Latitudinal but not elevational variation in blood glucose level is linked to life history across passerine birds

Oldřich Tomášek[1,2,*], Lukáš Bobek[1,3], Tereza Kauzálová[1], Ondřej Kauzál[1,4], Marie Adámková[1,3], Kryštof Horák[1], Sampath Anandan Kumar[1,3], Judith Pouadjeu Manialeu[5], Pavel Munclinger[2], Eric Djomo Nana[6], Télesphore Benoît Nguelefack[5], Ondřej Sedláček[4], Tomáš Albrecht[1,2]

[1]Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czechia
[2]Department of Zoology, Faculty of Science, Charles University, Prague, Czechia
[3]Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czechia
[4]Department of Ecology, Faculty of Science, Charles University, Prague, Czechia
[5]Laboratory of Animal Physiology and Phytopharmacology, Faculty of Science, University of Dschang, Dschang, Cameroon
[6]Agricultural Research Institute for Development (IRAD), Nkolbisson-Yaoundé, Cameroon

[*]**Author for correspondence:**
Oldřich Tomášek, Institute of Vertebrate Biology CAS, Studenec 122, 675 02 Studenec, tomasek@ivb.cz, tel. +420 560 590 604

E-mail addresses: tomasek@ivb.cz (OT), bobek.l@volny.cz (LB), kralova.tereza@mail.muni.cz (TK), o.kauzal@gmail.com (OK), ma.adamkova@gmail.com (MA), horakkrystof@seznam.cz (KH), sampath.anandan@mail.muni.cz (SAK), judithpouadjeu@yahoo.com (JPM), pavel.munclinger@natur.cuni.cz (PM), ericnana2000@yahoo.com (EDN), nguelefack@yahoo.fr (TBN), zbrd@email.cz (OS), albrecht@ivb.cz (TA)

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ABSTRACT

Macrophysiological research is vital to our understanding of mechanisms underpinning global life history variation and adaptation under diverse environments. Birds represent an important model taxon in this regard, yet our knowledge is limited to only a few physiological traits, mostly studied in temperate and Neotropical species. Here, we examined latitudinal and elevational variation in an emerging biomarker of physiological pace of life, blood glucose concentration, collected from 61 European temperate and 99 Afrotropical passerine species. Our data suggest that the slow physiological pace-of-life syndrome, indicated by lower baseline glucose level and stronger stress response, evolves convergently in lowland tropical birds across continents and is shaped by their low fecundity. In contrast, elevational variation in blood glucose levels implied a unique montane pace-of-life syndrome combining slow-paced life histories with fast-paced physiology. The observed patterns suggest an unequal importance of life history in shaping physiological adaptations associated with latitude and elevation.

INTRODUCTION

Species maximise their fitness by adapting their life histories and phenotypes to the environments they inhabit. Consequently, latitudinal and elevational gradients in life-history strategies emerged through evolution, shaped by variation in several key abiotic and biotic environmental factors along these gradients. Considering that species with similar life histories are expected to evolve suites of convergent physiological adaptations termed pace-of-life syndromes (POLS), corresponding large-scale geographical patterns of variation in POLS should also emerge (Ricklefs & Wikelski 2002). Research into such macrophysiological patterns is pressingly needed as their knowledge is essential to our understanding of the mechanisms underpinning global variation in demographic rates and life histories, species distribution and abundance, or physiological adaptation under diverse environmental conditions (Ricklefs & Wikelski 2002; Chown *et al.* 2004; Chown & Gaston 2008, 2016; Boyce *et al.* 2020; Tomášek *et al.* 2021).

In birds, lowland tropical habitats select for slow life histories as indicated by smaller clutch size (Moreau 1944; Martin *et al.* 2000; Jetz *et al.* 2008b), flatter growth rate curve (Martin 2015), higher annual survival rates (Martin *et al.* 2017; Muñoz *et al.* 2018), and longer lifespan (Scholer *et al.* 2018) compared to higher latitudes. Following the predictions of the POLS hypothesis (Ricklefs & Wikelski 2002), the slow physiological pace of life in tropical birds have been supported by low metabolic rates at both the cellular (Jimenez *et al.* 2014b) and organismal level (Wiersma *et al.* 2007b, a; Stager *et al.* 2016; Boyce *et al.* 2020), high resistance to oxidative stress (Jimenez *et al.* 2013), and low baseline level of stress hormone corticosterone (Hau *et al.* 2010). However, no clear latitudinal patterns are apparent in immune responses (Versteegh *et al.* 2012) and stress-induced corticosterone level (Hau *et al.* 2010). Moreover, the intensity of stress response shows positive (Bókony *et al.* 2009; Jessop *et al.* 2013), rather than expected negative (Vitousek *et al.* 2019), correlation with latitude. This issue is further complicated by the existence of long-distance migration, with studies reporting contrasting results regarding the effects of migratory tendency on life history (Böhning-Gaese *et al.* 2000; Soriano-Redondo *et al.* 2020) or physiological pace of life (Wiersma *et al.* 2007b; Jetz *et al.* 2008a; Tomasek *et al.* 2019). Besides, any general conclusion about the slow physiological pace of life in tropical birds is prevented by the fact that tropical species from outside the Neotropics, particularly those from the Afrotropical region, have been heavily underrepresented in the macrophysiological studies published to date (Jessop *et al.* 2013; Jimenez *et al.* 2014a; Bushuev *et al.* 2018; Boyce *et al.* 2020).

The effect of elevation on avian pace of life is much less clear both from theoretical and empirical perspectives (Boyle *et al.* 2015; Hille & Cooper 2015; Laiolo & Obeso 2017). A recent meta-analysis indicated reduced fecundity in higher elevations, but no clear effect on adult survival or other life-history traits (Boyle *et al.* 2015). However, the extreme scarcity of relevant studies on elevational gradients in the tropics prevents any general conclusion in this regard (Boyle *et al.* 2015). To further complicate matters, the few available studies suggest that, in the tropics, the direction of the relationship between clutch size and elevation may vary among taxonomic orders (Boyce *et al.* 2015; Balasubramaniam & Rotenberry 2016).

