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Blood Variation Implicates Respiratory Limits on Elevational Ranges of Andean Birds

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ABSTRACT: The extent to which species ranges reflect intrinsic physiological tolerances is a major question in evolutionary ecology. To date, consensus has been hindered by the limited tractability of experimental approaches across most of the tree of life. Here, we apply a macrophysiological approach to understand how hematological traits related to oxygen transport shape elevational ranges in a tropical biodiversity hot spot. Along Andean elevational gradients, we measured traits that affect blood oxygen-carrying capacity—total and cellular hemoglobin concentration and hematocrit, the volume percentage of red blood cells—for 2,355 individuals of 136 bird species. We used these data to evaluate the influence of hematological traits on elevational ranges. First, we asked whether the sensitivity of hematological traits to changes in elevation is predictive of elevational range breadth. Second, we asked whether variance in hematological traits changed as a function of distance to the nearest elevational range limit. We found that birds showing greater hematological sensitivity had broader elevational ranges, consistent with the idea that a greater acclimatization capacity facilitates elevational range expansion. We further found reduced variation in hematological traits in birds sampled near their elevational range limits and at high absolute elevations, patterns consistent with intensified natural selection, reduced effective population size, or compensatory changes in other cardiorespiratory traits. Our findings suggest that constraints on hematological sensitivity and local genetic adaptation to oxygen availability promote the evolution of the narrow elevational ranges that underpin tropical montane biodiversity.

Keywords: phenotypic plasticity, hemoglobin, hypoxia, niche breadth, macrophysiology.

Introduction

When, if ever, are species ranges limited by intrinsic physiological tolerances? Correlative niche models have demonstrated the pervasive influence of climate on plant and animal distributions (reviewed in Elith and Leathwick 2009), but inferring the specific effects of abiotic variables on organismal fitness and population viability requires additional evidence (Bozinovic et al. 2011; Bozinovic and Naya 2015). Physiological tests paired with occurrence records have demonstrated that thermal tolerance is associated with geographic range size in groups as diverse as aquatic insects (Polato et al. 2018), *Mimulus* monkeyflowers (Sheth and Angert 2014), and dung and carrion beetles (Sheldon and

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Tewksbury 2014). In plants, reciprocal transplants suggest that geographic range limits frequently align with niche limits (Hargreaves et al. 2014; Lee-Yaw et al. 2016). Yet for the majority of species where experimental manipulation is impractical, clear links among physiological trait values, environmental factors, and distribution limits remain elusive. In these cases, macrophysiological data—measurements of physiological trait variation at large spatial and phylogenetic scales—can provide valuable insights into the functional underpinnings of biogeographic patterns (Chown et al. 2004; Gaston et al. 2009).

Few biogeographic patterns are as striking as pervasive elevational specialization in tropical vertebrates. In the Peruvian Andes, for example, birds have a median elevational range breadth of approximately 1,100 m, despite more than 5,000 m of habitable elevation gradient below the line of permanent snow and ice (Parker et al. 1996). This specialization contributes to extraordinary beta diversity: along a single surveyed transect near Manu, Peru, 66 hummingbird species occur, but no more than 30 at a single elevation (Walker et al. 2006). Because rapid species turnover is correlated with a dramatic environmental gradient (McNew et al. 2021), biologists have long hypothesized that narrow breadth of tolerance to one or more abiotic variables contributes to range limits (von Humboldt 1838; Janzen 1967; Terborgh 1971; Jankowski et al. 2013). Yet to date, research on the proximate causes of range limits has largely focused on biotic interactions (Terborgh and Weske 1975; Freeman et al. 2016), complemented by a handful of comparative studies of metabolic and thermal physiology (Londoño et al. 2015; Londoño et al. 2017; Wolf et al. 2020; Gutierrez-Pinto et al. 2021).

One environmental variable with well-characterized consequences for organismal physiology is the partial pressure of oxygen (P_{O_2}), which systematically declines with increasing elevation. Lowland organisms show a variety of plastic acclimatization responses to low P_{O_2}, including interrelated changes in numerous respiratory, cardiovascular, and metabolic traits (Storz et al. 2010; Storz and Scott 2019). Among these short-term responses are a suite of gene expression changes driven by the hypoxia-inducible factor (HIF) pathway, including upregulation of erythropoietin and resulting increases in blood oxygen-carrying capacity (Semenza et al. 1991). As much as 99% of O_2 in vertebrate blood is bound to hemoglobin (Hb), a tetrameric, iron-containing metalloprotein synthesized in red blood cells, also known as erythrocytes. Under low ambient P_{O_2} conditions, animals experience reduced O_2 saturation of arterial blood (Sa_{O_2}). HIF-induced erythropoiesis can compensate for this shortfall by augmenting total red blood cell mass, thereby enhancing blood-O_2 transport capacity. In the absence of concurrent increases in plasma volume, erythropoiesis should lead to increases in both total blood Hb concentration ([Hb]) and hematocrit (Hct), the volume percentage of red blood cells. Because each Hb tetramer binds up to four O_2 molecules, [Hb] is proportional to blood oxygen-carrying capacity per unit volume of blood. Hct is a function of the quantity and size of Hb-containing erythrocytes. Another trait that is relevant to blood-O_2 transport—mean cellular Hb concentration (MCHC)—is simply the scaled quotient of [Hb] and Hct.

Over longer timescales, evolutionary shifts in elevation are often associated with predictable changes in Hb-O_2 affinity that help optimize pulmonary O_2 uptake and tissue O_2 delivery according to changes in inspired P_{O_2} (Projecto-Garcia et al. 2013; Natarajan et al. 2016; Storz 2016; Storz 2019). The fitness trade-offs associated with physiological adaptation to a particular P_{O_2} regime could promote the evolution of elevational specialization in vertebrates. If so, variation in blood phenotypes across the elevational range of species may bear the signature of this evolutionary process. For example, individual plasticity in Hb concentration could affect overall acclimatization capacity and, by extension, the environmental niche breadth of species. Similarly, the relationship between local (elevation-specific) variation in Hb concentration and relative position within a species' elevational range could indicate stronger trait-specific selection near range limits (Hoffman and Blows 1994; Pennington et al. 2021). Two recent phylogenetic comparative analyses of compiled data on Hb concentration and Hct showed that both parameters tended to increase with elevation, even when data were derived from a wide variety of bird taxa and collected by various investigators without any specific intent to test elevation effects (Yap et al. 2019; Minias et al. 2020).

Here, we apply a macrophysiological approach to understand how commonly studied hematological traits associated with blood-O_2 transport capacity might shape elevational distributions in a biodiversity hot spot, using original data. We measured [Hb] and Hct and calculated MCHC for 2,355 individuals of 136 bird species. Assuming that all three hematological traits would respond similarly to environmental hypoxia without systematic variation among species, we addressed two hypotheses related to the relationship between physiology and elevational range using multivariate linear models in a Bayesian framework.

