Smaller beaks for colder winters: Thermoregulation drives beak size evolution in Australasian songbirds

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Abstract

Birds' beaks play a key role in foraging, and most research on their size and shape has focused on this function. Recent findings suggest that beaks may also be important for thermoregulation, and this may drive morphological evolution as predicted by Allen's rule. However, the role of thermoregulation in the evolution of beak size across species remains largely unexplored. In particular, it remains unclear whether the need for retaining heat in the winter or dissipating heat in the summer plays the greater role in selection for beak size. Comparative studies are needed to evaluate the relative importance of these functions in beak size evolution. We addressed this question in a clade of birds exhibiting wide variation in their climatic niche: the Australasian honeyeaters and allies (Meliphagoidea). Across 158 species, we compared species' climatic conditions extracted from their ranges to beak size measurements in a combined spatial-phylogenetic framework. We found that winter minimum temperature was positively correlated with beak size, while summer maximum temperature was not. This suggests that while diet and foraging behavior may drive evolutionary changes in beak shape, changes in beak size can also be explained by the beak's role in thermoregulation, and winter heat retention in particular.

Introduction

Many phenotypic traits are multifunctional, and thus understanding their evolution in terms of adaptation and constraint can be a challenge (Gould and Lewontin 1979; Wainwright 2007). Even if a trait is critically important for maintaining one function, trait divergence among species may be driven by selection on a second, sometimes less obvious, function (Cox et al. 2003; Ellers and Boggs 2003). Bird beaks exhibit a stunning diversity in size and shape (Cooney et al. 2017), and no doubt this diversity reflects the functional importance of beaks in a variety of selective contexts (Willson et al. 1975; Gill 2007). Bird beaks are obviously critical for foraging, and the relationship between beak size, shape and diet in Darwin's finches (Grant et al. 1976; Grant and Grant 2002) is now the cornerstone of many introductory lectures on natural selection. However, beaks have also been shown to function as thermoregulatory structures. They can function akin to radiators as they shed heat through convection without losing water (Tattersall et al. 2009; Greenberg et al. 2012a), an effect that can be augmented by vasodilation or reduced by vasoconstriction (Hagan and Heath 1980). Thus a large beak may be adaptive in hot environments, but present a problem for heat retention in cold environments (Danner and Greenberg 2015). Given these different functions – heat radiation, heat retention and foraging – it is unclear which factors are most responsible for the evolution of bird beak size during diversification.

Thermal constraints are known to lead to large-scale variation in morphology. In particular, Allen's rule (Allen 1877) is a classic ecogeographic pattern for endothermic organisms that describes a gradient in extremity length varying with climate. It predicts that selection will lead to individuals in higher elevations and latitudes exhibiting relatively smaller extremities as a means of thermoregulation (Lomolino et al. 2006). Researchers have long documented Allen's rule as a geographic gradient in limb length among individuals of many bird and mammal species (Yom-Tov and Nix 1986; Bried et al. 1997; VanderWerf 2012). While Allen's Rule was earlier thought to be limited to local adaptation within species (Mayr 1956), several studies have since established this pattern as one that applies also across species (Cartar and Guy Morrison 2005; Nudds and Oswald 2007). However, these focused on limb length as the object of selection for thermoregulation.

Examinations of Allen's rule in beak length have been rare until recently, as R. Greenberg and colleagues have established the repeated adaptation of North American sparrow species' beak size to salt marsh habitats (Greenberg and Droege 1990; Grenier and Greenberg 2005). Both among and within salt marsh sparrow populations, summer temperatures are good predictors of beak surface area (Greenberg and Danner 2012; Greenberg et al. 2012b; Greenberg and Danner 2013). However, it is apparent that winter temperatures can also influence beak size, and a framework was presented by Greenberg et al. (2012; Danner and Greenberg 2015) to test which season is the critical period for thermoregulation. As with limb length, Allen's rule in beak size has recently been extended as a pattern observed across species (Symonds and Tattersall 2010).

However, the key question remains: If thermal effects are important, is winter or summer the critical season driving interspecific variation in beak size?

Australia provides a testing ground uniquely suited to disentangle the critical season hypothesis, as its central arid and semi-arid zones exhibit challenging high temperatures in summer and relatively low temperatures in winter. Thus, if selection acts primarily on individuals unable to shed heat during summer, we should expect to see larger beak sizes among species exposed to hot summers. However, if selection acts primarily on individuals unable to conserve heat during winter, we should expect to see smaller beak sizes among species exposed to cold winters. To test these hypotheses, we focused on a diverse clade of Australasian songbirds, the honeyeaters and allies (Meliphagoidea). These species are ideal for such analyses, as they are widespread across Australasia, but largely confined to it (Marki et al. 2017). Thus their in situ diversification across the different climate regimes of Australasia (e.g. Miller et al. 2013) provides a natural experiment of the effects of these regimes on beak morphology.

Methods

Measurements and Metrics

We used measurements taken from vouchered museum specimens of Meliphagoidea at the Australian National Wildlife Collection (ANWC). We measured beak length (culmen base to tip), beak depth (at distal end of nares), and beak width (at distal end of nares). NRF performed all measurements, sampling an average of 5 adult male specimens in breeding condition per recognized species (estimated from skull pneumatization and gonad size), with attempts to sample at least two individuals per subspecies group (Schodde and Mason 1999). We included those species for which we were able to obtain data on beak morphology, spatial distribution, and position on a multi-locus phylogeny. This led to a taxonomic sampling of 94 species in Meliphagidae, 40 species in Acanthizidae, and 24 species in Maluridae. We estimated beak surface area using the conical equation described in (Danner and Greenberg 2015). Body mass was included from measurements taken at the time of collection and reported in the ANWC specimen database (Schodde and Mason 1999); in the few cases when these were unavailable we used the median of estimates from the Handbook of the Birds of the World (hereafter HBW; del Hoyo et al. 2007, 2008).

Most honeyeater species exhibit some degree of curvature in their beak, potentially decreasing the conical estimate of beak surface area's accuracy. To support the accuracy of our beak size and area estimates, we used 2D geometric morphometric data from specimens photographed at the Natural History Museum in Tring, UK to provide supplementary estimates of size and surface area that account for curvature. We placed five landmarks and 19 semi-landmarks around in the outline of each species' beak in *tpsDig* (Rohlf 2004) using the arrangement shown in Figure 5. We used the R package *geomorph* (Adams and Otarola-Castillo 2013) to measure the area of this shape and the scaling factor of its Generalized Procrustes alignment as independent estimates.

Climate

We calculated climate averages for each species as the mean of all raster values contained within a species' range (Birdlife International and NatureServe 2011) using the R package *raster* (Hijmans 2015). As a measure of winter minimum and summer maximum temperatures, we used bioclim data at a resolution of ten minutes (bio5 and bio6 in Hijmans et al. 2005). These represent the maximum temperature of a region's warmest month and the minimum temperature of its coldest month, averaged across years from 1950 to 2000. For migratory species, we used the breeding range to calculate summer climate variables, and the non-breeding range to calculate winter climate variables. As the importance of convective versus evaporative heat exchange is likely to change depending on the availability of water during summer heat, we included a metric of aridity (hereafter "summer heat stress"). For this metric, we extracted summer precipitation (bio18 in Hijmans et al. 2005) to express "summer heat stress" as its statistical interaction with summer maximum temperature (see below).