Comparative studies of elevational variation in physiological pace of life are also rare. Theory and some evidence suggest that montane species evolve elevated metabolic rates (McNab 2009; Hille & Cooper 2015) due to higher metabolic demands associated with cold temperatures and low partial pressure of atmospheric oxygen in high elevations (Cheviron *et al.* 2012). However, elevation was not associated with basal metabolic rate (BMR) in neotropical birds (Londoño *et al.* 2015) and New Guinean passerines (McNab 2013).

To advance the understanding of large-scale geographical variation in physiological pace of life, we here analysed latitudinal and elevational patterns of variation in baseline and stress-induced blood glucose levels across 160 passerine species sampled in temperate Europe and tropical Africa. Blood glucose is a promising biomarker of physiological pace of life owing to its major role in energy metabolism, senescence-promoting effects (Holmes & Austad 1995; Braun & Sweazea 2008; Polakof *et al.* 2011, 2012), and relative ease of measurement in the field (Tomasek *et al.* 2019). In birds, baseline blood glucose level correlates negatively with body mass and positively with fecundity, suggesting that it is a POLS-associated trait underpinning life-history evolution (Tomasek *et al.* 2019). Hence, we predicted low baseline level in lowland tropical birds given their slow pace of life and low thermogenic demands compared to their temperate counterparts. Based on our previous observation (Tomasek *et al.* 2019), we further predicted lower baseline blood glucose level in migratory species.

During the stress response, stress hormones mediate an increase in blood glucose concentration to mobilise energy and promote survival (Viblanc *et al.* 2018). Therefore, lowland tropical species may have stronger blood glucose stress response, underpinning their higher survival rates. Alternatively, a weaker stress response in tropical birds was suggested by previous studies of latitudinal variation in corticosterone (Bókony *et al.* 2009; Jessop *et al.* 2013). Elevational variation in blood glucose may be determined by the interplay between opposing effects of life history and environmental demands. In montane habitats, higher thermogenic demands and lower partial concentration of oxygen may select for higher glucose levels (Schippers *et al.* 2012), whereas lower glucose levels may be selected for if montane passerines have reduced fecundity.

MATERIAL AND METHODS

Study species and field sites

We mist-netted passerine birds in both the temperate and tropical sites from 2013–2018. Sampling of the temperate-zone-breeding species took place at multiple sites in Czechia (48°41'–50°16'N, 13°56'–17°10'E; elevation 180–730 m) from March to October. Tropical species were sampled at the two areas in the Cameroon volcanic line in the Gulf of Guinea, west-central Africa: (i) an active volcano Mount Cameroon (4°07'N 9°04'E) and its surroundings, located on the coast of the Bight of Biafra (elevation 10–2280 m); and (ii) around the town Babanki in the Bamenda Highlands (6°05'N 10°17'E; 1960–2100 m). We conducted the fieldwork in both the dry season (spanning November–March; sampling in November, December, and February) and the rainy season (April–November; sampling in August, and September). To cover both taxonomically and ecologically broad spectrum of passerine species, we mist-netted in various habitats, including streams, reed beds, shrublands, primary and secondary lowland forests, montane forests, forest plantations, agricultural land, gardens, parks, and urban areas.

Field data

To measure baseline blood glucose level (G_0), we collected blood (max. ~0.5% of body mass) from the jugular vein of adult birds within four minutes (median time 109 s, 5th–95th percentile range 62–180 s) of hitting the mist net. There was no significant stress-induced increase in blood glucose level within this time period. To determine both the intensity and dynamics of blood glucose stress response, we measured glucose in the second (a drop of blood from the brachial vein) and third blood sample (jugular vein; max. ~0.5% of body mass) collected 15 min (G_{15}; 5th–95th percentile range 14–18 min) and 30 min (G_{30}; 5th–95th percentile range 29–33 min) from capture, respectively. Upon blood sampling, we quantified glucose concentrations using FreeStyle Freedom Lite portable glucose metres (Abbott Diabetes Care, Alameda, USA; linear range: 1.1–27.8 mmol/L), previously shown to be reliable devices (Breuner *et al.* 2013; Tomasek *et al.* 2019). In all but 34 individuals with a single measurement, we performed the measurements in duplicate using two independent glucose metres and averaged them subsequently. When the difference between the duplicate measurements exceeded 1.0 mmol/L, we repeated the measurements. The resulting measurement repeatability of G_0 was 0.968 (95% CI: 0.965–0.971; N = 1671 individuals).

We stored a drop of blood in ethanol for molecular analyses. In males, we also visually checked cloacal protuberance and attempted to collect sperm samples by gentle cloacal massage to aid both sex and breeding season determination (Albrecht *et al.* 2013). Before releasing the birds, we marked them with unique metal rings (Czech Ringing Centre, Natural History Museum, Prague; SAFRING, Cape Town) and weighed them to the nearest 0.1 g.

Molecular sex determination

We extracted DNA from blood samples preserved in 96% ethanol using the DNeasy Blood & Tissue Kit (Qiagen, Germany). To identify sex, we PCR-amplified two genes: CHD1 (Griffiths *et al.* 1998); and ATP5A1 (Bantock *et al.* 2008); and examined the amplicons using agarose gel electrophoresis, followed by capillary electrophoresis in unclear cases (Synek *et al.* 2016).

Literature and citizen science data

We compiled data on species-specific breeding seasons from literature (Šťastný & Hudec 2011; del Hoyo *et al.* 2019) and supplemented them with our data on sperm production in tropical birds. To determine elevational distributions of species populations living around the capture latitudes, we extracted species occurrence data from the citizen science project eBird (Sullivan *et al.* 2009; Strimas-Mackey *et al.* 2020) and selected observations from areas delimited by ±3 latitudinal and

