Do thermoregulatory costs limit altitude distributions of Andean forest birds?

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Summary

1. Along tropical mountains, species often occupy narrow altitude ranges. Numerous biotic and abiotic factors have been proposed as determinants of altitude occupancy.

2. We measured several aspects of thermal physiology of 215 bird species across a 2·6-km altitude gradient in the Peruvian Andes. We predicted that highland species would show adaptation to the colder high-altitude climate and that energy costs of thermoregulation might limit upslope dispersal of lowland natives.

3. We found reductions in thermal conductance, body temperature and lower critical temperature in highland birds compared with lowland species. These combine to make highland natives more resistant to heat loss.

4. We did not find convincing evidence that acute thermal limits or energy costs of thermoregulation constrained altitude distributions. Heat-budget models predicted low-to-moderate long-term costs at native altitudes. Costs increased for lowland natives modelled in the highland climate, but for all but a few species, costs remained within putative expenditure limits.

5. Although we did not test heat tolerances, we measured all species at temperatures similar to the hottest air temperatures at the lowland site. There was no evidence that high lowland temperatures preclude downslope movements of highland birds.

6. While thermal tolerances probably do not directly determine altitude occupancy by most species, the additional energy cost of thermoregulation experienced by lowland species moving upslope may trade off against investment in important life-history components such as breeding, and thereby affect altitude range limits.

Key-words: Andes, body temperature, elevation, lower critical temperature, thermal conductance

Introduction

The tropical Andes are among the most species-rich regions on earth, particularly for birds (Rahbek & Graves 2001; Cadena *et al.* 2012). That diversity reflects both high species numbers within habitats and rapid turnover across elevations (Melo, Rangel & Diniz-Filho 2009), with most Andean birds restricted to narrow elevation zones (e.g. Jankowski *et al.* 2013). Understanding the mechanisms underlying that zonation has long been a focus of research. Many studies implicate competition or aggression as barriers to occupancy of particular altitudes (e.g. Terborgh & Weske 1975; Jankowski, Robinson & Levey 2010). Strong

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habitat preferences, coupled with abrupt habitat transitions across altitudes, may also affect distributions (Terborgh 1971; Jankowski *et al.* 2009, 2013). Other studies suggest predation, parasites or pathogens as limiting factors (e.g. van Riper *et al.* 1986; Boyle 2008; Atkinson & Samuel 2010; G.A. Londoño, unpublished data).

Abiotic factors may also be important. Janzen (1967) proposed that stable tropical climates, coupled with minimal temperature overlap among elevations separated by more than ~600 m, lead to thermal physiologies optimized for specific altitudes. This results in low tolerance for temperatures outside the optimal range, inhibiting up- or downslope dispersal. Some tropical ectotherms fit Janzen's predictions: these species have narrow temperature optima within which performance is maximal, and their thermal

tolerances are often closely matched to environmental temperatures (Huey *et al.* 2009). Therefore, species adapted to cool mid- and high altitudes risk lethal hyperthermia if they move downslope, whereas warm-adapted lowland species risk compromised performance if they move upslope.

Whether similar constraints apply to endotherms – which almost by definition are more tolerant of temperature variation than ectotherms – is unclear (Ghalambor *et al.* 2006). For Andean birds, altitude distributions are unavoidably associated with temperature due to the adiabatic lapse rate of 5–6 °C per 1000 m of elevation (Rapp & Silman 2012). However, distribution models based on occupancy generally cannot reveal the causal factors underlying altitude limits and hence do not indicate if a species can tolerate conditions at other elevations. That requires knowledge of relevant physiological traits in combination with microclimate.

For endotherms such as birds, thermal constraints could range from acute physiological limits to indirect limits derived from life-history trade-offs. Acute limits arise from environmental temperatures that exceed a species' ability to keep body temperature within tolerable bounds. Indirect limits result from energy costs or behavioural restrictions imposed by stressful but non-lethal thermal conditions. At temperatures outside of thermoneutrality, homeothermic endotherms increase metabolism above basal metabolic rate (BMR) to power thermogenesis (in cold) or evaporative cooling (in heat). Those costs reduce the energy available for crucial life-history functions (growth, maintenance, reproduction) and may be accompanied by behavioural shifts (e.g. microhabitat use or activity time) that impose further trade-offs. Acute limits for maximal heat production have been described for a number of tropical and temperate birds (McKechnie & Swanson 2010), but temperature tolerances *per se* are less studied. Little is known about long-term limits, particularly how regulatory costs or thermal constraints on behaviour impact life history. However, daily energy expenditures during the breeding season (presumably a time of high energy demand) seldom exceed 4 × BMR (Drent & Daan 1980), and this is often interpreted as an upper limit to energy flux over extended periods. Costs of thermoregulation can comprise only part of that total and still allow for energy allocation to other functions; therefore, long-term limits for thermoregulatory expenditures are likely closer to 2–2·5 × BMR. Estimated metabolic rates at the northern boundaries of winter ranges are similar to these values (Canterbury 2002).

Our study of Andean birds had two main objectives. The first was to determine whether thermal physiology varied along a 2·6-km altitude gradient encompassing a 12 °C range of annual temperature means (11–23 °C; Londoño *et al.* 2014a,b). Most birds maintain body temperatures (T_b) of 38–41 °C (Prinzinger, Pressmar & Schleucher 1991), and small species typically have lower critical temperatures (LCT; below which elevated heat production is

necessary) of 25–30 °C (Calder & King 1974). Therefore, a decrease in environmental temperature from 23 to 11 °C should greatly increase costs of regulatory thermogenesis. Given the selection that should impose, we expected differentiation of thermal physiology across altitudes. Previous work revealed no effect of altitude on BMR (Londoño *et al.* 2014a,b). However, BMR is largely irrelevant to thermoregulatory costs at temperatures below the LCT, and we predicted that thermal conductance, LCT and T_b would be reduced in high-altitude natives, because those changes would decrease heat loss rates.

Our second goal was to test whether thermal biology restricts altitude ranges. To do this, we combined physiological and microclimate data in a heat-budget model to produce species- and habitat-specific estimates of energy costs of thermoregulation. Our prediction was that lowland species exposed to the high-altitude climate would require thermoregulatory expenditures exceeding the putative maxima for acute exposure (4–7 × BMR) or over extended periods (2–4 × BMR). Finally, we considered potential barriers to downslope movements of highland birds.

Materials and methods

FIELD SITES

We worked at three sites near Parque Nacional del Manu in south-eastern Peru. Pantiacolla (mean elevation ~400 m; 12°39′S, 71°13′W) is in lowland rain forest. San Pedro (1500 m; 13°03′S, 71°32′W) is in montane cloud forest. Wayqecha (3000 m; 13°10′S, 71°35′W) is in highland cloud forest. San Pedro and Wayqecha are on steep mountain slopes; Pantiacolla is on more level terrain. The sites are roughly aligned along a north-east–south-west transect extending about 70 km.

Physiological data were obtained between June and December in 2011–2014; environmental temperatures were measured between September 2011 and December 2014.

