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Spatial and temporal variation in morphology in Australian whistlers and shrike-thrushes: is climate change causing larger appendages?

ISABELLE R. ONLEY[1,*], JANET L. GARDNER[2] and MATTHEW R. E. SYMONDS[1]

[1]*Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Burwood, VIC 3125, Australia*
[2]*Division of Ecology & Evolution, Research School of Biology, The Australian National University, Canberra, ACT 0200, Australia*

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Allen's rule is an ecogeographical pattern whereby the size of appendages of animals increases relative to body size in warmer climates in order to facilitate heat exchange and thermoregulation. Allen's rule predicts that one consequence of a warming climate would be an increase in the relative size of appendages, and evidence from other bird species suggests that this might be occurring. Using measurements from museum specimens, we determined whether spatio-temporal variation in bills and legs of Australian Pachycephalidae species exhibits within-species trends consistent with Allen's rule and increases in temperature attributable to climatic warming. We conducted regression model analyses relating appendage size to spatio-temporal variables, while controlling for body size. The relative bill size in four of the eight species was negatively associated with latitude. Tarsus length showed no significant trends consistent with Allen's rule. No significant increases in appendage size were found over time. Although bill size in some species was positively correlated with warmer temperatures, the evidence was not substantial enough to suggest a morphological response to climatic warming. This study suggests that climate change is not currently driving adaptive change towards larger appendages in these species. We suggest that other adaptive mechanisms might be taking place.

ADDITIONAL KEYWORDS: Allen's rule – bird morphology – climate change – evolutionary ecology – museum specimens – Pachycephalidae – thermoregulation.

INTRODUCTION

Bird bills have many functions, including feeding, defence and communication, while the legs assist with locomotion and foraging (Suthers, 1994; Bennett, 1996; Grant, 1999; Chappell & Kacelnik, 2002; Zeffer *et al.*, 2003; Podos & Nowicki, 2004; Froggatt & Gill, 2016). One of the less appreciated functions of both appendages is their role in thermoregulation, one of the major components of homeostasis. By pumping blood through the bill, heat can be lost to the external environment through radiation, a process which also occurs to a lesser extent through the tarsus (Midtgård, 1984; Nudds & Oswald, 2007; Greenberg *et al.*, 2012a; Tattersall *et al.*, 2017). For example, the toco toucan (*Ramphastos toco*) can dissipate ≤ 400% of its resting heat production to the external environment by controlling blood flow through the bill when it needs to dissipate heat load (Tattersall *et al.*, 2009). Likewise, Steen & Steen (1965) found that herons and gulls increase the amount of metabolic heat lost through the legs as ambient temperature increases, again by control of blood flow to these appendages. Certain behaviours have also been observed that limit heat loss from the bill and legs in cold climates, such as 'back rest' behaviour, where the bill is nestled within the plumage while roosting to provide insulation, and unipedal standing, where one leg is tucked up against the body while standing (Ryeland *et al.*, 2017, 2019).

The thermoregulatory function of these exposed, non-feathered appendages could have consequences for variation in avian morphology over latitudinal

*Corresponding author. E-mail: isabelle.onley@adelaide.edu.au

clines. The link between appendage size and geographical location, known as Allen's rule, states that the appendages of endothermic species tend to increase in size (relative to body size) as the average temperature of their environment increases (Allen, 1877). Underlying this theory is the argument that appendages with larger surface areas have increased capacity for heat dissipation and, therefore, provide a more effective cooling mechanism in hot conditions. Conversely, organisms experiencing colder climates will generally have smaller appendages relative to body size to reduce the loss of body heat to the environment (Tattersall *et al.*, 2012). Allen's rule trends in bird bills and tarsi have been correlated with geographical location and climatic conditions across avian taxa, with larger appendages being found at lower latitudes and elevations (Cartar & Guy Morrison, 2005; Nudds & Oswald, 2007; Symonds & Tattersall 2010; Friedman *et al.*, 2017; Tattersall *et al.* 2017).

Since the industrial revolution, anthropogenic greenhouse gases have been released into the atmosphere at an unprecedented rate. As a result, global temperatures are being driven upward. The most recent report by the Intergovernmental Panel on Climate Change (IPCC) found that combined land and ocean surface temperatures worldwide had demonstrated a linear warming trend of 0.85 °C from 1880 to 2012 (IPCC, 2014), and similar trends have been documented specifically for Australia (Hennessy *et al.*, 2007).

For species impacted by climate change, a number of notable ecological responses have been recorded; these include range shifts and contractions, changes to predator–prey interactions, alterations in phenology (such as the timing of breeding and migration) and changes in body size (McCarty, 2001; Walther *et al.*, 2002; Parmesan, 2006; Gardner *et al.*, 2011). Given the existing morphological trends often observed across climatic and spatial gradients (i.e. evidence for smaller body sizes and larger appendages in hotter climates), it can be hypothesized that rising temperatures over time in recent decades might have given rise to similar patterns on a temporal scale, with birds adapting to warmer climatic conditions by having relatively larger bills and/or longer legs. Evidence for such effects comes from Australian parrots, in which four of five species studied have exhibited 4–10% increases in relative bill size over the past century, concomitant with warming temperatures (Campbell-Tennant *et al.*, 2015). However, that study was unable to rule out the possibility that the observed increases in bill size were driven by alternative ecological pressures, such as changes in habitat or food availability. Consequently, equivalent studies across taxa with different foraging and habitat requirements might help to provide more convincing evidence that climatic warming is driving changes in appendage size.

Here, we examine whether similar predicted trends in bill and leg (tarsus) length occur in a different group of birds, the Australian Pachycephalidae (whistlers and shrike-thrushes). These are small (~20–70 g), predominantly insectivorous birds found throughout Australia in woodland and scrubland environments. Following the same protocols as the previous study in parrots by Campbell-Tennant *et al.* (2015), we use bill, tarsus and body size (wing length) measurements from museum skins of wild birds collected over a period of ~150 years throughout Australia and test for associations between the appendage size and corresponding climate data.

Our study has two main aims: (1) to determine whether geographical variation of bill and tarsus size exists in accordance with Allen's rule, whereby larger appendages, relative to body size, correspond to warmer climates and lower latitudes; and (2) to examine whether rising temperatures in the past 100 years, caused by anthropogenic climate change, are associated with an increase in bill and leg size, relative to body size, consistent with predictions arising from Allen's rule.

MATERIAL AND METHODS

Bird data collection

Measurement data were obtained from adult bird museum skin specimens taken from archive collections at four Australian Museums: the Melbourne Museum, the Australian Museum (Sydney), the South Australian Museum (Adelaide) and the Commonwealth Scientific and Industrial Research Organisation's Australian National Wildlife Collection (Canberra). Measurements focused on the following eight species of the family Pachycephalidae: grey shrike-thrush (*Colluricincla harmonica*; $N = 554$), little shrike-thrush (*Colluricincla megarhyncha*; $N = 164$), rufous whistler (*Pachycephala rufiventris*; $N = 650$), Australian golden whistler (*Pachycephala pectoralis*; $N = 614$), Gilbert's whistler (*Pachycephala inornata*; $N = 115$), grey whistler (*Pachycephala simplex*; $N = 89$), olive whistler (*Pachycephala olivacea*; $N = 108$) and crested shrike-tit (*Falcunculus frontatus*; $N = 184$). The specimens were collected over a period between 1857 and 2013. All specimens collected after 2013 were excluded to prevent confounding effects attributable to shrinkage in bills that may occur within the first couple of years of collection (Summers, 1976; Totterman, 2016). Measurements were conducted in May–July 2017. Juvenile individuals were not included in this analysis.