Spatial Distribution

To visualize spatial distributions of beak size traits, we accounted for allometry using residuals of their regression against body mass (these characters were not used for comparative analyses described below). We used the *Spatial Analysis in Macroecology* software package (Rangel et al. 2010) to estimate both species richness and average trait values for each taxonomic family at every grid cell (0.5^{o} x 0.5^{o}). These grids were then trimmed to include only cells with at least two species present. We used *QGIS* to produce choropleth figures describing species richness and the spatial distribution of beak size traits (QGIS Development Team 2015).

Comparative Methods

Correcting for phylogenetic non-independence is critical to the comparative method. We conducted a separate analysis for each family by taking advantage of their recent multi-locus phylogeny (Lee et al. 2012; Nyári and Joseph 2012; Joseph et al. 2014). This approach has the advantage of both using high-quality molecular phylogeny and at the same time assessing between-family heterogeneity in evolutionary patterns. To estimate time-calibrated branch lengths for these trees, we used penalized likelihood in *ape* to constrain branch lengths by divergence time estimates in the references listed above (Paradis et al. 2004; Paradis 2013).

Just as closely related species are not phylogenetically independent, they are not spatially independent either (Freckleton and Jetz 2009). Indeed, even when using correction for spatial autocorrelation spurious correlations often result from comparisons of species' climate variables and traits, apparently due to an autocorrelation of ecology and historical biogeography (Tello and Stevens 2012; Warren et al. 2014; Friedman and Remeš 2016). To avoid these pitfalls, we used a Phylogenetic Generalized Least Squares framework that combines correction for both phylogenetic and spatial relationships (Freckleton and Jetz 2009; hereafter "spatial PGLS"). This model includes estimates of both a phylogenetic effect parameter (λ) and a spatial effect parameter (Φ). Scripts to run this analysis in the R programming environment are available from R. Freckleton upon request as stated in the original publication (Freckleton and Jetz 2009). We used this spatial PGLS method to test for significant relationships between morphological characters and environmental factors in each family using a multivariate analysis. In this analysis, we included winter minimum temperature, summer maximum temperature and precipitation as predictors.

To correct for allometric scaling of beak size, we included body mass as a covariate in each analysis. This approach is preferred among contemporary phylogenetic comparative studies because the use of residuals may cause collinearity issues (Freckleton 2009; Symonds and Tattersall 2010; Baab et al. 2014; Benson-Amram et al. 2015). To display allometric relationships outside the context of our phylogenetically-corrected analyses, we used residuals from the regression of $\log_{10}$ beak size against $\log_{10}$ body mass (for linear beak measurements, body mass was raised to the 1/3 power; for beak surface area body mass was raised to the 2/3 power).

To estimate the effect size of each predictor as the standardized regression coefficient, we scaled each climate predictor by its standard deviation so that its variance equaled 1. Below, we present results from bivariate analyses, as well as analyses using multivariate models.

Results and Discussion

Our analyses illuminate the roles of different selection pressures in the evolution of beak size in Australasian honeyeaters and allies. In particular, we found a consistent evolutionary correlation between winter temperature and beak size (Figure 1, Figure 2A). In areas with low winter minimum temperature, bird species tend to have low beak surface area, length and width (Figure 2C; weaker effects were observed for depth). The relationship between winter temperature and beak size was consistent in its direction and was significant across each clade examined in this study. In contrast, we found little evidence that beak size was associated with summer maximum temperatures (Figure 2B,D).

Among multivariate analyses including summer heat stress (i.e., interaction between summer maximum temperature and summer precipitation), beak size measures were significantly correlated with winter minimum temperatures in most models (Table 1). Five out of twelve comparisons, and at least one from each clade we examined, showed significant relationships between low winter temperatures and small beak sizes. In particular, winter temperatures were associated with beak surface area and beak length in Meliphagidae and Maluridae, and with beak width in Acanthizidae. On the contrary, summer heat stress was not significantly associated with beak size in models that included winter temperatures (Figure 3, Table 1). Maps of average beak sizes across Australasia showed a similar pattern: species of each family tended to exhibit relatively larger beaks in northern Australia and New Guinea, and smaller beaks in central and southern Australia (Figure 4).

We observed the greatest effect of winter temperatures on beak length in Meliphagidae and Maluridae, and on beak width in Acanthizidae (Figure 2C). The relationship between winter temperature and beak surface area, the most important beak characteristic in terms of thermoregulation, was consistent across all families for univariate analyses. Furthermore, measurements of beak area and scale accounting for curvature in Meliphagids showed similar results (Figure 5). These results provide support for the evolutionary relationship between climate and extremity length, particularly beak size (see also Campbell-Tennant et al. 2015; Gardner et al. 2016). Furthermore, our results show support for winter and not summer temperatures driving beak size evolution across several clades adapting to a broad range of climates. This pattern is not predicted by proximate explanations for Allen’s rule that rely on a direct effect of temperature on skeletal development (Serrat et al. 2008; Burness et al. 2013), as developing songbirds grow exceptionally fast and thus reach their adult size prior to the onset of winter temperatures (Starck and Ricklefs 1998). However, birds can exhibit seasonal variation in size of the keratin-based rhamphotheca, resulting in a slight increase in beak length during the breeding season (Morton and Morton 1987; Greenberg et al. 2012). In our study we measured specimens in breeding condition, thus we can to some extent control for – but cannot describe – seasonal variation of the rhamphotheca.

Among seasons, we found that winter temperatures were often significantly correlated with beak size, while summer temperatures and summer heat stress were never correlated with beak size. This suggests that selection in winter produces the most detectable effect at a broad phylogenetic scale, not that summer temperature has no impact on beak size. The evidence from North American sparrows clearly supports a gradient in beak size driven by summer heat stress (Greenberg et al. 2012b). However, this summer effect was observed most prominently in coastal regions where winters are relatively mild (Danner and Greenberg 2015). Similarly, we observed large beak sizes for species in coastal northern Australia, where summers are hot and winters tend to be mild (Figure 4). However, we observed small beak sizes for species in inland Australia, where summers are still hot but winter temperatures can be harsh as well. While selection on beak size in winter and summer appears to interact negatively across Australia's broad aridity gradient, these effects may interact positively along steep altitudinal gradients, where regions tend to be either hot or cool year-round. This could explain the drastic contrasts in beak size we observed between the central highlands and coastal lowlands of New Guinea in Meliphagidae and Acanthizidae (Figure 4), as well as those observed in Hawaiian elepaios (VanderWerf 2012). Path analysis studies (Hardenberg and Gonzalez-Voyer 2013) are needed to describe the effects of complex interactions between seasonal climates and elevation on morphological evolution.

In this study we cannot rule out a major role for diet in the evolution of beak size and shape in Meliphagoidea. While preferences for seeds, insects, or the nectar in flowers are available in most species descriptions, these dietary categories may be less important than the size of preferred items in driving the evolution of divergent beak sizes (Grant et al. 1976). The manner in which these items are procured (e.g., pursuit and hawking vs. probing and gleaning) and the substrate they occupy likely also influence adaptive beak evolution (Miller et al. 2017). Such detailed descriptions are typically unavailable for taxa in remote regions like central Australia and New Guinea, making comparative studies difficult. Lastly, particular food sources may increase or decrease in availability due to climate, making it difficult to rule out diet in explaining differences in beak size between regions. Thus, our results highlight the importance and need for thorough descriptions of foraging niche at a broad taxonomic scale to better tease out the relative roles of diet and thermoregulation in driving beak evolution.

