



Adaptive variation in the upper limits of avian body temperature

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Edited by Scott McWilliams, University of Rhode Island, Kingston, RI; received September 10, 2021; accepted May 5, 2022 by Editorial Board Member Scott V. Edwards

Physiological performance declines precipitously at high body temperature (T_b), but little attention has been paid to adaptive variation in upper T_b limits among endotherms. We hypothesized that avian maximum tolerable T_b ($T_{b,max}$) has evolved in response to climate, with higher $T_{b,max}$ in species exposed to high environmental heat loads or humidity-related constraints on evaporative heat dissipation. To test this hypothesis, we compared $T_{b,max}$ and related variables among 53 bird species at multiple sites in South Africa with differing maximum air temperature (T_{air}) and humidity using a phylogenetically informed comparative framework. Birds in humid, lowland habitats had comparatively high $T_{b,max}$ (mean \pm SD = 45.60 ± 0.58 °C) and low normothermic T_b ($T_{b,norm}$), with a significantly greater capacity for hyperthermia ($T_{b,max} - T_{b,norm}$ gradient = 5.84 ± 0.77 °C) compared with birds occupying cool montane (4.97 ± 0.99 °C) or hot arid (4.11 ± 0.84 °C) climates. Unexpectedly, $T_{b,max}$ was significantly lower among desert birds (44.65 ± 0.60 °C), a surprising result in light of the functional importance of hyperthermia for water conservation. Our data reveal a macrophysiological pattern and support recent arguments that endotherms have evolved thermal generalization versus specialization analogous to the continuum among ectothermic animals. Specifically, a combination of modest hyperthermia tolerance and efficient evaporative cooling in desert birds is indicative of thermal specialization, whereas greater hyperthermia tolerance and less efficient evaporative cooling among species in humid lowland habitats suggest thermal generalization.

maximum body temperature | heat tolerance limit | adaptive thermoregulation | climatic variation | endotherm

Body temperature (T_b) has pervasive effects on physiological function (1, 2), and performance declines when T_b deviates below or above optimal values, constraining the ranges of environmental temperatures animals can tolerate (3, 4). Among ectotherms, considerable adaptive variation correlated with climate in lower thermal limits contrasts with phylogenetic and geographical conservatism in upper thermal limits [e.g., (3, 5, 6)]. Less attention has focused on the adaptive significance of inter- and intraspecific variation in T_b among endotherms, with hypotheses concerning avian and mammalian physiological adaptation to climate typically tested via comparative analyses of metabolic rate [MR; e.g., (7–9)] or evaporative heat loss [EHL; e.g., (10, 11)]. Historically, endotherm T_b was viewed as a nonadaptive constant (12), and most comparative analyses of avian or mammalian T_b focused on scaling with body size (13–15). However, the last decade has seen increasing interest in adaptive thermoregulation among endotherms, focusing on whether endotherm thermal performance curves show a continuum from thermal generalization to specialization and whether optimality models can predict patterns of T_b (16–18). Several studies have reported patterns of inter- or intraspecific T_b patterns broadly consistent with predictions arising from this conceptual framework (19–22).

Most investigations of adaptive variation in T_b among endotherms have focused on normothermic T_b [$T_{b,norm}$; e.g., (23–25)]. Adaptive variation in maximum tolerable T_b [$T_{b,max}$; the highest T_b reached before rapid declines in performance and broadly analogous to critical thermal maximum in ectotherms (26)], on the other hand, has received almost no attention, likely on account of the technical challenges associated with accurately quantifying gas exchange at very high air temperatures ($T_{air,s}$) while still maintaining low humidity levels in metabolic chambers. Selection favoring high endotherm $T_{b,max}$ might be expected among taxa that regularly experience environmental temperatures approaching or exceeding $T_{b,norm}$ and for which larger thermal safety margins have obvious adaptive value. Selection for hyperthermia tolerance would also be predicted for diurnal taxa occupying hot, arid environments where heat storage is vital for water conservation (27, 28). In addition, pronounced hyperthermia tolerance

Significance

We compared body temperatures (T_b) and the associated thermoregulatory traits of 53 bird species from three climatically distinct areas to test the idea that maximum T_b and hyperthermia tolerance evolve in response to climate-related thermoregulatory demands and constraints. The notion of adaptive variation in T_b among endothermic animals has gained traction recently, but the potential role of climate as a correlate of interspecific variation in upper T_b limits has received little attention. Our finding that both maximum tolerable T_b and normothermic T_b vary significantly among birds occupying sites that vary in humidity and maximum air temperatures provides insights into avian adaptive thermoregulation.

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Author contributions: M.T.F., Z.J.C., and A.E.M. designed research; M.T.F., Z.J.C., and K.S. performed research; M.T.F. and Z.J.C. analyzed data; and M.T.F., Z.J.C., and A.E.M. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission. S.M. is a guest editor invited by the Editorial Board.

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This article contains supporting information online at <http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2116645119/-/DCSupplemental>.

Published June 21, 2022.

likely confers thermal benefits in warm, humid habitats where high atmospheric humidity constrains evaporative heat dissipation (29–32).

Among birds and small mammals, T_bmax ranges from $\sim 38^\circ\text{C}$ to 46°C , with arid-zone birds among the most heat-tolerant taxa in terms of their capacity to maintain T_b at sublethal levels at very high environmental temperatures (33). Avian T_b typically increases above active-phase normothermic values of 39°C to 41°C during heat exposure or following intense activity (34–36). Avian T_bmax values associated with thermoregulatory failure or the loss of coordinated locomotor capacity are typically 42°C to 44°C among nonpasserines and 44°C to 45°C in passerines [reviewed by McKechnie et al. (35)]. Occasionally, however, higher T_bmax has been reported, with Weathers (29) documenting individual T_bmax as high as 47.0°C in a small passerine from the tropical lowlands of Panama, leading him to hypothesize that elevated T_bmax confers adaptive advantages in humid environments where EHL is constrained. More recently, even higher T_bmax (mean for 20 individuals = 48.0°C ; highest individual value = 49.1°C) has been reported for an African passerine that forms vast flocks (37). Overall, however, relatively little is known about interspecific variation in avian T_bmax beyond ~ 60 arid-zone species [e.g., (38–40)].