First, we asked whether the rate of increase in hematological trait values per unit elevation (hereafter, "hematological sensitivity") is predictive of elevational range breadth, using an approach similar to a previous study of leaf trait sensitivity to elevation (Sides et al. 2014). We assumed within-species genetic homogeneity among the populations we sampled, a plausible assumption given the high vagility of birds and short linear distances across elevational gradients. Hematological sensitivity can be interpreted as a proxy for the species-wide mean of individual trait plasticity measurements. Because a hypoxia-induced increase in [Hb] can contribute to hypoxia acclimatization by increasing arterial O_2 content—augmenting convective O_2 transport when paired with other cardiovascular adjustments (Gonzalez et al. 1994; Tate et al. 2017; Gonzalez and Kuwahira 2018; Stembridge et al. 2019; Tate et al. 2020; Storz and Bautista 2022)—[Hb] sensitivity could be positively correlated with elevational range breadth. However, [Hb] is a highly plastic trait that reflects overall levels of tissue O_2 delivery and stimulus-response characteristics of the erythropoietin system. Under hypoxia, a reduced oxygenation of renal tissue stimulates the synthesis and release of erythropoietin, which in turn activates red blood cell production. Thus, species that can maintain adequate tissue O_2 delivery in spite of environmental hypoxia may exhibit little change in [Hb] with increasing elevation. By contrast, species that suffer greater impairments of tissue O_2 delivery at high elevation (and that therefore experience a stronger hypoxia-induced stimulus of erythropoiesis) will show a higher rate of increase in [Hb] as a function of elevation. If hypoxia tolerance is an important determinant of upper elevational range limits, we might expect a negative correlation between hypoxia-induced variation in [Hb] and elevational range breadth.

Second, we asked whether variance in hematological traits changed as a function of distance from the nearest elevational range limit and as a function of absolute elevation. At least three non-mutually-exclusive mechanisms could lead to nonrandom patterns of variance in trait values across elevation: changes in effective population size at range limits, a history of directional selection on the genetic component of trait variance, and/or hypoxia-induced changes in the environmental component of trait variance (plasticity). Without attempting to discriminate among these mechanisms, we predicted that optimal respiratory performance occurred near the midpoint of a species' elevational range and that departures from this optimum would lead to a positive correlation between both distance from the nearest elevational range limit and absolute elevation and local variation in hematological phenotypes. We further tested for the presence of an interaction between these predictors, expecting that the increasingly severe ambient hypoxia at higher absolute elevation might diminish the positive effect of increasing distance from the nearest elevational range limit (i.e., increasing proximity to elevational range midpoint and optimal respiratory performance) on local hematological trait variance.

Methods

Hematological Measurements

From 2006 to 2020, we measured [Hb] and Hct for birds captured during collaborative fieldwork in Peru by the Museum of Southwestern Biology (MSB) in New Mexico and the Centro de Ornitología y Biodiversidad (CORBIDI) in Lima, Peru. We conducted this work with assistance from numerous researchers associated with these institutions—whose contributions we gratefully acknowledge here and describe in detail elsewhere (Witt, Linck, Williamson, et al., in prep.)—and under the following permits from Peru's management authorities: 004-2007-INRENA-IFFS-DCB, 135-2009-AG-DGFFS-DGEFFS, 0377-2010-AG-DGFFS-DGEFFS, 0199-2012-AG-DGFFS-DGEFFS, 006-2013-MINAGRI-DGFFS/DGEFFS, 280-2014-MINAGRI-DGFFS-DGEFFS, and 2022-RDG 405-2017-SERFOR-DGGSPFFS.

As quickly as possible after capture, we punctured the brachial vein on the underside of each bird's wing and collected whole blood using heparinized microcapillary tubes (for Hct) or spectrophotometer cuvettes (for [Hb]). We centrifuged microcapillary tubes for 5 min at 13,000 rpm to separate red blood cells from plasma and used digital calipers to quantify the relative volume of each, averaging two samples per bird to arrive at our final estimate of Hct as a percentage. We measured [Hb] (g/dL blood) for an ~5-μL blood sample with the Hemocue HB201 analyzer and associated Hb photometer. As this proprietary method generates values ~1.0 g/dL higher than measures from a direct cyanomethaemoglobin spectrophotometer (Simmons and Lill 2006), we corrected the resulting estimates by subtracting this quantity (DuBay and Witt 2014). Specimens and tissues are housed at the MSB and CORBIDI, and specimen data are detailed in a separate data publication (Witt et al., in prep.).

Data Filtering and Preparation

To ensure only high-quality measurements were included in our analyses, we applied a series of filters to the full data set of hematological traits. We first removed all records that lacked a measurement for either [Hb] or Hct and then used these values to calculate MCHC ([Hb]/Hct(%) × 100; Campbell and Ellis 2007). We then removed outliers using a set of thresholds for the minimum and maximum allowable value of each hematological trait, which were derived by visualizing distributions and quantile-quantile (Q-Q) plots and assuming significant deviations from normality represented measurement error, unhealthy individuals, or otherwise unusable data. These thresholds were [Hb] values ≤ 11 or ≥ 24, Hct values ≤ 0.3 or ≥ 0.8, and MCHC values ≤ 22 or ≥ 42. After applying outlier filters, we dropped any species with less than five remaining records and merged the resulting data set with estimates of elevational range breadth compiled from the literature and verified by expert opinion (Parker et al. 1996; Schulenberg et al. 2010), expanding these estimates as necessary to encompass our own observations when they fell outside the bounds of previously published data sets.

Because our metric of hematological sensitivity to elevation was calculated as the rate of change in each hematological trait per unit elevation and we wished to avoid biasing estimates through the influence of a single erroneous measurement, we next ran simple linear regressions of [Hb], Hct, and MCHC against the elevation they were recorded at and removed all points with a Cook's distance of $4/n$ (where n is sample size; Cook's distance is an estimate of the influence of a data point on least squares regression results). We applied a more conservative Cook's distance cutoff of $3.5/n$ for juveniles, as they may exhibit anomalous hematological trait variation (Fair et al. 2007). (Juveniles were identified by the presence of bursa of Fabricius during specimen preparation, an organ necessary for B cell development.) We then regressed [Hb], Hct, and MCHC against elevation a second time and generated a new data set where each row referenced a single species, with estimates of the slope of each hematological trait and its associated standard error. After dropping species lacking data from at least two elevations >200 m apart—a span chosen to reflect evidence of fine-scale physiological sensitivity to $\mathrm{PO_2}$ in vertebrates (Gassmann et al. 2019)—we combined this data set of hematological plasticity and elevational range breadth values with estimates of the median elevation of each species' range (calculated as $\mathrm{Elev_{min}} + \mathrm{Elev_{breadth}}/2$) and its average mass (calculated as the arithmetic mean of the recorded mass of all individual birds in our final filtered data set). Last, we calculated the proportion of each species' total elevational range represented by individuals in our data set, a metric we included to evaluate the possible effects of sampling bias on our data.