±20 longitudinal degrees around approximate centres of our field areas (49°N 15°E and 5°N 10°E for temperate and tropical species, respectively). We assigned elevation to the eBird observations using their GPS coordinates and elevation data from the GeoNames database (www.geonames.org; retrieved September 21, 2020; Chamberlain *et al.* 2020). To eliminate a potential bias due to species misidentification in extreme elevations, we calculated the centre of species elevational range (hereafter as species-specific elevation) as the midpoint between the first (lower range boundary) and 99th (upper range boundary) elevational percentile within each species. To control for potential effects of diet, we used diet categories *herbivory/granivory*, *insectivory, nectarivory/*frugivory, and *omnivory* from Wilman *et al.* (2014). We also determined migration distance of temperate species to control for its association with baseline blood glucose in temperate songbirds (Tomasek *et al.* 2019). To this end, we obtained the data on wintering areas of bird populations breeding in Czechia from del Hoyo et al. (2019) and Cepák *et al.* (2008) and measured the distances between the geographical centre of Czechia (49°44′38″N 15°20′19″E) and the approximate centres of species wintering areas (zero distance assigned to sedentary species) using ArcGIS (Esri, Redlands, USA) software (e.g. Kumar *et al.* 2021). To control for the effect of rainfall (Vaughn *et al.* 2020), we prepared a binary variable with the *low* level including the observations from the temperate zone and the tropical dry season (average monthly precipitation below 92 mm), and the *high* level including the observations from the tropical rainy season (average monthly precipitation over 232 mm; climateknowledgeportal.worldbank.org; retrieved July 7, 2021). Species clutch size was obtained from Myhrvold et al. (2015). To control for phylogenetic inertia, we used the most complete molecular phylogeny of extant bird species to date (BirdTree.org; Hackett *et al.* 2008; Jetz *et al.* 2012). We generated a set of 1,000 trees and summarised them into a single consensual tree using TreeAnnotator implemented in BEAST 2.3.0 software (Bouckaert *et al.* 2014).

Statistical analysis

Using the baseline (G_0) and stress (G_{15}, G_{30}) blood glucose levels, we calculated the intensity of blood glucose stress response as the concentration change between G_0 and G_{15} ($G_{0\text{-}15}$), G_{30} ($G_{0\text{-}30}$), or G_{max} (the higher value of both stress time points; $G_{0\text{-}max}$). We also calculated the concentration change between G_{15} and G_{30} ($G_{15\text{-}30}$), which reflects the speed of the blood glucose stress response with negative values indicating that the peak concentration is closer to G_{15}. These measures represented our dependent variables.

We first analysed the data with Bayesian phylogenetic mixed models based on the Hamiltonian Monte Carlo algorithm using the brms package (Bürkner 2017) in R 4.0.2 (R Core Team 2021). This approach allowed us to fit individual measurements of blood glucose measures as dependent variables while controlling for phylogeny. We fitted breeding zone (*temperate*/*tropical*) and species-specific elevation (km) as the focal fixed effects. We included capture elevation as a covariate to differentiate the effect of within-species elevational plasticity from macroevolutionary change (cf. van de Pol & Wright 2009). At the species level, the models also included mean body mass, migration distance (thousands of km) and diet. The models further controlled for individual-level covariates including sex, season (*breeding*/*non-breeding*), precipitation (*low*/*high*), time of day (time from sunrise to the capture in hours), and sampling latency (time from capture to blood sampling in minutes). We also fitted interactions of breeding zone with species body mass, species-specific elevation, capture elevation, breeding season, and sex to allow for possible differences between temperate and tropical species. In contrast to some previous studies (Kaliński *et al.* 2014, 2015; McGraw *et al.* 2020), there was no effect of individual-level body mass (see also Tomasek *et al.* 2019); hence, we excluded this predictor from our analyses, which allowed us to include individuals lacking body mass measurement. We coded categorical factors as dummy variables to enable their centring and obtaining mean effects of other predictors across factor levels (Schielzeth 2010). To estimate mean differences in blood glucose measures between lowland temperate and tropical species, we centred elevational variables at their approximate means for lowland species (defined as species with lower range boundary ≤500 m and upper range boundary ≤2000 m, i.e., species occupying considerable part of the lowland forest zone and not extending to the montane forest zone; Bussmann 2006). Specifically, species-specific elevation was centred at 700 m and capture elevation at 450 m to represent an average lowland species in this latitudinal contrast analysis. Nonetheless, we also show non-centred model coefficients suitable for predictions in Tables S3–S4. Phylogeny and species identity were included in all the models as random effects to control for phylogenetic non-independence and non-independence due to factors unrelated to phylogeny, respectively (Garamszegi 2014; Bürkner 2018). We used default priors defined in the brms package and ran the models in 25 chains, each with 20,000 iterations, warm-up of 8000, and thinning of 10. Potential scale reduction factor was ≈1 in all cases indicating good model convergence (Gelman & Rubin 1992). We present results as posterior means with quantile-based 95% credible intervals (CrI_{95}) and consider the support for an effect to be significant when CrI_{95} does not contain zero.

Subsequently, we examined whether the effects of latitude and elevation on G_0 are direct or mediated by clutch size using phylogenetic path analysis implemented in the phylopath package (Bijl 2018), which combines phylogenetic generalised least-square models (PGLS) with *d*-separation method (Hardenberg & Gonzalez-Voyer 2013). Using species-specific mean values of G_0, we designed eight alternative path models that differed in whether latitude or elevation influenced G_0 directly, through clutch size, or by a combination of both effects. The *d*-separation method combines *P* values of underlying PGLS models to calculate Fisher's *C* statistic for each path model. We further calculated *C* statistic information criterion with a correction for small sample sizes (CICc) and CICc weight to rank the path models and assess their probability. To estimate path model coefficients, we used model averaging based on all plausible models ($P > 0.05$). Finally, we assessed the relative support for the direct and indirect (i.e., independent of and mediated by clutch size, respectively) effects of latitude and elevation on G_0 by comparing sums of CICc weights of all the plausible models containing these paths of causation.

RESULTS

We measured blood glucose levels in 1705 individuals of 160 passerine species (950/99 tropical, 755/61 temperate; Fig. 1), representing 39 families. Blood glucose levels at all three time points, as well as the strength and speed of the stress response showed significant repeatability at the species level (Table 1). We used G_{0-30} as the measure of blood glucose stress response in our models because of its higher species-level repeatability compared to both G_{0-15} and G_{0-max}, and because the species-level repeatability of the stress response speed (G_{15-30}) was very low (Table 1). For this reason, and given almost complete lack of association with the species-level predictors (Table S1), we do not discuss $G_{15\text{-}30}$ further.

Baseline blood glucose level

G_0 declined with body mass in temperate (*b* = −0.89 [−1.54, −0.24]), but not in tropical birds (*b* = −0.13 [−0.74, 0.47]), although the difference in slopes between zones was only weakly supported with CrI_{95} containing zero (Fig. 2; Table 2). The resultant main effect of body mass across zones was also only weak.