It has long been clear that bird species employ a diverse range of specialized beak shapes that are a close fit to their diets (Beecher 1951; Wooller and Richardson 1988). However, our results from a phylogenetic comparative study of Australasian songbirds suggest that such structures likely also scale with the demands of climate. Shorebirds provide an excellent example, where bill size has dramatically increased with probing behavior (Barbosa and Moreno 1999). But such species can be observed with their bills tucked into insulating plumage when not in use, likely mitigating their thermoregulatory costs (Midtgård 1978).

For decades, the evolution of beak size in Galapagos finches has been an instructive model system for the study of adaptation, and findings using this system have largely highlighted the importance of trophic processes in the evolution of beak size (Grant et al. 1976; Grant and Grant 2002). However as island endemics, these species are only subject to the climates present in a narrow (if dynamic) geographic range (Grant and Boag 1980). We predict that if our study were repeated in that clade it would be difficult to find a similar effect of climate on beak size evolution; this contrast underscores the importance of broad comparative studies in evolutionary research. In contrast, our study of honeyeaters and allies across Australasia spans both tropical and temperate zones including a continental gradient in precipitation and an extended range of elevations up to above 4.5 km of elevation (Figure 4). We interpret our findings to suggest that at greater spatial or temporal scales (Meliphagoidea originated 25-30 Mya in early Oligocene; Moyle et al. 2016, Marki et al. 2017), the beak's thermoregulatory role may explain a more considerable amount of evolutionary change than previously thought. The relative importance of this mechanism versus foraging niche divergence in explaining beak evolution will be an exciting avenue of future research. Ultimately this finding highlights the diversity of selective pressures acting on species morphological traits (Schluter et al. 1991) and the contrasting patterns they may produce at different spatial and temporal scales (Carroll et al. 2007).

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Figure Titles

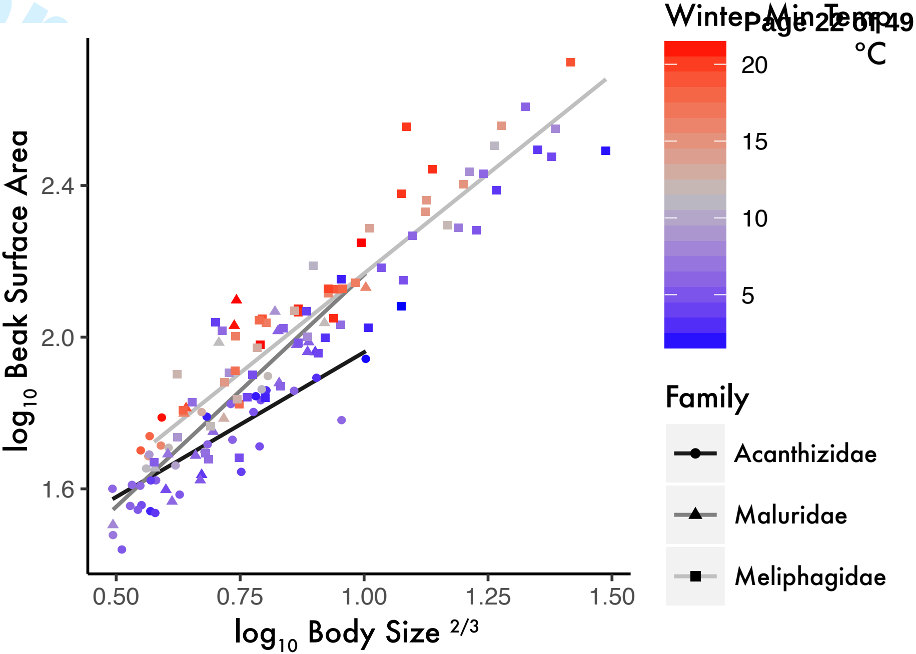
Figure 1: Allometric plot of $\log_{10}$ beak surface area against $\log_{10}$ body mass, with body mass raised to the $^{2/3}$ power to account for the dimensional difference between area and volume. Minimum winter temperatures, averaged across species ranges, are shown as the color of the points in the scatterplot, while taxonomic families are depicted with different symbols. Ordinary least squares linear models are shown to aid visualization of climate patterns relative to allometry relationships, with lines colored by family. Most species in regions with warm winters have large beaks for their body mass, whereas most species in regions with cold winters have small beaks for their body mass.

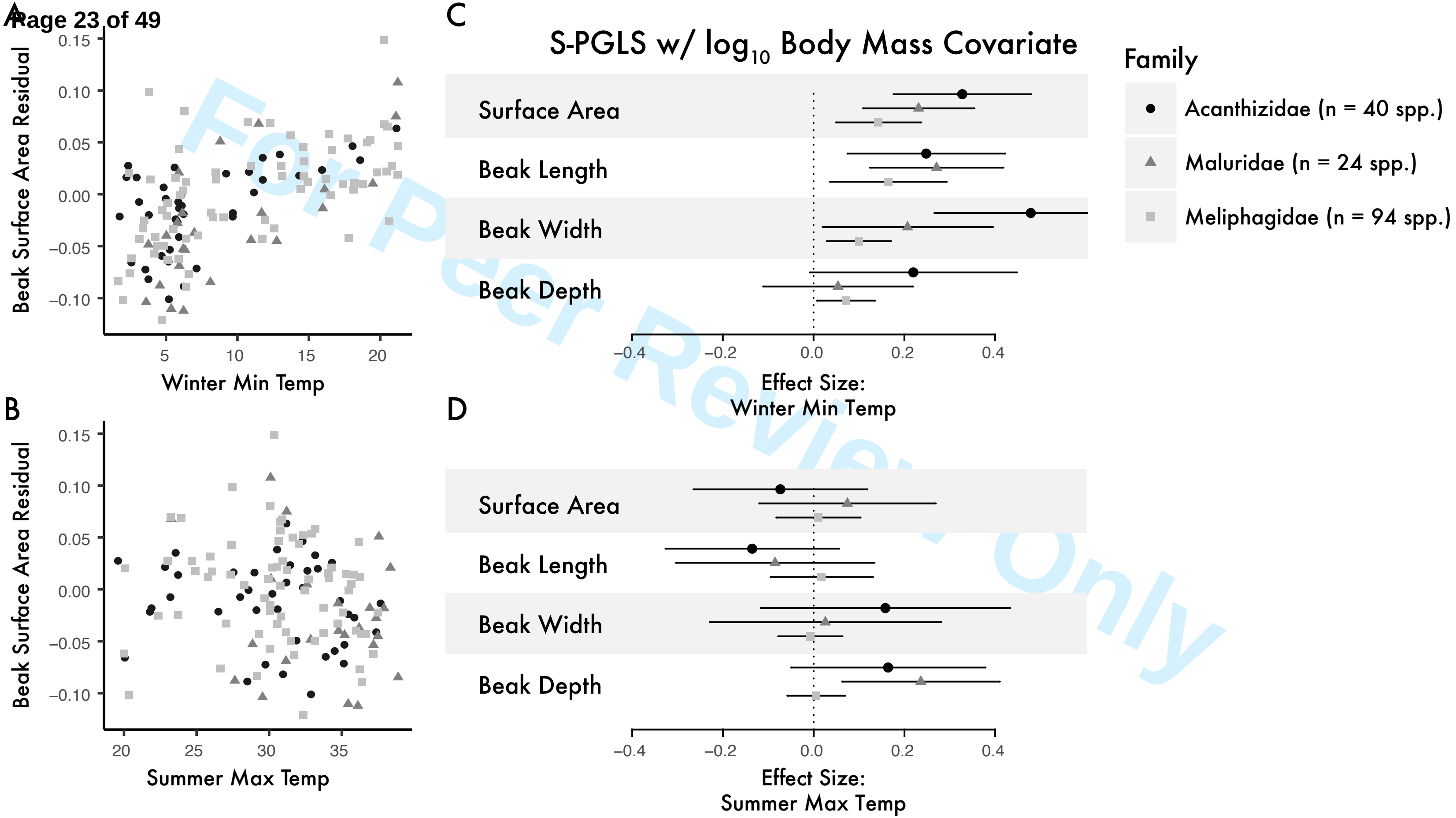
Figure 2: (A-B) Comparisons of beak surface area, as residuals of the relationship shown in Figure 1, to climate averages. (C-D) Effects of climate averages on four measures of beak size, shown as effect sizes (standardized β) with 95% confidence intervals. These effect sizes describe bivariate spatial PGLS models comparing trait values across species and including body mass as a covariate to account for allometry. Taxonomic families are represented by the symbols shown in the legend in the upper right.