To understand variation in hematological traits within species at a given approximate elevation, we generated a second data set using the above outlier filters and minimum per-species sample size. We then divided the sampled elevational range of each species into 100-m bins, discarding the remainder in the event of a noninteger quotient. In each bin with a minimum of six records we calculated the coefficient of variation (CoV) as $\hat{c}_v = s/\bar{x}$, where s is the standard deviation of the sample and $\bar{x}$ is the arithmetic mean. This resulted in a data set where each row referenced a bin-specific CoV value, which we associated with the mean elevation of all individuals sampled within that 100-m bin, their species, and the relative distance of the mean elevation of each bin from the nearest elevational range limit. As only 23 of the 73 species in this data set were sampled at two or more elevational bins, our model heavily depends on interspecies variation. However, we found no evidence of systematic or substantial interspecies variation (phylogenetic or otherwise) in CoV for hematological traits, all of which are constrained similarly by functional considerations and vary over modest ranges in all birds.

Bayesian Models

To evaluate the hypothesis that hematological sensitivity was predictably related to elevational range characteristics, we built a set of generalized multivariate linear models in the R package brms (ver. 2.13.5; Bürkner 2017), which is a wrapper for the statistical programing language Stan. For each hematological trait ([Hb], Hct, and MCHC), we modeled sensitivity (S) using a Student's t distribution for outcome variables and regularizing priors:

$$\begin{aligned}
S_{\mathrm{EST},i} &\sim \mathrm{Student}(\nu, \mu_i, \alpha),\\
\mu_i &= \alpha + \alpha_j + \beta_R R_i + \beta_E E_i + \beta_P P_i\\
&\quad + \beta_M M_i + \beta_{R\times E} R_i E_i,\\
P_{\mathrm{OBS},i} &\sim \mathrm{Student}(\nu, P_{\mathrm{EST},i}, P_{\mathrm{SE},i}),\\
\alpha &\sim \mathrm{Normal}(0, 0.5),\\
\alpha_j &\sim \mathrm{Normal}(\alpha, \sigma_A),\\
\beta_R &\sim \mathrm{Normal}(0, 0.5),\\
\beta_E &\sim \mathrm{Normal}(0, 0.5),\\
\beta_M &\sim \mathrm{Normal}(0, 0.5),\\
\beta_P &\sim \mathrm{Normal}(0, 0.5),\\
\beta_{R\times E} &\sim \mathrm{Normal}(0, 0.5),\\
\sigma &\sim \mathrm{Cauchy}(0, 0.5).
\end{aligned}$$

Here, the predictor R is elevational range breadth, E is median range elevation, P is proportion of elevational range sampled, M is mass, and A is a covariance matrix of phylogenetic distance among taxa. We generated this matrix by applying the function maxCladeCred() from the R package phangorn (ver. 2.5.5) to 10,000 trees from the Hackett "backbone" phylogeny downloaded from https://birdtree.org/ and pruned it to the subset of species present in our data set using the keep.tip() function in the R package ape (ver. 5.3; Paradis et al. 2004; Schliep 2011; Jetz et al. 2012). Because our metric of hematological sensitivity is associated with substantial uncertainty stemming from sample size and data collection methods, we model measurement error by treating the outcome variable as a vector of parameters with the likelihood $P_{\mathrm{EST},i} \sim \mathrm{Student}(\nu, \mu_i, \alpha)$, which is given a prior that treats our observed data as drawn from a Student's t distribution with unknown mean $P_{\mathrm{EST},i}$ and the calculated standard error of the regression ($P_{\mathrm{SE},i}$). In this way, uncertainty in the outcome variables is incorporated into regression parameters but is itself influenced by the linear model (McElreath 2020).

This "full" model (1) reflects the more specific hypothesis that all predictors and phylogeny influence S. To evaluate whether predictive power was improved by simplifying the model structure, we built reduced models that (2) included the interaction term between R and E but did not include phylogenetically correlated intercepts, (3) did not include either the interaction term between R and E or phylogenetically correlated intercepts, and (4) included phylogenetically correlated intercepts but did not include the interaction term between R and E. We then created a null model (5) where outcome variables were solely influenced by phylogenetically correlated intercepts. As these five model backbones were repeated for each of the three outcome variables, we fitted a total of 15 sensitivity models.

We built similar models to evaluate whether variance in hematological traits changed as a function of distance from the midpoint of the elevational range. We modeled the CoV (V) in [Hb], Hct, and MCHC within a given elevational band using a logarithmic distribution and without modeling measurement error:

$$\begin{aligned}
V_i &\sim \text{Lognormal}(\mu_i, \alpha),\\
\mu_i &= \alpha + \alpha_j + \beta_E E_i + \beta_D D_i + \beta_{E\times D} E_i D_i,\\
\alpha &\sim \text{Normal}(0, 0.5),\\
\alpha_j &\sim \text{Normal}(\alpha, \sigma_A),\\
\beta_E &\sim \text{Normal}(0, 0.5),\\
\beta_D &\sim \text{Normal}(0, 0.5),\\
\beta_{E\times D} &\sim \text{Normal}(0, 0.5),\\
\sigma &\sim \text{Cauchy}(0, 0.5).
\end{aligned}$$

In the full model (1) above, predictor E is the mean elevation of samples in a given bin, and D is the mean distance of samples from the nearest elevational range limit, scaled from 0 (samples located at the elevational range midpoint) to a maximum of 0.5 (samples located at either the upper or lower range limit). Intercept terms are defined as previously described. We created three reduced models by (2) excluding phylogenetically correlated intercepts, (3) excluding the interaction term between E and D, and (4) excluding both phylogenetically correlated intercepts and the interaction term between E and D. We compared all four models with predictors to a null model (5) that included a phylogenetically correlated intercept alone. As with sensitivity models, this created a total of 15 additional models, with five models each for the outcome variables [Hb], Hct, and MCHC.

Prior to fitting brms models, we standardized each predictor and scaled sensitivity estimates to the same order of magnitude. We fitted all models using two Markov chain Monte Carlo (MCMC) runs with 5,000 generations of warm-up and 5,000 generations of sampling each. We evaluated convergence and stationarity by examining trace plots, ensuring effective sample sizes were sufficiently high (>1,000), and verifying for each model that the Gelman-Rubin diagnostic $\hat{R}$ was less than or equal to 1. We further evaluated model fit by performing a posterior predictive check of the distribution of each outcome variable and screened for sampling issues by creating scatterplots of the relationships among variables from MCMC draws.

To compare the predictive ability of alternate sensitivity and CoV models for the same outcome variables, we applied leave-one-out cross-validation information criteria (LOOIC) and assessed differences in the expected log pointwise predictive density (ELPD) using the function loo() in brms. For the full model for each outcome variable and summary statistic, we used the function median_hdi() in the R package tidybayes (ver. 2.1.1) to calculate the 95%, 80%, and 50% credible intervals of the posterior probability distribution of each predictor and interaction. We visualized these distributions using tidybayes and ggplot (ver. 3.3.2).