In light of increasing evidence for adaptive thermoregulation in endotherms, we hypothesized that avian hyperthermia tolerance has evolved in response to climate, with high environmental temperatures or constraints on evaporative cooling selecting for higher T_bmax among inhabitants. To test this hypothesis, we evaluated T_bmax associated with thermal endpoints in 53 species at three sites at similar latitudes, but substantially different climate. We predicted that a) T_bmax is high among arid-zone birds, on account of their evolutionary history of high T_{air} and reduced water access; b) T_bmax is high in birds inhabiting humid lowlands, reflecting constraints imposed by high levels of humidity on evaporative cooling (29); and c) T_bmax is lower among birds occupying mesic montane regions where T_{air} and humidity values are low and where less selection pressure might be expected for hyperthermia tolerance relative to deserts and humid lowlands. To elucidate the ecological significance and physiological mechanisms underlying variation in T_bmax , we also quantified traits related to EHL and metabolic heat production (MHP) at high T_{air} .

1. Results

1.1. T_b . Among the 53 study species, T_bmax ranged from 43.2°C to 48.0°C . The top multivariate model for T_bmax (SI Appendix, Table S5; phylogenetic generalized least-squares regression [PGLS]/GLS: $F_{8,51} = 19.21$, $P < 0.001$, $R^2 = 0.72$) revealed the following significant predictors: climate ($P < 0.001$), heat tolerance limit (HTL; the maximum T_{air} tolerated before the onset of severe hyperthermia; $P < 0.001$), maximum evaporative cooling capacity [MaxEHL/MHP—calculated as maximum EHL/MHP; $P < 0.001$], the slope of T_b as a function of T_{air} above thermoneutrality (T_bslope ; $P < 0.001$), and T_bnorm ($P < 0.001$) as well as the interaction between HTL and climate ($P < 0.001$). The T_bmax of arid-zone birds ($\bar{x} = 44.65 \pm 0.60^\circ\text{C}$) was significantly lower (by $\sim 0.9^\circ\text{C}$) compared with those of birds from the montane ($\bar{x} = 45.42 \pm 0.78^\circ\text{C}$; phylogenetic analysis of variance [PhyANOVA] with post hoc tests: $t = -3.72$, $P < 0.01$; Tukey: honest significant difference [HSD] = -0.77 , $P < 0.001$) and lowland ($\bar{x} = 45.60 \pm 0.58^\circ\text{C}$; PhyANOVA: $t = -4.79$, $P < 0.01$; Tukey: HSD = -0.95 , $P < 0.001$) sites. Montane and lowland birds did

not differ in T_bmax (PhyANOVA: $t = -0.83$, $P = 0.45$; Tukey: HSD = -0.18 , $P = 0.69$) (Fig. 1) in the overall dataset. However, the exclusion of two species with atypically high T_bmax values (*Quelea quelea*, 48.0°C and *Euplectes orix*, 46.4°C) resulted in T_bmax among montane birds ($\bar{x} = 45.18 \pm 0.24^\circ\text{C}$) becoming significantly lower than those of lowland birds (PhyANOVA: $t = -2.36$, $P = 0.02$; Tukey: HSD = -0.42 , $P = 0.56$), but still significantly higher compared with arid-zone birds (PhyANOVA: $t = 3.06$, $P = 0.03$; Tukey: HSD = 0.53 , $P < 0.01$). When included in the multivariate model, body mass (M_b) was not a significant predictor ($t = -1.88$, $P = 0.07$) of T_bmax [model 3.2 (PGLS/GLS: $F_{9,50} = 14.51$, $P < 0.001$, $R^2 = 0.70$); SI Appendix, Table S5] and was auto-correlated with other predictor variables (SI Appendix, Table S7). The rate of increase in T_b (i.e., T_bslope) was significantly higher among lowland birds ($\bar{x} = 0.40 \pm 0.07^\circ\text{C}$) than in arid-zone ($\bar{x} = 0.29 \pm 0.07$, PhyANOVA: $t = 4.07$, $P = 0.003$; Tukey: HSD = 0.11 , $P < 0.001$) and montane birds ($\bar{x} = 0.34 \pm 0.09^\circ\text{C}$, PhyANOVA: $t = 2.26$, $P = 0.02$; Tukey: HSD = 0.06 , $P = 0.07$). No significant difference in T_bslope was detected between montane and arid-zone birds (PhyANOVA: $t = 2.2$, $P = 0.15$; Tukey: HSD = 0.05 , $P = 0.08$; Fig. 2B).