In accordance with the slow pace of life in the tropics, lowland tropical species had lower G_0 compared to their lowland temperate counterparts (Fig. 2). The mean difference was around 2.1 mmol/L (temperate: 12.6 [11.5, 13.7]; tropical: 10.5 [9.4, 11.6]; Table 2) as estimated at the approximate mean values of species-specific elevation (700 m) and capture elevation (450 m) for lowland birds. Latitude influenced G_0 both directly and through clutch size, with both effects included in the best supported path analysis model and obtaining similar sums of CICc weights across all the plausible models (Fig. 3 and 4). Model-averaged standardised effect sizes of the latitudinal effects related and unrelated to clutch size were −0.41 and −0.52, respectively.

Migration distance was only weakly negatively associated with G_0 in the general model, with CrI_{95} marginally containing zero (Table 2). Nevertheless, this association was significantly supported when sedentary tropical species were excluded from the model (*b* = −0.16 [−0.30, −0.03]).

Across zones, G_0 was higher in montane species as evidenced by the positive main effect of species-specific elevation and no difference in slopes between the tropical and temperate zone (Fig. 2; Table 2). Within zones, this effect was more strongly supported in tropical (*b* = 1.12 [0.62, 1.61]) than in temperate species (*b* = 0.63 [−0.84, 2.08]), possibly due to smaller range of elevations covered in the latter. Higher G_0 in montane species was not mediated by clutch size, with sums of CICc weights strongly supporting a direct effect of species-specific elevation (Fig. 4).

To control for the within-species effect of elevation, we included capture elevation as an individual-level fixed effect in the model. Nonetheless, neither the main effect nor its interaction with breeding zone were supported by our data (Table 2).

Controlling for potentially important covariates revealed seasonal variation in G_0 with elevated concentrations during non-breeding season in both tropical and temperate species and during the tropical rainy season. G_0 was further higher in females (Table 2), although a significant interaction between sex and zone suggested more pronounced intersexual difference in temperate ($b = -0.56$ [−0.84, −0.29]) than in tropical species ($b = -0.20$ [−0.44, 0.04]). Diet was not associated with G_0 (Fig. S1)

Blood glucose stress response

Both $G_{0\text{-}30}$ and G_{30} declined with body mass with no difference between zones (Fig. 2; Table 2). Lowland tropical species showed more intense $G_{0\text{-}30}$ stress response compared to their lowland temperate counterparts (temperate: 1.83 [0.59, 3.06]; tropical: 3.38 [2.09, 4.53]), which, combined with their lower G_0, resulted in no association of G_{30} stress level with latitude (Fig. 2; Table 2). Migration distance had no effect on any of the stress response measures. The tendency towards positive association with $G_{0\text{-}30}$ was not significant even when tropical birds were excluded from the model ($b = 0.13$ [−0.09, 0.34]).

Both $G_{0\text{-}30}$ and G_{30} increased with species-specific elevation indicating more intense stress response and higher stress-induced blood glucose level in montane species (Fig. 2; Table 2). Nonetheless, a significant interaction with breeding zone in the case of $G_{0\text{-}30}$ suggested that the increase in stress response intensity with species-specific elevation is much steeper in the temperate zone (3.55 [1.33, 5.79]) than in the tropics (0.48 [−0.30, 1.27]; Fig 2d). The increase in G_{30} with species-specific elevation also tended to be steeper in temperate ($b = 4.07$ [1.55, 6.59]) than in tropical species ($b = 1.67$ [0.80, 2.54]; Fig. 2), although the CrI_{95} of the interaction term marginally contained zero (Table 2). Neither $G_{0\text{-}30}$ nor G_{30} was associated with capture elevation at the within-species level.

$G_{0\text{-}30}$ stress response was further associated with diet (Fig. S1) and precipitation (Table 2). G_{30} stress concentration was higher in females, whereas a similar tendency in $G_{0\text{-}30}$ was not significantly supported (Table 2). The $G_{0\text{-}30}$ model did not change qualitatively when G_0 was added as a covariate (Table S2).

DISCUSSION

Blood glucose has so far been neglected in comparative and macrophysiological research, despite its essential role in organismal energetics and senescence-promoting effects. We found recently that G_0 co-evolves with species' life history, thereby constituting a component of pace-of-life syndromes (Tomasek *et al.* 2019). Building on this finding and using data collected from 160 temperate and tropical passerine species, we show here that baseline and stress-induced blood glucose levels vary predictably with latitude and elevation, suggesting that these functional traits play an important role in life-history evolution and adaptation to varying environmental conditions on large geographical scales.

In lowland species, our data showed lower G_0 in the tropics compared to the temperate zone, supporting the conclusion of the slow physiological pace of life in tropical lowlands. The European and Afrotropical origin of our data strengthens the generality of such a conclusion, which until now was mostly based on macrophysiological data from the Americas (Jimenez *et al.* 2014a) and more recently from Southeast Asia (Bushuev *et al.* 2018; Boyce *et al.* 2020). The path analysis indicated that the effect of latitude on G_0 is both related and unrelated to fecundity. These results suggest that the slow physiological pace of life of tropical species is driven by a combination of their slow life histories and other unmeasured effects, possibly involving reduced energy demands of thermogenesis.

G_0 was further explained by species-specific elevation, with montane species exhibiting higher G_0. A potential confounding effect of within-species plasticity (Sweazea *et al.* 2020) was controlled for by fitting capture elevation along with species-specific elevation in the model. Hence, the positive coefficient of species-specific elevation indicates that montane species evolved higher G_0, supporting the hypothesis that high elevations select for fast physiological pace of life (Hille & Cooper 2015).

Our path analysis showed that elevated G_0 in montane species was not due to higher fecundity as clutch size did not change with elevation. Moreover, available studies usually report decrease in fecundity with elevation in both tropical (Kleindorfer 2007; Boyce *et al.* 2015) and temperate passerine species (Badyaev 1997; Boyle *et al.* 2015). Therefore, high fecundity is probably not the main selection force behind evolution of high G_0 in montane species. Our results thus support the recent hypothesis that montane bird species exhibit a unique pace of life combining slow-paced life-histories with fast-paced physiology (Hille & Cooper 2015).