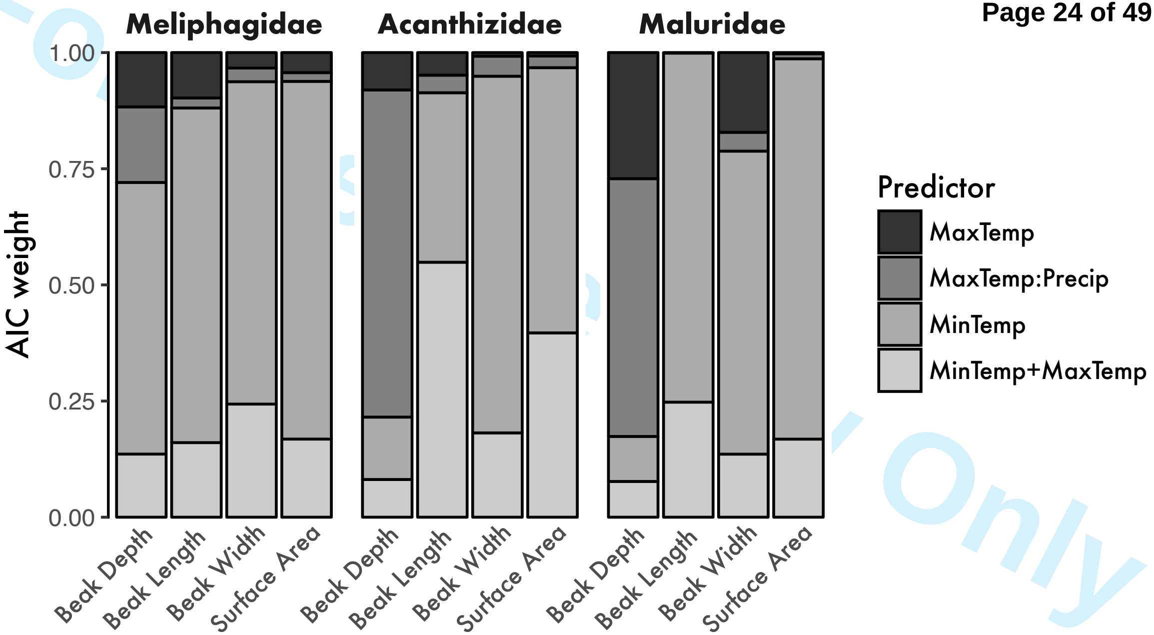
Figure 3: Comparison of model support across spatial PGLS analyses. Bar plots show Akaike weights for models including combinations of predictor variables that reflect a priori hypotheses. Models that include minimum temperature have majority support in all analyses explaining beak surface area. Summer heat stress (MaxTemp:Precip) models are a better fit as explanations of beak depth in Acanthizidae and Maluridae. However, these associations with summer heat stress were not statistically significant when winter minimum temperature was included in multivariate analyses (Table 1).

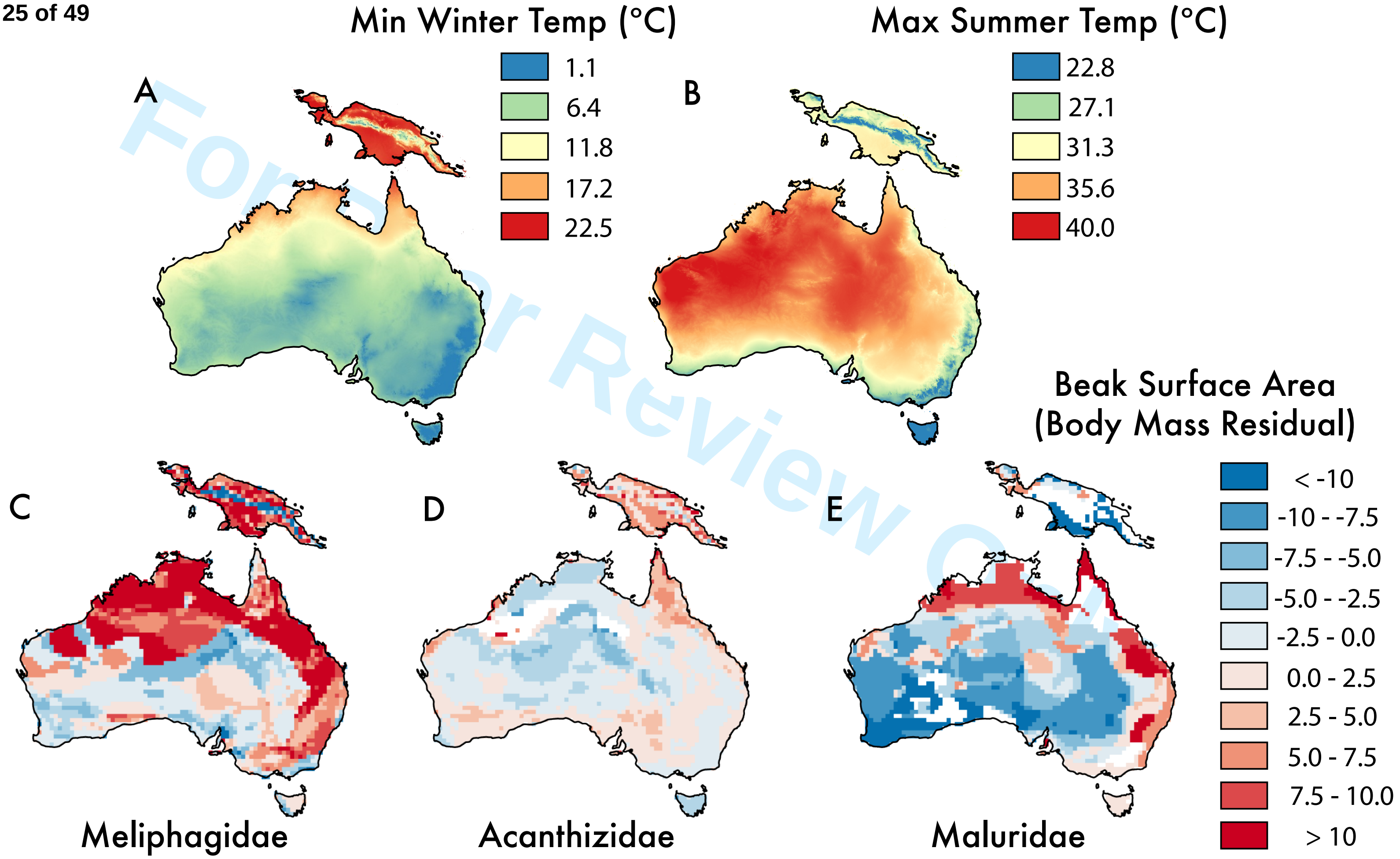
Figure 4: Geographic distribution of minimum winter temperature (a) and maximum summer temperature (b) from the Bioclim dataset (Hijmans et al. 2005). Beak surface area averaged across species present in 0.5° grid cells (as residuals of regression with body mass) for Meliphagidae (c), Acanthizidae (d) and Maluridae (e). Larger beaks are shown in red and smaller beaks are shown in blue. Cells with less than two species present were excluded (white).

