

CONTINENT-WIDE PATTERNS OF SONG VARIATION PREDICTED BY CLASSICAL RULES OF BIOGEOGRAPHY

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Abstract

Physiological constraints related to atmospheric temperature pose a limit to body size in endothermic animals. This relationship has been summarized by two classical principles of biogeography: Bergmann's and Allen's rules. Body size may also constrain other phenotypic traits important in ecology, evolution and behaviour, and such effects have seldom been investigated at a continental scale. Through a multilevel-modelling approach, we demonstrate that continent-wide morphology of related African barbets follows predictions of both ecogeographic rules, and that body size mirrors variation in song pitch, an acoustic trait important in species recognition and sexual selection. Bergmann's rule effects on song dwarf those of acoustic adaptation at a continental scale. Our findings suggest that macroecological patterns of body size can influence phenotypic traits important in ecology and evolution, and provide a baseline for further studies on the effects of environmental change on bird song.

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**CONTINENT-WIDE PATTERNS OF SONG VARIATION PREDICTED BY
CLASSICAL RULES OF BIOGEOGRAPHY**

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Data accessibility statement: Data and R code used for analysis are available on Dryad online repository (<https://doi.org/10.5061/dryad.905qftn2>). A small fraction of the acoustic data (85 / 1747 recordings) included in this study is available at Figshare (DOI: 10.6084/m9.figshare.1294832) resulting from Sebastianelli *et al.*, 2021

ABSTRACT

Physiological constraints related to atmospheric temperature pose a limit to body size in endothermic animals. This relationship has been summarized by two classical principles of biogeography: Bergmann's and Allen's rules. Body size may also constrain other phenotypic traits important in ecology, evolution and behaviour, and such effects have seldom been investigated at a continental scale. Through a multilevel-modelling approach, we demonstrate that continent-wide morphology of related African barbets follows predictions of both ecogeographic rules, and that body size mirrors variation in song pitch, an acoustic trait important in species recognition and sexual selection. Bergmann's rule effects on song dwarf those of acoustic adaptation at a continental scale. Our findings suggest that macroecological patterns of body size can influence phenotypic traits important in ecology and evolution, and provide a baseline for further studies on the effects of environmental change on bird song.

INTRODUCTION

Diverse organisms respond analogously to environmental pressures at a global scale, providing evidence that geographical variation in phenotypes arises from natural selection (Guillaumet *et al.* 2005; Kelly 2006; Novembre & Di Rienzo 2009). Thermal homeostasis represents a major constraint in endothermic animals that is critical for survival (McKechnie & Wolf 2010). This is because an organism's activity is constrained by its capacity to exchange heat with the atmosphere (Speakman & Król 2010), a process that is tightly linked with morphology (Mitchell *et al.* 2018).

The relationship between morphology and the physiological need to maintain constant temperature becomes more evident when scrutinising macroecological patterns of variation in body and appendage size. Two major ecogeographical rules have been formulated in an attempt to describe such trends of morphological variation at a global scale: Bergmann's (Bergmann 1847) and Allen's rules (Allen 1877). Bergmann's rule posits that larger body sizes are found at higher latitudes and elevation, because larger bodies facilitate retention of body heat in cooler climates as a result of a smaller body surface area to body volume ratio. Based on similar principles, Allen's rule states that larger appendages (i.e. beaks, tails and unfurred limbs) relative to body size help to exchange body heat with the atmosphere and therefore relatively larger appendages are more advantageous at lower latitudes (and at low altitudes), where temperatures are on average higher, than towards the poles. Both ecogeographic rules have been supported by studies on birds (Ashton 2002; Nudds & Oswald 2007; Olson *et al.* 2009; Romano *et al.* 2020, 2021), mammals (Meiri & Dayan 2003; Alhajeri *et al.* 2020) as well as other taxa (Alho *et al.* 2011; Osorio-Canadas *et al.* 2016; Tseng & Soleimani Pari 2019). However, many exceptions have also been reported (Ashton & Feldman 2003; Gutiérrez-Pinto *et al.* 2014; Freeman 2017;

Nunes *et al.* 2017), possibly because of complex ecological and evolutionary trade-offs between body and limb size and resource availability, predation risk, dispersal ability, resistance to starvation or other environmental factors that affect species richness (Greve *et al.* 2008; Nunes *et al.* 2017; Romano *et al.* 2021).