The direct effects of latitude and elevation on G_0 supported by our path analysis indicate that high G_0 in both temperate and montane species may have evolved, at least in part, due to evolutionary mechanisms unrelated to species fecundity. Such mechanisms may include the fuel shift towards proportionally higher use of carbohydrates to save oxygen under hypoxic conditions in higher elevations (Schippers *et al.* 2012) or more intense thermogenesis in higher latitudes and elevations (Wiersma *et al.* 2007a; Hille & Cooper 2015). Although the few available avian studies indicated that the contribution of glucose to elevational hypoxia adaptation or variation in thermogenic rate is rather minor within species (Marsh & Dawson 1989a, b; Qu *et al.* 2013; but see Sweazea *et al.* 2020), our comparative data suggest its potential importance at the macroevolutionary level.

Following our prediction, blood glucose stress response measured as G_{0-30} was stronger in tropical species and this effect was independent of their lower G_0 (Table S2). Owing to the stronger stress response, tropical species reached G_{30} stress level similar to their temperate counterparts despite having lower G_0. Given the higher survival rates in tropical birds (Martin *et al.* 2017), their stronger blood glucose stress response supports the importance of this functional trait in promoting survival. Nevertheless, our results challenge the role of stress hormones in mediating stronger blood glucose stress response in tropical species because no corresponding latitudinal decrease in corticosterone stress response has been observed by available studies (Bókony *et al.* 2009; Hau *et al.* 2010; Jessop *et al.* 2013).

G_{0-30} further increased with species-specific elevation indicating stronger stress response in montane birds. Since G_0 level also increased with species-specific elevation, the resulting elevational increase in G_{30} level was relatively steep. This implies there is no optimal environment-independent G_{30} level, which might have been inferred from the absence of its latitudinal variation. The stronger stress response in montane birds might suggest their higher survival rates; however, adult survival was not associated with elevation in a recent meta-analysis (Boyle *et al.* 2015). This invalidates survival rate as the main driver of the observed increase in stress response with elevation, especially when considering that the meta-analysis was mostly based on temperate species, i.e., the ones showing the steeper elevational increase in G_{0-30} in our study. More probably, stronger mobilisation of blood glucose and its higher stress-induced concentration may suggest higher energy demands of stress response (Sokolova 2013) or fuel shift towards higher use of carbohydrates in hypoxic montane environments (Schippers *et al.* 2012).

Supporting our previous results from a smaller subset of temperate songbirds (Tomasek *et al.* 2019), G_0 decreased with migration distance. Considering that long-distance flight is primarily fuelled by fatty acids, it seems plausible that migratory species may have evolved energy metabolism relying less on glucose and more on fatty acids. Alternatively, migrants may have lower metabolic rates; however, this is not supported by most studies (Jetz *et al.* 2008a; McNab 2009; Gavrilov 2014). Migration was not associated with any other blood glucose measure, suggesting that migratory behaviour does not impose a strong directional selection on stress response.

Our data partly supported the negative scaling of G_0 with body mass reported previously (Braun & Sweazea 2008; Tomasek *et al.* 2019); however, it was only significant in temperate but not in tropical species. Whether such a difference between zones results from differences in allometric scaling of daily energy expenditure, shifts in the use of energy substrates (glucose vs. fatty acids), or other causes remains to be determined. $G_{0\text{-}30}$ showed negative association with G_0 but this may, at least partly, represent regression to the mean (Verhulst *et al.* 2013). Interestingly, both $G_{0\text{-}30}$ and G_{30} declined with body mass, indicating weaker stress response in larger species. In birds, similar relationship has also been observed in the case of stress-induced corticosterone level in at least one study (Hau *et al.* 2010), but not in others (Bókony *et al.* 2009; Jessop *et al.* 2013; Vitousek *et al.* 2019). Such a negative relationship with body mass challenges the prediction based on life history theory, namely that stronger stress response evolves in long-lived large species (Vitousek *et al.* 2019).

Our models further controlled for several potentially relevant covariates, including diet. Although previous studies on malachite sunbird (*Nectarinia famosa*; Downs *et al.* 2010) and two species of hummingbirds (Beuchat & Chong 1998) suggested that nectarivores have relatively high blood glucose level, G_0 showed no association with diet in our study. In contrast, diet predicted $G_{0\text{-}30}$, with herbivores/granivores mounting stronger $G_{0\text{-}30}$ stress response compared to insectivores and nectarivores/frugivores and omnivores falling in between. To our knowledge, our study is the first one to link stress response intensity and diet at the species level. A possible clue for why herbivores/granivores might have a stronger stress response may come from a recent study revealing higher baseline levels of stress hormones in herbivorous vertebrates (Edwards *et al.* 2020; unfortunately, stress-induced hormone levels were not tested). The authors argued that herbivores need elevated levels of stress hormones because their tissues are more resistant to hormones due to regular consumption of phytochemicals with allelopathic or endocrine disrupting effects. Such a possibility represents an interesting topic for future research.

Blood glucose further showed seasonal variation. First, G_0 was somewhat surprisingly higher outside the breeding season, possibly due to elevated energy demands associated with moulting (Remage-Healey & Romero 2000). Second, individuals captured during the tropical rainy season, characterised by intense precipitation, showed elevated G_0, reduced G_{0-30} and no difference in G_{30}. This pattern suggests that baseline glucose is the primary trait affected by precipitation. The elevated G_0 during periods of heavy rainfall may result from the prolonged stress response, increased activity during short rainless parts of the day, or hyperphagia induced by increased unpredictability of food availability (Fokidis *et al.* 2012; Vaughn *et al.* 2020).

Blood glucose level emerges as an important functional trait and biomarker in ecology, owing to its associations with energy metabolism (Scanes & Braun 2013), individual fitness (Kaliński *et al.* 2014, 2015; Récapet *et al.* 2016; Montoya *et al.* 2018; McGraw *et al.* 2020), and species life history (Tomasek *et al.* 2019). Here, we demonstrate that blood glucose is also a promising biomarker of physiological pace of life on large geographical scales. Our data revealed patterns of its latitudinal and elevational variation supporting the conclusion that physiological pace of life increases with both latitude (Jimenez *et al.* 2014a) and elevation (Hille & Cooper 2015) in passerine birds. The inclusion of species from the Afrotropical region, which has largely been overlooked in previous macrophysiological studies, indicates that slow physiological pace of life in the tropics is a universal macroevolutionary pattern observable across continents. Our data further indicate that latitudinal but not elevational variation is associated with species life history, supporting the hypothesis that montane species evolved a unique pace of life combining slow-paced life histories with fast-paced physiology (Hille & Cooper 2015). The patterns observed in our study also identified interesting topics for future research such as the potential importance of glucose for avian thermogenic capacity and hypoxia adaptation at the macroevolutionary scale. Overall, our results show that macrophysiological research into baseline and stress-induced blood glucose levels has a great potential to advance the much needed (Chown & Gaston 2016; Boyce *et al.* 2020; Tomášek *et al.* 2021) understanding of mechanisms underpinning both life history variation and adaptation to diverse environmental conditions at large geographical scales.