T_bnorm ranged from 38.5°C to 42.2°C . The T_bnorm of lowland birds ($\bar{x} = 39.76 \pm 0.60^\circ\text{C}$) was significantly lower by $\sim 0.7^\circ\text{C}$ than those of arid-zone ($\bar{x} = 40.55 \pm 0.71^\circ\text{C}$; PhyANOVA: $t = -4.27$, $P = 0.03$; Tukey: HSD = -0.79 , $P < 0.001$) and montane birds ($\bar{x} = 40.45 \pm 0.56^\circ\text{C}$; PhyANOVA: $t = -3.31$, $P = 0.003$; Tukey: HSD = -0.69 , $P < 0.001$). Montane and arid-zone birds' T_bnorm did not differ significantly (PhyANOVA: $t = -0.48$, $P = 0.51$; Tukey: HSD = -0.1 , $P = 0.88$). The difference between T_bmax and T_bnorm was significantly larger among lowland birds ($\bar{x} = 5.84 \pm 0.77^\circ\text{C}$) compared with montane ($\bar{x} = 4.97 \pm 0.99^\circ\text{C}$; PhyANOVA:

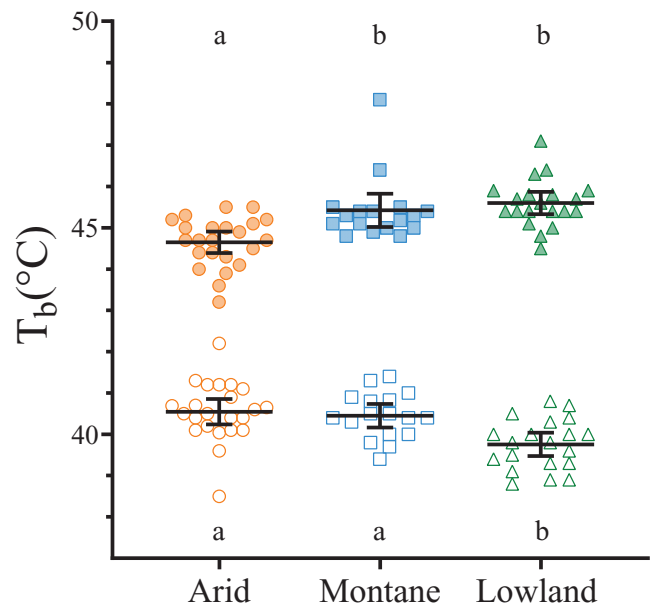


Fig. 1. T_bmax (filled symbols) and T_bnorm (white filled symbols) varied significantly among 53 South African bird species across multiple climatic study sites with differing maximum T_{air} and humidity. Horizontal lines represent mean values and vertical lines 95% CIs. Letters above plots denote significant differences ($\alpha < 0.05$) in T_bmax values between sampling localities; letters at the bottom denote significant differences ($\alpha = 0.05$) in T_bnorm values. Significant differences are derived from phylogenetic ANOVA post hoc multiple comparison assessments and conventional Tukey multiple comparison assessment regressions. Climate categories are hot arid (orange circles, $n = 23$), mesic montane (blue squares, $n = 17$), and humid lowland (green triangles, $n = 20$).

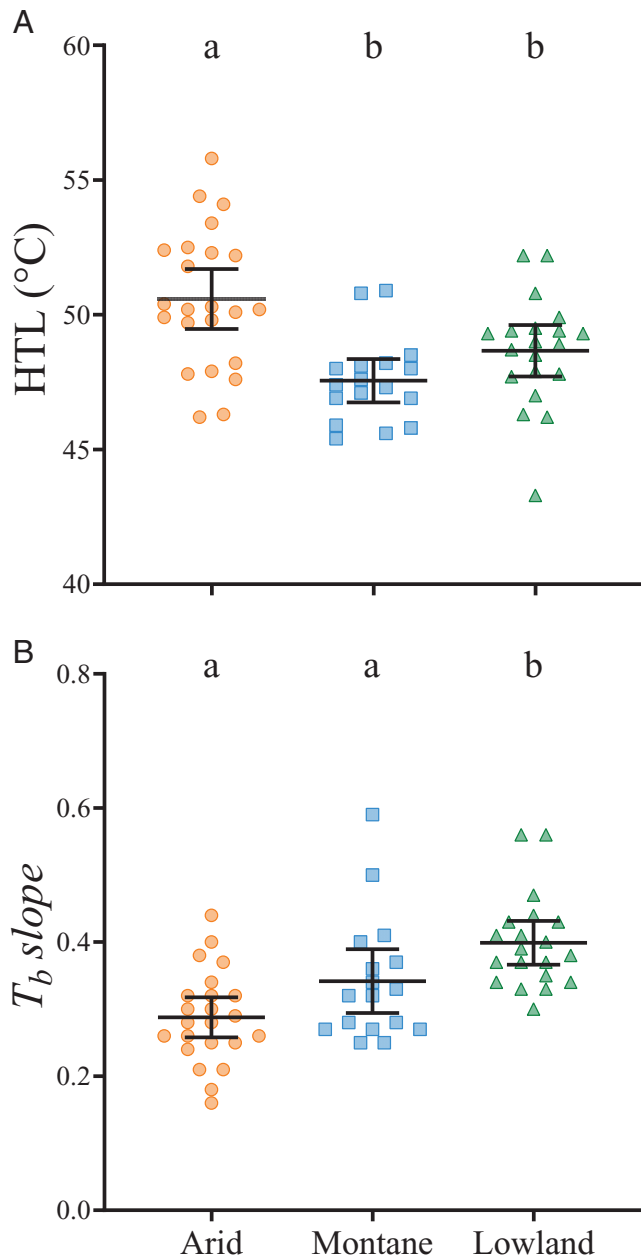


Fig. 2. (A and B) HTLs [i.e., maximum T_{air} tolerated; (A)] and the slope of T_b above the upper critical limit of thermoneutrality [T_b slope; (B)] among 53 South African bird species across a longitudinal gradient in T_{air} and humidity. Horizontal lines represent mean values and vertical lines 95% CIs. Letters above plots denote significant differences ($\alpha = 0.05$) as derived from phylogenetic ANOVA post hoc multiple comparison assessments. Climate categories are hot, arid (orange circles, $n = 23$), mesic montane (blue squares, $n = 17$), and humid lowland (green triangles, $n = 20$).