Although these rules have been widely tested both within (Romano *et al.* 2020, 2021) and between species of terrestrial animals (Ashton 2002; Alhajeri *et al.* 2020), with Bergmann's rule being tested even on marine mammals (Torres-Romero *et al.* 2016) and ectothermic organisms (Berke *et al.* 2013; Campbell *et al.* 2021), it remains unclear whether biogeographically-driven variation in morphology might drive continental-scale variation in behavioural traits important in ecology and evolution. Physiologically constrained variation in body size likely affects animal ecology and as a consequence its evolution. But it might also influence other selected traits through correlational selection (McGlothlin *et al.* 2005). Bird song is a labile trait that mediates key interactions such as mate attraction (Catchpole & Slater 1995), and facilitates assortative mating driven by female choice (Slabbekoorn & Smith 2002a). Because of this, it plays an active role in reproductive isolation between related species, as has been demonstrated both in birds capable of vocal learning (Qvarnström *et al.* 2006; Brambilla *et al.* 2008) and those that develop innate songs (Seddon & Tobias 2007). By consequence, any mechanism that leads to variation in acoustic traits may have implications for speciation (West-Eberhard 1983).

Bird song has been shown to vary because of numerous mechanisms. For instance, there is much evidence of background noise shaping acoustic signals, including from both biotic and abiotic natural sounds (Slabbekoorn & Smith 2002b; Kirschel *et al.* 2009a, 2011; Smith *et al.* 2013; Sebastianelli *et al.* 2021), and anthropogenic noise (Slabbekoorn & Peet 2003; Nemeth & Brumm 2009). Related to this idea is the effect of interactions between closely related species,

which may drive divergent character displacement in vocal signals facilitating species recognition (Kirschel *et al.* 2009b) or even convergent character displacement facilitating competitor recognition (Kirschel *et al.* 2019). Acoustic adaptation to the sound transmission properties of the environment is another well tested hypothesis, albeit with mixed support. Densely vegetated habitats are associated with attenuation of higher frequencies and greater reverberation of sounds (Morton 1975; Wiley & Richards 1982; Slabbekoorn *et al.* 2002) and therefore, as summarised in the sensory drive hypothesis, acoustic signals in dense habitats are predicted to have lower frequencies, slower pace and simpler structure (Seddon 2005; Kirschel *et al.* 2009a; Tobias *et al.* 2010), although a recent analysis on more than 5,000 passerine species found no support for the acoustic adaptation hypothesis in shaping peak song frequency in passerines (Mikula *et al.* 2021). Morphology is also strongly correlated with some acoustic traits. Specifically, there is an indirect relationship between body and beak size with song pitch (Ryan & Brenowitz 1985; Friedman *et al.* 2019; Kirschel *et al.* 2020c; Uribarri *et al.* 2020) and pace respectively (Podos 2001; Derryberry *et al.* 2012). This is because body size is associated with the size of the syrinx membrane and by consequence to the fundamental frequency of song (Ryan & Brenowitz 1985). Similarly, due to biomechanical constraints, larger beaks are associated with slower-paced songs (Podos 2001; Derryberry *et al.* 2012), but are also associated with lower frequencies, because the suprasyrinx trait, which includes the trachea, glottis and buccal cavity, has resonating properties that affect song frequency (Nowicki 1987; Palacios & Tubaro 2000). Lastly, although body mass, background noise and the sensory drive hypothesis might predict song patterns across space, such trends might be offset by the ability to learn songs from conspecific individuals by means of auditory feedback – i.e. vocal learning (Jarvis 2004; Nowicki & Searcy 2014). Indeed, vocal learning can lead to the development and fixation of new

dialects in relatively short periods of time (Mennill *et al.* 2018; Logue & Leca 2020), an ability that has been attributed almost exclusively to oscine passerines, parrots and hummingbirds (Catchpole & Slater 1995; Mennill *et al.* 2018).

Although there is some mixed support for the role of transmission properties in shaping acoustic signals (Ey & Fischer 2009), and proximity to certain noise profiles might lead to predictable signal structure patterns (Kirschel *et al.* 2011, Smith *et al.* 2013), no study has examined whether broad biogeographical rules might underpin continent-wide variation in acoustic signals. Here, we assess the extent to which continental-scale variation in the body size, beak size, and acoustic signals of four related species of bird whose songs develop innately follow predictions of Bergmann's and Allen's rules. Using a mixed-modelling approach, we investigated this question using data collected both from museum specimens and during 17 years of fieldwork across Sub-Saharan Africa. First, we tested whether body and beak size varied with latitude and elevation as predicted by Bergmann's and Allen's rules, incorporating environmental remote sensing data in our models to account for potential effects of niche specialization. We then tested the extent to which variation in the peak frequency of song varied across the continent in a predictable way following Bergmann's and Allen's rules or in accordance with the acoustic adaptation hypothesis (AAH).