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TABLES AND FIGURES

Table 1

Table 1 Variability, species-level repeatability and sample sizes of baseline and stress-induced blood glucose level parameters (mmol/L) in songbirds.

	G_0	G_{15}	G_{30}	G_{max}	$G_{0\text{-}15}$	$G_{0\text{-}30}$	$G_{0\text{-}max}$	$G_{15\text{-}30}$
Mean	12.0	15.3	15.2	16.3	3.3	3.3	4.4	0.5
SD	1.7	2.6	2.8	2.8	2.1	2.6	2.4	1.4
Min	7.9	9.6	9.3	10.4	−0.9	−4.2	−0.9	−3.3
Max	17.1	24.6	23.9	24.6	12.5	10.7	12.7	5.7
Repeatability	0.35	0.24	0.23	0.26	0.19	0.23	0.21	0.10
Rpt $LCrI_{95}$	0.28	0.18	0.17	0.19	0.13	0.17	0.15	0.06
Rpt $UCrI_{95}$	0.42	0.32	0.30	0.33	0.25	0.29	0.29	0.15
N_{ind}	1705	1221	1553	1178	1221	1553	1178	1178
N_{spec}	160	154	160	154	154	160	154	154

Table 2

Table 2 Models of baseline blood glucose level, the intensity of blood glucose stress response, and stress-induced blood glucose level (mmol/L). To estimate differences in blood glucose measures between typical lowland temperate and lowland tropical species, elevational variables were centred at their approximate means for lowland species. Specifically, species-specific elevation was centred at 700 m and capture elevation at 450 m. Body mass, breeding zone, sex and diet (factors coded as binary dummy variables) were centred at their means to estimate mean effects across sexes and diet types and to ensure biologically meaningful interpretation of main effects included in interactions. Breeding season was centred at 0 (breeding), meaning that the differences between breeding zones were estimated for the breeding season. The estimated latitudinal differences for non-breeding season were qualitatively similar (G_0: $b = -2.00$ [−3.01, −0.99]; $G_{0\text{-}30}$: $b = 2.06$ [0.36, 3.78]; G_{30}: $b = -0.12$ [−2.00, 1.79]). Values with CrI_{95} not containing zero are highlighted in bold and regarded as significant support for an effect.

	Baseline level (G_0)			Stress response intensity (G_{0-30})			Stress-induced level (G_{30})		
Predictor	b	LCrI	UCrI	b	LCrI	UCrI	b	LCrI	UCrI
ln-species body mass	−0.47	−0.94	0.01	−0.76	−1.41	−0.11	−1.23	−1.99	−0.44
breeding zone (tropical)	−2.13	−2.93	−1.33	1.54	0.37	2.70	−0.56	−1.88	0.74
species-specific elevation (km)	0.90	0.20	1.61	1.79	0.75	2.84	2.69	1.53	3.88
capture elevation (km)	−0.39	−0.96	0.17	−0.45	−1.51	0.60	−0.78	−1.94	0.38
breeding season (non-breeding)	0.44	0.10	0.77	0.09	−0.55	0.74	0.32	−0.38	1.02
precipitation (high)	0.76	0.39	1.14	−1.07	−1.78	−0.37	−0.44	−1.20	0.34
migration distance (1000 km)	−0.13	−0.28	0.02	0.16	−0.07	0.38	0.03	−0.22	0.29
diet									
insectivory	0.19	−0.82	1.22	−1.95	−3.29	−0.65	−1.50	−3.12	0.09
nectarivory & frugivory	0.52	−0.83	1.88	−2.27	−4.19	−0.44	−1.69	−3.93	0.46
omnivory	0.09	−0.94	1.13	−1.27	−2.68	0.08	−0.90	−2.59	0.72
sex (male)	−0.36	−0.54	−0.18	−0.28	−0.63	0.07	−0.64	−1.04	−0.26
time from sunrise (h)	−0.04	−0.08	0.002	0.11	0.03	0.19	0.08	−0.01	0.16
time from sunrise^2	0.02	0.01	0.03	0.017	0	0.035	0.03	0.01	0.05
blood sampling latency (min)	−0.14	−0.31	0.02	−0.11	−0.43	0.20	−0.17	−0.52	0.18
blood sampling latency^2	0.11	−0.07	0.29	0	−0.35	0.36	0.05	−0.34	0.43
zone×ln-species body mass	0.76	−0.06	1.59	0.16	−1.04	1.37	0.59	−0.77	1.96
zone×species-specific elevation	0.49	−1.05	2.04	−3.07	−5.46	−0.70	−2.40	−5.05	0.25
zone×capture elevation	0.16	−1.10	1.42	0.51	−1.94	2.95	0.67	−2.05	3.37
zone×breeding season	0.12	−0.56	0.81	0.52	−0.84	1.87	0.44	−1.06	1.94
zone×sex	0.37	0.01	0.73	−0.51	−1.22	0.20	−0.11	−0.89	0.67