BIRD CAPTURE AND HANDLING

We mist-netted birds in the afternoon (15:00–17:30 local time). Individuals in reproductive condition were released; others were held in quiet cloth-covered cages with water but no food. After dusk, birds were weighed and placed in metabolism chambers. Small nectar-feeding species (flowerpiercers, hummingbirds) were fed to repletion with sugar water (~25% sucrose) prior to insertion in chambers to reduce stress. Metabolic measurements did not begin until 1–2 h after birds were placed in chambers, sufficient time for sugar to be absorbed (Karasov *et al.* 1986). We first tested at 10 °C; subsequently, all individuals were tested at 30 °C; most were tested at 32–35 °C and some at 20 °C. Ambient temperatures (T_a) were maintained for at least 1 h before sampling began. Immediately after the 10 °C test, T_b was measured within 1 min of initial disturbance with a fine-gauge thermistor inserted at least 5–10 mm into the cloaca. Measurements at warm T_a, intended to elicit basal metabolism, occurred >5 h after the last feeding opportunity; accordingly, we assume birds were postabsorptive. A final T_b and body mass were obtained at the conclusion of measurements; we used the mean mass in calculations. Birds were banded and released at the site of capture the following morning.

Procedures were approved by the Institutional Animal Care and Use Committees at the University of California Riverside (protocol # 0408026, 20070023, 20100048) and the University of Florida (Protocol #: 201106068), and by permits from the government of Peru (0239-2013 MINAGRI-DGFFS/DGEFFS 2013).

THERMAL PHYSIOLOGY

We used open-circuit respirometry to measure rates of oxygen consumption ($\dot{V}O_2$) as previously described (Londoño *et al.* 2014a,b; Appendix S1, Supporting Information). We converted $\dot{V}O_2$ to metabolic heat production (MHP; watts) using an oxycaloric value of 19·8 J mL^{-1} O$_2$.

Barometric pressure averaged 521 torr at Wayqecha and 723 torr at Pantiacolla. To determine whether lower O$_2$ partial pressure at Wayqecha affected thermoregulation of lowland birds, we tested a subset of Pantiacolla species in acute hypobaria. We used a small vacuum pump to pull air through the chamber against the resistance of the upstream flow controller. Chamber pressure was measured with a manometer (Sper Scientific 840081, Scottsdale, AZ, USA). We adjusted pump power and the flow controller until the desired pressure differential and flow were achieved. Other aspects of the system were as in Londoño *et al.* (2014a,b). During tests, we cooled the chamber to 10 °C at ambient pressure, measured $\dot{V}O_2$ after 1 h of acclimation, then quickly reduced pressure by 200 torr and measured $\dot{V}O_2$ again. Results were compared with paired t-tests.

THERMAL CONDUCTANCE AND LOWER CRITICAL TEMPERATURE

We calculated thermal conductance (C; watts per °C) at 10 °C from MHP and the gradient between body and ambient temperatures:

$$C = MHP/(T_b - T_a) \qquad \text{eqn 1}$$

We estimated lower critical temperature (LCT) from C, basal metabolic rate (BMR, watts) and body temperature at BMR:

$$LCT = T_b - (BMR/C) \qquad \text{eqn 2}$$

In a few large species, we could not compute C or LCT because there was no increase in MHP at 10 °C (i.e. 10 °C was within thermoneturality).

These calculations assume that T_b is constant and C is minimal at temperatures below the LCT, as in the classic 'Scholander model' (Scholander *et al.* 1950). In our species, the change in T_b across T_a of 10 °C to 30–34 °C averaged 0·9 °C (see Results). Assuming a linear change, the T_b at the LCT would differ from that at BMR by ~0·5 °C or less. Also, conductance at 20 °C did not differ from conductance at 10 °C ($N = 435$, $P = 0.28$; Appendix S1). Accordingly, we believe the computed LCT are acceptably accurate.

ENVIRONMENTAL TEMPERATURES

We measured thermal conditions at our field sites as shade air temperature (T_a) and the temperature of grey-painted hollow aluminium spheres that integrated the thermal effects of T_a, wind and sunlight (operative environmental temperature T_e; Bakken 1976; Walsberg & Weathers 1986; Chappell, Morgan & Bucher 1990). T_e and T_a were recorded with data loggers, and results were processed into 30-min mean values (Appendix S1).

ENERGY COSTS OF THERMOREGULATION

The energy an endotherm spends to thermoregulate is a function of physiology (C, T_b, BMR), environment (T_a, wind, sun), behaviour (microhabitat, posture, activity, use of torpor) and other factors (e.g. plumage wetting or damage). Precise calculation of thermoregulatory costs requires detailed data on all these factors, many of which were unavailable. We modelled expenditures with the 'Thermoregulatory Costs' heat-budget algorithm in LabAnalyst (http://warthog.ucr.edu/). For each microclimate datum (i.e. every 30 min), this computes the heat production necessary to maintain T_b (Fig. S1). Over the duration of microclimate records, it provides each species' mean cost of thermoregulation, highest single 30 min value, highest 6-, 12- and 24-h costs, and the fraction of time in thermoneutrality. Because the quantitative details of sun use and torpor are unknown in the species we studied, we modelled heat budgets with and without these factors to estimate upper and lower bounds of thermoregulatory costs.

MINIMUM TOLERABLE T_E

Maximal cold-induced metabolic rate ('summit metabolism', MR$_{sum}$; McKechnie & Swanson 2010) is a common index of cold resistance. Together, C, minimum T_b and MR$_{sum}$ are the main determinants of an endotherm's lowest tolerable T_e ($T_e$min). Power and logistical constraints precluded measurement of MR$_{sum}$. However, in 19 lowland bird species from Panama, MR$_{sum}$ averaged 4·5 ± 0·3 × higher than BMR (Wiersma, Chappell & Williams 2007). Panamanian and Peruvian birds have similar BMR (Londoño *et al.* 2014a,b), so we assumed PMR$_C$ was also similar and estimated $T_e$min as:

$$T_e min = T_b - ((BMR * 4 \cdot 5)/C) \qquad \text{eqn 3}$$

We measured C and T_b during the rest phase (night). Because active-phase C is about 1·4-fold higher than rest-phase C (Aschoff 1981; Schleucher & Withers 2001), and active-phase T_b averages 2 °C higher than rest-phase T_b (Prinzinger, Pressmar & Schleucher 1991), we estimated daytime minimal T_e as:

$$T_e min = (T_b + 2) - ((BMR * 4 \cdot 5)/(C * 1 \cdot 4)) \qquad \text{eqn 4}$$

In some small non-passerines, circadian T_b changes may exceed 2 °C (Aschoff 1982). If applicable to Peruvian birds, this would affect estimates of daytime LCT and $T_e$min. The impact on heat budgets is minor, because the highest costs occur at night, when T_e is lowest (Fig. 1) and we directly measured T_b. Accordingly, and because circadian T_b variance in Peruvian birds is unknown, we did not include mass scaling of T_b changes in our analyses.

ANALYSIS AND STATISTICS

Species means were used for all analyses except hypobaric and seasonality tests. Body mass (M_b), BMR and conductance were log-transformed to account for allometric scaling. To normalize distributions, we converted $T_e$min to K and applied either a log or square-root transform, and if necessary a further Box–Cox transform. Comparisons were performed with ordinary least-squares analysis of covariance (OLS ANCOVA) with altitude as a fixed factor and M_b as the covariate. Time in thermoneutrality was analysed with nonparametric tests (Wilcoxon and Tukey–Kramer HSD). Tests were performed with JMP Pro 11 (SAS Institute, Cary, NC, USA).