MATERIAL AND METHODS

Study taxa

Tinkerbirds (*Pogoniulus* spp.) are small African barbets (Family: Lybiidae) distributed throughout Sub-Saharan Africa (Short and Horne 2001). Four species are included in our analysis: *P. bilineatus* and *P. subsulphureus*, which inhabit mostly tropical and subtropical

forests, and *P. chrysoconus* and *P. pusillus*, which primarily occupy woodland savanna (Short & Horne 2002). The distribution of the study species covers a gradient of vegetation density, thus enabling us to test the effects of vegetation structure on tinkerbird vocalization (i.e. sensory drive hypothesis). *Pogoniulus* tinkerbirds are Piciformes and their songs are innate (Kirschel *et al.* 2009b, Lukhele *et al.* unpublished ms). Their song is therefore unlikely to vary geographically by means of cultural learning, even though it might vary at a local scale as a consequence of specific adaptive pressures (Sebastianelli *et al.* 2021) or interactions with conspecifics (Kirschel *et al.* 2009b, 2019, 2020b).

Morphometric data

We obtained continent-wide morphometric data (Fig.1A) from 678 tinkerbird specimens (*P. bilineatus*: 329; *P. chrysoconus*: 144; *P. pusillus*: 121 and *P. subsulphureus*: 84) most of which were measured for previous studies (Nwankwo *et al.* 2018; Kirschel *et al.* 2020b, 2021), from the ornithology collections of the American Museum of Natural History, Natural History Museum of Los Angeles, Louisiana State University Museum of Natural Science, National Museum of Natural History, Yale Peabody Museum of Natural History, Donald R. Dickey bird collection at UCLA and the British Natural History Museum. In parallel with our morphometric measurements from museum study skins, we also took biometrics of 669 tinkerbirds of the four species that were ringed between 2004 and 2021 during extensive fieldwork across the African continent (*P. bilineatus*: 135; *P. chrysoconus*: 215; *P. pusillus*: 270 and *P. subsulphureus*: 49) (see Kirschel *et al.* 2009b; Nwankwo *et al.* 2019) (Fig. 1B). *P. chrysoconus* and *P. pusillus* hybridise extensively in contact zones where many of our field measurements were obtained (Nwankwo *et al.* 2019; Kirschel *et al.* 2020a), and many individuals thus had mixed ancestry of varying proportions, meaning that they could not reliably be assigned to either species.

Furthermore, the monophyly of each currently recognised species has recently been brought into question (Kirschel *et al.* 2021). We thus pooled all data from these two species into a single taxon, hereafter *chrysopus*, for statistical analysis, and for consistency used the same approach for the museum data. From museum specimens, we measured wing, tarsus, tail and beak length (exposed maxilla); from the field we took those same measurements as well as body mass using a pesola or digital scale. Although museum specimens were all measured by a single observer (ANGK), field biometrics were taken by five observers following standard protocols (Kirschel *et al.* 2009b; Nwankwo *et al.* 2019). As a result, some observer bias is inevitable, and we assessed potential observer bias in our measurements using a two-step approach (see Supplemental Methods). The process resulted in the removal of some outliers, with a final field biometrics dataset of 655 observations remaining.

[FIG. 1]

Acoustic data and song analysis

A total of 1582 tinkerbird songs were recorded (Fig. 1C) between 2004 and 2021 (*P. bilineatus*: 379, *P. chrysoconus*: 385, *P. pusillus*: 532 and *P. subsulphureus*: 286, as identified in the field based on plumage) as part of studies focusing on specific regions or species (Kirschel *et al.* 2009b, 2020b, 2021; Nwankwo *et al.* 2018; Sebastianelli *et al.* 2021) using a Sennheiser microphone (ME67, ME88, MKH 8020 with Telinga parabola, MKH 8050 or MKH 8070) attached to a Marantz PMD 670 or 661 digital solid-state recorders or Sony TCD5M cassette recorder and saved as 16-bit, 44.1 or 48 kHz WAV files. We added a further 165 recordings from online digital repositories (i.e. <http://www.xeno-canto.org>) and from private collections. We acknowledge that compiling recordings from different sources involves different observers, devices and file format, thus raising the possibility that our measurements are biased. However, a