From each specimen, the following morphological measurements were taken using Digimatic Mitutoyo digital callipers (accuracy ±0.02 mm): (1) length of the bill from the feathering at the base of the upper mandible to the bill tip; (2) width of the bill, by measuring the distance from the posterior edge of the nares (nostrils) on one side to the same on the other; (3) depth of the bill from the posterior edge of the nares on the upper mandible to the base of the lower mandible, at right angles to the tomia (cutting edge of the mandible); and (4) length of the tarsometatarsus from the intertarsal joint to the lower edge of the last undivided scute (scale) before the toes diverge. All measurements were conducted by the same individual (I.R.O.) to provide consistency and eliminate inter-observer error. The bill measurements were then used to estimate bill surface area, using the following formula (derived from the calculation for the surface area of an elliptical cone; Greenberg *et al.*, 2012b):

$$\frac{(\text{depth} + \text{width})}{4} \times \text{length} \times \pi$$

A recent study that assessed measurement repeatability in museum specimens of Meliphagides (honeyeaters, fairy wrens, thornbills and their allies) found that wing and tarsus length measurements were highly repeatable (wing, mean intraclass correlation coefficient > 0.982 across 78 species (N = 6709 specimens); and tarsus, mean intraclass correlation coefficient > 0.956 across 31 species (N = 2062 specimens) (Subasinghe K, Symonds MRE, Gardner JL, unpublished data). Moreover, bill surface area estimated from linear measurements using the formula for surface area of a cone was associated significantly and strongly with bill surface area based on digital computed tomography scans (N = 93 individuals from ten species of passerines).

Wing length was used as an index of structural body size, because a measurement of body weight at the time of collection was not available for most specimens, and wing length has been determined to be the best single linear predictor of body size in passerines (Gosler *et al.*, 1998; Ashton, 2002; but see also Gosler & Harper, 2000). The wing length (flattened wing chord) was recorded using a butt-ended ruler. This measurement was taken from the top of the carpal joint of the folded wing to the tip of the longest primary feather, while holding the wing flat and parallel to the body.

In addition to the morphological measurements for each specimen, metadata were collected from the specimen tags and collection databases on the sex, collection date and location (latitude and longitude; Fig. 1) of the specimens. Where no data were recorded for sex, individuals were classified on the basis of plumage colour, which is sexually dimorphic in the *Pachycephala* species studied (Higgins, 2006).

Climate data

Along with latitude and longitude, a number of spatial variables were calculated for use in the analysis. The elevation of each collection site was determined using the mapping tool GEOPLANER v.2.7 (available at www.geoplaner.com) and Google Earth (Google Earth 7.1.4.1529). The shortest distance from the collection location to the coastline (distance to coast) was also extracted using the Point Distance tool in the program ArcMap (v.10.3), in addition to Google Earth.

Date-specific climate data were extracted for the collection location of each specimen in order to identify associations between morphology and specific climate variables. Historical climate data for the collection location were averaged over the 5 years up to and including the collection year of the specimen (consistent with the previous study by Campbell-Tennant *et al.*, 2015; although similar analyses using both 1 and 30 year averages yielded the same conclusions). The climate variables analysed for each time period were as follows: mean maximum summer (December–February) temperature (in degrees Celsius); mean minimum winter (June–August) temperature (in degrees Celsius); mean annual rainfall (in millimetres); and total annual number of days with thermal maxima > 35 °C. The last variable was included as an indicator of extreme heat stress, because temperatures > 35 °C are considered to incur severe fitness costs (McKechnie & Wolf, 2010). These data were obtained through the Australian Bureau of Meteorology's advanced spatial climate analyses datasets available from 1911 onwards, and therefore, only specimens collected from the years 1915–2013 were used in this second stage of analysis to allow for the generation of 5 year climate averages, necessitating the removal of 540 specimens collected before 1915. The resolution of the climate data was approximately 5 km × 5 km (Jones *et al.*, 2009).

Statistical analysis

All analyses were carried out using R (v.3.4.1). Analyses sought to identify the best predictors of two response variables, bill surface area and tarsus length. To do this, a model averaging approach was taken using Akaike's information criterion (AIC). This approach was implemented using the R packages *MuMIn* (Barton, 2016) and *AICcmodavg* (Mazerolle, 2017). These were used to create a 'dredge' (Barton, 2016) of all possible models combining the predictor variables to produce a ranked table identifying the best models predicting the response variable (where lower AIC scores represented 'better' models; Symonds & Moussalli, 2011). Model averaging was then used to identify the importance of individual predictors,

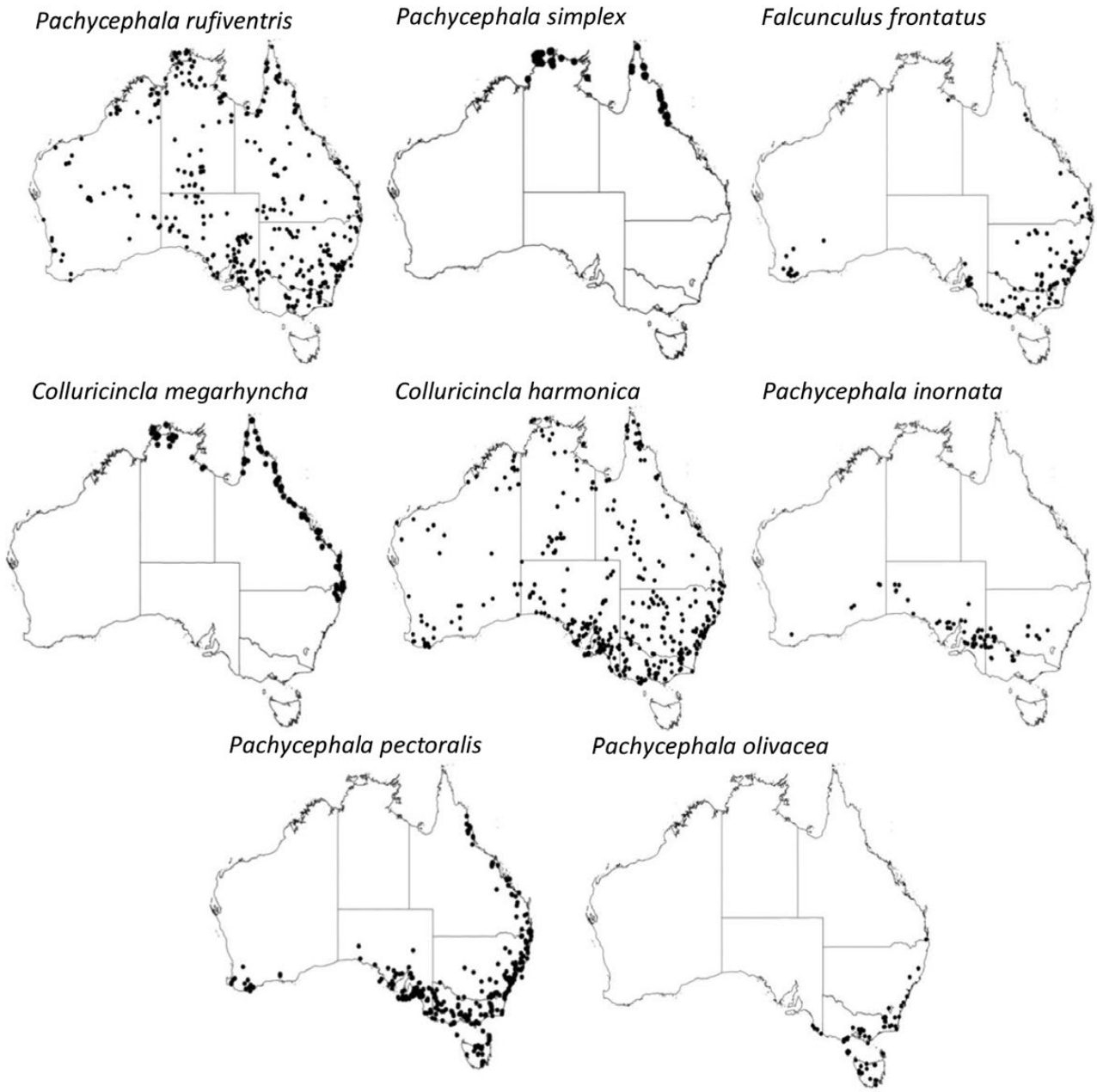

Figure 1. Collection locations of the specimens of each species used in the analysis.