For each model with a predictor whose 80% credible interval did not overlap zero, we used the predict() and fitted() functions in brms to generate fitted curves with probability distributions, with and without incorporating residuals, respectively. To do so, we used parameter estimates from the best-fit model (table 1) to predict new observations of a given outcome variable across 3 standard deviations of the credible predictor while holding all other predictors constant at their mean value. For interaction terms that were credible at the 80% threshold, we repeated this approach three times for a single predictor in the interaction, holding the remaining predictor at −2 standard deviations, its mean value, and +2 standard deviations. In this way, we were able to visualize the effect of one predictor on the change in the slope of the

Table 1: Relative performance of models for (1) hematological trait sensitivity to elevation (indicated by Δ) and (2) local variation in hematological traits at a given elevation (indicated by CoV)

Model Description				Response					
No.	Predictors	Interaction	Phylogeny	Δ[Hb]	ΔHct	ΔMCHC	CoV [Hb]	CoV Hct	CoV MCHC
1	✗	✗	✗	−1.8 (1.0)	−1.5 (.8)	−2.9 (1.9)	−.5 (.8)	−1.2 (1.0)	−1.6 (.6)
2	✗	✗		−1.1 (.4)	−1.1 (2.1)	−2.2 (1.9)	**0**	−.2 (.9)	−.7 (.6)
3	✗			**0**	−.8 (2.1)	−1.8 (1.6)	−.6 (1.3)	**0**	**0**
4	✗		✗	−1.0 (.8)	−1.1 (.8)	−2.6 (1.7)	−1.0 (1.5)	−.1 (.6)	−.9 (.4)
5			✗	−1.7 (3.0)	**0**	**0**	−3.3 (3.0)	−6.4 (3.3)	−3.8 (3.2)

Note: Values represent the change in expected log pointwise predicted density (ELPD) from leave-one-out cross validation of Bayesian models, with associated standard errors reported in parentheses. Variable-specific predictors and interaction terms are described in "Methods." ELPD values reflect the difference between each model for a given response variable and the best-fit model among all comparisons (highlighted here in boldface type). CoV = coefficient of variation; [Hb] = total blood hemoglobin concentration; Hct = hematocrit; MCHC = mean cellular hemoglobin concentration.

relationship between a second predictor and its outcome variable.

Results

Data Filtering

Our initial data set included hematological trait measurements from 5,927 individual birds representing 656 unique species, with sampling from 107 unique localities ranging from 39 to 4,578 masl. After filtering, the reduced data set used to model hematological sensitivity to elevation included 2,355 hematological trait values from 136 species (fig. 1), collected from 39 to 4,578 masl, with a minimum sample size of $n = 4$ and a maximum sample size of $n = 115$. Our reduced variance data set included 118 CoV estimates for each hematological trait from 73 species, of which 23 included measurements for two or more elevational bins. The minimum median elevation of the bins used to calculate CoV values was 250 m, and the maximum median elevation was 4,350 m.

Sensitivity and Variance of Hematological Traits

In general, all three hematological traits were positively correlated with elevation within species following data filtering (figs. 1–3). The median value of the slope of [Hb] regressed against elevation (m) was 0.7 g/dL/1,000 m, with a 50% interquartile range (IQR) of −0.4 to 1.7; for Hct, the median slope was 2% per 1,000 m, with a 50% IQR of 1% to 5%. MCHC was less sensitive to elevation, with a mean slope of 0.07 g/dL/1,000 m and a 50% IQR of −0.00123 to 0.00121. The CoVs of [Hb] and Hct were comparable, with median values of 0.0752 (50% IQR: 0.0617 to 0.0983) and 0.0743 (0.0579 to 0.0982), respectively; the CoV of MCHC was relatively reduced (median: 0.0497; 0.0387 to 0.0665).

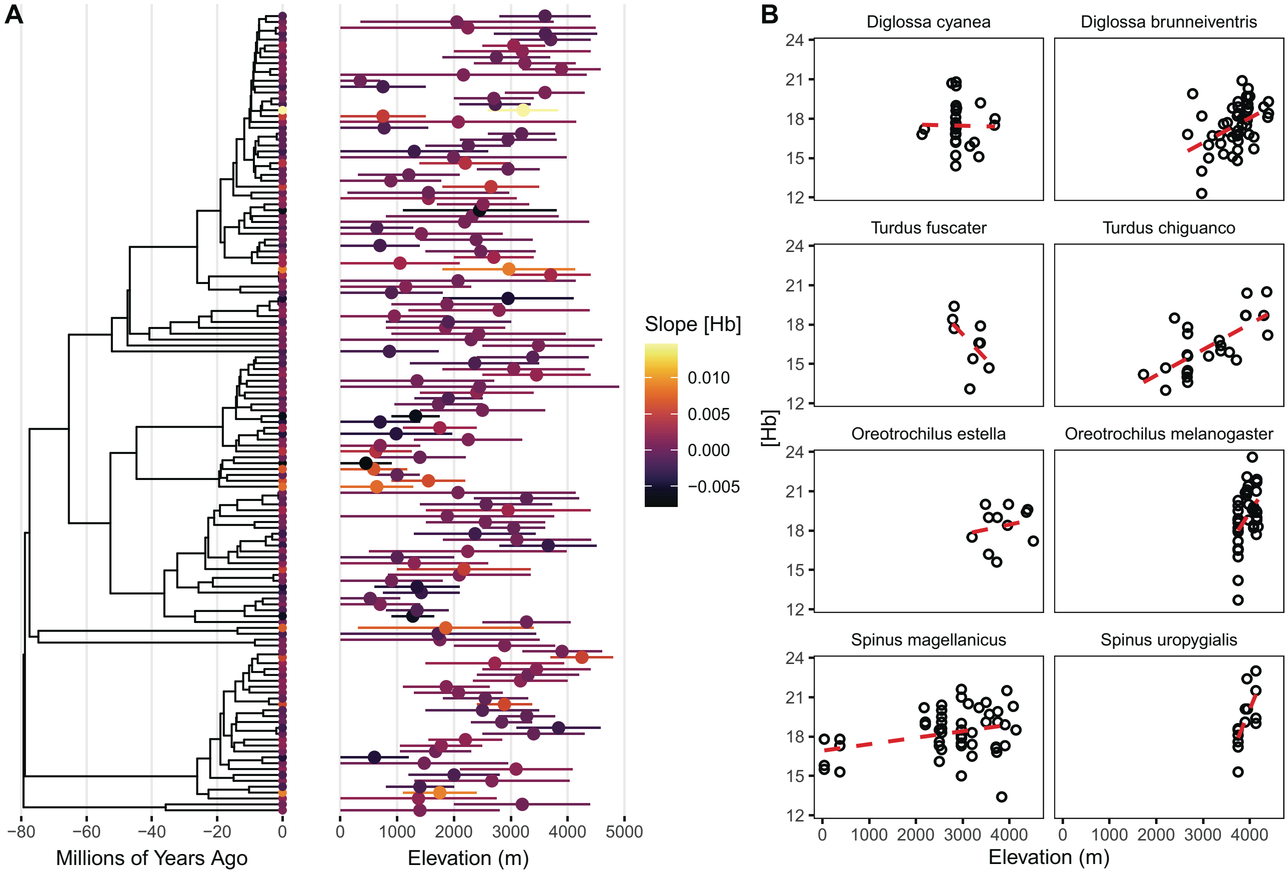

Figure 1: *A*, Low phylogenetic signal ($\lambda = 0.03$) in sensitivity of total blood hemoglobin concentration ([Hb]) to change in elevation across 136 species of Andean birds. Elevational range breadth and median range elevation (masl) are plotted adjacent to their corresponding species and shaded according to [Hb] sensitivity; values on the *x*-axis of the phylogeny represent millions of years before present. *B*, Examples of interspecific variation within genera in sensitivity of [Hb] to elevation.