recent study has shown that sound compression (e. g. MP3 format) does not affect commonly used acoustic measurements, even though it can have an effect on extreme value parameters (Araya-Salas *et al.* 2019). We avoided potential pitfalls in measurement bias by following recommendations therein. Furthermore, the use of different recording equipment is expected to affect attenuation of high and low frequency but not mean peak frequency, with several recent studies analysing peak frequency differences at continental or global scales using sound recordings from an assortment of sources, recording equipment and file compression formats (e.g. Kirschel *et al.* 2019; Mikula *et al.* 2021).

Because *P. chrysoconus* and *P. pusillus* hybridize where they meet, species identification based on plumage phenotype might not reflect the genotype of individual birds, and their songs are also very similar (see Fig. 1D) (Kirschel *et al.* 2021), even described by some as indistinguishable (Short & Horne 2001, 2002). As for morphology, we thus treat *P. chrysoconus* and *P. pusillus* as a single taxon (i.e. *chrysopus*) in all our statistical models on song pitch as well.

Field recordings of *P. bilineatus* and *P. subsulphureus* were measured previously using a protocol described therein for those species (Kirschel *et al.* 2009b; Nwankwo *et al.* 2018). Briefly, peak frequency was calculated from several song bouts per individual using power spectra for each 1-3 s song bout, produced using a Fast Fourier transformation of 4096 with a 10.8 Hz frequency resolution in Raven Pro v.1.3 – 1.6 (Center for Conservation Bioacoustics 2019). The recordings attributed to *P. chrysoconus* and *P. pusillus* based on forecrown plumage, as well as those from other sources for *P. bilineatus* and *P. subsulphureus*, were imported into Raven Pro 1.6 and individual notes detected using the built-in band limited automated energy detector (BLED), which detects sounds when the amplitude in a specified band of frequency

exceeds a specific signal to noise ratio of background noise (in dB). Note detections were visually inspected for false positives (e.g. anthropogenic or natural sounds with similar structure) and modified where necessary to match the actual begin and end time of each note. During this process, we used the waveform view as a reference to avoid potential bias associated with visual interpretation of the spectrogram. The latter approach was also used to annotate notes that were missed by the BLED. For these measurements, we set the spectrogram view as in Sebastianelli *et al.* (2021) to obtain a high-frequency resolution for spectral measurements (Discrete Fourier Transform (DFT) size: 65,536) using Hann windows and 50% overlap. We then extracted peak frequency from each detection. Although we used different approaches to measure tinkerbird songs, differences in frequency resolution (e.g. different spectrogram settings between recent and historical measurements) are negligible and unlikely to affect model inference.

Environmental remote sensing data

We used ArcMap 10.7 (ESRI 2011) to extract environmental remote sensing data to assess the potential effect of vegetation structure on bird song (i.e. sensory drive hypothesis) and body size (i.e. niche adaptation). From the Moderate-Resolution Imaging Spectroradiometer (MODIS), we obtained enhanced vegetation index (EVI), normalized difference vegetation index (NDVI) (MODIS/Terra Vegetation Indices 16-Day L3 Global 250m Grid V061), leaf area index (LAI) (MODIS/Terra Leaf Area Index/FPAR 8-Day L4 Global 500m Grid V061) and percent tree cover (VCF) (MODIS/Terra Vegetation Continuous Fields Yearly L3 Global 250m Grid V006). Elevation data were obtained at 30 m resolution (1-arc second) from the global Shuttle Radar Topography Mission (SRTM) (Farr *et al.* 2007).