$t = 0.87$, $P < 0.01$; Tukey: HSD = 0.74, $P < 0.01$) and arid-zone birds ($\bar{x} = 4.11 \pm 0.84$ °C; PhylANOVA: $t = 1.74$, $P < 0.001$; Tukey: HSD = 1.48, $P < 0.01$) and was also significantly higher for montane (PhylANOVA: $t = 0.87$, $P < 0.01$; Tukey: HSD = 0.68, $P = 0.03$) compared with arid-zone birds (Fig. 1).

1.2. HTLs. HTLs ranged from 43.3 °C to 56.0 °C ($\bar{x} = 49.09 \pm 2.47$ °C; Fig. 3A and SI Appendix, Tables S3.1 and S4.1). Regression analysis revealed T_b max was positively correlated with HTL at our montane site (PGLS/GLS: $F_{1,15} = 13.21$, $P < 0.001$, $R^2 = 0.47$), but not at our arid (PGLS: $F_{1,21} = 0.04$,

$P = 0.84$, $R^2 = 0.002$; GLS: $F_{1,21} = 0.03$, $P = 0.86$, $R^2 = 0.01$) or lowland sites (PGLS/GLS: $F_{1,18} = 0.84$, $P = 0.36$, $R^2 = 0.001$), explaining the interaction between HTL and climate (study area) in model 3.3 (SI Appendix, Table S5). After controlling for the effects of M_b , post hoc multiple comparative analysis revealed arid-zone birds ($\bar{x} = 50.59 \pm 2.57$ °C) had significantly higher HTL by ~ 2.5 °C than birds from the montane ($\bar{x} = 47.55 \pm 1.56$ °C; PhylANOVA: $t = 4.59$, $P < 0.003$; Tukey: HSD = 3.06, $P < 0.001$) or lowland ($\bar{x} = 48.67 \pm 2.04$ °C; PhylANOVA: $t = 3.25$, $P = 0.004$; Tukey: HSD = 2.06, $P < 0.001$) sites. No significant difference in HTL occurred between montane and lowland birds (PhylANOVA: $t = -1.45$, $P = 0.16$; Tukey: HSD = -0.99 , $P = 0.32$; Fig. 2A and SI Appendix, Table S6).

1.3. EHL and MHP. Maximum ratios of EHL and MHP were significantly ($\sim 26\%$) higher in arid-zone birds ($\bar{x} = 1.91 \pm 0.25$) compared with montane ($\bar{x} = 1.57 \pm 0.25$; PhylANOVA: $t = 3.03$, $P = 0.003$; Tukey: HSD = 0.34, $P = 0.01$) or lowland birds ($\bar{x} = 1.46 \pm 0.26$; PhylANOVA: $t = 4.3$, $P = 0.003$; Tukey: $t = 0.45$, $P < 0.001$), but did not differ between montane and lowland birds (PhylANOVA: $t = -1.05$, $P = 0.33$; Tukey: HSD = 0.12, $P = 0.55$) (Fig. 3A). Conventional and phylogenetic regression analysis revealed T_b max and maximum EHL/MHP were significantly and negatively correlated among arid-zone birds (PGLS: $F_{1,21} = 4.99$, $P = 0.04$, $R^2 = 0.15$; GLS: $F_{1,21} = 3.77$, $P = 0.07$), but not among montane (PGLS/GLS: $F_{1,15} = 0.55$, $P = 0.47$, $R^2 = 0.04$) or lowland birds (PGLS/GLS: $F_{1,18} = 2.48$, $P = 0.13$, $R^2 = 0.07$; Fig. 3B).

Evaporative scope (EvapScope; maximum evaporative water loss [EWL]/minimum thermoneutral EWL) was not a significant predictor of T_b max in model 3.1 ($t = 1.33$, $P = 0.19$) and increased Akaike information criterion (AIC) values when included in our multivariate regression model (SI Appendix, Table S5). Multiple comparison analysis revealed montane birds ($\bar{x} = 7.86 \pm 1.67$) had significantly ($\sim 21\%$) lower EvapScope than arid-zone ($\bar{x} = 9.07 \pm 2.60$; PhylANOVA: $t = -2.09$, $P = 0.02$; Tukey: HSD = -1.99 , $P = 0.1$) or lowland ($\bar{x} = 10.86 \pm 2.78$; PhylANOVA: $t = -2.67$; $P = 0.01$; Tukey: HSD = -2.62 , $P = 0.03$) birds. There was no significant difference in EvapScope between lowland and arid-zone birds (PhylANOVA: $t = -0.69$, $P = 0.51$; Tukey: HSD = 0.63, $P = 0.77$; Fig. 3C). Multiple comparison analysis of the metabolic cost of evaporative cooling (MetabCost; maximum MR/thermoneutral MR) suggested that lowland birds ($\bar{x} = 1.94 \pm 0.33$) had significantly ($\sim 20\%$) higher MetabCost than arid-zone ($\bar{x} = 1.62 \pm 0.20$; PhylANOVA: $t = 2.09$, $P = 0.02$; Tukey: HSD = 0.38, $P < 0.001$) and montane birds ($\bar{x} = 1.61 \pm 0.24$; PhylANOVA: $t = 1.97$, $P = 0.04$; Tukey: HSD = 0.38, $P < 0.001$). MetabCost did not differ significantly between arid-zone and montane birds (PhylANOVA: $t = 0.26$, $P = 0.77$; Tukey: HSD = 0.01, $P = 0.99$) (Fig. 3D).