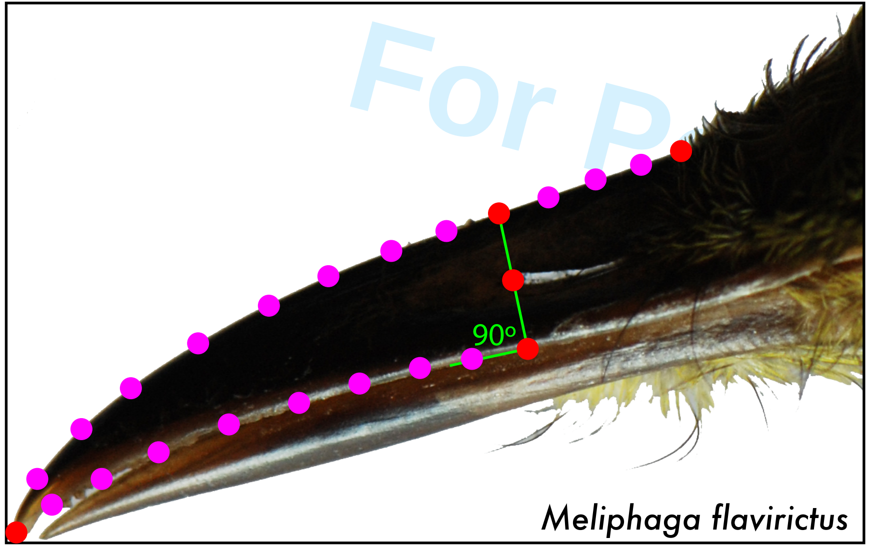
Figure 5: At left, landmark (red) and sliding semi-landmark (magenta) positions used in scoring beak area and size. Semi-landmarks were set at equal intervals between landmarks. At right, effect sizes (standardized β) of spatial PGLS models comparing climate predictor variables to landmark-based measures of beak area and size; lines represent 95% confidence intervals.

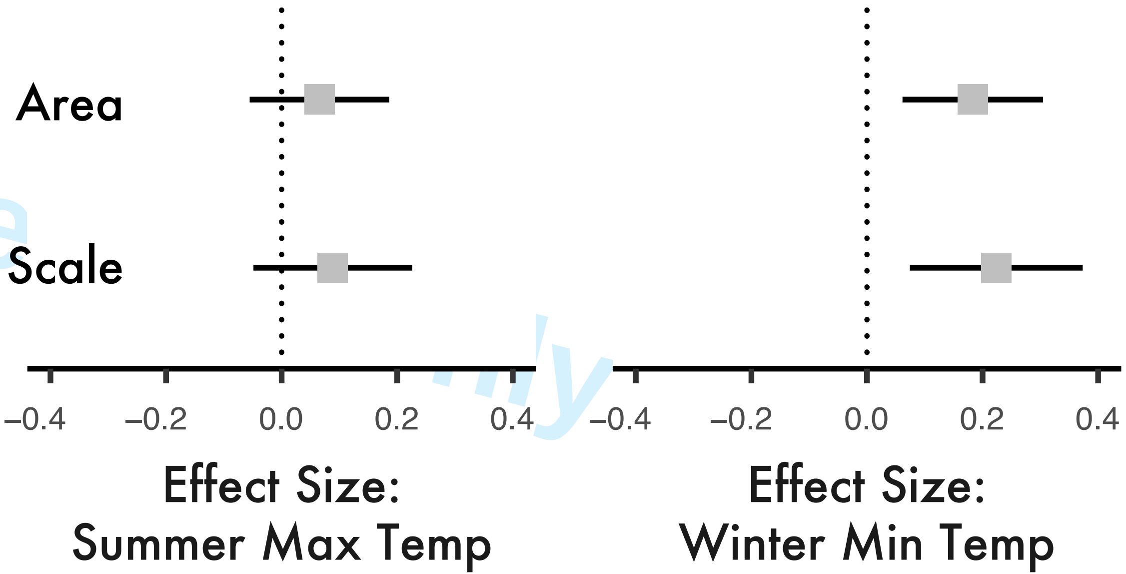

Winter Min Temp
°C
20
15
10
5
Family
Acanthizidae
Maluridae
Meliphagidae
$\log_{10}$ Beak Surface Area
2.4
2.0
1.6
0.50
0.75
1.00
1.25
1.50
$\log_{10}$ Body Size $^{2/3}$


A
Beak Surface Area Residual
0.15
0.10
0.05
0.00
−0.05
−0.10
5
10
15
20
Winter Min Temp
B
Beak Surface Area Residual
0.15
0.10
0.05
0.00
−0.05
−0.10
20
25
30
35
Summer Max Temp
C
S-PGLS w/ $\log_{10}$ Body Mass Covariate
Surface Area
Beak Length
Beak Width
Beak Depth
−0.4
−0.2
0.0
0.2
0.4
Effect Size:
Winter Min Temp
D
Surface Area
Beak Length
Beak Width
Beak Depth
−0.4
−0.2
0.0
0.2
0.4
Effect Size:
Summer Max Temp
Family
● Acanthizidae (n = 40 spp.)
▲ Maluridae (n = 24 spp.)
■ Meliphagidae (n = 94 spp.)