Statistical analysis

We tested our hypotheses using generalized linear mixed models (GLMMs) implemented in the *glmmTMB* (Brooks *et al.* 2017) package in R (v. 4.0.5.; R Core Team 2020). To test for variation in body size as predicted by Bergmann's rule, we first conducted principal component analyses (PCA) separately on the morphological measurements collected from live individuals in the field and from museum study skins. In the PCA analysis, we included body mass, tarsus, tail and wing length for the field collected data, though body mass was unavailable for the museum specimen based PCA. Principal components (PCs) were selected based on eigenvalues > 1 and used as dependent variables in gaussian GLMMs as indicators of body size. Species, absolute latitude, elevation, and the vegetation structure variables (EVI, NDVI, LAI and VCF) were included as fixed factors, with individual bird ID nested in location as random factors to account for differences between individuals and among populations, and observer as a crossed random effect to account for possible observer bias. The observer's random effect was excluded from the models with museum data, since all study skins were measured by one observer (ANGK). Because many museum specimens were collected before 1960 (the earliest date MODIS data are available), we did not use remote sensing data as fixed factors in the models on museum morphometrics. To test for Allen's rule, we included an allometric scaling measurement (log-transformed) as response variable in our GLMMs. We used beak length in relation to wing length (hereafter beak ratio), as beaks are known to play an important role in bird thermoregulation (Tattersall *et al.* 2009; Ryding *et al.* 2021) and wing length reliably represents body size (Salewski & Watt 2017; Romano *et al.* 2021). Notwithstanding, because it has been recently suggested that wing length might vary in response to changing climate in some tropical birds (Jirinec *et al.* 2021), we also tested the beak length:body mass ratio on our field-based biometrics (Table S1). The models included the above-mentioned covariates plus observer as

fixed factors as well as bird ID nested in location as random factor. We then tested for the effects of absolute latitude, elevation and the remotely sensed environment variables on peak song frequency (log-transformed) in each species separately, again with individual bird ID nested in location included as random factors.

We selected our best fit models via backward selection from full models and selecting different combinations of fixed and random factors until the lowest corrected Akaike Information Criterion score was obtained (Mazerolle 2019). All continuous response variables were standardized to aid model comparison (Schielzeth 2010) and collinearity among spatial variables (i.e. latitude) and the other covariates checked (Zuur *et al.* 2010). Model assumptions were also checked and fit confirmed using the *DHARMA* (Harting 2019) and *performance* (Lüdecke *et al.* 2021) packages in R. To visualise model effects, we used the *effects* package (Fox & Weisberg 2018).

To obtain a measure of the relative effect of each factor, we computed the proportion of variance explained by both fixed and random factors (conditional R^2) and estimated the relative contribution of each predictor to the total variance of the best fit models. This was achieved with an analysis of the relative importance and partitioning of variation of the continuous covariates. Following Essl *et al.* (2019), we fitted linear mixed-effect models (LMMs) that were equivalent to our initial GLMMs. We then constructed linear models including all fixed effects of the GLMMs after deducting the contribution of the random effects from the response variable. Subsequently, we assessed the relative importance of the continuous predictors by computing an R^2 partitioning by averaging over orders (Lindeman *et al.* 1980) using the *relaimpo* package in R (Grömping 2006). To determine whether the greatest effects attributed to latitude were reflected

either side of the Equator, we also visualised the respective raw data against latitude (see Supplemental Methods).

Detecting phylogenetic signal

Phylogenetic relationships affect trait variation. For instance, traits of closely related species tend to have more similar trait values than those of distantly related lineages. To account for this similarity (i.e. phylogenetic signal), we first downloaded the phylogenetic tree (<http://www.birdtree.org>) that better reflected the relationships among our study taxa based on previously published phylogenies (Kirschel *et al.* 2018, 2020b, 2021; Nwankwo *et al.* 2018, 2019), and then calculated Blomberg's K (Blomberg *et al.* 2003) for peak frequency and each morphological measurement (on both field and museum body measurements separately) using the *picante* package in R (Kembel *et al.* 2010). Values of $K=1$ indicate that variation across the phylogenetic tree equals the one deriving from Brownian motion, whereas values of $K < 1$ or $K > 1$ will indicate that phylogeny predicts less or more trait variation than expected under Brownian motion respectively.

RESULTS

Geographical variation in body and appendage size

Variation in body and appendage size in all tinkerbird taxa closely followed predictions of both Bergmann's (Fig.2A-D) and Allen's rules (Fig.2E-H). PC1 extracted from PCA on both the field and museum collected measurements (negatively associated with body mass and wing length in the field dataset and with tail and wing length in the museum data, Table S2) was significantly negatively related with absolute latitude and elevation, thus demonstrating that the tinkerbirds are smaller towards the equator and at lower elevation, with a stronger effect of latitude.

Strikingly, body size decreased from the southern hemisphere towards the equator and increased going north from there (Fig 1SA-B). We also found a significant difference among taxa, with *P. subsulphureus*, but not *chrysopus*, smaller than *P. bilineatus*.