in addition to average parameter estimates with associated 95% confidence intervals. Predictors having a parameter weight of ≥ 0.70 and confidence intervals that did not cross zero were considered to be 'significant'.

Data analysis consisted of two stages, conducted separately for each of the eight species in the study. The first stage involved use of linear regression models to test for an association between the morphological data and the spatial variables. In this analysis, the predictor variables were the spatial variables of latitude (degrees South with minutes expressed as a decimal value), elevation (in metres) and distance from the coast (in kilometres), and the temporal variables of season and year of collection. Although year of collection was the main temporal predictor, season of collection was also included because seasonal variation in bill size is common, owing to factors such as dietary wear and thickening or thinning of the outer sheath, or ramphotheca (Davis, 1954; Tattersall *et al.*, 2017). Finally, as covariates, wing length (as an indicator of body size) and sex were included.

A linear regression model was also run to determine whether a Bergmann's rule trend was occurring, wherein body size is expected to increase with increasing latitude. This model included all predictor variables as above, with sex included as a covariate and wing length as the response variable.

Analyses using samples from a geographical distribution may potentially exhibit spatial autocorrelation, which can confound results (Koenig, 1999). However, statistical approaches for dealing with spatial autocorrelation can be problematic for analysis of geographical clines such as Allen's rule, because there is a of risk 'controlling' for the very pattern one is seeking to discover (Diniz-Filho *et al.*, 2003). Instead, we sought to establish whether the samples were taken evenly from across the geographical distribution of the species. Therefore, in order to account for spatial autocorrelation, before conducting this analysis the location data for each species were inspected against spatial variables to identify geographical outliers that might be overly influential and confound the results. Analysis was then conducted twice, once with outliers included and once with outliers removed. Removal of these outliers did not affect the main conclusions of the analysis; therefore, we report the values using the complete dataset.

To identify any non-linear relationships between appendage size and year, a generalized additive model (GAM) analysis was also conducted using the R package *mgcv* (Wood, 2006), with the same predictor variables as above. The GAM analysis allowed a smoothing function to be fitted to the year of collection term, thereby indicating fluctuations over time. An EDF value was calculated, indicating the effective degrees of freedom for the model terms (Wood, 2006). Correlation tests between latitude and year of collection for each species revealed no significant relationship, with the exception of *P. inornata*. However, given that *P. inornata* encompasses a relatively small geographical range, it is unlikely to affect the analysis.

The second stage of the analysis involved relating the morphological variables to historical climate data (maximum summer temperature, minimum winter temperature, annual rainfall and days > 35 °C; Campbell-Tennant *et al.*, 2015; Gardner *et al.*, 2016). Before undertaking analysis using climatic factors, correlation tests were conducted to determine whether predictors were linked. It was found that maximum summer temperature and minimum winter temperature were highly correlated; therefore, any models containing both of these variables were excluded from AIC analysis. As with the spatial analysis, a model averaging approach was taken to evaluate the relationship of bill size and tarsus length to historical climate data variables (with sex and wing length again included as covariates). Again, a dredge was used for model selection, and model averaging was used to calculate the relative parameter weights, parameter estimates and confidence intervals.

Given that bill size has sometimes been observed to decline at very high temperatures, contrary to Allen's rule (Greenberg & Danner, 2012), we also investigated whether the 'best' climate models were improved by fitting a curvilinear trend and including a quadratic term for environmental temperature (maximum summer temperature, minimum winter temperature or days > 35 °C). In only three of 24 models was the AIC score better with a quadratic term included, and in these cases examination of partial regression plots revealed no obvious decline in bill surface area at high temperature. Consequently, we present only the results for the simpler models.

RESULTS

Analysis of the data using only spatio-temporal variables found that the bill size of four out of eight species (*P. simplex*, *P. pectoralis*, *F. frontatus* and *C. harmonica*) tended to decrease significantly with increasing latitude (degrees south), as predicted by Allen's rule (Table 1; Fig. 2). In these cases, latitude was an important predictor of bill size, having a parameter weight of ≥ 0.95 and confidence intervals on the estimates that did not cross zero. In one further case (*C. megarhyncha*), latitude was an important predictor of relative bill size, but in the opposite direction to that predicted by Allen's rule (i.e. larger appendages at higher latitudes).

The effect of latitudinal gradients on tarsus length was weaker (Table 2); latitude was an important predictor in determining tarsus length of only three out of eight species (*P. rufiventris*, *P. olivacea* and *C. harmonica*), all of which demonstrated a positive relationship between tarsus length and latitude (i.e. larger legs at more southerly latitudes in Australia), contradicting Allen's rule. Elevation had an important influence over bill size in two species, but the trends were not consistent, and elevation was not a strong predictor of tarsus length in any species. Distance from the coast was generally not associated with bill size except for *F. frontatus* (for which smaller bills were associated with greater distance from the coast), but tarsus length and distance from the coast were strongly related in three species: in *P. rufiventris* and *C. megarhyncha* tarsus length increased with distance from coast, whereas in *P. pectoralis* it decreased. Little seasonal variation was found in either bill size or tarsus length, but sexual dimorphism was common;

Table 1. Results of spatio-temporal linear regression analysis of bill size for eight species of Australian shrike-thrushes and whistlers, containing the Akaike parameter weight (Weight), model-averaged estimates (Estimate) and upper and lower 95% confidence intervals (Lower 95% CI/Upper 95% CI)