We estimated phylogenetic signal (Pagel 1999; Blomberg, Garland & Ives 2003) based on the avian 'tree of life' (Jetz *et al.* 2012; Londoño *et al.* 2014a,b). When phylogenetic signal was present, we used phylogenetic independent contrasts (Felsenstein 1985;
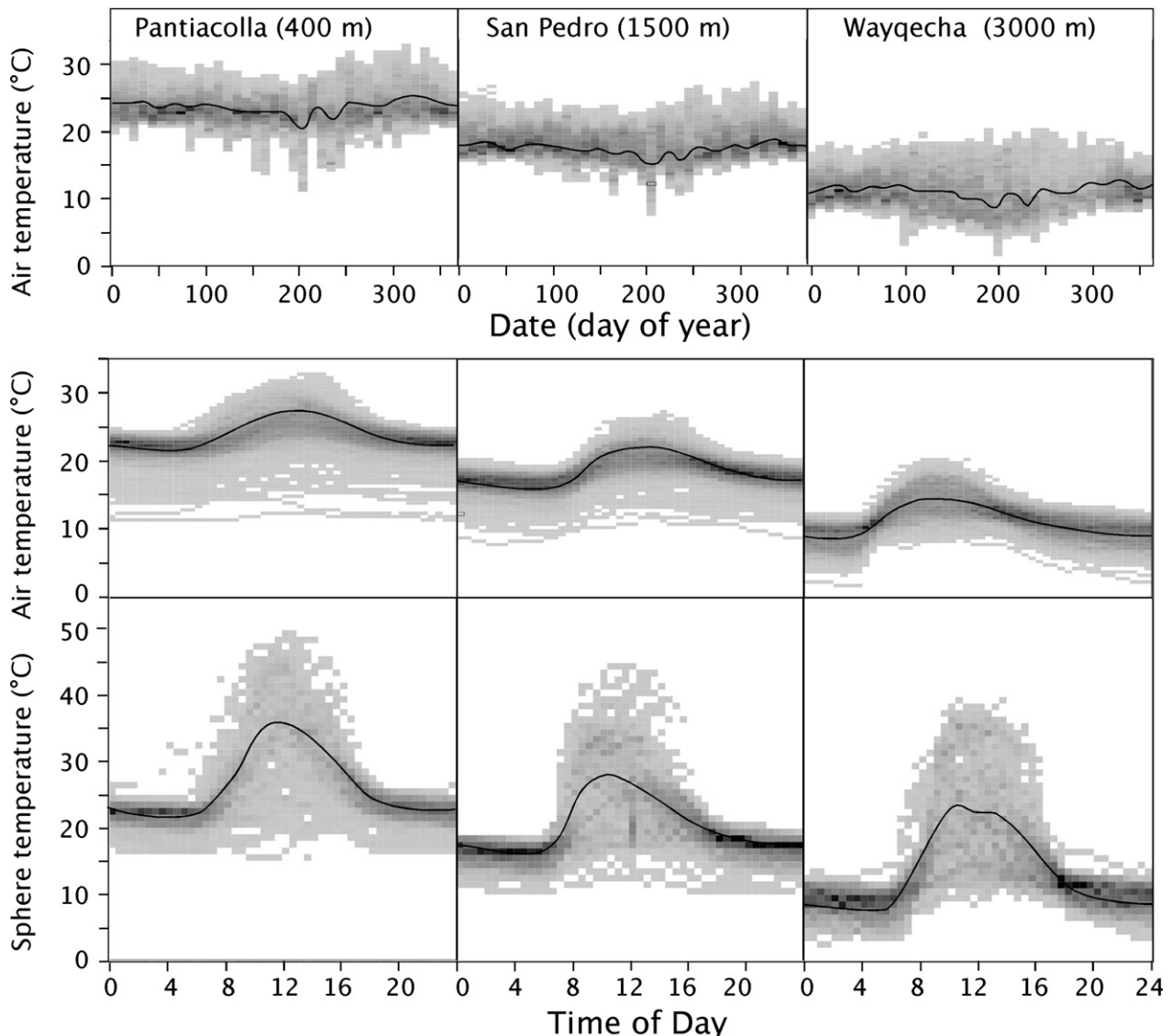
Fig. 1. Temperatures at our three field sites in southeastern Peru. Air temperature is shade temperature; sphere temperature is the temperature of bird models (gray-painted aluminum spheres) placed in open areas to receive sunlight and wind. The frequency of particular temperatures is indicated by shading (pale = few, dark = many). Solid lines are smoothed daily means (annual plots) or 30-min means (24-h plots). Data were obtained in 2011–2014. Mean air temperatures differ by about 12 °C between Pantiacolla and Wayqecha (Table S1).

Garland, Harvey & Ives 1992) and phylogenetic generalized least squares (PGLS) as implemented in ʀ (R Core Team 2013; Appendix S1). For all tests, the significance level was 0·05.

Results

ENVIRONMENTAL TEMPERATURES

Seasonal variation was small (Fig. 1), but air and sphere temperatures and high temperature maxima averaged about 12 °C cooler at Wayqecha than at Pantiacolla (Table S1). Temperatures at San Pedro were intermediate. Differences were smaller for low temperature minima (Table S1). Air and sphere temperatures differed substantially when sunlight was present, and daily temperature cycles were larger for spheres than for T_a (Fig. 1).

THERMAL PHYSIOLOGY

For T_b and LCT, we included data from birds with stable $\dot{V}O_2$ and $T_b \geq 35$ °C. Individuals with lower T_b were considered to be in torpor (e.g. Bech *et al.* 1997). For calculation of C, we used data from birds with stable $\dot{V}O_2$ and $T_b \geq 33$ °C. Applying these criteria, we obtained BMR, BMR T_b, LCT, C and minimum tolerable T_e for 215 species, and 10 °C T_b for 206 species. In all analyses, phylogenetic signal was present in M_b ($\lambda = 1\cdot00$, $P < 0\cdot0001$) and most physiological variables (Table 1). There was no evidence of seasonal variation in any physiological trait (Appendix S1).

We found no effect of altitude on BMR with either OLS ANCOVA ($P = 0\cdot27$) or PGLS ($P = 0\cdot91$; Table 1; Londoño *et al.* 2014a,b).

Table 1. Phylogenetic signal and effects of body mass and native altitude on physiological variables

	Body mass	Native altitude	Phylogenetic signal	Phylogenetic signal (residuals)	PGLS best fit model
BMR	$F_{1,211} = 626$ $P < 0.0001$	$F_{2,210} = 0.09$ $P = 0.91$	$\lambda = 0.953$ $P < 0.0001$	$\lambda = 0.304$ $P = 0.0004$	Pagel
C	$F_{1,211} = 957$ $P < 0.0001$	$F_{2,210} = 21.5$ $P < 0.0001$	$\lambda = 0.956$ $P < 0.0001$	$\lambda \sim 0$ $P \sim 1$	Ornstein–Uhlenbeck $(\alpha = 5)$
T_b10	$F_{1,202} = 11.4$ $P = 0.0009$	$F_{2,201} = 6.03$ $P = 0.0029$	$\lambda = 0.301$ $P = 0.0014$	$\lambda = 0.156$ $P = 0.062$	Grafen
T_bBMR	$F_{1,211} = 23.9$ $P < 0.0001$	$F_{2,210} = 11.7$ $P < 0.0001$	$\lambda = 0.219$ $P = 0.0012$	$\lambda = 0.068$ $P = 0.21$	Pagel
LCT	$F_{1,211} = 0.075$ $P = 0.78$	$F_{2,210} = 10.1$ $P = 0.0001$	$\lambda = 0.31$ $P = 0.0027$	$\lambda = 0.31$ $P = 0.0026$	Pagel

Body mass and conductance were log10-transformed prior to analysis; a further Box–Cox transform was needed to normalize body mass. Analyses were performed with phylogenetic least-squares methods (PGLS), with phylogenetic signal and its mass residuals tested with Pagel's λ. Five evolutionary models were evaluated, and the best fit was determined using AIC (see text). Significant results ($P < .05$) are boldfaced.