Beak length:wing length ratio from field collected data was also significantly related to absolute latitude and elevation in the model including all taxa, with longer beaks in relation to wing length at lower latitudes (again the strongest effect) and at lower altitudes. *Chrysopus* had significantly smaller and *P. subsulphureus* a significantly larger beak to wing length ratio than *P. bilineatus* (Table 1c). The best selected model had observer as a fixed factor, and revealed significant differences among observers. The museum collected data corroborate results from field data, with the ratio of beak length:wing length decreasing significantly with both absolute latitude and elevation meaning that beaks were smaller compared to wings further from the equator and at higher elevation. As with the field data, specimens of *P. chrysoconus* and *P. pusillus* (pooled together) had significantly lower, and *P. subsulphureus* a significantly higher, beak:wing ratio than *P. bilineatus* (Table 1d).

[Table 1]

Geographical variation in tinkerbird song

We found that *P. bilineatus* and *P. chrysoconus* with *P. pusillus* combined (*chrysopus*) sing higher frequency songs at lower latitudes, while elevation is negatively correlated with peak frequency in all taxa (Table 2, Fig. 3). The effect of latitude was strongest in *chrysopus* (Table 2), again reflected by a striking pattern of increasing frequency from South Africa northwards towards the equator followed by decreasing frequency towards the northernmost populations in

the Horn of Africa (Fig. 1SC). Taken together, our models show that tinkerbirds sing higher pitched songs at lower elevation, and except for *P. subsulphureus*, at lower latitudes.

Furthermore, the models also show that vegetation structure had an effect in two taxa as well, with EVI negatively associated with song peak frequency in *P. subsulphureus* and *chrysopus*, but the effect size of vegetation was much lower than for latitude and elevation, with the former having the strongest effect on *chrysopus* and the latter on *P. bilineatus* and *P. subsulphureus* song frequencies.

[Table 2]

Phylogenetic signal

We detected no significant phylogenetic signal in song peak frequency ($K = 0.32$, $z = 1.30$, $p = 0.742$). Similarly, we found no phylogenetic signal either from field collected data [beak ratio ($K = 1.43$, $z = -1.273$, $p = 0.259$), mass ($K = 0.46$, $z = 0.35$, $p = 0.742$), tarsus ($K = 0.50$, $z = 0.198$, $p = 0.411$), tail ($K = 0.85$, $z = -0.670$, $p = 0.273$) and wing length ($K = 1.02$, $z = -0.95$, $p = 0.232$)] or museum study skin measurements [beak ratio ($K = 0.61$, $z = -0.20$, $p = 0.583$), wing ($K = 0.70$, $z = -0.43$, $p = 0.584$), tarsus ($K = 0.32$, $z = 1.33$, $p = 0.742$) and tail lengths ($K = 0.61$, $z = -0.13$, $p = 0.611$)], which all exhibited non-significant phylogenetic signals.

[Fig.3]

DISCUSSION

Body size varies across a latitudinal gradient as predicted by Bergmann's rule, and this may underpin continental-scale song frequency variation in tinkerbirds. Consistent with the strong negative relationship between body size and peak frequency found within several taxa (Kirschel *et al.* 2020c; Uribarri *et al.* 2020), tinkerbird song pitch is lower at higher latitudes and elevation,

where birds are on average larger, than at lower latitudes and elevation. Furthermore, vegetation density, as measured by EVI, had a significant negative effect on *P. subsulphureus* and *chrysopus* frequency, providing support for the sensory drive hypothesis in shaping acoustic signals in birds, and especially those with innate songs, though effect sizes were an order of magnitude lower than those predicted by Bergmann's rule.

We also found a pattern of relatively smaller tinkerbird beaks at higher latitudes and higher elevation. In other words, tinkerbirds have longer beaks relative to body size toward the tropics and at lower elevation. This provides evidence for variation in beak size according to Allen's rule in tinkerbirds. Nowicki (1987) proposed that the suprasyringeal vocal tract in birds has resonating properties: the longer the tract, which includes the buccal cavity, the lower its resonating frequency. However, in contrast to Allen's rule predictions, we observed that peak frequency was higher towards the equator and at lower elevation, where the vocal tract is relatively longer. Predictions according to Bergmann's rule thus have a stronger effect than those of Allen's rule on song pitch. Furthermore, tinkerbirds do not open their beaks when they sing (ANGK, MS, SL *personal observation*), and instead project their tonal pulses by producing airflow in an expiratory direction through the syrinx from inflated air sacs, in a mechanism similar to other birds that lack vocal learning, such as doves (Suthers 2004). Beak length may thus not play any role in resonating frequencies in such birds. Instead, peak frequency is likely more intimately related with air sac pressure variation, musculature and hence body size (Beckers *et al.* 2003).