Predictor	Parameter	*Pachycephala rufiventris*	*Pachycephala simplex*	*Pachycephala olivacea*	*Pachycephala inornata*	*Pachycephala pectoralis*	*Falcunculus frontatus*	*Colluricincla harmonica*	*Colluricincla megarhyncha*
Latitude	Weight	0.47	**1**	0.51	0.37	**1**	**0.95**	**1**	**1**
	Estimate	0.0208135	**–0.589469**	–0.1407	–0.159974	**–0.2227279**	**–0.354196**	**–0.3618450**	**0.284384**
	Lower 95% CI	–0.01074643	**–0.828397730**	–0.333390939	–0.549799555	**–0.271041964**	**–0.6002634**	**–0.432927968**	**0.146779956**
	Upper 95% CI	0.05237346	**–0.350541048**	0.051986688	0.2298511	**–0.1744138015**	**–0.1081281**	**–0.290761994**	**0.421987254**
Year	Weight	**1**	0.36	**0.99**	0.27	0.30	**0.95**	0.42	0.31
	Estimate	**–0.0124873**	–0.007319	**–0.02533**	–0.002064	–0.0016809	**0.035147**	–0.0087587	0.007008
	Lower 95% CI	**–0.01727244**	–0.021345597	**–0.039433558**	–0.014109718	–0.007810038	**0.01074322**	–0.023535999	–0.011150730
	Upper 95% CI	**–0.007702095**	0.006708277	**–0.011452944**	0.009816101	0.0044483121	**0.05955144**	0.006018574	0.025165887
Elevation (m)	Weight	**0.81**	0.49	0.30	0.72	0.41	**0.78**	0.28	0.35
	Estimate	**0.0008726**	–0.002421	–0.00004464	–0.004302	–0.0004301	**–0.003288**	–0.0003961	–0.001620
	Lower 95% CI	**0.00008914247**	–0.005866072	–0.001774583	–0.008764569	–0.001186311	**–0.006304731**	–0.002285318	–0.005076187
	Upper 95% CI	**0.001656127**	0.001023695	0.001685308	0.0001599986	0.0003260376	**–0.0002709643**	0.001493214	0.001836145
Distance from coast (km)	Weight	0.68	0.27	0.48	0.35	0.46	**0.92**	0.28	0.40
	Estimate	–0.0008142	–0.002472	–0.01013	–0.002161	–0.0026481	**–0.009395**	–0.0004925	0.019127
	Lower 95% CI	–0.001675544	–0.067814097	–0.025259662	–0.006567768	–0.006634641	**–0.01632152**	–0.002006806	–0.014849803
	Upper 95% CI	0.00004721919	0.062871044	0.004997839	0.002245712	0.0013384007	**–0.002468936**	0.002991884	0.053102907
Sex	Weight	0.92	0.82	0.34	0.29	0.72	1	0.99	0.28
	Estimate	–	–	–	–	–	–	–	–
	Lower 95% CI	–	–	–	–	–	–	–	–
	Upper 95% CI	–	–	–	–	–	–	–	–
Season	Weight	0.77	0.12	0.53	0.16	0.31	0.21	0.40	0.37
	Estimate	–	–	–	–	–	–	–	–
	Lower 95% CI	–	–	–	–	–	–	–	–
	Upper 95% CI	–	–	–	–	–	–	–	–
Wing length	Weight	**0.99**	0.29	0.35	0.71	0.30	**1**	**0.98**	**1**
	Estimate	**0.1230874**	0.064307	0.09740	0.176195	0.0187115	**0.957257**	**0.2026238**	**0.791477**
	Lower 95% CI	**0.05504413**	–0.155332901	–0.102058863	–0.004097633	–0.067771364	**0.7212777**	**0.075263000**	**0.615077522**
	Upper 95% CI	**0.1911307**	0.283947448	0.296857695	0.3564874	0.1051943219	**1.193436**	**0.329984669**	**0.967876653**
R^2 (%) (global model)		12.69	32.07	17.97	12.5	15.68	53.3	19.69	45.59

Parameters are as follows: year of collection (Year); latitude; elevation; distance from coast; season of collection (Season); sex; and wing length (used as an index of body size). Season and sex are categorical predictors; therefore, only parameter weights are displayed here for these variables. The R^2 values indicate the percentage of variance explained by the global model. Values in bold represent parameter weights with confidence intervals that do not cross zero.

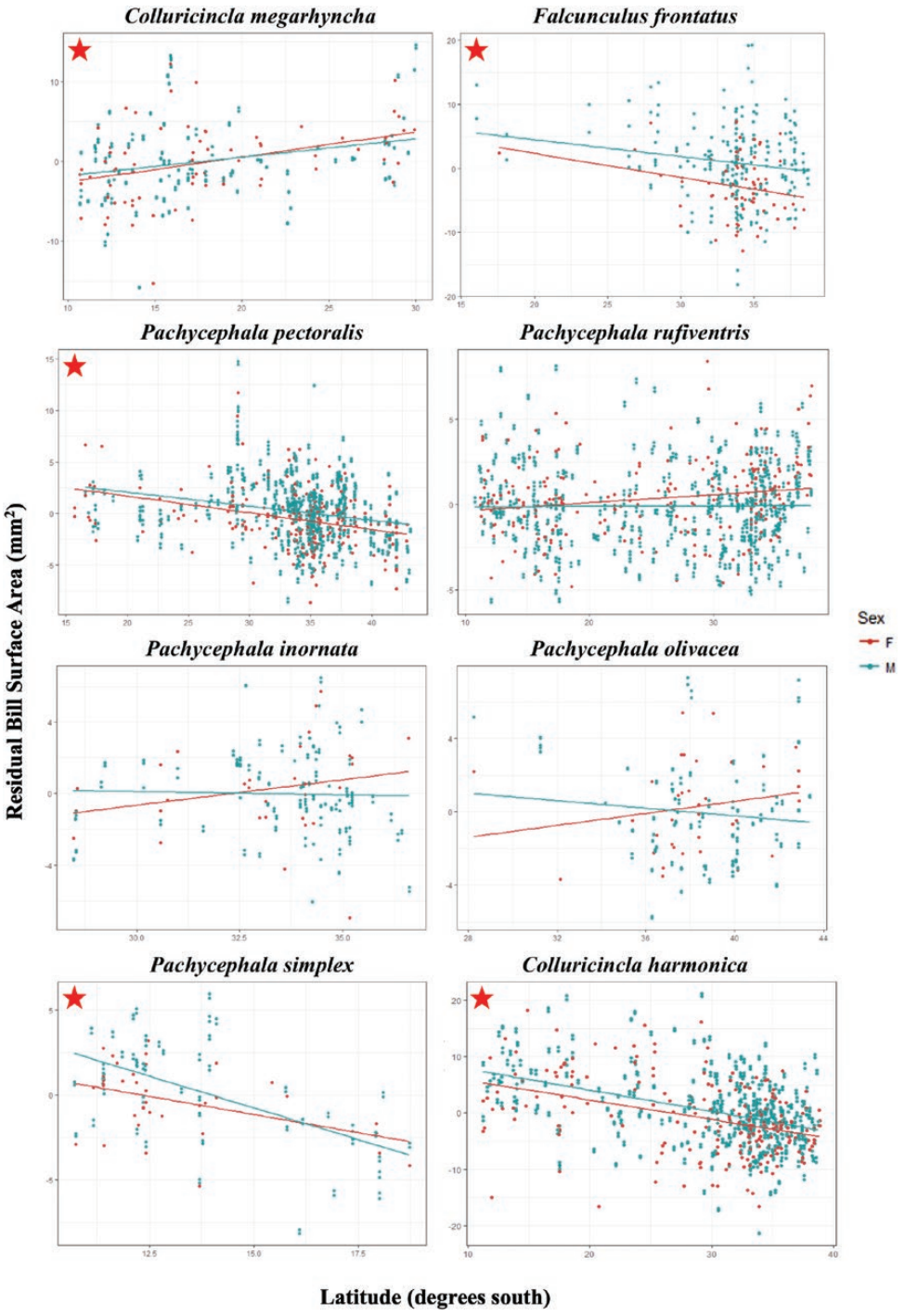

Figure 2. Linear regression relationships between residual bill surface area (in square millimetres) and latitude (degrees south) for Pachycephalidae species. Red stars indicate statistically significant relationships.

five species showed sex as a strong predictor for bill size, and three species for tarsus length (males typically had larger bills than females).

There was no consistent evidence of a trend towards increasing bill size over time. The GAM analysis showed significant temporal variation in bill size over time in a number of species; however, there were no consistent non-linear patterns to indicate that there were specific aspects of climatic variance over time that were consistent across multiple taxa (Fig. 3). The correlation between latitude and year of collection in *P. inornata* did not affect the results.