Meliphagidae
Acanthizidae
Maluridae
AIC weight
1.00
0.75
0.50
0.25
0.00
Beak Depth
Beak Length
Beak Width
Surface Area
Predictor
MaxTemp
MaxTemp:Precip
MinTemp
MinTemp+MaxTemp


Min Winter Temp (°C)
1.1
6.4
11.8
17.2
22.5
Max Summer Temp (°C)
22.8
27.1
31.3
35.6
40.0
A
B
Beak Surface Area
(Body Mass Residual)
< -10
-10 - -7.5
-7.5 - -5.0
-5.0 - -2.5
-2.5 - 0.0
0.0 - 2.5
2.5 - 5.0
5.0 - 7.5
7.5 - 10.0
> 10
C
D
E
Meliphagidae
Acanthizidae
Maluridae


90°
Meliphaga flavirictus


Area
Scale
−0.4
−0.2
0.0
0.2
0.4
−0.4
−0.2
0.0
0.2
0.4
Effect Size:
Summer Max Temp
Effect Size:
Winter Min Temp

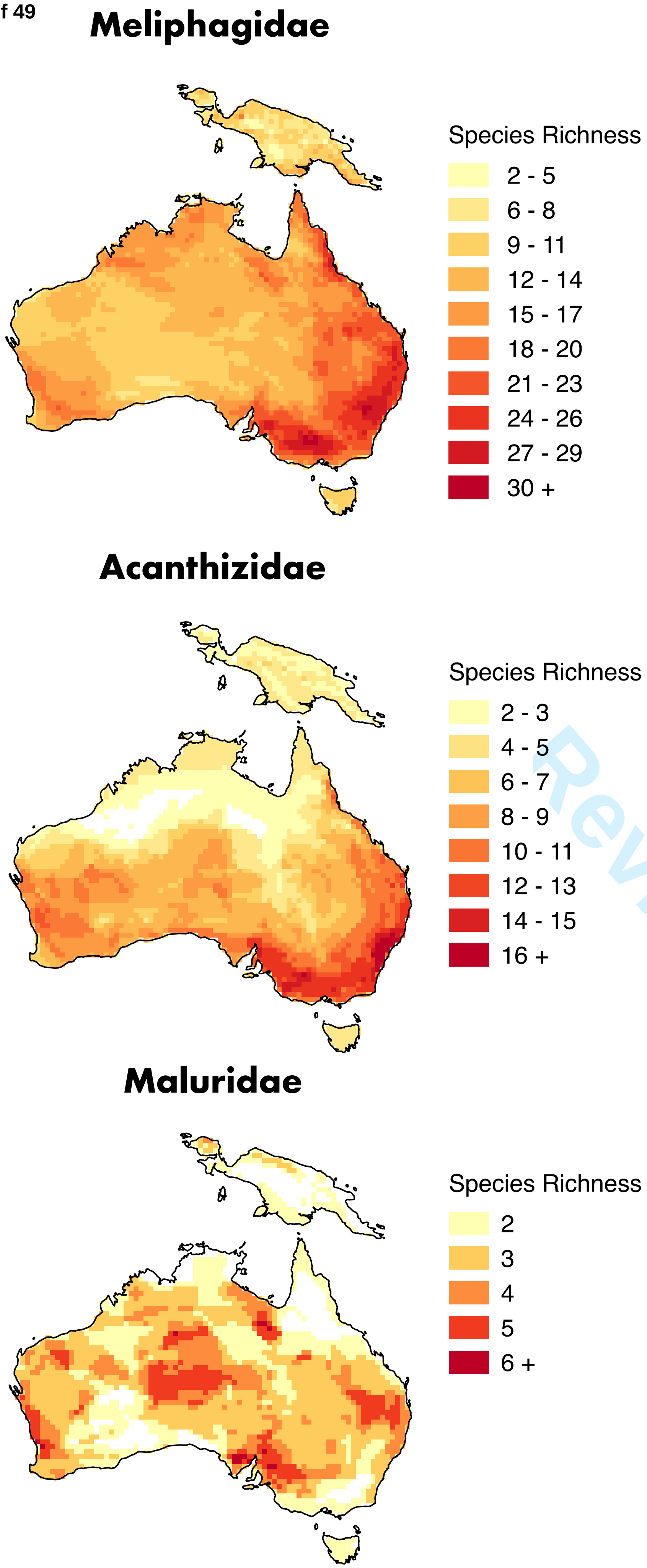


Figure S1: Species richness of three Australasian songbird clades, estimated from the sampling used in this study. Note that this sampled richness may differ from estimates of species richness assembled from all recognized taxa, particularly in New Guinea.

~~R. R.~~**Abstract**

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Birds' beaks play a key role in foraging, and most research on their ~~morphology~~size and shape has focused on this function. Recent findings suggest that beaks may also be important for thermoregulation, and this may drive morphological evolution as predicted by Allen's rule. However, the role of thermoregulation in the evolution of beak size ~~during diversification~~across species remains largely unexplored. In particular, it remains unclear whether the need for retaining heat in the winter or dissipating heat in the summer plays the greater role in selection for beak size. Comparative studies are needed to evaluate the relative importance of these functions in ~~the~~beak size evolution ~~of beak size~~. We addressed this question in a clade of birds exhibiting wide variation in their climatic niche: the Australasian honeyeaters and allies (Meliphagoidea). Across 158 species, we compared species' ~~climate~~climatic conditions extracted from their ranges to beak size measurements in a combined spatial-phylogenetic framework. We found that winter minimum temperature was positively correlated with beak size, while ~~broad dietary categories were~~summer maximum temperature was not. This suggests that while diet and foraging behavior may drive evolutionary changes in beak shape, changes in beak size can also be explained by the beak's role in thermoregulation, and winter heat retention in particular.

Introduction

Many phenotypic traits are multifunctional, and thus understanding their evolution in terms of ~~selection~~adaptation and constraint can be a challenge (Gould and Lewontin 1979; Wainwright 2007). Even if a trait is critically important for maintaining one function, trait divergence ~~and variation~~ among species may be driven by selection on a second, sometimes less obvious, function (Cox et al. 2003; Ellers and Boggs 2003). Bird beaks exhibit a stunning diversity in size and shape~~,~~ (Cooney et al. 2017), and no doubt this diversity reflects the functional importance of beaks in a variety of selective contexts (Willson et al. 1975; Gill 2007). Bird beaks are obviously critical for foraging, and the relationship between beak size, shape and diet in Darwin's finches (Grant et al. 1976; Grant and Grant 2002) is now the cornerstone of many introductory lectures on natural selection. However, beaks have also been shown to function as thermoregulatory structures. They can function akin to radiators as they shed heat through convection without losing water (Tattersall et al. 2009; Greenberg et al. 2012a), an effect that can be augmented by vasodilation or reduced by vasoconstriction (Hagan and Heath 1980). Thus a large beak may be adaptive in hot environments, but present a problem for heat retention in cold environments (Danner and Greenberg 2015). Given these different functions – heat radiation, heat retention and foraging – it is unclear which factors are most responsible for the evolution of bird beak size during diversification.

Thermal constraints are known to lead to large-scale variation in morphology. In particular, Allen's rule (Allen 1877) is a classic ecogeographic pattern for endothermic organisms that describes a gradient in extremity length varying with climate. It predicts that selection will lead to individuals in higher elevations and latitudes exhibiting relatively smaller extremities as a means of thermoregulation (Lomolino et al. 2006). Researchers have long documented Allen's rule as a geographic gradient in limb length among individuals of many bird and mammal species (Yom-Tov and Nix 1986; Bried et al. 1997~~), and most recently in Hawaiian elepaios (~~; VanderWerf 2012). While Allen's Rule was earlier thought to be limited to local adaptation within species (Mayr 1956), several studies have since established this pattern as one that applies also across species (Cartar and Guy Morrison 2005; Nudds and Oswald 2007). However, these focused on limb length as the object of selection for thermoregulation.