The power of the association between Bergmann's rule and song frequency is especially evident when examining the relative contribution of latitude and elevation in model variance. Latitude plays a major role in determining the pattern of variation of peak frequency in both *P.*

chrysoconus and *P. pusillus* combined, where it explains ~70% of the variance, and *P. bilineatus* (26%) song. Latitude had no significant effect on *P. subsulphureus* song frequency, but this could be a consequence of the much smaller latitudinal range of this species (see also Kirschel *et al.* 2020b), which strictly occurs in Afrotropical rainforest across the equator, with latitudinal patterns in accordance with Bergmann's rule within the tropics not expected (Huston & Wolverton 2011), and especially restricted latitudinal range of our song data (all within 6.83° to -1.00 °), except for three recordings from Angola. On the other hand, elevation had significant effects on the song pitch of all three taxa, with a major effect on *P. bilineatus* (73%) and *P. subsulphureus* (93%), but also on *chrysopus* (23%). Vegetation structure (EVI), by contrast, explains a small, though significant, portion of variance in *chrysopus* (6%) and *P. subsulphureus* (5%) song pitch. Taken together, these results provide compelling evidence of a primary role of latitude and elevation in shaping peak frequency in tinkerbirds, which we suggest is a by-product of body size variation predicted by Bergmann's rule.

Bird song plays a crucial role in bird ecology. Efficient communication is essential in several contexts in a bird's life cycle, ranging from mate attraction, territory defence, synchronization of breeding behaviour and avoidance of predation (Catchpole & Slater 1995; Bradbury & Vehrencamp 2011). Song frequency is one of the more important features of bird song. Given that this acoustic trait is negatively correlated with body size, it could be sexually selected if individuals with lower-pitched songs are perceived as more competitive (Mikula *et al.* 2021). Several studies have supported this assumption, showing that individuals with lower-pitched songs dominate intrasexual territorial contests, are more attractive to females, and have higher fitness (Hardouin *et al.* 2007; Brumm & Goymann 2017; Kirschel *et al.* 2020c).

Therefore, any mechanisms that lead to consistent variation in peak frequency might shape interactions among and within species over time, with potential evolutionary consequences.

Body size is just one of the mechanisms that have been proposed to contribute substantially to the evolution of song pitch in birds. Another is the interaction among related species, and in *P. bilineatus* and *P. subsulphureus* they have led to divergence of song pitch in populations where the two species coexist to facilitate species recognition (Kirschel *et al.* 2009b). Although those species' songs diverge where they coexist, the pattern emerges after controlling for the effect of their relative distributions either side of an elevational gradient, but at a continental scale, the climatic effects predicted by Bergmann's rule outweigh any local community effects. Song pitch has also been widely hypothesised to adapt to the acoustic properties of the environment (Wiley & Richards 1978; Wiley & Richards 1982). Although the hypothesis has been supported across a different range of taxa, including birds (Ey & Fischer 2009), a recent global study has brought this into question (Mikula *et al.* 2021). Yet, we found a significant negative association between EVI - a proxy for vegetation cover and greenness (Fernández-Martínez *et al.* 2015) - and peak frequency in two of our study taxa, suggesting adaptation of song pitch to habitat structure over a continental scale. Given the innate nature of tinkerbird song, our results point towards stronger environmental pressure on vocalization of birds with limited vocal flexibility, as suggested by other studies on birds that lack vocal learning (McCracken & Sheldon 1997; Bertelli & Tubaro 2002). Mikula *et al.* (2021) focused on passerines, the vast majority of which develop songs through cultural learning, and that ability may obscure acoustic adaptation. But those effects can be precluded in this study system, because tinkerbirds are unable to learn and shape the pitch of their songs by means of auditory feedback. Although songs are genetically determined in tinkerbirds, we detected only a weak

non-significant phylogenetic signal in peak frequency, meaning that sister species might not sing at more similar frequencies and instead selection on body size is implicated in driving continent-wide patterns in song pitch.

