). Constitutive immune defences correlate with life-history variables in tropical birds. *Journal of Animal Ecology*, 77, 356–363. <https://doi.org/10.1111/j.1365-2656.2007.01347.x>
- Lindström, K. M., Foutopoulos, J., Pärn, H., & Wikelski, M. (2004). Immunological investments reflect parasite abundance in island populations of Darwin's finches. *Proceedings of the Royal Society of London B*, 271, 1513–1519. <https://doi.org/10.1098/rspb.2004.2752>
- Lochmiller, R. L., & Deerenberg, C. (2000). Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, 88, 87–98. <https://doi.org/10.1034/j.1600-0706.2000.880110.x>
- MacColl, E., Vanesky, K., Buck, J. A., Dudek, B. M., Eagles-Smith, C. A., Heath, J. A., ... Downs, C. J. (2017). Correlates of immune defenses in golden eagle nestlings. *Journal of Experimental Zoology Part A*, 327, 243–253.
- Martin, L. B. II, Pless, M., Svoboda, J., & Wikelski, M. (2004). Immune activity in temperate and tropical house sparrows: A common-garden experiment. *Ecology*, 85, 2323–2331. <https://doi.org/10.1890/03-0365>
- Merino, S., Moreno, J., Vázquez, R. A., Martínez, J., Sánchez-Monsálvez, I., Estades, C. F., ... McGehee, S. (2008). Haematzoa in forest birds from southern Chile: Latitudinal gradients in prevalence and parasite lineage richness. *Austral Ecology*, 33, 329–340. <https://doi.org/10.1111/j.1442-9993.2008.01820.x>
- Minias, P. (2019). Data from: Evolution of heterophil/lymphocyte ratios in response to ecological and life-history traits: A comparative analysis across the avian tree of life. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.s3cc172>
- Minias, P., Pikus, E., Whittingham, L. A., & Dunn, P. O. (2019). Evolution of copy number at the MHC varies across the avian tree of life. *Genome Biology and Evolution*, 11, 17–28.
- Minias, P., & Podlaskczuk, P. (2017). Longevity is associated with relative brain size in birds. *Ecology and Evolution*, 7, 3558–3566. <https://doi.org/10.1002/ece3.2961>
- Minias, P., Whittingham, L. A., & Dunn, P. O. (2017). Coloniality and migration are related to selection on MHC genes in birds. *Evolution*, 71, 432–441. <https://doi.org/10.1111/evo.13142>
- Minias, P., Włodarczyk, R., & Meissner, W. (2018). Leukocyte profiles are associated with longevity and survival, but not migratory effort: A comparative analysis of shorebirds. *Functional Ecology*, 32, 369–378. <https://doi.org/10.1111/1365-2435.12991>
- 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. <https://doi.org/10.2307/3546966>
- Møller, A. P. (2007). Senescence in relation to latitude and migration in birds. *Journal of Evolutionary Biology*, 20, 750–757. <https://doi.org/10.1111/j.1420-9101.2006.01236.x>

- Møller, A. P., Arriero, E., Lobato, E., & Merino, S. (2009). A meta-analysis of parasite virulence in nestling birds. *Biological Reviews*, 84, 567–588. <https://doi.org/10.1111/j.1469-185X.2009.00087.x>
- Møller, A. P., & Erritzøe, J. (1996). Parasite virulence and host immune defense: Host immune response is related to nest reuse in birds. *Evolution*, 50, 2066–2072. <https://doi.org/10.1111/j.1558-5646.1996.tb03592.x>
- Møller, A. P., & Erritzøe, J. (1998). Host immune defence and migration in birds. *Evolutionary Ecology*, 12, 945–953. <https://doi.org/10.1023/A:1006516222343>
- Møller, A. P., Merino, S., Brown, C. R., & Robertson, R. J. (2001). Immune defense and host sociality: A comparative study of swallows and martins. *The American Naturalist*, 158, 136–145. <https://doi.org/10.1086/321308>
- Moreno, J., Sanz, J. J., & Arriero, E. (1999). Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. *Proceedings of the Royal Society of London B*, 266, 1105–1109. <https://doi.org/10.1098/rspb.1999.0750>
- Müller, C., Jenni-Eiermann, S., & Jenni, L. (2011). Heterophils/Lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings. *Functional Ecology*, 25, 566–576. <https://doi.org/10.1111/j.1365-2435.2010.01816.x>
- Norris, K., & Evans, M. R. (2000). Ecological immunology: Life history trade-offs and immune defense in birds. *Behavioral Ecology*, 11, 19–26. <https://doi.org/10.1093/beheco/11.1.19>
- Nunn, C. L., Altizer, S. M., Sechrest, W., & Cunningham, A. A. (2005). Latitudinal gradients of parasite species richness in primates. *Diversity and Distributions*, 11, 249–256. <https://doi.org/10.1111/j.1366-9516.2005.00160.x>
- O'Connor, E. A., Cornwallis, C. K., Hasselquist, D., Nilsson, J.-Å., & Westerdahl, H. (2018). The evolution of immunity in relation to colonization and migration. *Nature Ecology and Evolution*, 2, 841. <https://doi.org/10.1038/s41559-018-0509-3>