Examinations of Allen's rule in beak length have been rare until recently, as R. Greenberg and colleagues have established the repeated adaptation of North American sparrow species' beak size to salt marsh habitats (Greenberg and Droege 1990; Grenier and Greenberg 2005). Both among and within salt marsh sparrow populations, summer temperatures are ~~strong~~good predictors of beak surface area (Greenberg and Danner 2012; Greenberg et al. 2012b; Greenberg and Danner 2013). However, it is apparent that winter temperatures can also influence beak size, and a framework was presented by Greenberg et al. (2012; Danner and Greenberg 2015) to test which season is the critical period for thermoregulation. As with limb length, Allen's rule in beak size has recently been extended as a pattern observed across species (Symonds and Tattersall 2010). ~~However, several key questions remain: 1) Is the effect of climate on beak size~~

~~detectable during evolutionary diversification? 2)~~However, the key question remains: If thermal effects are important, is winter or summer the critical season driving interspecific variation in beak size?

Australia provides a testing ground uniquely suited to disentangle the critical season ~~hypotheses~~hypothesis, as its central arid and semi-arid zones exhibit challenging high temperatures in summer and relatively low temperatures in winter. Thus, if selection acts primarily on individuals unable to shed heat during summer, we should expect to see larger beak sizes among ~~arid-adapted~~ species exposed to hot summers. However, if selection acts primarily on individuals unable to conserve heat during winter, we should expect to see smaller beak sizes among ~~arid-adapted~~ species exposed to cold winters. To test these hypotheses, we focused on a diverse clade of Australasian songbirds, the honeyeaters and allies (Meliphagoidea). These species are ideal for such analyses, as they are widespread across Australasia, but largely confined to it~~.~~ (Marki et al. 2017). Thus their in situ diversification across the different climate regimes of Australasia (e.g. Miller et al. 2013) provides a natural experiment of the effects of these regimes on beak morphology.

Methods

Measurements and Metrics

We used measurements taken from vouchered museum specimens of Meliphagoidea at the Australian National Wildlife Collection (ANWC). We measured beak length (culmen base to tip), beak depth (at distal end of nares), and beak width (at distal end of nares). NRF performed all measurements, sampling an average of 5 adult male specimens in breeding condition per recognized species (estimated from skull pneumatization and gonad size), with attempts to sample at least two individuals per subspecies group (Schodde and Mason 1999). We included those species for which we were able to obtain data on beak morphology, ~~diet,~~ spatial distribution, and position on a multi-locus phylogeny. This led to a taxonomic sampling of 94 species in Meliphagidae, 40 species in Acanthizidae, and 24 species in Maluridae. We estimated beak surface area using the conical equation described in (Danner and Greenberg 2015). Body mass was included from measurements taken at the time of collection and reported in the ANWC specimen database~~;~~ (Schodde and Mason 1999); in the few cases when these were unavailable we used the median of estimates from the Handbook of the Birds of the World (hereafter HBW; del Hoyo et al. 2007, 2008).

Most honeyeater species exhibit some degree of curvature in their beak, potentially decreasing the conical estimate of beak surface area's accuracy. To support the accuracy of our beak size and area estimates, we used 2D geometric morphometric data from specimens photographed at the Natural History Museum in Tring, UK to provide supplementary estimates of size and surface area that account for curvature. We placed five landmarks and 19 semi-landmarks around in the outline of each species' beak in *tpsDig* (Rohlf 2004) using the arrangement shown in Figure 5. We used the R package *geomorph*

(Adams and Otarola-Castillo 2013) to measure the area of this shape and the scaling factor of its Generalized Procrustes alignment as independent estimates.

~~*Diet*~~

~~To formulate broad descriptions of species' diets, we used reports of species' feeding habits from HBW (del Hoyo et al. 2007, 2008). The coarse nature of those data requires that we interpret them with caution and as a first approximation only. We established seven food type categories that matched those reported for species in HBW: "plant material", "fruit", "nectar", "seeds", "insects and other invertebrates", "fish", and "other vertebrates" (equivalent to estimates in Wilman et al. 2014). We scored species' diet by assigning the proportion of each category used in the diet according to text descriptions, such that the sum of all category values for any species was equal to five. This approach enabled us to convert information in the text according to predefined and fixed terminology into a proportional use of diet categories. To test whether axes of diet variation affected beak size, we performed a phylogenetically-corrected PCA (Revell 2009) of diet scores (Figure S1).~~

Climate

We calculated climate averages for each species as the mean of all raster values contained within a species' range (Birdlife International and NatureServe 2011) using the R package *raster* (Hijmans 2015). As a measure of winter minimum and summer maximum temperatures, we used bioclim data at a resolution of ten minutes (bio5 and bio6 in Hijmans et al. 2005). These represent the maximum temperature of a region’s warmest month and the minimum temperature of its coldest month, averaged across years from 1950 to 2000. For migratory species, we used the breeding range to calculate summer climate variables, and the non-breeding range to calculate winter climate variables. As the importance of convective versus evaporative heat exchange is likely to change depending on the availability of water during summer heat, we included a metric of aridity (hereafter “summer heat stress”). For this metric, we extracted summer precipitation (bio18 in Hijmans et al. 2005) to express “summer heat stress” as its statistical interaction with summer maximum temperature (see ~~Results~~below).

Spatial Distribution

To visualize spatial distributions of beak size traits, we accounted for allometry using residuals of their regression against body mass (these characters were not used for comparative ~~methods~~analyses described below). We used the *Spatial Analysis in Macroecology* software package (Rangel et al. 2010) to estimate both species richness and average trait values for each taxonomic family at every grid cell (0.5° x 0.5°). These grids were then trimmed to include only cells with at least two species present. We used *QGIS* to produce choropleth figures describing species richness and the spatial distribution of beak size traits (QGIS Development Team 2015).

Comparative Methods

Correcting for phylogenetic non-independence is critical to the comparative method~~, and requires accurate and complete information on phylogenetic~~

~~relationships among species. Among avian lineages, the honeyeaters and allies are among the most phylogenetically under-sampled. Consequently, it was necessary to conduct~~. We conducted a separate analysis for each family ~~to take~~by taking advantage of their ~~most~~ recent multi-locus phylogeny (Lee et al. 2012; Nyári and Joseph 2012; Joseph et al. 2014). This approach has the advantage of both using high-quality molecular phylogeny and at the same time assessing between-family heterogeneity in evolutionary patterns. To estimate time-calibrated branch lengths for these trees, we used penalized likelihood in *ape* to constrain branch lengths by divergence time estimates in the references listed above (Paradis et al. 2004; Paradis 2013).

Just as closely related species are not phylogenetically independent, they are not spatially independent either (Freckleton and Jetz 2009). Indeed, even when using correction for spatial autocorrelation spurious correlations often result from comparisons of species' climate variables and traits, apparently due to an ~~autocorrelations~~autocorrelation of ecology and historical biogeography (Tello and Stevens 2012; Warren et al. 2014; Friedman and Remeš 2016). To avoid these pitfalls, we used a Phylogenetic Generalized Least Squares framework that combines correction for both phylogenetic and spatial relationships (Freckleton and Jetz 2009; hereafter "spatial PGLS"). This model includes estimates of both a phylogenetic effect parameter (λ) and a spatial effect parameter (Φ). ~~A series of scripts for~~Scripts to run this analysis in the R programming environment ~~to run this model~~ are available from R. Freckleton upon request as stated in the original publication (Freckleton and Jetz 2009). We used this spatial PGLS method to test for significant relationships between morphological characters and environmental factors in each family using a multivariate analysis. In this analysis, we included ~~diet,~~ winter minimum temperature~~ and~~, summer maximum temperature and precipitation as predictors.