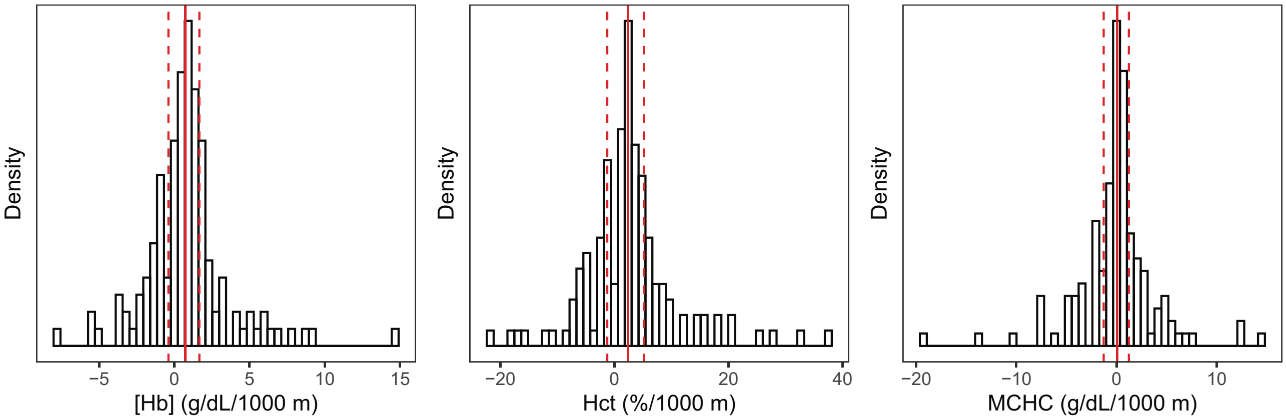

Figure 2: Observed distributions of hematological sensitivity to elevation, for 136 species. Solid lines indicate the median value and are flanked by dashed lines that mark the 50% interquartile range (IQR). [Hb] = total blood hemoglobin concentration; Hct = hematocrit; MCHC = mean cellular hemoglobin concentration.

Bayesian Models

A model of [Hb] sensitivity that included fixed effects for elevational range breadth, median range elevation, mass, and proportion of elevational range sampled—but not a term for the interaction between elevational range breadth and median range elevation or phylogenetically correlated intercepts—had the highest ELPD, albeit with substantial standard error associated its improved performance over a null model (ΔELPD $<$ 2 × SE; table 1). Elevational range breadth had a positive effect, credible at the 80% level. No predictors had a credible influence on Hct sensitivity, which was best predicted by phylogenetically correlated intercepts alone, although again uncertainly (ΔELPD $<$ 2 × SE; table 1). While a null model of MCHC sensitivity also outperformed all others, elevational range breadth had a positive effect on MCHC sensitivity with predictors, credible at the 80% level (fig. 3). As estimated in models that included all predictors and interaction terms, phylogenetic signal (λ) was 0.03 (95% credible interval: 0.00–0.01) for [Hb], 0.19 for Hct (95% credible interval: 0.02–0.43) for Hct, and 0.04 for MCHC (95% credible interval: 0.00–0.12).

Models of the CoV of hematological traits that included fixed effects were universally better fits for our data than a phylogeny-only null model, although standard errors for differences in ELPD among models were large (all ΔELPD $<$ 2 × SE). The CoV of [Hb] within a given elevational band was best predicted by a model with fixed effects for elevation, distance from the nearest elevational range limit, and their interaction (table 1). The mean elevation of samples had a negative influence on the CoV of a given 100-m bin, with a 95% credible interval that did not overlap with zero. Distance from the nearest elevational range limit had a positive influence on CoV, credible at the 95% level (fig. 3). The interaction of elevation and distance from elevational range limit had a negative effect, with an interval that was credible at the 80% level, but not at the 95% level (fig. 4).

The sign of the effect of these predictors was identical for the full model of CoV of Hct at a given elevation (fig. 3), although the model with the highest ELPD did not include an interaction term (table 1). In the full model, elevation had a negative effect on the CoV of Hct, credible at the 95% level. Distance from the nearest elevational range limit was positively associated with CoV and was also credible at the 95% level; the effect of the interaction of elevation and distance from nearest elevational range limit was negative, with a 95% credible interval that did not overlap zero. Last, the best model for the CoV of MCHC included fixed effects (but not an interaction term or phylogenetically correlated intercepts; table 1); distance from nearest elevational range limit had a positive effect, credible at the 80% level (fig. 3). The phylogenetic signal in models of the local CoV of hematological traits with all predictors and an interaction term was 0.01 (95% credible interval: 0.00–0.03) for [Hb], 0.01 for Hct (95% credible interval: 0.00–0.03) for Hct, and 0.01 for MCHC (95% credible interval: 0.00–0.04).

Discussion

The role of physiological tolerances in shaping species ranges remains poorly known for the vast majority of taxa, making it difficult to discern whether there are functional underpinnings for prominent biogeographic patterns. In a large data set of intraspecific physiological measurements in Andean birds, we found that hematological sensitivity and local variation in hematological trait phenotypes were predictably associated with elevational range characteristics (figs. 3, 4). Our study suggests that plasticity and genetic adaptation of hematological traits play a role in determining