OLS ANCOVA indicated that C was affected by both M_b ($F_{1,214} = 978$, $P < 0.0001$) and altitude ($F_{2,213} = 4.09$, $P = 0.018$; Fig. 2a). Mass-adjusted C (least-squares mean) was about 9% higher at 400 m than at 3000 m. There was no $M_b \times$ altitude interaction ($P = 0.28$). Post hoc Tukey–Kramer comparisons revealed a difference between high- and low-altitude sites ($P = 0.0146$); no other comparison was significant ($P > 0.2$). The pooled C data were correlated with M_b: C (watts per °C) = 0.00359 $M_b^{0.515 \pm 0.015}$ SE ($r^2 = 0.85$, $P < 0.0001$). That scaling is consistent with previous analyses of avian conductance (mass exponent 0.48 to 0.52; Schleucher & Withers 2001), and there was no difference in C ($P = 0.265$) between the

Peru species and rest-phase measurements of 35 temperate-zone species from Aschoff (1981), recommended as the best predictor of avian C by Schleucher & Withers (2001).

Accounting for phylogeny, conductance and M_b were positively correlated ($r^2 = 0.58$; $P < 0.0001$; 95% CI = 0.49–0.66) and PGLS revealed effects of M_b and altitude on C ($P < 0.0001$; Table 1), with lower C at high altitude and at large M_b.

In OLS ANCOVA, body temperature at $T_a = 10$ °C (T_b10) was positively correlated with M_b ($F_{1,205} = 22.6$, $P < 0.0001$) and varied among altitudes ($F_{2,204} = 7.74$, $P = 0.0006$; Fig. 2c). There was no $M_b \times$ altitude
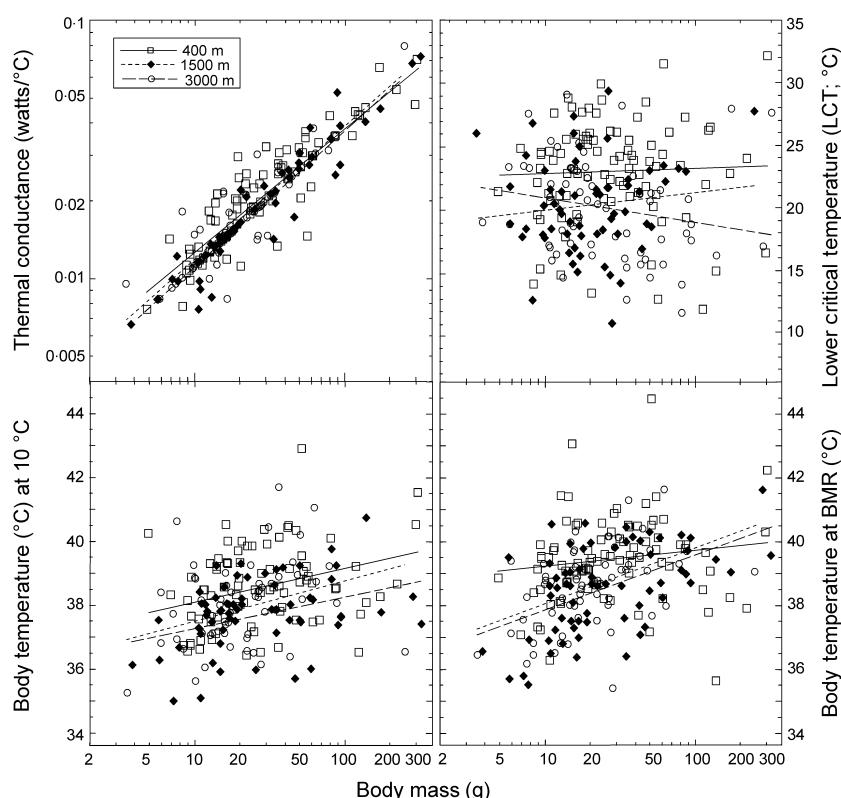

Fig. 2. Thermal physiology of Andean birds from three fields sites at different altitudes (Pantiacolla, 400 m; San Pedro, 1500 m; Wayqecha, 3000 m). (a) thermal conductance; (b) lower critical temperature; (c) body temperature at an ambient temperature of 10° C; (d) body temperature at BMR (ambient temperature 30–34 °C). All traits differ among altitudes; all but lower critical temperature are affected by body mass (Table 1). Basal metabolic rates (BMR, not shown) are strongly positively correlated with body mass but are not affected by altitude.

interaction ($P = 0.96$). Tukey–Kramer tests revealed differences between high- and low-altitude sites ($P = 0.0004$), but not for other altitude comparisons ($P > 0.07$). Mass-adjusted T_b10 at high altitude was 37.7 ± 0.16 °C (mean ± SE), or 0·8 °C less than that of lowland species (38.5 ± 0.14 °C); the value for the mid-altitude site was intermediate (38.0 ± 0.17 °C).

Accounting for phylogeny, body mass and T_b10 were positively correlated ($r^2 = 0.16$; $P = 0.015$; 95% CI = 0·03–0·30). PGLS revealed the same qualitative results as OLS: T_b10 varied with M_b and altitude ($P = 0.0009$ and $P = 0.0029$, respectively; Table 1), increasing as M_b increased and declining as altitude increased.

Body temperatures at BMR were obtained at T_a between 30 and 34 °C. There was no effect of test temperature on T_b at BMR ($P = 0.86$, ANCOVA with M_b and altitude as covariates); therefore, we pooled these data as 'T_bBMR'. OLS ANCOVA revealed effects of M_b and altitude on T_bBMR, with higher T_bBMR at low altitude ($F_{2,210} = 9.99$, $P < 0.0001$) and at large M_b ($F_{1,211} = 33.3$, $P < 0.0001$; Fig. 2d). The M_b × altitude interaction did not reach significance ($F_{2,210} = 2.9$, $P = 0.056$). Tukey–Kramer comparisons revealed differences between high- and low-altitude sites ($P < 0.0001$) and low- and mid-altitude sites ($P = 0.0047$), but not mid- and high-altitude sites ($P = 0.69$). Mass-adjusted T_bBMR at high altitude averaged 38.5 ± 0.15 °C (mean ± SE), or 0·9 °C cooler than at low altitude (39.4 ± 0.13 °C); T_bBMR at the mid-altitude site was intermediate (38.7 ± 0.16 °C).

PGLS revealed the same qualitative results as OLS: T_bBMR was positively correlated with body mass ($r^2 = 0.19$; $P = 0.006$; 95% CI = 0·05–0·32) and decreased with increasing altitude ($P < 0.0001$; Table 1).

OLS ANCOVA indicated that LCT was not affected by M_b ($F_{1,211} = 0.20$, $P = 0.65$) but decreased with increasing altitude ($F_{2,210} = 12.9$, $P < 0.0001$; Fig. 2b). There was no M_b × altitude interaction ($P = 0.19$). Tukey–Kramer tests revealed differences between high- and low-altitude sites ($P < 0.0001$) and low- and mid-altitude sites ($P = 0.0002$) but not mid- and high-altitude sites ($P = 0.98$). Mass-adjusted LCT at high altitude (19.4 ± 0.51 °C; mean ± SE) averaged 3·0 °C lower than at low altitude (22.4 ± 0.44 °C); the LCT at San Pedro (19.6 ± 0.53 °C) was close to that at high altitude.