Table 2. Results of spatio-temporal linear regression analysis of tarsus length for eight species of Australian shrike-thrushes and whistlers, containing Akaike parameter weight (Weight), model-averaged estimates (Estimate) and upper and lower 95% confidence intervals (Lower 95% CI/Upper 95% CI)

Predictor	Parameter	*Pachycephala rufiventris*	*Pachycephala simplex*	*Pachycephala olivacea*	*Pachycephala inornata*	*Pachycephala pectoralis*	*Falcunculus frontatus*	*Colluricincla harmonica*	*Colluricincla megarhyncha*
Latitude	Weight	**1**	0.29	**0.81**	0.28	0.32	0.26	**1**	0.31
	Estimate	**0.01991**	−0.02777	**0.0766064**	0.02079	0.0049269	−0.0005440	**0.02717**	0.01086
	Lower 95% CI	**0.0091451567**	−0.112767471	**0.0076176770**	−0.096393059	−0.0160540329	−0.0372627811	**0.0138090767**	−0.0182480896
	Upper 95% CI	**0.03066696**	0.057021002	**0.1455952**	0.137966823	0.02590778	0.0361748557	**0.0405288109**	0.03997422
Year	Weight	**0.81**	0.26	0.34	0.36	0.57	0.29	0.38	0.28
	Estimate	**0.001888**	−0.001056	0.0023152	−0.002211	−0.0016778	−0.0009947	0.001400	0.001049
	Lower 95% CI	**0.0002458894**	−0.006341177	−0.0028020435	−0.006620310	−0.0037433827	−0.0041498682	−0.0012724545	−0.0028425906
	Upper 95% CI	**0.003529457**	0.004231241	0.007432494	0.002199068	0.0003878637	0.0021603968	0.0040723351	0.004940654
Elevation (m)	Weight	0.27	0.25	0.28	0.26	0.44	0.26	0.31	0.27
	Estimate	0.00003294	0.00008492	0.0001160	0.000006625	−0.0001626	−0.00006888	0.0001245	0.00007571
	Lower 95% CI	−0.0002452875	−0.001134348	−0.0003634858	−0.001646016	−0.00042433827	−0.0005076827	−0.0002279892	−0.0007189223
	Upper 95% CI	0.0003111631	0.001303113	0.0005954173	0.001659267	0.00009960901	0.0003699147	0.0004770751	0.0008703396
Distance from coast (km)	Weight	**1**	0.25	0.26	0.27	0.94	0.29	0.27	**0.92**
	Estimate	**0.001154**	0.001761	0.0001313	−0.0003543	−0.0019286	0.0002973	−0.00006569	**0.009019**
	Lower 95% CI	**0.0008537847**	−0.017389398	−0.0054120163	−0.002062797	−0.0033100035	−0.0007101157	−0.0005376708	**0.0023698456**
	Upper 95% CI	**0.001453912**	0.020916862	0.005674554	0.001354289	−0.0005471357	0.0013048168	0.0004062867	**0.01566814**
Sex	Weight	0.40	0.25	0.30	0.27	0.89	0.96	0.98	0.34
	Estimate	–	–	–	–	–	–	–	–
	Lower 95% CI	–	–	–	–	–	–	–	–
	Upper 95% CI	–	–	–	–	–	–	–	–
Season	Weight	0.97	0.15	0.19	0.09	0.14	0.70	0.33	0.13
	Estimate	–	–	–	–	–	–	–	–
	Lower 95% CI	–	–	–	–	–	–	–	–
	Upper 95% CI	–	–	–	–	–	–	–	–
Wing Length	Weight	**0.98**	0.26	0.58	0.48	**0.91**	0.59	**1**	**1**
	Estimate	**0.03776**	0.008396	0.0594631	0.04758	**0.0332806**	0.03069	**0.06218**	**0.06662**
	Lower 95% CI	**0.0139666493**	−0.062370660	−0.0125737523	−0.020306389	**0.0087623812**	−0.0063715319	**0.0379932669**	**0.0292726223**
	Upper 95% CI	**0.06135792**	0.079162658	0.1314999	0.115470783	**0.05779879**	0.0677550100	**0.0863702474**	**0.1039722**
R^2 (%) (global model)		22.2	5.445	10.41	5.101	3.128	14.17	8.317	14.59

Parameters are as follows: year of collection (Year); latitude; elevation; distance from coast; season of collection (Season); sex; and wing length (used as an index of body size). Season and sex are categorical predictors; therefore, only parameter weights are displayed here for these variables. The R^2 values indicate the percentage of variance explained by the global model. Values in bold represent parameter weights with confidence intervals that do not cross zero.