Figure 1

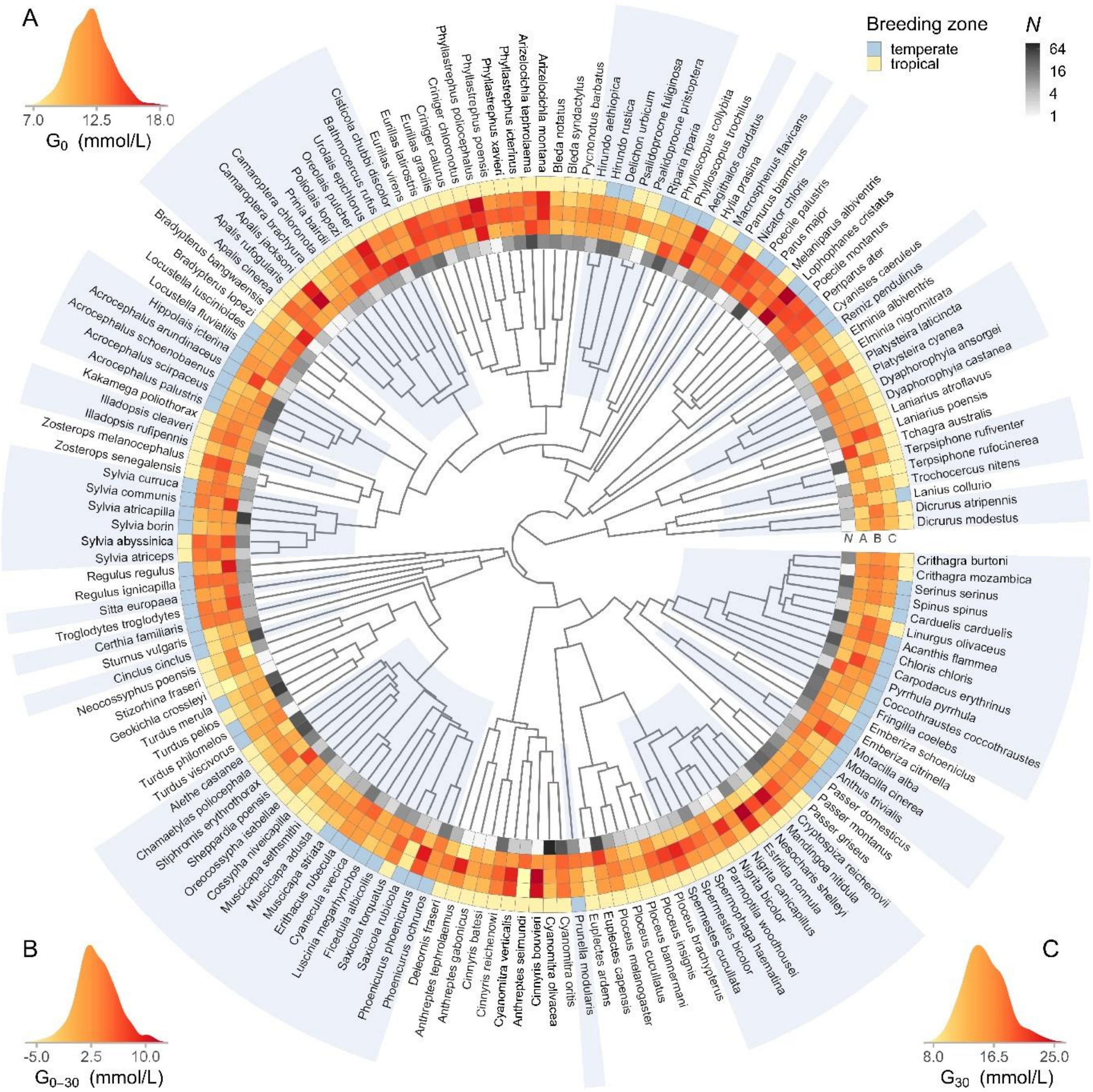


Figure 1 Distribution of species mean values of baseline blood glucose level (A), the intensity of blood glucose stress response (B), and stress-induced blood glucose level (C) across passerines. The grey-scale heat map represents sample size per species (*N*). Taxonomic families are separated by alternate background shading.