As with OLS results, PGLS indicated that LCT was not correlated with M_b but declined with increasing altitude ($P = 0.78$ and $P = 0.0001$, respectively; Table 1).

PRESSURE EFFECTS ON LOWLAND SPECIES

In 86 individuals of 57 Pantiacolla species tested at 10 °C at both the *in situ* barometric pressure and the Wayqecha barometric pressure, there was no difference in $\dot{V}O_2$ ($P = 0.84$, paired *t*-test; Fig. S2).

MINIMUM TOLERABLE T_E

Minimum tolerable T_e (T_emin) lacked phylogenetic signal. Night-time T_emin was inversely related to altitude ($F_{2,210} = 5.61$; $P = 0.0042$) and declined as M_b increased ($F_{1,211} = 4.96$; $P = 0.0269$). Mass-adjusted T_emin was -47.0 °C ± 2·4 (SE) at the mid-altitude site, -45.5 ± 3.0 °C at the high-altitude site, and -37.6 ± 2.0 °C at the low-altitude site. There was no M_b × altitude interaction ($P = 0.06$). Daytime T_emin (where T_b and C were higher) was affected by altitude ($F_{2,210} = 6.01$; $P = 0.0029$) and M_b ($F_{1,211} = 4.32$; $P = 0.0389$), with no interaction ($P = 0.07$). Daytime mass-adjusted T_emin was -20.0 ± 1.7 °C at the mid-altitude site, -19.5 ± 1.6 °C at the high-altitude site and -13.6 ± 1.4 °C at the low-altitude site.

At night, distributions of shade T_a had essentially no overlap with T_emin at any altitude (Fig. 3). During the day, T_emin was roughly 23 °C higher, and for a few species, shade T_a at Wayqecha was, on rare occasions, below T_emin (Fig. 3).

HEAT TOLERANCE

We exposed all species to T_a of 30 °C and most to 32–35 °C for several hours when measuring BMR. Those T_as are similar to the highest T_a at the low-altitude site (Fig. 1) and therefore simulate the warmest conditions likely to be experienced in this habitat (exclusive of sun exposure, which can be avoided behaviourally). Chamber relative humidity during BMR measurements (mean $44.9 \pm 16.4\%$
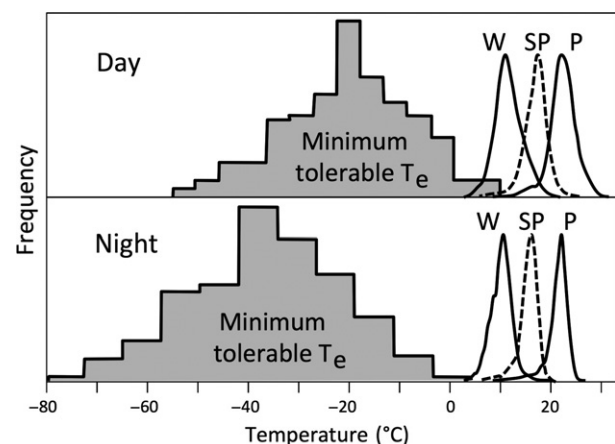

Fig. 3. Relationship between the estimated minimum tolerable environmental temperature (T_emin; gray shading) and shade temperature distributions (right) for 215 Andean bird species at three field sites, Wayqecha (W, 3000 m), San Pedro (SP, 1500 m), and Pantiacolla (P, 400 m). We computed T_emin from body temperature, conductance, and a maximal thermogenic heat production of $4.5 \times$ BMR (Wiersma, Chappell & Williams 2007). T_emin was affected by native altitude (see text) but data from different altitudes are pooled in this figure. T_emin differed between night and day because both body temperature and conductance are higher during the active phase (day) of the diurnal cycle (Prinzinger, Pressmar & Schleucher 1991; Schleucher & Withers 2001).

SD) varied between stations ($F_{2,834} = 109$, $P < 0.0001$), ranging from 34·6 ± 10·5% at San Pedro to 51·4 ± 16·6% at Pantiacolla. There was no indication of heat stress (panting, escape attempts, hyperthermia) in any mid- or high-altitude species and only one instance of T_b higher than 44 °C (44·4 °C in *Syndactyla ucayalae*, a lowland native).

THERMOREGULATORY COSTS AT NATIVE ALTITUDES

Phylogenetic signal was significant for all cost estimates ($P < 0.038$). Most costs, with or without sun exposure and torpor, increased with increasing altitude and (expressed as multiples of BMR) decreased with increasing M_b (Table 2). The fraction of time in thermoneutrality was not affected by M_b, but declined as altitude increased (Table 2).

At all altitudes, annual mean costs averaged below 2 × BMR (Fig. 4, Table 2). The highest 24, 12 and 6 h costs averaged below 2·4 × BMR at Pantiacolla and were somewhat greater at higher elevations. A few small species at Wayqecha had predicted costs exceeding 4 × BMR and one exceeded 5 × BMR for the highest 30 min. Pantiacolla birds experienced thermoneutral conditions almost 50% of the time, declining to 15% at Wayqecha.

Exclusion of sun exposure and torpor had little effect (Table 2). Differences were substantial only for time in thermoneutrality, which decreased considerably.

THERMOREGULATORY COSTS IN THE HIGH-ALTITUDE CLIMATE

Thermoregulatory costs in the Wayqecha climate showed no phylogenetic signal and no effect of M_b, but increased for low- and mid-altitude species (Figs 1 and 4; Tables 2 and 3). Native altitude affected costs, which averaged 11–20% larger for lowland species than for highland natives (Table 3). Nevertheless, annual mean costs averaged <2·1 × BMR across all native altitudes, and even the highest 30 min costs averaged <3 × BMR (Fig. 4). No species' annual mean exceeded 4 × BMR; only five species exceeded 4 × BMR for intervals of 6 or 12 h (one high-altitude native), and only three species, including one high-altitude native, exceeded 4 × BMR over 24 h. Highest 30-min costs equalled or exceeded 5 × BMR for four species (one high-altitude native).

Exclusion of sun exposure and torpor had minor effects on costs (Table 3). Differences were substantial only for time in thermoneutrality.