Predictable patterns in behavioural traits based on biogeographical and ecological predictors can help us predict how such traits may respond to increasing environmental change (Trisos *et al.* 2020). Bergmann's rule defines how endothermic animals may evolve because of climatic differences primarily in temperature. The acoustic adaptation hypothesis posits that frequencies are expected to increase in more open habitats, such as where deforestation reduces vegetation density. Coupling the effects of AAH with those of temperature, we expect global warming to further increase song pitch in these birds in predictable ways. Because tinkerbird songs are innate, continent-wide patterns of variation in their song pitch may serve as a baseline for future studies on the effects of environmental change on the communication signals of birds and other endotherms around the world.

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Table 1. Best fit GLMMs' output showing the effects of absolute latitude, taxon, elevation and observer on a) body PC1 from field collected data, b) body PC1 from museum measurements, c) beak ratio from ringing data and d) beak ratio from museum study skins. In the table, the relative importance of the continuous predictors (Lmg R²) and the proportion of variance explained by both fixed and random factors in each model (conditional R²) are also reported.

	Estimate	Std. Error	<i>z</i>	<i>p</i>	Lmg R ²	Cond. R ²
a) Response						
Field PC1 (63.66%)						0.96
Intercept	1.334	0.152	8.759	<0.001		
Absolute latitude	-0.108	0.007	-13.688	<0.001	0.92	
Taxon: <i>chrysopus</i>	0.131	0.126	1.037	0.3		
Taxon: <i>P. subsulphureus</i>	2.105	0.154	13.663	<0.001		
Elevation	-0.371	0.067	-5.519	<0.001	0.07	
b) Response						
Museum PC1 (68.65%)						0.91
Intercept	0.432	0.070	6.162	<0.001		
Absolute latitude	-0.108	0.006	-16.155	<0.001	0.61	
Taxon: <i>chrysopus</i>	0.021	0.100	0.213	0.831		
Taxon: <i>P. subsulphureus</i>	2.078	0.145	14.301	<0.001		

Elevation	-0.591	0.044	-13.434	<0.001	0.38
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c) Response

Log₁₀-field beak ratio					0.97
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Intercept	-1.491	0.0135	-110.49	<0.001	
Absolute latitude	-0.003	0.0007	-4.70	<0.001	0.61
Taxon: <i>chrysopus</i>	-0.284	0.014	-19.45	<0.001	
Taxon: <i>P. subsulphureus</i>	0.040	0.017	2.34	<0.001	
Elevation	-0.028	0.006	-4.43	<0.001	0.38
Observer 2	0.331	0.018	18.23	<0.001	
Observer 3	-0.067	0.015	-4.32	<0.001	
Observer 4	0.108	0.015	7.09	<0.001	
Observer 5	0.109	0.013	8.05	<0.001	

d) Response

Log₁₀-museum beak ratio					0.47
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Intercept	-1.513	0.004	-367.4	<0.001	
Absolute latitude	-0.001	0.0004	-3.5	<0.001	0.68
Taxon: <i>chrysopus</i>	-0.058	0.006	-9.3	<0.001	
Taxon: <i>P. subsulphureus</i>	0.060	0.008	6.9	<0.001	
Elevation	-0.012	0.002	-4.6	<0.001	0.31

Table 2. Best fit GLMM output on the effect of latitude, vegetation density (EVI) and elevation on song peak frequency (log-transformed) on each separate taxon. The relative importance of the continuous predictors (Lmg R²) and the proportion of variance explained by both fixed and random factors (conditional R²) are also shown.

Response: Log-peak frequency	Estimate	Std. Error	z	p	Lmg R²	Cond. R²
a) <i>P. bilineatus</i>						
Intercept	6.985	0.005	1329.0	<0.001		0.91
Absolute latitude	-0.001	0.0006	-2.9	0.003	0.25	
Elevation	-0.020	0.003	-5.7	<0.001	0.74	
b) <i>chrysopus</i>						
Intercept	7.204	0.005	1398.5	<0.001		0.88
Absolute latitude	-0.003	0.0003	-10.1	<0.001	0.69	
Elevation	-0.021	0.002	-7.5	<0.001	0.23	
EVI	-0.008	0.001	-5.2	<0.001	0.06	
c) <i>P. subsulphureus</i>						
Intercept	7.228	0.010	705.6	<0.001		0.98
Absolute latitude	0.004	0.002	1.5	0.124	0.23	
Elevation	-0.023	0.005	-4.1	<0.001	0.71	
EVI	-0.006	0.003	-2.0	0.043	0.05	