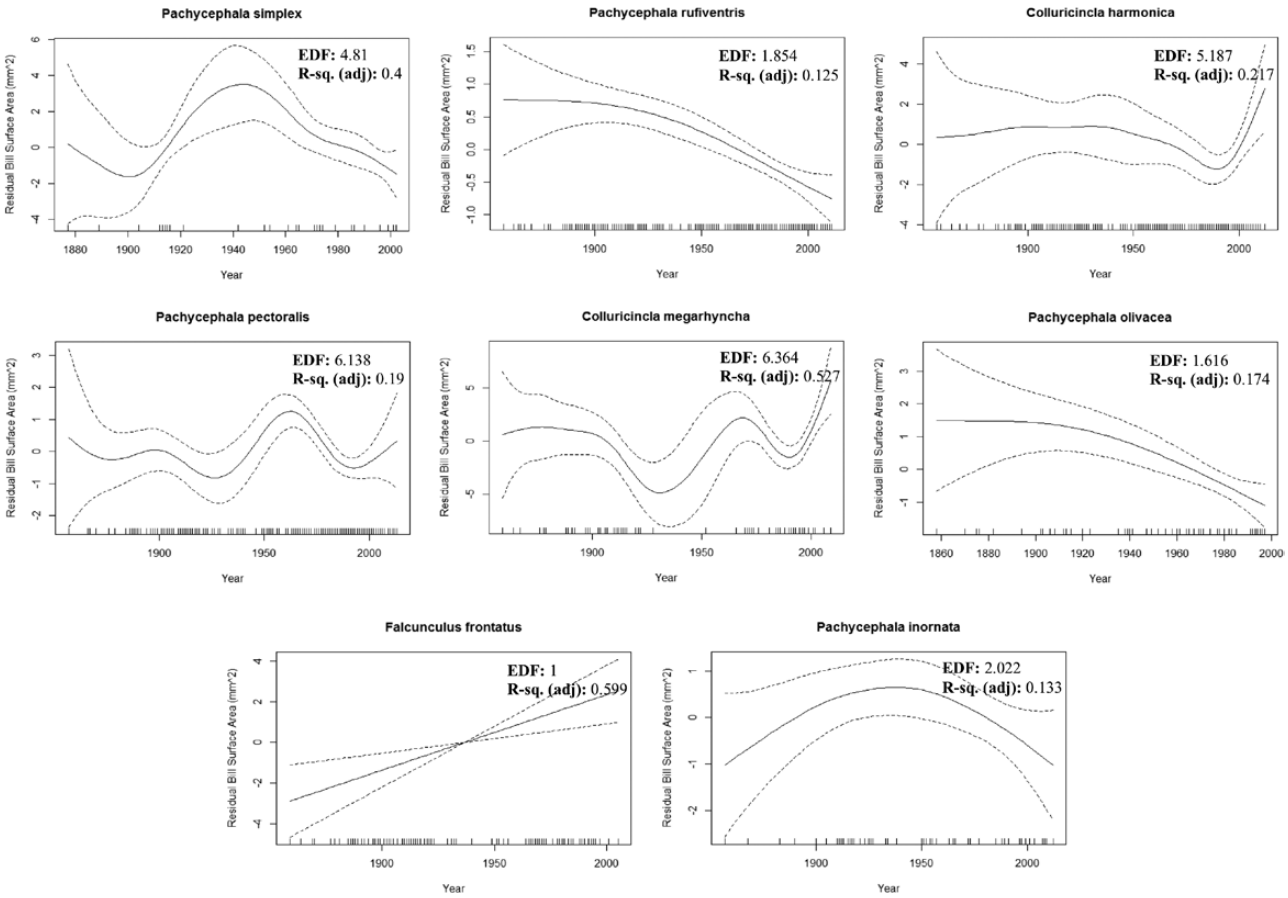

Figure 3. Ggeneralized additive model (GAM) analysis showing the non-linear relationships between residual bill surface area (in square millimetres) and year for each species studied. Dotted lines represent 95% confidence intervals. Effective degrees of freedom (EDF) and R^2 values are also included. An EDF of one is equal to a linear trend, and less than three is near linear (Gardner *et al.*, 2014). For *Falcunculus frontatus*, *Pachycephala olivacea* and *Pachycephala rufiventris*, the relationship is best described as linear (EDF value less than two).

There were no strong consistent patterns relating historical climate data to bill size across each of the species. Four species (*P. pectoralis*, *P. simplex*, *P. rufiventris* and *C. megarhyncha*) showed significant relationships with various climatic factors, but these responses were varied and there were no consistent trends identified across species (Supporting Information, Appendix S1).

Across species, tarsus length appeared to be most strongly predicted by rainfall, although once again the responses were varied (Supporting Information, Appendix S2). The remaining climatic predictors were of little significance to tarsus length overall, with no clear patterns evident between species. Those species that did show strong relationships with temperature did not show any consistent direction to indicate an Allen's rule trend.

A Bergmann's rule trend was observed in four of the eight species studied (*P. rufiventris*, *P. inornata*, *P. pectoralis* and *F. frontatus*; Supporting Information, Appendix S3), with each showing an increase in body size at higher latitudes. A negative correlation between body size and year of collection was also observed in *P. rufiventris*, *P. pectoralis* and *C. harmonica*.

DISCUSSION

Significant negative relationships between bill size and latitude were observed in four of the eight species of Australian whistlers and shrike-thrushes studied: *P. simplex*, *P. pectoralis*, *F. frontatus* and *C. harmonica*. This pattern is consistent with Allen's rule, with relative bill surface decreasing as distance from the equator increases, indicating that, in these species, birds have adapted to colder environments by having smaller bills relative to body size, consistent with the hypothesis that this helps to mitigate heat loss.

Although we did not observe such patterns across all eight species (although two others tended towards an Allen's rule pattern), this proportion is roughly in line with patterns across other bird species, where ~60% of species are estimated to show Allen's rule in bill size (Tattersall *et al.*, 2017).

Conversely, however, no species demonstrated a strong negative relationship between tarsus length and latitude in the present study. This is consistent with the findings of Symonds & Tattersall (2010), who also did not observe strong Allen's rule patterns in tarsus size across bird species (but see Nudds & Oswald, 2007). Symonds & Tattersall (2010) suggested that the absence of an Allen's rule pattern for tarsi might be attributable to physiological differences in the control of heat loss, when compared with the bill. Vascular networks within tarsi are believed to be more well organized (e.g. with countercurrent mechanisms) and are thus more highly adapted for heat retention and loss than those of bird bills (Steen & Steen, 1965; Midtgard, 1984; Symonds & Tattersall, 2010), which might mean that there is less requirement for them to adapt in morphological size to adjust to climatic variation.

Changes in air density at higher altitudes may influence morphology, and increased levels of aridity, changes in habitat structure and feeding preferences relating to distance from the coast may also trigger selection in some species (Recher, 2006; Greenberg *et al.*, 2012; Gutiérrez-Pinto *et al.*, 2014; Campbell-Tennant *et al.*, 2015). We found that elevation and distance from the coast had more influence over tarsus length than latitude, in addition to being strongly related to bill size in a couple of species; however, there were no consistent patterns that would suggest a universal response to these spatial factors.

When we analysed the specific climate-related variation in morphology, we found a mixture of significant correlations between appendage size and temperature, but with no consistent patterns across species. Notably, two of the species that demonstrated clear Allen's rule patterns in their responses to increased temperatures were also among those with the largest geographical range (*Colluricincla harmonica* and *Pachycephala pectoralis*). The wider range of climatic conditions experienced by these species might make it easier to detect intraspecific morphological variation consistent with Allen's rule (Van Valen, 1965; Romano *et al.*, 2020). However, other widely distributed species also showed negative relationships between bill size and increased temperature, a result inconsistent with Allen's rule.

The majority of species with smaller, coastal distributions did not demonstrate a strong relationship between bill size and temperature. This reflects the results of the study of yellow warblers by Luther & Greenberg (2014), who found that those tropical, mangrove-restricted subspecies living in narrow temperature ranges showed no relationship between bill size and ambient temperature, at odds with their inland congeners, which demonstrated a strong Allen's rule trend. For Pachycephalidae species living in narrow ranges, a similar trend might be occurring.

It is interesting that the Allen's rule trends between latitude and bill size observed for four species are supported in the analysis of temperature variables for only two species. This might suggest that the latitudinal Allen's rule patterns have different underlying explanations in the other two species, such as changes in habitat or diet over the range. Additionally, recent analyses of factors underlying patterns in bill size and shape in Australian passerines suggest that there are complex interplays between climate and ecological niche variables that might ultimately obscure direct trends (Gardner *et al.*, 2016; Friedman *et al.*, 2019).

Contrary to the second prediction of the present study, we found no evidence for overall increases in appendage size over the past 160 years that might be explained by climatic warming. For most species, year of collection did not clearly predict the size of either bills or tarsi. Of the three species that demonstrated a significant linear relationship between bill surface area and year (*F. frontatus*, *P. rufiventris* and *P. olivacea*), the last two showed a negative correlation (i.e. decreasing bill size over time). Year had even less influence over tarsus length, with only one species (*P. rufiventris*) demonstrating a strong relationship between tarsus length and year, although here the relationship was positive, consistent with our prediction. Paradoxically, however, relative tarsus length showed a negative relationship with temperature in this species;, therefore, it is unlikely that increases in temperature over time are driving this increase in tarsus length.

There are a number of confounding effects that might explain why temporal trends in appendage size were not evident. First, the increase in average temperature caused by climate change over the last century might not be significant enough to trigger selection in these species. One degree Celsius of warming in Australia (IPCC, 2014) might not be enough to result in heat stress and therefore a need for more efficient thermoregulation, meaning that climate change is having no effect on whistler and shrike-thrush morphology at this stage. A recent analysis of changes in body size in Australian honeyeaters over the past 50 years (Gardner *et al.*, 2019) found that only species that have experienced > 0.011 °C temperature increase per year (1.1 °C per 100 years) show declines in body size as would be predicted by Bergmann's rule and warming climates.

Second, it is possible that any morphological patterns in widespread species, such as *P. rufiventris*, have

been confounded by varying environmental conditions across their geographical range. Individuals living in the tropics, for example, face more intense and frequent rainfall events under climate change than their congeners further from the equator (Chou *et al.*, 2012). This increase in humidity might have implications for changes in appendage size; a recent study by Gardner *et al.* (2016) on the bill morphology of Australian passerines found that bill size was positively correlated with humidity during the summer months. It can therefore be expected that climate change is likely to affect populations of widespread species differently based on their geographical location.