Discussion

The goals of our study are aligned with Janzen's (1967) concept that the striking altitudinal zonation characteristic of tropical mountains occurs because up- or downslope range expansion is challenging for tropical animals. Janzen assumed that: (i) temperature differences are effective barriers to dispersal; (ii) seasonal stability in the tropics minimizes the temperature 'overlap' between altitudes (i.e. temperatures differ across altitudes but have little variation within altitudes); and (iii) organisms are unlikely to evolve tolerances to temperature they do not experience. Compared with high-latitude species that experience strong seasonality, tropical organisms should have narrow ranges of thermal tolerances and limited capacities for acclimatization. Accordingly, dispersing up- or downslope would bring them into intolerable temperature regimes. Climates at our sites conform to expectations of seasonal stability

Table 2. Estimated thermoregulatory costs and percentage of time in thermoneutral conditions for Andean bird species from three altitudes (400, 1500 and 3000 m) exposed to weather conditions at their native altitudes recorded in 2011–2014

	Pantiacolla (400 m; N = 90)	San Pedro (1500 m; N = 64)	Wayqecha (3000 m; N = 68)	*P* (body mass)	*P* (native altitude)
Annual mean cost	1·29 ± 0·037	1·47 ± 0·045	1·86 ± 0·042	**0·042**	**<0·0001**
	1·31 ± 0·044	1·55 ± 0·052	2·01 ± 0·049	**0·0452**	**0·0049**
Highest 24-h cost	1·97 ± 0·057	2·07 ± 0·069	2·26 ± 0·064	0·0748	**<0·0001**
	2·15 ± 0·061	2·19 ± 0·073	2·36 ± 0·068	**0·0324**	**0·0040**
Highest 12-h cost	2·19 ± 0·064	2·32 ± 0·077	2·44 ± 0·072	0·0684	**<0·0001**
	2·41 ± 0·067	2·41 ± 0·080	2·55 ± 0·075	**0·0285**	**0·0472**
Highest 6-h cost	2·27 ± 0·066	2·38 ± 0·066	2·54 ± 0·073	0·0521	**0·0001**
	2·44 ± 0·068	2·47 ± 0·082	2·64 ± 0·076	**0·0048**	0·126
Highest 30-min cost	2·34 ± 0·072	2·47 ± 0·086	2·97 ± 0·080	0·0734	**<0·0001**
	2·50 ± 0·072	2·57 ± 0·087	2·91 ± 0·081	**0·0419**	**< 0·0001**
% of time thermoneutral	47·6 ± 3·2	25·3 ± 2·4	15·4 ± 1·0		**<0·0001**
	43·5 ± 3·4	13·7 ± 2·7	1·7 ± 0·5		**< 0·0001**

In each cell, the upper value incorporates thermal effects of sun exposure and energy savings from use of torpor (in species that exhibited nocturnal hypothermia) and the lower value excludes the thermal and energetic benefits of sun exposure and torpor. Costs are expressed as factorial increases above basal metabolic rate (BMR) and are shown as least-squares mean ± SE, obtained from ANCOVA with log mass as covariate. Significant phylogenetic signal was present in all thermoregulatory cost variables ($P < 0.039$), and P values for across-altitude cost comparisons are from PGLS models. The percentage of time thermoneutral is shown as unadjusted means ± SE; comparisons among different altitudes were performed with nonparametric methods (Tukey Kramer HSD); there was no effect of mass ($P > 0.4$). Significant results ($P < .05$) are boldfaced.
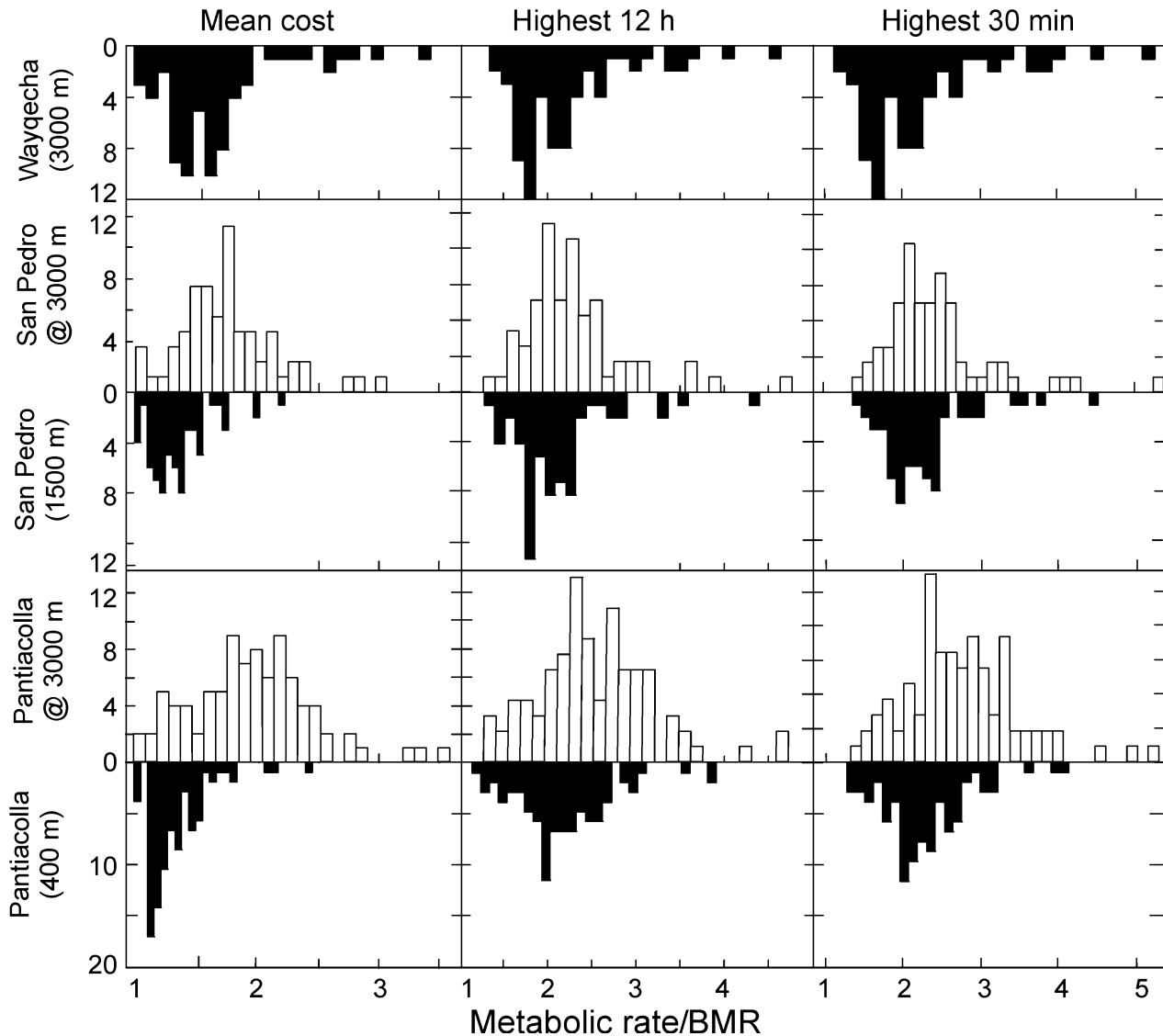
Fig. 4. Estimated thermoregulatory costs of bird species from low-, mid-, and high-elevation sites (N = 88, 61, and 66 species, respectively). Costs (x-axis) are expressed as factorial increases above basal metabolic rate (BMR). Black bars show the number of species for each metabolic cost at native altitudes; open bars show the number of species for each cost at the high-altitude site. The three columns show the mean thermoregulatory cost (left), the highest cost averaged over 12 h (middle) and the highest cost averaged over 30 min (right). Costs were computed with a heat-budget model (Fig. S1) and the weather data in Fig. 1.

within altitudes but differences across altitudes (Fig. 1), and we hypothesized that (i) species native to different altitudes (and hence different T_e) will show adaptive specialization in thermal physiology and (ii) such specialization, combined with the ~12 °C across-altitude temperature gradient, precludes occupation of non-native altitudes.