2. Discussion

Our data support the hypothesis that avian hyperthermia tolerance has evolved in response to climate. Our prediction of high T_b max in species occupying humid lowlands was confirmed, but the direction of differences between arid and montane species was opposite to what we predicted; arid-zone species had lower T_b max compared with their montane and lowland counterparts. The combination of comparatively high T_b max and low T_b norm in lowland species reveals larger thermal safety margins, permitting greater increases in T_b above normothermic setpoints before the

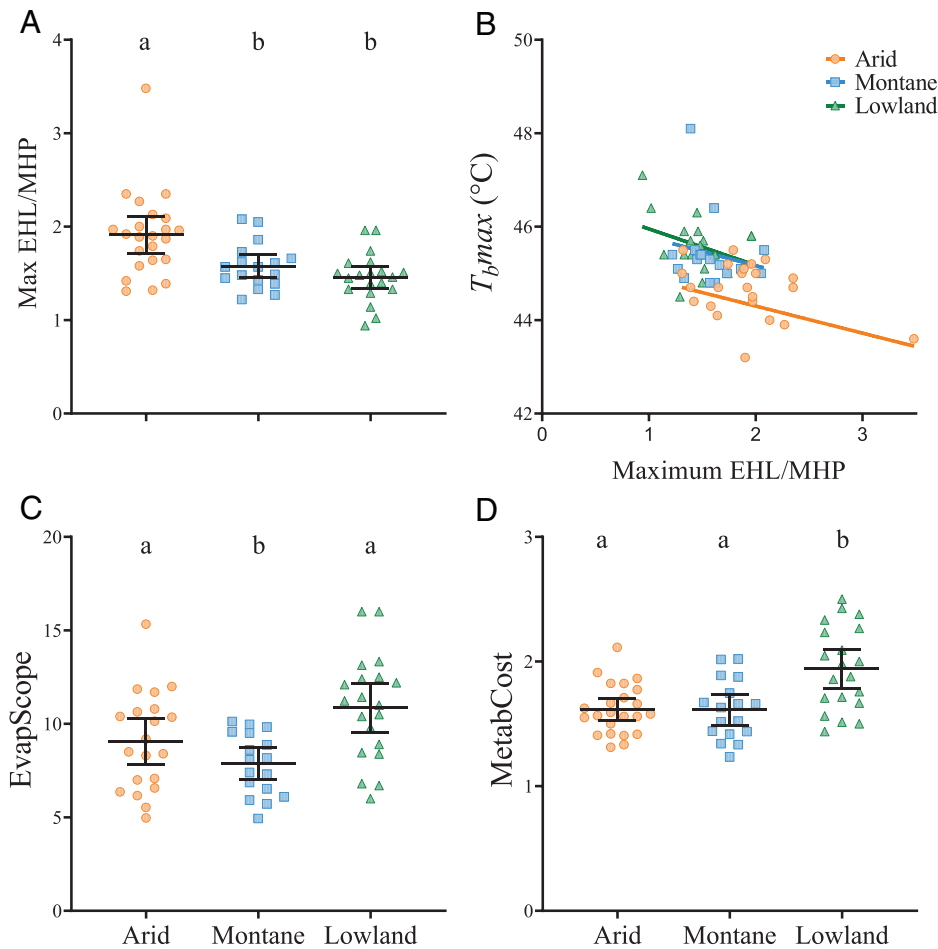


Fig. 3. (A–D) Variation in maximum ratio of EHL and MHP [Max EHL/MHP; (A)], phylogenetic regressions (PGLS) of the relationships between T_bmax and maximum EHL/MHP (B), the ratio of maximum to minimum thermoneutral evaporative water loss [EvapScope; (C)], and the MetabCost calculated as the ratio of maximum to minimum thermoneutral MR [MetabCost; (D)] among 53 South African bird species inhabiting hot arid (orange circles, $n = 23$), mesic montane (blue squares, $n = 17$), or humid lowland (green triangles, $n = 20$) climates. Horizontal lines represent mean values, and vertical lines 95% CIs. Letters above plots denote significant differences ($\alpha = 0.05$) as identified using phylogenetic ANOVA post hoc multiple comparison assessments. Max EHL/MHP was significantly higher among arid-zone birds than among those from montane and lowland localities, whereas EvapScope was significantly higher in lowland and arid-zone birds than in montane birds. MetabCost was significantly higher in lowland birds than in species inhabiting arid or montane climates.

onset of loss of coordinated locomotory capacity and thermoregulatory failure. The climate-correlated variation in T_bmax and T_bnorm and the gradient between these two variables that we report here represents a macrophysiological pattern for endotherms, reflecting broad quantitative differences in interactions between EHL and metabolic costs during acute heat exposure.

The T_bmax values of some lowland and montane birds in this study were unexpectedly high. Although reports of birds tolerating $T_b \geq 46^\circ\text{C}$ without any adverse effects are rare [e.g., (29, 37, 41, 42)] and lethal T_b limits are generally thought to be 46°C to 48°C (43–45), many species from the lowland site had T_bmax in the 45.5°C to 46.0°C range, and five (three lowland, two montane) had $T_bmax > 46^\circ\text{C}$ (Fig. 1). The combination of high T_bmax and low EHL/MHP in lowland species supports Weathers' (29) argument that among species in humid habitats, the capacity for pronounced hyperthermia tolerance plays a major role in thermoregulation during hot weather. However, the similarly high T_bmax in species inhabiting cooler, drier montane areas is puzzling. This finding is at least partly on account of the inclusion of *Q. quelea* and *E. orix* in the montane dataset. Both of these species are widespread habitat generalists and, when excluded from the montane dataset, T_bmax became significantly lower than that of lowland species but remained higher than that of arid-zone species. The atypically high T_bmax

in both these euplectids might be the evolutionary product of selection for dehydration tolerance in individuals foraging in large flocks, rather than historical and current climate (37). Regardless, our data reiterate that tolerance of $T_bmax \geq 48^\circ\text{C}$ by *Q. quelea* (37) is extreme compared with most birds, rivaled only by recent findings of T_bmax up to 48°C in common nighthawk chicks (*Chordeiles minor*) (42).