Figure 2

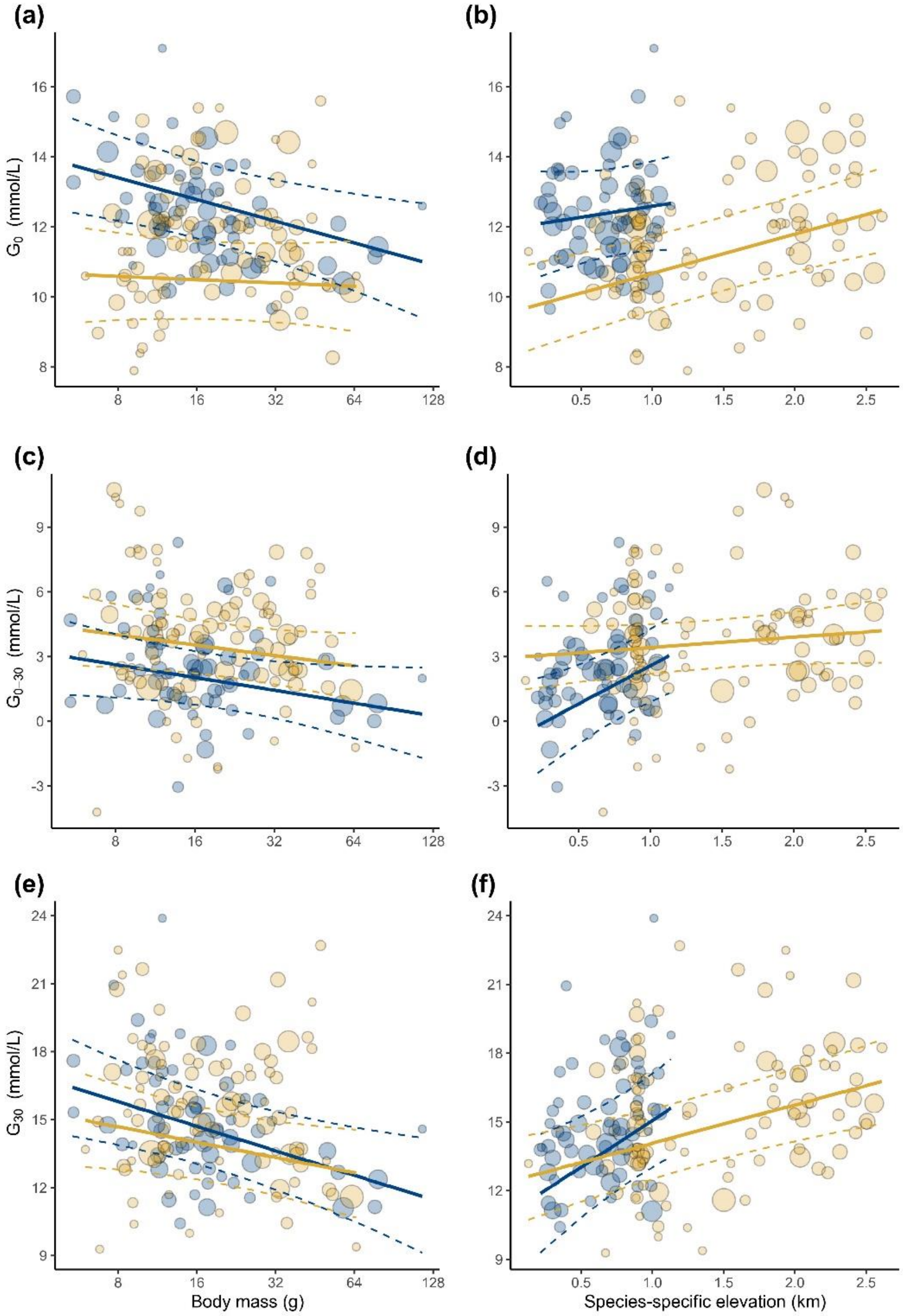


Figure 2 Allometric scaling (left) and elevational variation (right) of baseline blood glucose level (a, b), the intensity of blood glucose stress response (c, d), and stress-induced blood glucose level (e, f) in temperate (blue) and tropical (yellow) passerines. Predicted values and their CrI_{95} were obtained from the presented Bayesian phylogenetic models and are controlled for all the included covariates. Body mass is plotted on the natural logarithmic scale. The points represent species mean values calculated from the raw data and the point area is proportional to species sample size.

Figure 3

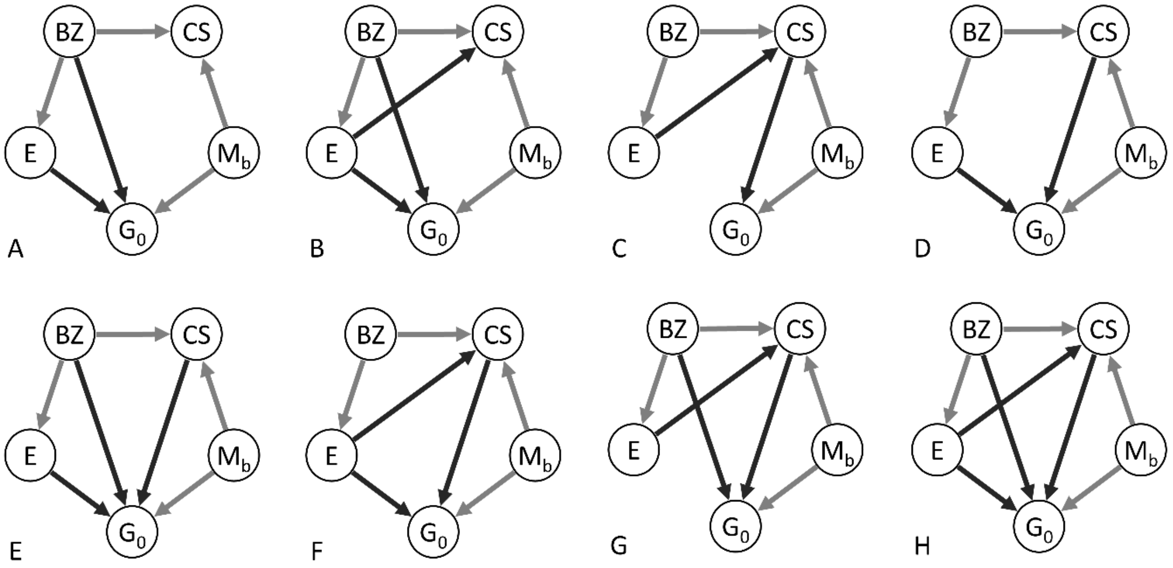
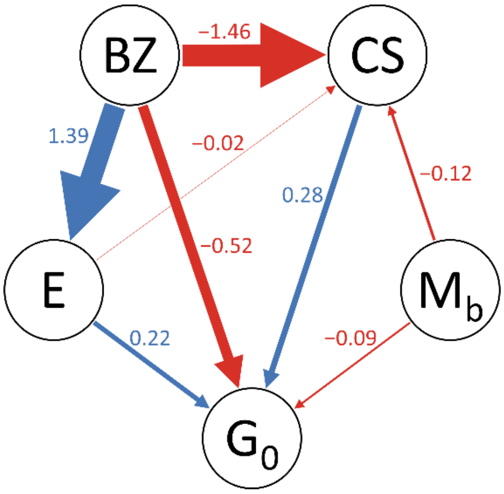


Figure 3 Directed acyclic graphs depicting our candidate path models. The path analysis tested whether the effects of latitude (breeding zone; BZ) and species-specific elevation (E) on baseline blood glucose are direct and/or mediated by fecundity (clutch size; CS), while controlling for the effects of body mass (M_b). The grey arrows are established relationships that were present in all the models. The black arrows represent relationships that were tested in phylogenetic path analysis and thus varied among the models. The link between breeding zone and elevation was included to control for the differences in species-specific elevation between tropical and temperate species in our data set.

Figure 4



Model	*k*	*C*	*P*	CIC_c	ΔCIC_c	Weight
E	3	3.53	0.74	29.8	0	0.344
D	4	7.09	0.53	31.0	1.20	0.189
H	2	2.73	0.60	31.3	1.59	0.155
A	4	7.62	0.47	31.5	1.73	0.144
F	3	6.28	0.39	32.5	2.75	0.087
B	3	6.82	0.34	33.0	3.29	0.066
C	4	12.93	0.11	36.8	7.04	0.010
G	3	12.34	0.055	38.6	8.81	0.004

Effect of BZ on G_0	Σ weight
Mediated by CS	0.789
Direct	0.714

Effect of E on G_0	Σ weight
Mediated by CS	0.257
Direct	0.986

Figure 4 The results of phylogenetic path analysis testing the causal relationships between latitude (breeding zone; BZ), species-specific elevation (E), clutch size (CS) and baseline blood glucose level, while controlling for the effects of body mass (M_b). Blue and red arrows represent positive and negative relationships, respectively, with arrows pointing in the direction of causality. The arrow width is proportional to the log-transformed standardised effect size (untransformed form shown as a decimal) resulting from model averaging of all the path models. The sum of weights of each causality chain consists of weights of all the path models that included the complete focal chain, i.e., BZ → G_0 or E → G_0 for direct effects and BZ → CS → G_0 or E → CS → G_0 for effects mediated by clutch size.