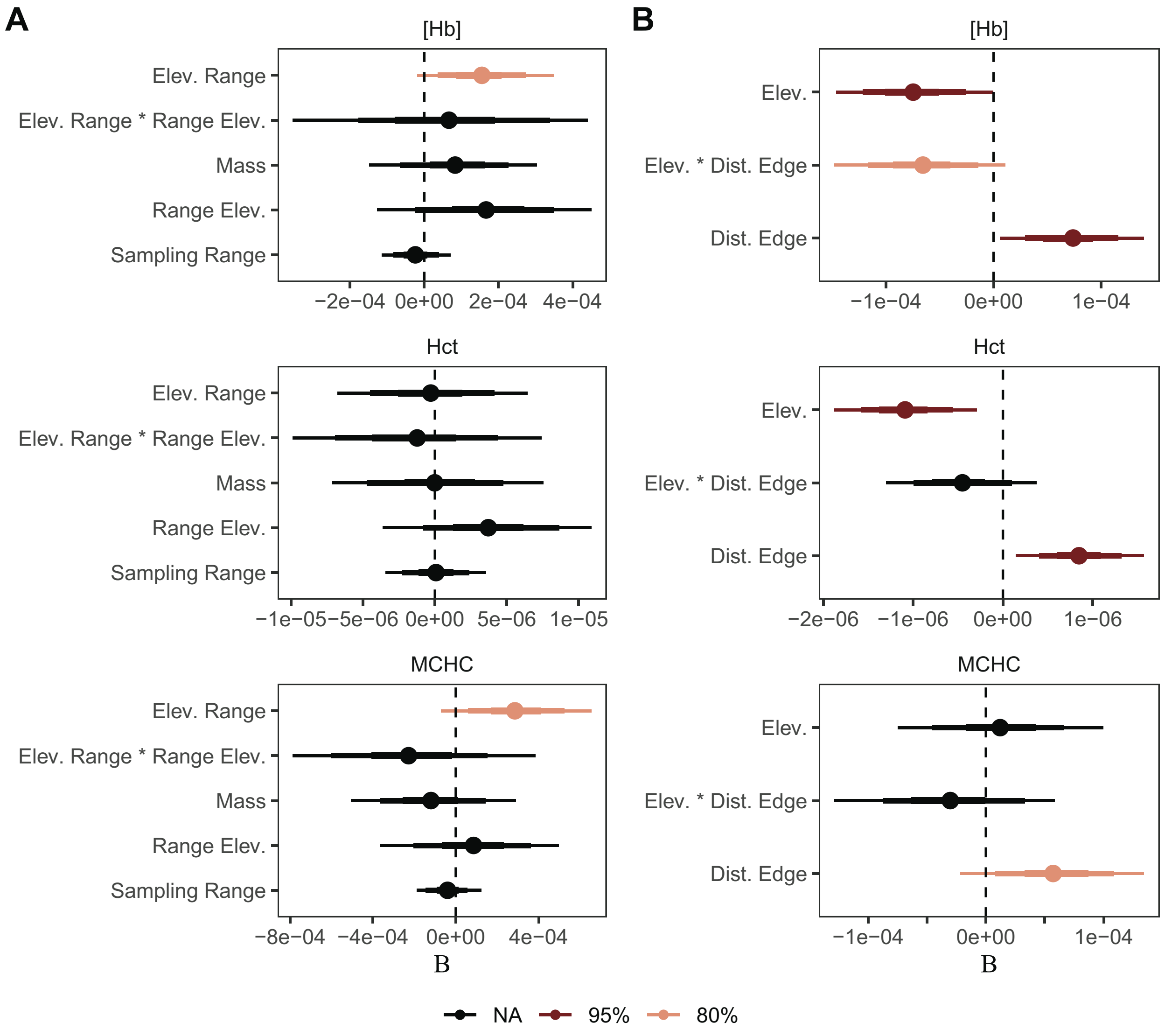

Figure 3: Posterior effect sizes for predictors in Bayesian models of hematological trait sensitivity across elevation (*A*) and the coefficient of variation in hematological traits within a given 100-m elevational bin (*B*). Median estimates are indicated by points; decreasing line thickness away from the median indicates the 50%, 80%, and 95% credible intervals for each estimate. Predictors with 95% and 80% credible intervals that fall entirely above or below zero are indicating by shading in the key below the figure. In *A*, "Elev. Range" is the predictor elevational range breadth, "Mass" is average species mass, "Range Elev." is median range elevation, and "Sampling Range" is proportion of the species' elevational range that was represented by our data. In *B*, "Elev." is the mean elevation of samples in a given bin and "Dist. Edge" is the distance from the sampling point to the nearest upper or lower elevational range limit for the species. [Hb] = total blood hemoglobin concentration; Hct = hematocrit; MCHC = mean cellular hemoglobin concentration.

species elevational niches, linking Po_2 with realized geographic ranges.

Across 136 species, sensitivity of both [Hb] and MCHC were positively correlated with elevational range breadth. Because our sensitivity metric is calculated per unit elevation and vertebrate species may be sensitive to Po_2 differences at elevations as little as 300 m apart (Gassmann et al. 2019), increased hematological sensitivity in elevational generalists appears to reflect higher plasticity in the measured traits rather than increased opportunity for acclimatization alone. This pattern supports the hypothesis that enhanced acclimatization capacity through population-level plasticity may facilitate range expansion into novel Po_2 regimes (fig. 3*a*). An important caveat here is that local adaptation to elevation may influence apparent hematological sensitivity, and such local adaptation could be more