Our most surprising finding is the comparatively low T_bmax of arid-zone birds, despite them experiencing the highest T_{air} maxima, greatest water scarcity, and thus strong selection for water conservation (27, 46). Moreover, compared with species from the other two sites, arid-zone species had lower T_bmax , yet tolerated significantly higher T_{air} values (Fig. 3). It is difficult to offer an adaptive explanation for low T_bmax . Instead, we suggest it reflects some physiological constraint associated with arid habitats. One possibility is that cellular heat shock responses involving the rapid synthesis of heat shock proteins (HSPs) in response to high temperatures are blunted in desert birds on account of the energetic costs involved [reviewed by (47)]. This notion is indirectly supported by the reductions in HSP expression associated with experimentally limited resource availability in some plants (48–51) and invertebrates (52). The energetic costs of heat shock responses have not, to the best of our knowledge, been directly quantified, but HSP expression

tightly tracking current and past conditions implies that if HSPs were produced cheaply, they could always be generously expressed (53). Comparatively low T_b^{max} among arid-zone birds, despite the adaptive value of hyperthermia for water conservation (27, 28), suggests that extreme ($T_b > 45^\circ\text{C}$) hyperthermia tolerance has substantial costs.

The comparatively low T_b^{norm} of lowland birds was also unexpected. We argue that reduced T_b^{norm} increases the scope for hyperthermia, permitting greater increases in T_b during hot, humid weather (Fig. 1) (29, 30, 32). Our findings support this idea, with the gradient between T_b^{max} and T_b^{norm} among lowland birds being significantly larger than among arid-zone species and montane species by 1.74°C and 0.84°C , respectively. The ability of lowland species to accommodate larger increases in T_b above baseline levels therefore seems to arise from both lower T_b^{norm} and higher T_b^{max} . These differences are also reflected in the more rapid increases in T_b (Fig. 3B, T_b^{slope}) compared with species from montane and arid-zone sites. The variation in thermal physiology among lowland and arid-zone species suggests an avoidance versus tolerance continuum, whereby lowland birds appear to have experienced selection for hyperthermia tolerance, but arid-zone birds evolved hyperthermia avoidance through more efficient evaporative cooling. The higher metabolic costs and consequently lower maximum evaporative cooling efficiency achievable by birds occupying humid, lowland habitats further support the notion of a greater degree of thermal generalization evolving in birds where high humidity constrains heat dissipation. Generally lower activity levels among desert birds, reflected in the $\sim 50\%$ lower daily energy expenditure of desert birds compared with nondesert birds (10), likely also play a crucial role in minimizing heat loads associated with activity and hence the likelihood of T_b increasing far above normothermic levels.

The data included in the present analysis were collected under standardized conditions of very low humidity so as not to impede evaporative heat dissipation, facilitating direct comparisons across taxa. For lowland birds, the efficiency of evaporative cooling would have been higher under the present experimental conditions than under typical summer conditions at our study site (Fig. 1), so it is very unlikely that their higher T_b^{max} values represent an experimental artifact. Avian evaporative cooling efficiency decreases substantially under humid conditions (30, 32, 54), and a recent heat-related avian mortality event in eastern South Africa at $T_{air} = 43^\circ\text{C}$ to 45°C and a water vapor pressure of ~ 1.8 kPa (55) underscores the thermoregulatory challenges faced by birds during hot, humid conditions. It is thus likely that birds inhabiting humid lowlands have to rely on cool microsites provided by closed canopy forests where T_{air} is low relative to surrounding areas (56, 57). Human-induced landscape transformations driving the loss of climatic refugia in coastal forests (58, 59) are therefore a concern in light of recent and predicted future increases in T_{air} (60).

3. Conclusions

The variation in avian thermal physiology at high environmental temperatures and hyperthermia avoidance versus tolerance spectrum that we report here reveal that the upper limits of endotherm T_b have evolved in response to climate. Although we did not quantify thermal performance curves, our data support the existence of a continuum from thermal specialization to thermal generalization among endotherms (16, 17). The combination of reduced capacity for hyperthermia and more efficient evaporative cooling in desert birds versus greater capacity for hyperthermia and reduced evaporative cooling capacity in humid lowland birds

reveals some of the complex ways in which climate can influence endotherm thermal physiology. The counterintuitive finding of comparatively modest hyperthermia tolerance in desert birds compared with birds from more mesic areas raises questions regarding the costs of hyperthermia tolerance and suggests that the historical and recent focus on desert species in the avian thermal physiology literature (27, 35, 44, 61) may have underestimated hyperthermia tolerance among birds in general.

Finally, our findings reiterate that a clearer understanding of adaptive thermoregulation among endotherms is needed for modeling responses to climate change (16). For instance, biophysical models of heat and water exchange (62, 63) need to be parameterized using species-specific upper and lower boundaries to T_b , which, as shown here, vary geographically as well as phylogenetically. The role of adaptive variation in hyperthermia tolerance in determining the nature of behavioral trade-offs between thermoregulation and foraging and the associated missed-opportunity costs (64) also deserves attention, especially as sublethal fitness costs are anticipated to be the major driver of declines among southern African arid-zone birds (65). All else being equal, species that accommodate larger increases in T_b above normothermic levels before the onset of rapid declines in physiological function may be more buffered from behavioral trade-offs during hot weather.

4. Materials and Methods

4.1. Study Areas. We obtained data for bird assemblages occurring in three climatically distinct areas (hot arid, mesic montane, and humid lowland) at latitudes of $S\ 29.25^\circ$ to $S\ 29.25^\circ$ in South Africa (SI Appendix, Fig. S1). We measured T_b^{max} and related variables for birds at mesic montane and humid lowland sites and used published data collected using almost identical experimental protocols for a hot, arid region (38, 39, 66, 67). Climatic data (SI Appendix, Fig. S1) for all study sites were obtained for the period 1970 to 2000 from the WorldClim2 database (68).