Our results support the first hypothesis: we found significant differences across altitudes in several important indices of thermal physiology (C, T_b and LCT; Table 1). These 'match' the thermal gradient, with highland birds more resistant to heat loss (Fig. 2). The potential energetic benefit is shown in cost calculations for the highland climate (Table 3): the combination of lower T_b and lower C in highland species produced savings of 11–20% compared with lowland natives. In the context of a bird's overall energy budget (daily energy expenditure; DEE), benefits are smaller because DEE includes substantial activity costs in addition to expenditures for BMR and thermoregulation (Weathers & Sullivan 1993), but even a 5–10% reduction in DEE could be ecologically important.

Those findings make intuitive sense, but there are caveats. First, trait differences between altitudes may not be genetic adaptations, but instead may result from phenotypic plasticity or flexibility. If so, lowland species moving upslope would acclimatize and attain physiological phenotypes similar to those of highland natives. Similarly, we have not excluded seasonal acclimatization, which can induce substantial changes in cold tolerance (e.g. Cooper & Swanson 1994; Piersma, Cadée & Daan 1995; Liknes, Scott & Swanson 2002; McKechnie & Lovegrove 2002;

McKechnie 2008). Many of those studies concerned species that experience strong seasonality; in contrast, the Peruvian birds are exposed to little annual T_e change (Fig. 1). We found no seasonal physiological variation in 16 species for which we have measurements across 4–6 months (winter to late spring; Appendix S1), but because we lack data for almost half the year (January–May), some changes may not have been detected.

Secondly, due to technical constraints and ethical considerations (the need to release birds the morning after capture), we could not test at the coldest T_a measured at our high-altitude sites. About 15% of Wayqecha T_a were below our minimum test temperature of 10 °C (<0·5% were colder than 5 °C; Fig. 1); at San Pedro, <0·1% of T_a were lower than 10 °C. However, BMR testing (at 30–35 °C) encompassed the highest T_a we observed: no Pantiacolla T_a exceeded 33 °C and no T_a at San Pedro or Wayqecha exceeded 30 °C.

Also, we could not measure summit metabolism (MR_{sum}), which generally requires near- or subzero temperatures and a helium–oxygen atmosphere (Cooper & Swanson 1994; Liknes, Scott & Swanson 2002). This was not feasible at our sites, but it seems questionable if that level of thermogenesis is relevant to the majority of our species. Estimated cold tolerances ($T_e$min), based on C, T_b and the MR_{sum} of $4·5 \times$ BMR for Panamanian forest birds (Wiersma, Chappell & Williams 2007), are generally far colder than night-time T_a, even at Wayqecha (Fig. 3). Paradoxically, there was a small overlap between daytime $T_e$min and shade T_a (Fig. 1), due to higher T_b and C during the daytime active phase. Such overlap occurred rarely in a few species in the Wayqecha climate, and for many of these, higher T_e were achievable behaviourally via sun exposure (Fig. 1).

Finally, high-altitude hypoxia could limit the power output of lowland birds, rendering them unable to generate sufficient heat to withstand T_e they tolerate at their native altitude. Ambient oxygen partial pressure (pO_2) in dry air was about 38% higher at Pantiacolla than at Wayqecha (152 vs. 109 torr); the difference was 43% for moisture-saturated gas at lung temperatures of ~38 °C (140 torr at Pantiacolla; 98 torr at Wayqecha). However, we found no difference in 10 °C $\dot{V}O_2$ in Pantiacolla species tested at both pO_2 (Fig. S2). Since most Wayqecha T_e are warmer than 10 °C and nearly all are warmer than 5 °C (Fig. 1), that suggests the highland pO_2 would not be a barrier to upslope movement of lowland species.

To summarize, we found altitude variation in all measured thermophysiological traits except BMR. While the differences are not large and their causality (genetic adaptation vs. phenotypic plasticity) is unknown, they combine to provide highland species substantial savings in costs of regulatory thermogenesis. One unexpected finding was absence of an effect of mass on LCT. Other factors being equal, the surface to volume ratio decreases and, presumably, plumage thickness increases at larger M_b (Calder & King 1974). Both factors reduce the potential for heat loss

per unit mass, so we expected an inverse relationship between M_b and LCT. However, LCT depends on T_b and BMR as well as C (eqn 2). Body temperature varies only slightly with M_b (Fig. 2d), while in our data set BMR scales to $M_b^{0·551}$ and C scales to $M_b^{0·515}$. Therefore, LCT should scale approximately to $M_b^{0·036}$. Given the variance in LCT (Fig. 2B), that value did not statistically differ from scaling to M_b^0 (no effect of mass). Presumably, LCT would decline at larger body sizes than the ~300 g maximum M_b in our data (Calder & King 1974).

We found little support for our second hypothesis: that thermal physiology is so specialized that birds cannot disperse to non-native altitudes. As expected, thermoregulatory costs increase with increasing altitude (Table 2), but even at Wayqecha, these costs are generally below levels expected to be restrictive. In birds from all latitudes, short-term limits to metabolic heat production are usually $4–7 \times$ BMR (McKechnie & Swanson 2010). Among the Peruvian species, very few approached that limit even for brief periods (the largest estimated cost, about $5 \times$ BMR for the highest 30 min, was for a small highland hummingbird). Similarly, estimated minimum tolerable temperatures, with rare exceptions, were much colder than measured T_e (Fig. 3). We conclude that limits to acute cold tolerance are unlikely to preclude upslope movement for all but a few lowland natives, and even for these, intolerably low temperatures occur infrequently.

Long-term constraints on metabolic power output (over days or weeks) may be more important than acute cold tolerance in determining altitude occupancy, but are poorly understood. Based largely on measurements during breeding, long-term maxima are often assumed to be ~$4 \times$ BMR (e.g. Drent & Daan 1980; Peterson, Nagy & Diamond 1990). Because thermoregulatory costs cannot comprise the entire energy budget, we assumed they can be at most $2·5 \times$ BMR, similar to cost estimates for wintering temperate-zone birds (Canterbury 2002). Some insight into the possibility that long-term costs preclude occupation of non-native altitudes comes from modelling lowland birds in the Wayqecha climate (Table 3, Fig. 4). Lowland species have less resistance to heat loss than highland birds, so it is unsurprising that their costs were greater than those of highland natives (Table 3) and that costs for lowland species increased considerably in the highland climate (e.g. 60% greater annual means than at their native altitude). Nevertheless, maximal costs for lowland species exceeded $2·5 \times$ BMR only for short intervals (12 h or less), and annual mean costs averaged only slightly above $2 \times$ BMR – again, inconsistent with the hypothesis that lowland species cannot tolerate Wayqecha temperatures. Moreover, if high regulatory costs inhibit upward migration, then lowland species should be disproportionately represented among species with the highest long-term costs in the Wayqecha climate. This was not the case. In species for which costs were $2·5 \times$ BMR or greater, there was no effect of native altitude on mean annual costs ($N = 23$; $P = 0·86$), maximal

Table 3. Estimated thermoregulatory costs and percentage of time in thermoneutral conditions for Andean bird species from three native altitudes (400, 1500 and 3000 m) exposed to weather conditions at Wayqecha, Peru (3000 m) recorded in 2011–2014