A third possibility is that the increases in temperature caused by climate change are happening too rapidly for selection to act on these species. Many species have already been driven to extinction under climate change, and the apparent lack of adaptive evolution in Australian shrike-thrushes and whistlers might be an early warning sign that these birds are on a similar trajectory. *Pachycephala inornata*, *P. olivacea* and *F. frontatus* are all currently listed as 'Decreasing' under the IUCN Red List of Threatened Species (BirdLife International, 2016).

A fourth possibility is that shrike-thrushes and whistlers in Australia have developed alternative mechanisms for adapting to climate change. A number of responses have already been observed in avifauna, including behavioural thermoregulation, changes in body size and physiology and shifts in range (Lenoir *et al.*, 2010; Cahill *et al.*, 2012; Teplitsky & Millien, 2014), and although decreases in body size and increases in relative appendage size have been observed, the responses of species to climate change might be more variable and complex than those predicted by Allen's and Bergmann's rules (Kingsolver *et al.*, 2012; Teplitsky & Millien, 2014). This wide variation in adaptive responses might offer some explanation for the lack of Allen's rule trend observed over time in the present study.

Finally, it is possible that the variation in responses of bill size over time is attributable to competition over food sources. For example, a mix of selective pressures relating to both competitor species and food supply has prompted rapid evolutionary responses in bill size in Darwin's finches (Grant & Grant, 2006). Further to this, bill size may also be influenced by changes in preferred food sources of species to increase foraging efficiency, as observed by Benkman (1993) and Dehling *et al.* (2014); both studies found that functional traits of bird species were closely related to those of their food sources across geographical and temporal gradients. Similar selection pressures might have influenced the fluctuations in Pachycephalidae bill size over time, which are inconsistent between species.

The factor with the strongest influence on tarsus length appears to be rainfall, although again the responses were varied. This suggests that environmental factors driven by rainfall, such as habitat quality and food availability, might be stronger drivers for morphological changes than temperature and the need to use tarsi as tools for heat exchange (Rubenstein & Lovette, 2007).

Although this study provided evidence of Allen's rule in bird bills along latitudinal gradients in half the species studied, there was no evidence of an Allen's rule trend in response to climate change (i.e. increasing bill or tarsus size over time) to support the findings of Campbell-Tennant *et al.* (2015) for Australian parrots. Similar studies on a variety of Australian bird groups with differing life histories would provide clarification as to whether climate change is resulting in morphological changes in other avian taxa. In addition, further studies on the behavioural and physiological responses of Pachycephalidae to heat stress, and on range shifts and contractions over time, might indicate other adaptive mechanisms by which these species might be accounting for heat stress.

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REFERENCES

Allen JA. 1877. The influence of physical conditions on the genesis of species. *Radical Review* **1:** 108–140.

Ashton KG. 2002. Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography* **11:** 505–523.

Barton K. 2016. *MuMIn: multi-model interference. R package 1.15.6 ed*. Available at: https://CRAN.R-project.org/package=MuMIn

Benkman CW. 1993. Adaptation to single resources and the evolution of crossbill (*Loxia*) diversity. *Ecological Monographs* **63:** 305–325.

Bennett MB. 1996. Allometry of the leg muscles of birds. *Journal of Zoology* **238:** 435–443.

Birdlife International. 2016. *The IUCN red list of threatened species*. Available at: doi.10.2305/IUCN.UK.2016-3.RLTS.T22705437A94018781.en

Cartar RV, Guy Morrison RI. 2005. Metabolic correlates of leg length in breeding arctic shorebirds: the cost of getting high. *Journal of Biogeography* **32:** 377–382.

Cahill AE, Aiello-Lammens ME, Fisher-Reid MC, Hua X, Karanewsky CJ, Ryu HY, Sbeglia GC, Spagnolo F, Waldron JB, Warsi O, Wiens JJ. 2012. How does climate change cause extinction? *Proceedings of the Royal Society B: Biological Sciences* **280:** 20121890.

Campbell-Tennant DJE, Gardner JL, Kearney MR, Symonds MRE. 2015. Climate-related spatial and temporal variation in bill morphology over the past century in Australian parrots. *Journal of Biogeography* **42:** 1163–1175.

Chappell J, Kacelnik A. 2002. Tool selectivity in a non-primate, the New Caledonian crow (*Corvus moneduloides*). *Animal Cognition* **5:** 71–78.

Chou C, Chen C-A, Tan P-H, Chan KT. 2012. Mechanisms for global warming impacts on precipitation frequency and intensity. *Journal of Climate* **25:** 3291–3306.

Davis J. 1954. Seasonal changes in bill length of certain passerine birds. *The Condor* **56:** 142–149.

Dehling DM, Töpfer T, Schaefer HM, Jordano P, Böhning-Gaese K, Schleuning M. 2014. Functional relationships beyond species richness patterns: trait matching in plant–bird mutualisms across scales. *Global Ecology and Biogeography* **23:** 1085–1093.

Diniz-Filho JAF, Bini LM, Hawkins BA. 2003. Spatial autocorrelation and red herrings in geographical ecology. *Global Ecology and Biogeography* **12:** 53–64.

Friedman NR, Harmáčková L, Economo EP, Remeš V. 2017. Smaller beaks for colder winters: Thermoregulation drives beak size evolution in Australasian songbirds. *Evolution* **71:** 2120–2129.

Friedman NR, Miller ET, Ball JR, Kasuga H, Remeš V, Economo EP. 2019. Evolution of a multifunctional trait: shared effects of foraging ecology and thermoregulation on beak morphology, with consequences for song evolution. *Proceedings of the Royal Society B: Biological Sciences* **286:** 20192474.

Froggatt JMA, Gill BJ. 2016. Bill morphology reflects adaptation to a fibrous diet in the kākāpō (*Strigops*: Psittaciformes). *New Zealand Journal of Zoology* **43:** 138–148.

Gardner JL, Amano T, Backwell PRY, Ikin K, Sutherland WJ, Peters A. 2014. Temporal patterns of avian body size reflect linear size responses to broadscale environmental change over the last 50 years. *Journal of Avian Biology* **45:** 529–535.

Gardner JL, Amano T, Peters A, Sutherland WJ, Mackey B, Joseph L, Stein J, Ikin K, Little R, Smith J, Symonds MRE. 2019. Australian songbird bird size tracks climate variation: 82 species over 50 years. *Proceedings of the Royal Society B: Biological Sciences* **286:** 20192258.

Gardner JL, Peters A, Kearney MR, Joseph L, Heinsohn R. 2011. Declining body size: a third universal response to warming? *Trends in Ecology & Evolution* **26:** 285–291.

Gardner JL, Symonds MRE, Joseph L, Ikin K, Stein J, Kruuk LEB. 2016. Spatial variation in avian bill size is associated with humidity in summer among Australian passerines. *Climate Change Responses* **3:** 11.

Gosler AG, Greenwood JJD, Baker JK, Davidson NC. 1998. The field determination of body size and condition in passerines: a report to the British Ringing Committee. *Bird Study* **45:** 92–103.

Gosler AG, Harper DGC. 2000. Assessing the heritability of body condition in birds: a challenge exemplified by the great tit *Parus major* L. (Aves). *Biological Journal of the Linnean Society* **71:** 103–117.

Grant PR. 1999. *Ecology and evolution of Darwin's finches*. Princeton University Press.