Our mesic montane study site was located near the town of Harrismith ($28^\circ 11'S$, $29^\circ 10'E$), Free State province, South Africa. Situated in a mountainous area at the eastern edge of the South African escarpment, two main vegetation types prevail: Basotho montane shrublands on basalt and sandstone mountains and eastern Free State sandy grasslands in valleys (69), although the latter are heavily transformed by agriculture. Mean austral spring/summer (October to March) maximum T_{air} at the site is 26.4°C , with mean annual precipitation of ~ 713 mm (68) (SI Appendix, Fig. S1).

The humid lowland study site was located near the town of Richards Bay ($28^\circ 46'S$, $32^\circ 2'E$), KwaZulu-Natal, South Africa. The area consists of a mosaic of natural grasslands, woodlands, and coastal lowland forest embedded in a matrix of human-modified land use types (59). The climate is humid and subtropical, with a mean spring/summer maximum T_{air} of 28.2°C and mean annual precipitation of $\sim 1,126$ mm (SI Appendix, Fig. S1).

We used published data for arid-zone species investigated at multiple sites in the southern Kalahari [(38) $-27^\circ 04'S$, $21^\circ 23'E$, (67) $26^\circ 58'S$, $21^\circ 50'E$ and (39) $26^\circ 06'S$, $22^\circ 52'E$] and the Koa River valley south of the town of Aggeney, Northern Cape province, South Africa [(39) and (66), $29^\circ 18'S$, $18^\circ 51'E$]. All arid-zone study sites fall within the arid savanna and Nama Karoo biomes. The mean austral spring/summer maximum T_{air} for all three southern Kalahari sites is 34.9°C , with mean annual precipitation of ~ 210 mm (SI Appendix, Fig. S1). At the Aggeney site, mean austral spring/summer maximum T_{air} is 31.0°C and mean annual precipitation ~ 134 mm (Fig. 1).

4.2. Study Species. We measured T_b^{max} and quantified patterns of EHL and MHP at high T_{air} in 346 individuals representing 31 species (some of which occurred at more than one site) at our montane ($n = 16$ species) and lowland ($n = 20$ species) sites during the austral spring/summer of 2019 to 2020. We included published data for *Q. quelea* (37), collected at the same Harrismith study site. Published data from the arid sites included 199 individuals representing 23 species (SI Appendix, Table S1) for which T_b^{max} and all physiological trait

values relevant to this study were measured. M_b of the species included in this analysis ranged from 7 to 110 g and did not differ significantly among study areas. Overall, our analysis is based on 53 species, representing 6 orders [Apodiformes (swifts), Coliiformes (mousebirds), Passeriformes (songbirds), Piciformes (barbets and tinkerbirds), Coraciiformes (bee-eaters and kingfishers), and Cuculiformes (cuckoos)] and 22 families. For seven species, data were collected at multiple study areas, with only one species (*Lanius collaris*) investigated at all three study areas (SI Appendix, Table S1).

4.3. Experimental Protocol and Measured Response Variables. Measurements of gas exchange, T_{air} , and T_b and the experimental protocol involved methods identical to those described by Czenze et al. (39) (SI Appendix for details). These methods and data inclusion criteria were also used in other studies from which we obtained data (38, 66, 67); all data included were collected under standardized conditions. In brief, thermoregulatory responses were assessed using flow-through respirometry. Birds were placed individually in an airtight metabolic chamber fitted with a plastic mesh platform (on which birds could rest) elevated ~10 cm above a ~1-cm layer of mineral oil to prevent evaporation from excreta affecting water vapor pressure readings. An oil-free compressor provided atmospheric air, which was subsequently scrubbed of water vapor using a membrane dryer (ChampionCMD3 air dryer and filter; Champion Pneumatic), while a mass flow controller (Alicat Scientific Inc.) was used to regulate experimental channel flow rates, maintaining low humidity levels within the chamber and standardizing experimental chamber conditions experienced by all birds across study sites.

Measurements took place during the day. Relationships between T_b , EWL, MR, and EHL/MHP over T_{air} s ranging from 28 to 56 °C were quantified by exposing birds to a stepped T_{air} profile involving 4 °C increments between $T_{air} = 28$ and $T_{air} = 40$ °C and 2 °C increments at $T_{air} > 40$ °C. Temperature-sensitive passive integrated transponder tags (Biotherm; Biomark) injected intraperitoneally into each bird were used to measure T_b continuously (every second) and quantify T_bmax values and HTLs. We recorded T_b values using a portable transceiver system (HPR+; Biomark) connected to an antenna placed alongside the metabolic chamber.

Birds were monitored continuously during measurements using an infrared camera and were removed from the chamber only when T_bmax was deemed to have been elicited. Our criteria for T_bmax follow previously used methods (38); these authors identified thermal endpoints as loss of coordination/balance or rapid uncontrolled increases in T_b associated with declines in EHL or MHP. Birds' activity levels were closely monitored during measurements, and only data from calm birds were included in analyses.

This work was approved by the Animal Ethics Committee of the University of Pretoria (protocol NAS181/2019) and the Research and Scientific Ethics Committee of the South African National Biodiversity Institute (SANBI NZG/RES/P19/13). Birds were captured under permit JM 8,057/2019 from the Free State province's Department of Economic, Small Business Development, Tourism and Environmental Affairs and OP 4026/2019 from the Ezemvelo KwaZulu-Natal provincial wildlife authority.