	Pantiacolla (400 m; $N = 90$)	San Pedro (1500 m; $N = 64$)	Wayqecha (3000 m; $N = 68$)	P (body mass)	P (native altitude)
Annual mean cost	2·07 ± 0·051	1·73 ± 0·062	1·72 ± 0·059	0·418	**< 0·0001**
	2·11 ± 0·054	1·86 ± 0·064	1·88 ± 0·063	0·192	**0·0049**
Highest 24 h cost	2·40 ± 0·060	2·09 ± 0·072	2·11 ± 0·069	0·463	**0·0015**
	2·40 ± 0·062	2·12 ± 0·073	2·15 ± 0·071	0·145	**0·0068**
Highest 12 h cost	2·63 ± 0·067	2·28 ± 0·081	2·30 ± 0·078	0·204	**0·0009**
	2·66 ± 0·068	2·35 ± 0·082	2·38 ± 0·080	0·161	**0·0060**
Highest 6 h cost	2·67 ± 0·068	2·34 ± 0·083	2·37 ± 0·079	0·171	**0·0037**
	2·67 ± 0·069	2·35 ± 0·083	2·38 ± 0·079	0·161	**0·0060**
Highest 30 min cost	2·91 ± 0·075	2·57 ± 0·090	2·61 ± 0·087	0·138	**0·0137**
	2·98 ± 0·078	2·69 ± 0·095	2·75 ± 0·090	0·414	**0·0145**
% of time thermoneutral	4·1 ± 0·7	7·9 ± 0·8	15·4 ± 1·0		**< 0·0001**
	0·4 ± 0·2	0·7 ± 0·4	1·7 ± 0·5		**0·0187**

In each cell, the upper value incorporates thermal effects of sun exposure and energy savings from use of torpor (in species that exhibited nocturnal hypothermia) and the lower value excludes the thermal and energetic benefits of sun exposure and torpor. Costs are expressed as factorial increases above basal metabolic rate (BMR) and are shown as least-squares mean ± SE, obtained from ANCOVA with log mass as covariate. Significant phylogenetic signal was absent. The percentage of time thermoneutral is shown as unadjusted means ± SE; comparisons among different altitudes were performed with nonparametric methods (Tukey–Kramer HSD); there was no effect of mass ($P > 0.4$). Significant results ($P < .05$) are boldfaced.

24-h costs ($N = 54$; $P = 0.40$), maximal 12-h costs ($N = 77$; $P = 0.61$) or maximal 6-h costs ($N = 86$, $P = 0.49$).

It is worth noting that our proposed limit of $2.5 \times$ BMR is subject to several potentially important ameliorating factors. First, activity is a substantial portion of a typical bird's energy budget (Weathers & Sullivan 1993), and exercise-generated heat can be substituted for thermogenesis (Webster & Weathers 1990), lowering overall costs. Secondly, heat released during digestion is also substitutive for thermogenesis, at least for high-protein foodstuffs (Chappell, Bachman & Hammond 1997; Bech & Praesteng 2004). Thirdly, birds may save energy behaviourally during the day by sun-basking or selecting warm microhabitats and at night by using sheltered roosts (Buttemer 1985; Buttemer *et al.* 1987). Fourthly, in species that undergo torpor, the minimum observed T_b, which we used in calculations, may not be the lowest defended T_b in natural conditions, since we did not subject birds to torpor-inducing temperatures for more than a few hours. Our heat budgets did not account for most of these factors, so our cost estimates are probably worst-case scenarios.

We emphasize that while strong physiological constraints on upward dispersal are unlikely, an ability to tolerate highland thermal conditions is only part of the challenge lowland natives face if they move upslope. Higher heat-production costs would probably trade off against investment in critical life-history traits – for example, reducing the energy available for maintenance or reproduction, or requiring increased foraging effort. That is a much more complex issue than thermal biology *per se,* and a robust understanding of these interactions requires extensive knowledge of numerous factors, such as food availability and distribution, foraging efficiency, predation intensity, and reproductive physiology and behaviour.

Three additional points merit discussion. First, are lowland temperatures a barrier to downslope movement? We did not measure heat tolerance but our data speak to this possibility. Exposure to 30–35 °C for several hours during BMR tests did not elicit indications of heat stress in mid- or high-altitude species. After BMR testing, T_b averaged 0·9 °C cooler in highland natives than in lowland natives (suggesting these T_a were not more stressful to highland birds) (Fig. 2d). Test temperatures encompassed essentially all T_a at the lowland site (Fig. 1). While hotter T_e occur in direct sunlight (Fig. 1), shaded refugia are readily available in these forested habitats. Because we perfused metabolism chambers with dry air, evaporative cooling may have been facilitated compared with natural conditions. Chamber humidity during BMR tests averaged 36–50% (from ventilatory and transcutaneous water loss and evaporation from faeces), less than maximal humidity at Pantiacolla. However, daytime T_b is generally 40 °C or higher (Aschoff 1982) and because no Pantiacolla T_a exceeded 33 °C, there was always a gradient for heat loss of ~7 °C (>10 °C more than 99% of the time) and a substantial vapour pressure deficit between T_b and T_a (~2·3 kPa even at 100% RH at 33 °C). Therefore, even assuming that across-altitude physiological differences are genetically fixed, we found no indication that warm lowland temperatures prevent downslope dispersal. Moreover, we measured birds at night, when C is minimal (Aschoff 1981). During the day, when T_a are highest, C and T_b are elevated, so birds lose heat more readily and are better able to tolerate hot conditions (if T_e remains below T_b).

Secondly, our findings apply to adults, but thermal factors may have stronger effects on other life stages. Altitude limits could arise from tolerances of eggs or nestlings, or indirectly through impacts on the energy budgets of parents and offspring. For example, a cool climate may reduce

incubation or body temperatures and hence developmental and growth rates of eggs and young ectothermic nestlings, or require more parental time spent incubating or brooding. Later in development, energy trade-offs from a thermally demanding climate may slow the growth of endothermic nestlings or make it difficult for parents to find sufficient food for both themselves and their offspring.

Thirdly, our 3-year climate sample showed high seasonal stability, but does not exclude the possibility that rare weather events with extreme temperatures may impose stronger selection than the routine thermal milieu. We cannot address this possibility with our data, as the magnitude and duration of such events are difficult to predict, and it is unclear whether species from different altitudes diverge in their abilities to withstand them.

To our knowledge, no previous analysis of altitudinal distributions of tropical birds has integrated physiological data from a large number of species with extensive microclimate records. We found across-altitude differences in several key thermophysiological traits in Andean birds, consistent with thermal specialization as predicted by Janzen (1967). However, with few exceptions among 200+ tested species, we did not find convincing evidence that limits to thermal tolerance –acute or long term – are by themselves sufficient to preclude colonization of non-native altitudes. That said, we believe that thermoregulatory costs, even if not directly limiting, have important interactions with crucial ecological and life-history parameters that together determine altitude ranges.

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Data accessibility

Most data for this manuscript are deposited with the Dryad Digital Repository http://dx.doi.org/10.5061/dryad.52m30 (Londoño *et al.* 2014a,b).

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Appendix S1. Supplementary material.

Appendix S2. Maximum Clade Credibility (MCC) tree, using TreeAnnotator (Rambaut & Drummond, 2013), base on 2000 random trees including 215 species from the Global Phylogeny of Birds (http://birdtree.org/; Stay2MayrParSho Hackett backbone; Jetz *et al*. 2012, Hackett *et al*. 2008).

Fig. S1. Logic flow charts for the algorithms used to select environmental temperature (a) and calculate thermoregulatory costs (b).

Fig. S2. Effect of hypobaric hypoxia on rates of oxygen consumption ($\dot{V}O_2$) at 10 °C in lowland birds.

Table S1. Shade air temperatures (T_a; °C) and temperatures of bird models (gray-painted spheres) exposed to sunlight (T_e; °C) at three field sites during 2012–2014.