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature*, 401, 877–884. <https://doi.org/10.1038/44766>
- Palacios, M. G., Cunnick, J. E., Vleck, D., & Vleck, C. M. (2009). Ontogeny of innate and adaptive immune defense components in free-living tree swallows, *Tachycineta bicolor*. *Developmental & Comparative Immunology*, 33, 456–463. <https://doi.org/10.1016/j.dci.2008.09.006>
- Pap, P. L., Vágási, C. I., Vincze, O., Osváth, G., Veres-Szászka, J., & Cziráj, G. Á. (2015). Physiological pace of life: The link between constitutive immunity, developmental period, and metabolic rate in European birds. *Oecologia*, 177, 147–158. <https://doi.org/10.1007/s00442-014-3108-2>
- Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20, 289–290. <https://doi.org/10.1093/bioinformatics/btg412>
- Parejo, D., Silva, N., & Avilés, J. M. (2007). Within-brood size differences affect innate and acquired immunity in roller *Coracias garulus* nestlings. *Journal of Avian Biology*, 38, 717–725. <https://doi.org/10.1111/j.2007.0908-8857.04081.x>
- Pierotti, R., & Annett, C. (1994). Patterns of aggression in gulls: Asymmetries and tactics in different social categories. *Condor*, 96, 590–599. <https://doi.org/10.2307/1369461>
- Poulin, R., & Morand, S. (2000). The diversity of parasites. *The Quarterly Review of Biology*, 75, 277–293. <https://doi.org/10.1086/393500>
- R Development Core Team (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Råberg, L., Vestberg, M., Hasselquist, D., Holmdahl, R., Svensson, E., & Nilsson, J.-Å. (2002). Basal metabolic rate and the evolution of the adaptive immune system. *Proceedings of the Royal Society of London B*, 269, 817–821. <https://doi.org/10.1098/rspb.2001.1953>
- Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution*, 3, 217–223. <https://doi.org/10.1111/j.2041-210X.2011.00169.x>
- Revell, L. J., Harmon, L. J., & Collar, D. C. (2008). Phylogenetic signal, evolutionary process, and rate. *Systematic Biology*, 57, 591–601. <https://doi.org/10.1080/10635150802302427>
- Saino, N., Incagli, M., Martinelli, R., & Møller, A. P. (2002). Immune response of male barn swallows in relation to parental effort, corticosterone plasma levels, and sexual ornamentation. *Behavioral Ecology*, 13, 169–174. <https://doi.org/10.1093/beheco/13.2.169>
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, 21, 55–89.
- Snow, D. W., & Perrins, C. M. (1998). *The birds of the western palearctic (concise edition)*. Vol. 1–2. Oxford, UK: Oxford University Press.
- Stokes, D. L., & Boersma, P. D. (2000). Nesting density and reproductive success in a colonial seabird, the Magellanic penguin. *Ecology*, 81, 2878–2891. [https://doi.org/10.1890/0012-9658\(2000\)081\[2878:NDARSI\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2000)081[2878:NDARSI]2.0.CO;2)
- Tacutu, R., Craig, T., Budovsky, A., Wuttke, D., Lehmann, G., Taranukha, D., ... de Magalhães, J. P. (2013). Human Ageing Genomic Resources: Integrated databases and tools for the biology and genetics of ageing. *Nucleic Acids Research*, 41, D1027–D1033.
- Tella, J. L., Scheuerlein, A., & Ricklefs, R. E. (2002). Is cell-mediated immunity related to the evolution of life-history strategies in birds? *Proceedings of the Royal Society of London B*, 269, 1059–1066. <https://doi.org/10.1098/rspb.2001.1951>
- Tieleman, B. I., Williams, J. B., Ricklefs, R. E., & Klasing, K. C. (2005). Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. *Proceedings of the Royal Society of London B*, 272, 1715–1720. <https://doi.org/10.1098/rspb.2005.3155>
- Vágási, C. I., Pap, P. L., Vincze, O., Osváth, G., Erritzøe, J., & Møller, A. P. (2016). Morphological adaptations to migration in birds. *Evolutionary Biology*, 43, 48–59. <https://doi.org/10.1007/s11692-015-9349-0>
- Vincze, O. (2016). Light enough to travel or wise enough to stay? Brain size evolution and migratory behaviour in birds. *Evolution*, 70, 2123–2133. <https://doi.org/10.1111/evo.13012>
- Wilcoxon, T. W., Boughton, R. K., Morgan, G. M., & Schoech, S. J. (2013). Heritability of immunological characteristics in Florida Scrub-Jays (*Aphelocoma coerulescens*). *Canadian Journal of Zoology*, 91, 789–794. <https://doi.org/10.1139/cjz-2013-0075>
- Winkler, D. W., Billerman, S. M., & Lovette, I. J. (2015). *Bird families of the world: An invitation to the spectacular diversity of birds*. Barcelona, Spain: Lynx Edicions.

How to cite this article: Minias P. Evolution of heterophil/lymphocyte ratios in response to ecological and life-history traits: A comparative analysis across the avian tree of life. *J Anim Ecol*. 2019;88:554–565. <https://doi.org/10.1111/1365-2656.12941>