4.4 Data Analyses.

4.4.1. Within-species patterns of thermoregulation. We quantified physiological response variables for each individual and used these to calculate mean values per species. Species sample sizes (n) for most species were $n = 10$ individuals, but for seven species it varied between $n = 6$ and $n = 9$ (SI Appendix, Tables S2.1 and S3.1). All analyses were conducted in R 4.0.5 (R Core Team, 2020). The physiological response variables HTL, T_bnorm , T_bslope , EvapScope, MetabCost, maximum EHL/MHP, and MR were quantified for each individual. Respective inflection T_{air} values above which T_b , EWL, EHL/MHP, and MR increase rapidly were identified using the package *segmented.lme* (70), with individual identity included as a random predictor. We analyzed T_b , EWL, and MR above and below inflection points separately using linear mixed-effect models in the R package *nlme* (71), estimating the slopes for the relationships of thermoregulatory response variables as functions of T_{air} . The "dredge" function in the *MuMIn* package was used to conduct model selection (72). Our initial standardized model included T_{air} (or $T_{air} - T_b$), M_b , and the $T_{air} \cdot M_b$ interaction. M_b did not emerge as a significant ($P > 0.05$) predictor for any response variables of any species and did not improve model fit. It was subsequently excluded from analyses. We selected the model with the highest rank among competing models

using AIC values corrected for small sample size (AIC_c) and Akaike weights (73). If competing models were within $\Delta AIC_c < 2$, we retained the most parsimonious model. We accounted for pseudoreplication by including individual identity as a random factor in all analyses. Significance was assessed at $\alpha < 0.05$, and values are presented as mean \pm SD.

4.4.2. Among-site comparisons. To evaluate the influence of phylogeny on patterns of T_bmax and explanatory physiological variables, we downloaded 100 phylogenies from www.birdtree.org (74), using the Hackett phylogeny as a backbone (75). We constructed a maximum-likelihood tree including all study species using *Mesquite* (76). Branch-length transformations were determined using AIC by comparing an Ornstein-Uhlenbeck model (77) with a Brownian motion model of trait evolution (78). The Ornstein-Uhlenbeck model was retained as it yielded lower AIC scores.

We tested for phylogenetic signal by estimating Pagel's λ (79) in the residual error of our PGLS while simultaneously estimating regression parameters (80) and rescaled our models using the estimates of λ . Significant phylogenetic signal was detected in T_bmax and all related physiological variables when including M_b and locality as predictor variables (SI Appendix, Table S5). We also tested for phylogenetic signal within study areas. Within lowland and montane study sites, phylogenetic signal was not detected (Pagel's $\lambda = 0$), whereas it was for our arid study area (Pagel's $\lambda = 0.637$). The significant phylogenetic signal was driven by the inclusion of six closely related lark (Alaudidae) species (SI Appendix, Table S1). The exclusion of larks from the arid-zone dataset resulted in no phylogenetic signal being detected (Pagel's $\lambda = 0$). We therefore present results from both conventional generalized models (GLS) and post hoc multiple comparison tests (Tukey HSD) as well as PGLS analysis and phylogenetically informed post hoc tests (phyANOVA) where phylogenetic signal occurred. Whereas there were some differences between the results of phylogenetic and conventional analyses, conventional regression and multiple comparison analyses largely confirmed the results of phylogenetic analyses (SI Appendix, Tables S4 and S6). We included M_b when testing for λ to account for the allometric scaling of physiological traits such as basal MR (81, 82) and HTL (83).

The "pgls" function in the R package "*caper*" (84) was used to conduct all regression analyses. To detect differences in T_bmax between study areas and determine significance of physiological variables on patterns of T_bmax , we developed a multivariate additive linear model. We again used the *MuMIn* package and "dredge" function to conduct a model selection procedure (72) using AIC values and weights to identify the model that best explained observed patterns of T_bmax . In conjunction with the model selection approach, we also tested for auto-correlation among predictor variables (SI Appendix, Table S7, Durbin Watson test) and assessed the normality of residual distribution for model outputs using a Shapiro-Wilk test. Four competing models were within $\Delta AIC < 2$ (SI Appendix, Table S5). We selected model 3.3 ($T_bmax \sim$ Climate + HTL + Max-EHL/MHP + T_bslope + T_bnorm + HTL:climate; SI Appendix, Table S5), as it was most parsimonious. This model excluded EvapScope and M_b , but incorporated all other thermoregulatory variables. Since this model included an interaction between study locality and HTL (SI Appendix, Table S5), we investigated each study area separately for the relationship between T_bmax and HTL (PGLS) to unravel the drivers behind the interaction. Residuals for model 3.3 were found to be normally distributed (Shapiro-Wilk normality test: $P = 0.34$).

The *anova.pgls* function in the R-package "*caper*" (84) was applied to our multivariate model output to determine significance of predictor variables and assess whether values of T_bmax differed significantly among study localities (SI Appendix, Table S6). We subsequently conducted a post hoc multiple comparison taking into account phylogenetic relationships using the *PhyANOVA* function in the R package "*phytools*" (85) to obtain pairwise differences in T_bmax as well as predictor variables between study localities. The *PhyANOVA* function conducts a simulation-based phylogenetic ANOVA and performs all post hoc comparisons of means among groups providing a P value by phylogenetic simulation (86).

Data Availability. Digital repository data have been deposited in Dryad and are available at <https://doi.org/10.5061/dryad.pk0p2ngr2> (87).

ACKNOWLEDGMENTS. We thank Philip Pattinson for access to his farm and home, "Mooihoek," in Harrismith and all those who contributed to the fieldwork component of the study, specifically Anna Probert, Nevanya Lubbe, Ryno Kemp, and Barry van Jaarsveld. We also thank Tshepiso Lesedi Majelantle for valuable

discussions around statistical approaches used in this study, Barry Lovegrove and Henry Pollock for assistance with phylogenetic analysis, Mylene Mariette for discussions about the energy cost of heat shock responses, and two anonymous reviewers for constructive comments that improved the quality of the manuscript. This work is based on research supported by the DSI-NRF Centre of

Excellence at the FitzPatrick Institute and the National Research Foundation of South Africa (Grant No. 119754 to A.E.M.). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Research Foundation.

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