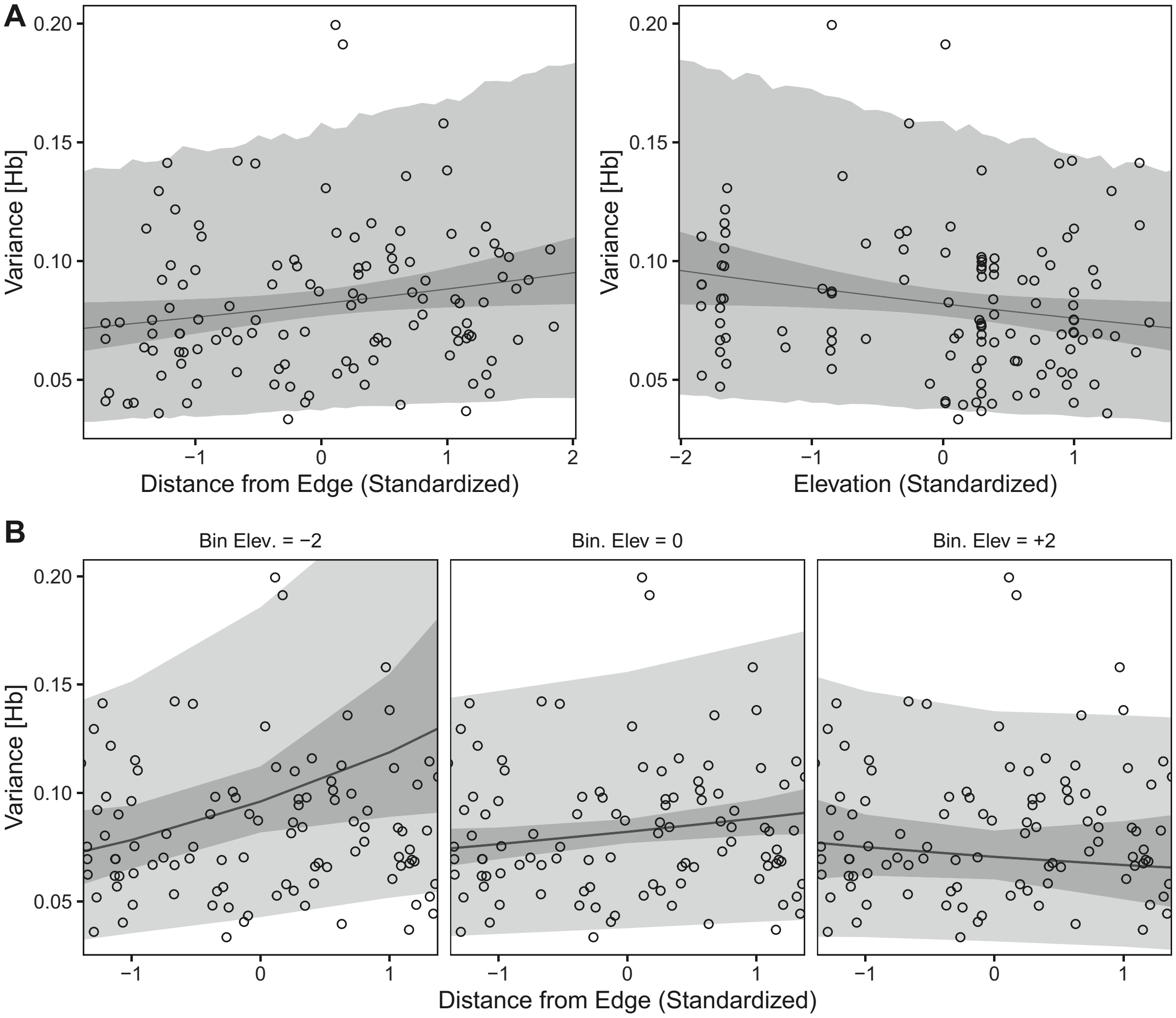

Figure 4: *A*, Predicted effects of distance from nearest elevational range limit ("Distance from Edge"; *left*) and elevation (*right*) on variance in total blood hemoglobin concentration ([Hb]). The curves in each plot represent the contribution of the variable on the *x*-axis to the outcome variable while holding all other predictors constant at their mean value. *B*, Effect of elevation on the contribution of distance from the nearest elevational range limit ("Distance from Edge") to variance in [Hb]. Panes from left to right show that increasing the standardized value of elevation decreases the slope of the relationship between the distance from the nearest elevational range limit and variance in [Hb].

likely in elevational generalists than specialists. However, Williamson et al. (2022) reported no effect of species adaptation to elevation on elevational trait variation for [Hb], Hct, or MCHC.

We consider the correlation between [Hb] and MCHC sensitivity and elevational range breadth an intuitive, if tentative, result, as niche breadth is often correlated with the degree of plasticity in other contexts (Williamson and Witt 2021). For example, an influential article by Van Valen (1965) compared populations of six species of passerine birds codistributed in island-mainland pairs, finding broad support for the hypothesis that functional morphological variation (as measured by the intraspecific coefficient of variation for a given trait) was "controlled to a significant extent by the adaptive diversity of the niche" (p. 378). While Van Valen assumed genetic control of the bill traits in his study, he acknowledged that under certain circumstances nonheritable plasticity might produce a similar pattern. In stickleback fishes, common garden experiments suggest that phenotypic plasticity has evolved repeatedly in generalist populations (Svanbäck and Schluter 2012), a finding consistent with both theory (Tienderen 1997) and other empirical work (Bradshaw 1965; Balaguer et al. 2001). In particular, plasticity appears to aid range expansion in many invasive species (Richards et al. 2006; Davidson et al. 2011; Knop and Reusser 2012).

Alternatively, if the broad ranges of elevational generalists are ephemeral products of recent population expansions and do not reflect a species' "equilibrium" niche breadth (Gadek et al. 2018), the positive correlation between elevational range breadth and hematological sensitivity might be a consequence of recent ephemeral range expansions. In other words, species with narrower, more stable ranges will have had greater time to evolve plasticity-suppressing adaptations in response to trade-offs (i.e., through genetic compensation; Storz and Scott 2021; for evidence of a negative correlation between plasticity and thermal tolerance, see Barley et al. 2021). Recently expanded "generalists" would be expected to retain this plasticity and continue to show correspondingly high values of hematological sensitivity. We think this scenario is unlikely: in species or lineages with long-term high-elevation ancestry, reduced hematological sensitivity with increasing elevation likely reflects adaptive changes in other convective and diffusive steps in the O_2 transport pathway that help sustain adequate levels of tissue oxygenation in spite of environmental hypoxia, dampening the hypoxic stimulus to increase [Hb] (via increased erythropoiesis, reduced plasma volume, or both; Stembridge et al. 2019; Storz and Bautista 2022). Furthermore, we would expect species with high median range elevations to show reduced hematological sensitivity if genetic compensation were an important predictor of trait values, but this was not the case (fig. 3*a*).

Reduced local variation in hematological traits near elevational range limits and at high absolute elevation (fig. 3*b*) is similarly intuitive but has more than one possible mechanistic interpretation. As hematological traits are highly labile and responsive to changes in tissue O_2 delivery, decreasing variance with increasing proximity to elevational range limits might primarily reflect an increased role for compensatory changes in other cardiorespiratory traits (such as ventilation and pulmonary O_2 diffusion) that affect the hypoxic stimulus for activating erythropoiesis or modulating plasma volume. Assuming that trait values at least partly reflect additive genetic variation, lower CoV values might also result from neutral demographic processes that lead to genome-wide reductions in effective population size (N_e). Low N_e is expected near range limits under the central-marginal hypothesis and its variants (Hengeveld and Haeck 1982; Brown 1984; Hoffman and Blows 1994; Gaston 2003; Vucetich and Waite 2003) because of declining habitat suitability and its effects on census population size. While this pattern appears far from universal (Eckert et al. 2008), especially across elevational gradients (Freeman and Beehler 2018), it remains a plausible alternative explanation for our findings.

Acknowledging these possibilities, the scenario we consider most intriguing is that lower CoV values at range limits reflect an increased intensity of directional selection on the traits in question, implicating hematological traits in the maintenance of elevational distributions in Andean birds (figs. 3*b*, 4*a*). Specifically, reduced variation in [Hb] and Hct near elevational range limits might suggest an increasing fitness cost for phenotype-environment mismatches. By extension, this would suggest that species' distributions may be partially limited by a failure to adapt or acclimatize to P_{O_2} conditions beyond their current elevational niche. Likewise, reduced variation in [Hb] and Hct at high absolute elevation (fig. 4*b*) would be expected if the nonlinear decline in arterial O_2 saturation with increasing elevation leads to stronger selection on oxygen transport functions regardless of relative range position. One way this might occur is if elevational variation in hematological trait phenotypes is influenced by locally adaptive genetic variation along the elevational gradient (Schweizer et al. 2019; Lim et al. 2021). Higher CoV values at the elevational range center in our models could therefore reflect increased heterozygosity of causal loci that are subject to variable selection across elevation or a mosaic of alleles that are favored at upper and lower elevations, respectively.

We assumed that patterns among [Hb], Hct, and MCHC would be comparable across taxa and elevation at the spatial and taxonomic scales of our study. However, their inconsistency across models suggests that their varied roles in oxygen transport may influence correlations with elevational range characteristics. Recent work on ecophysiological rules in blood oxygen-carrying capacity supports this hypothesis. For example, while most hummingbird species respond to changes in P_{O_2} by adjusting cell number and cell size similarly, adjustments to cell volume are proportionally more important for those at high or low—but not moderate—elevations (Williamson et al. 2022).