Grant PR, Grant BR. 2006. Evolution of character displacement in Darwin's finches. *Science* **313:** 224–226.

Greenberg R, Cadena V, Danner RM, Tattersall GJ. 2012a. Heat loss may explain bill size differences between birds occupying different habitats. *PLoS ONE* **7:** e40933.

Greenberg R, Danner RM. 2012b. The influence of the California marine layer on bill size in a generalist songbird. *Evolution* **66:** 3825–3835.

Gutiérrez-Pinto N, McCraken KG, Alza L, Tubaro P, Kopuchian C, Astie A, Cadena CD. 2014. The validity of ecographical rules is context-dependent: testing for Bergmann's and Allen's rules by latitude and elevation in a widespread Andean duck. *Biological Journal of the Linnean Society* **11:** 850–862.

Hennessy K, Fitzharris B, Bates BC, Harvey N, Howden SM, Hughes L, Salinger J, Warrick R. 2007. Australia and New Zealand. In: Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE, eds. *Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the fourth assessment report of the Intergovernmental Panel on Climate Change.* Cambridge: Cambridge University Press, 507–540.

Higgins P. 2006. *Handbook of Australian, New Zealand and Antarctic birds*. Melbourne: Oxford University Press.

IPCC. 2014. *Climate change 2014: synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change.* Geneva: IPCC.

Jones DA, Wang W, Fawcett R. 2009. High quality spatial climate data-sets for Australia. *Australian Meteorological and Oceanographic Journal* **58:** 233–248.

Kingsolver JG, Diamond SE, Siepielski AM, Carlson SM. 2012. Synthetic analyses of phenotypic selection in natural populations: lessons, limitations and future directions. *Evolutionary Ecology* **26:** 1101–1118.

Koenig WD. 1999. Spatial autocorrelation of ecological phenomena. *Trends in Ecology & Evolution* **14:** 22–26.

Lenoir J, Gégout JC, Guisan A, Vittoz P, Wohlgemuth T, Zimmerman NE, Dullinger S, Pauli H, Willner W, Svenning JC. 2010. Going against the flow: potential mechanisms for downslope range shifts in a warming climate. *Ecography* **33:** 295–303.

Luther D, Greenberg R. 2014. Habitat type and ambient temperature contribute to bill morphology. *Ecology and Evolution* **4:** 699–705.

Mazerolle MJ. 2017. *AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package v.2.1-1*. Available at: https://CRAN.R-project.org/package=AICcmodavg

McCarty JP. 2001. Ecological consequences of recent climate change. *Conservation Biology* **15:** 320–331.

McKechnie AE, **Wolf BO. 2010.** Climate change increases the likelihood of catastrophic avian mortality events during extreme heat waves. *Biology Letters* **6:** 253–256.

Midtgård U. 1984. Blood vessels and the occurrence of arterio-venous anastomoses in cephalic heat loss areas of mallards, *Anas platyrhynchos* (Aves). *Zoomorphology* **104:** 323–335.

Nudds RL, **Oswald SA. 2007.** An interspecific test of Allen's rule: evolutionary implications for endothermic species. *Evolution* **61:** 2839–2848.

Parmesan C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution and Systematics* **37:** 637–669.

Podos J, **Nowicki S. 2004.** Beaks, adaptation, and vocal evolution in Darwin's finches. *Bioscience* **54:** 501–510.

Recher HF. 2006. A hypothesis to explain why the south-western subspecies of the Crested Shrike-tit (*Falcunculus frontatus leucogaster*) is rare and declining. *Emu* **106:** 181–186.

Romano A, **Séchaud R**, **Roulin A. 2020.** Geographical variation in bill size provides evidence for Allen's rule in a cosmopolitan raptor. *Global Ecology and Biogeography* **29:** 65–75.

Rubenstein DR, **Lovette IJ. 2007.** Temporal environmental variability drives the evolution of cooperative breeding in birds. *Current Biology* **17:** 1414–1419.

Ryeland J, **Weston MA**, **Symonds MRE. 2017.** Bill size mediates behavioural thermoregulation in birds. *Functional Ecology* **31:** 885–893.

Ryeland J, **Weston MA**, **Symonds MRE. 2019.** Leg length and temperature determine the use of unipedal roosting in birds. *Journal of Avian Biology* **2019:** e02008.

Steen I, **Steen JB. 1965.** The importance of the legs in the thermoregulation of birds. *Acta physiologica Scandinavica* **63:** 285–291.

Summers RW. 1976. The value of bill lengths of museum specimens in biometric studies. *Wader Study Group Bulletin* **17:** 10–11.

Suthers RA. 1994. Variable asymmetry and resonance in the avian vocal tract: a structural basis for individually distinct vocalizations. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology* **175:** 457–466.

Symonds MRE, **Moussalli A. 2011.** A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behavioural Ecology and Sociobiology* **65:** 13–21.

Symonds MRE, **Tattersall GJ. 2010.** Geographical variation in bill size across bird species provides evidence for Allen's rule. *The American Naturalist* **176:** 188–197.

Tattersall GJ, **Andrade DV**, **Abe AS. 2009.** Heat exchange from the toucan bill reveals a controllable vascular thermal radiator. *Science* **325:** 468–470.

Tattersall GJ, **Arnaout B**, **Symonds MRE. 2017.** The evolution of the avian bill as a thermoregulatory organ. *Biological Reviews of the Cambridge Philosophical Society* **92:** 1630–1656.

Tattersall GJ, **Sinclair BJ**, **Withers PC**, **Fields PA**, **Seebacher F**, **Cooper CE**, **Maloney SK. 2012.** Coping with thermal challenges: physiological adaptations to environmental temperatures. *Comprehensive Physiology* **2:** 2151–2202.

Teplitsky C, **Millien V. 2014.** Climate warming and Bergmann's rule through time: is there any evidence? *Evolutionary Applications* **7:** 156–168.

Totterman SL. 2016. Random measurement error and specimen shrinkage in short-tailed shearwaters *Puffinus tenuirostris*. *Marine Ornithology* **44:** 11–20.

Van Valen L. 1965. Morphological variation and width of ecological niche. *The American Naturalist* **99:** 377–390

Walther GR, **Post E**, **Convey P**, **Menze A**, **Parmesan C**, **Beebee TJC**, **Fromentin JM**, **Hoegh-Guldberg O**, **Bairlein F. 2002.** Ecological responses to recent climate change. *Nature* **416:** 389–395.

Wood SN. 2006. *Generalized additive models: an introduction with R*. Boca Raton: Chapman & Hall/CRC.

Zeffer A, **Johansson LC**, **Marmebro Å. 2003.** Functional correlation between habitat use and leg morphology in birds (Aves). *Biological Journal of the Linnean Society* **79:** 461–484.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Linear regression analysis of bill surface area for eight species of Australian shrike-thrushes and whistlers, containing Akaike parameter weight (Weight), model-averaged estimates (Estimate) and upper and lower 95% confidence intervals (Lower 95% CI/Upper 95% CI).

Appendix S2. Linear regression analysis of tarsus length for eight species of Australian shrike-thrushes and whistlers, containing Akaike parameter weight (Weight), model-averaged estimates (Estimate) and upper and lower 95% confidence intervals (Lower 95% CI/Upper 95% CI).

Appendix S3. Results of spatio-temporal linear regression analysis of wing length (an index of body size) for eight species of Australian shrike-thrushes and whistlers, containing Akaike parameter weight (Weight), model-averaged estimates (Estimate), and upper and lower 95% confidence intervals (Lower 95% CI/Upper 95% CI).