The spatial pattern of variation in ecologically meaningful traits is a potentially rich source of information on range-limiting mechanisms. Although studies of range-limiting mechanisms remain rare outside a handful of well-studied taxa, a recent meta-analysis by Pennington et al. (2021) found that quantitative genetic variation declined from the geographic range center to range margins but increased toward niche limits. This finding is not consistent with our results if we assume that elevational ranges approximate species' realized niches. Teasing apart spurious patterns from causal mechanisms remains a formidable challenge as long as autocorrelation among geography, climate, and morphology run rampant. In systems that are unsuitable for experimental manipulation, careful scrutiny of patterns of trait variation can nonetheless be a powerful approach, particularly when those traits are closely linked with fitness and environmental variables.

Macrophysiology is inherently a Faustian bargain of accepting noisy, imperfect data in exchange for the ability to reveal general phenomena that might otherwise remain hidden. This study is no exception, and we wish to highlight several factors that complicate any interpretation of our results. First, we would be remiss to conclude an article on range limits without again emphasizing that they are multicausal phenomena: even if selection on hematological traits plays a major role in limiting elevational distributions, they are merely a handful of stars in the larger constellation of interrelated physiological and anatomical traits that contribute to organismal performance, a constellation itself positioned within the galaxy of other biotic and abiotic variables jointly constraining niche breadth. Second, variation in hematological trait values is itself influenced by numerous factors beyond elevation (Fair et al. 2007; Williamson and Witt 2021). These factors, as well as unavoidable measurement error, are likely responsible for low effect sizes for predictors in our models and correspondingly weak predictive power (table 1). Disentangling the genetic and environmental components of trait variance should be a research priority in future studies of niche breadth. Nonetheless, we find the presence of correlations between physiology and biogeography at relatively fine scales a heartening step in answering one of biology's most fundamental but challenging questions: why organisms live where they do.

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Statement of Authorship

E.B.L. and C.C.W. conceived the project, developed methods, and designed the study. J.L.W., E.B., E.J.B., P.M.B., S.G.D., L.M.F., C.R.G., A.B.J., M.R.J., J.N-Z., A.Q., C.J.S., D.S., J.T.C., K.V.-G., N.A.W., T.V., and C.C.W. collected data, with resources provided by C.C.W. and T.V. C.C.W. and E.B.L. obtained funding. E.B.L. conducted data validation, coded all analyses and visualizations, and drafted the manuscript. E.B.L. led the writing, with major contributions from C.C.W. and J.F.S. and minor contributions from J.L.W., P.M.B., E.J.B., C.J.S., and N.A.W.

Data and Code Availability

Specimen data are available from the Arctos database (https://arctosdb.org), associated with a forthcoming data article (Witt et al., in prep.). All data and code used in this study are available in the Dryad Digital Repository and Zenodo (https://doi.org/10.5061/dryad.1g1jwsv07; Linck et al. 2022).

APPENDIX

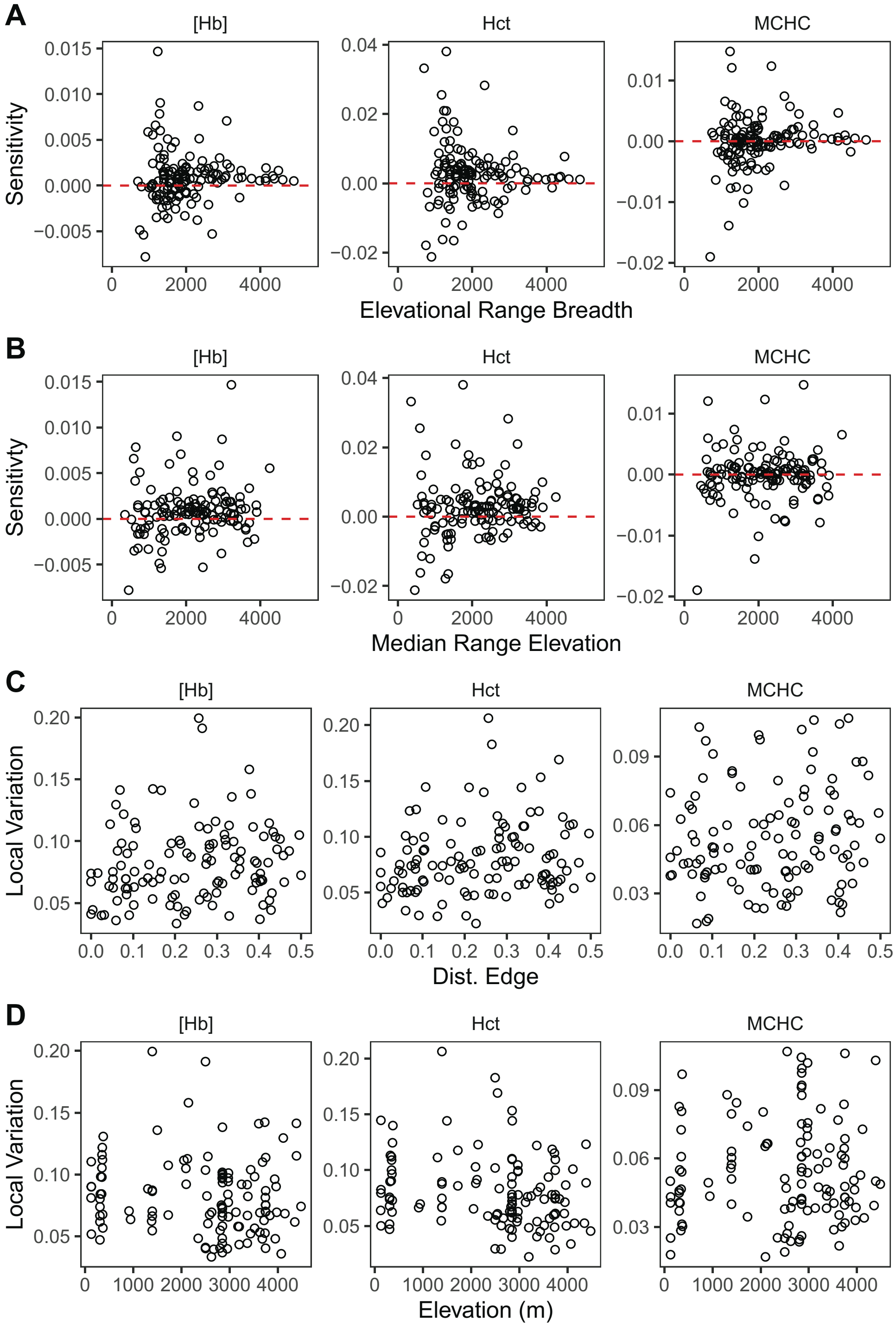

Figure A1: Data used in Bayesian models. The top two panels depict the relationship between the sensitivity of three hematological traits to elevation and elevational range breadth (*A*) or median range elevation (*B*) for 136 species. The bottom two panels depict the relationship between local variation in hematological trait values and distance to nearest elevational range limit (*C*) or mean elevation of samples (*D*).

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