To correct for allometric scaling of beak size, we included body mass as a covariate in each analysis. This approach is preferred among contemporary phylogenetic comparative studies because the use of residuals may cause collinearity issues (Freckleton 2009; Symonds and Tattersall 2010; Baab et al. 2014; Benson-Amram et al. 2015). To display allometric relationships outside the context of our phylogenetically-corrected analyses, we used residuals from the regression of $\log_{10}$ beak size against $\log_{10}$ body mass (for linear beak measurements, body mass was raised to the 1/3 power; for beak surface area body mass was raised to the 2/3 power).

To estimate the effect size of each predictor as the standardized regression coefficient, we scaled each climate predictor by its standard deviation so that its variance equaled 1. Below, we present results from bivariate analyses, as well as analyses using multivariate models.

Results and Discussion

Our analyses illuminate the roles of different selection pressures in the evolution of beak size in Australasian honeyeaters and allies. In particular, we found a ~~strong and~~ consistent ~~role of~~evolutionary correlation between winter temperature

~~in~~and beak size~~ evolution~~ (Figure 1, Figure 2A). In areas with low winter minimum temperature, bird species tend to have low beak surface area, length and width (Figure 2C; weaker effects were observed for depth). The ~~effect of~~relationship between winter temperature ~~on~~and beak size was consistent in its direction and was significant across each clade examined in this study. In contrast, we found little evidence that beak size was associated with summer maximum temperatures ~~or diet~~ (Figure 2B,D~~; Figure S1~~).

Among multivariate analyses including ~~diet~~summer heat stress (i.e., interaction between summer maximum temperature and summer ~~heat stress,~~precipitation), beak size measures were significantly correlated with winter minimum temperatures in most models (Table 1). Five out of twelve comparisons, and at least one from each clade we examined, showed significant relationships between low winter temperatures and small beak sizes. In particular, winter temperatures were associated with beak surface area and beak length in Meliphagidae and Maluridae, and with beak width in Acanthizidae. ~~Neither~~On the contrary, summer heat stress ~~nor diet~~ was not significantly associated with beak size in models that included winter temperatures (Figure 3, Table 1). Maps of average beak sizes across Australasia showed a similar pattern: species of each family tended to exhibit relatively larger beaks in northern Australia and New Guinea, and smaller beaks in central and southern Australia (Figure 4).

~~Our results show a prominent role for winter thermoregulation in driving beak size variation among species.~~ We observed ~~this~~the greatest effect ~~most strongly~~of winter temperatures on beak length in Meliphagidae and Maluridae, and on beak width in Acanthizidae (Figure 2C). ~~The effect on~~The relationship between winter temperature and beak surface area, the most important beak characteristic in terms of thermoregulation, was consistent across all families for univariate analyses. Furthermore, measurements of beak area and scale accounting for curvature in Meliphagids showed similar results (Figure 5). These results provide ~~strong~~ support for the evolutionary relationship between climate and extremity length, particularly beak size~~.~~ (see also Campbell-Tennant et al. 2015; Gardner et al. 2016). Furthermore, our results show support for winter and not summer temperatures driving beak size evolution across several clades adapting to a broad range of climates. This pattern is not predicted by proximate explanations for Allen's rule that rely on a direct effect of temperature on skeletal development (Serrat et al. 2008; Burness et al. 2013~~) for Allen's rule,~~), as developing songbirds grow exceptionally fast ~~(Starck and Ricklefs 1998)~~ and thus reach their adult size prior to the onset of winter temperatures~~.~~ (Starck and Ricklefs 1998). However, birds can exhibit seasonal variation in size of the keratin-based rhamphotheca, resulting in a slight increase in beak length during the breeding season (Morton and Morton 1987; Greenberg et al. 2012). In our study we measured specimens in breeding condition, thus we can to some extent control for – but cannot describe – seasonal variation of the rhamphotheca.

~~These findings suggest that selection in winter is the dominant~~Among seasons, we found that winter temperatures were often significantly correlated with beak size, while summer temperatures and summer heat stress were never correlated with beak size. This suggests that selection in winter produces the most detectable effect at a broad phylogenetic scale, not that summer temperature has no impact on beak size. The evidence from North American sparrows clearly supports a gradient in beak size driven by summer heat stress (Greenberg et al. 2012b). However, this summer effect ~~is~~was observed ~~only~~most prominently in coastal regions where winters are ~~universally~~relatively mild (Danner and Greenberg 2015). Similarly, we observed large beak sizes for species in coastal northern Australia, where summers are hot and winters tend to be mild (Figure 4). However, we observed small beak sizes for species in inland Australia, where summers are still hot but winter temperatures can be harsh as well. While selection on beak size in winter and summer appears to interact negatively across Australia's broad aridity gradient, these effects may interact positively along steep altitudinal gradients, where regions tend to be either hot or cool year-round. This could explain the drastic contrasts in beak size we observed between the central highlands and coastal lowlands of New Guinea in Meliphagidae and Acanthizidae (Figure 4), as well as those observed in Hawaiian elepaios (VanderWerf 2012). Path analysis studies (Hardenberg and Gonzalez-Voyer 2013) are needed to describe the effects of complex interactions between seasonal climates and elevation on morphological evolution.

~~Surprisingly, we found no evidence of a significant relationship between beak size and an approximation of diet; this observation was consistent across all clades we examined, using both bivariate and multivariate analyses (Figure S1, Table 1). Support for a relationship between beak size and diet was only observed for 2~~

~~comparisons out of 24 (both beak characters were also related to climate; Table 1). However,~~ In this study we cannot rule out a ~~strong~~major role for diet in the evolution of beak size and shape in Meliphagoidea. ~~While the dietary categories used in this study may be expected to reflect some broad changes in diet, they are not capable of describing many of the dietary~~While preferences ~~and foraging behavior that make up the key components of species' foraging niche. In particular, a preference~~ for seeds, insects, or the nectar in flowers are available in most species descriptions, these dietary categories may be less important than the size of ~~those objects~~preferred items in driving the evolution of divergent beak sizes (Grant et al. 1976). The manner in which these items are procured (e.g., pursuit and hawking vs. probing and gleaning) and the substrate they occupy likely also influence adaptive beak evolution~~.~~ (Miller et al. 2017). Such detailed descriptions are typically unavailable for taxa in remote regions like central Australia and New Guinea, making comparative studies difficult. Lastly, particular food sources may increase or decrease in availability due to climate, making it difficult to rule out diet in explaining differences in beak size between regions. Thus, our results highlight the importance and need for thorough descriptions of foraging niche at a broad taxonomic scale to better tease out the relative roles of diet and thermoregulation in driving beak evolution.

It has long been clear that bird species employ a diverse range of specialized beak shapes that are a close fit to their diets (Beecher 1951; Wooller and Richardson 1988). However, our results from a phylogenetic comparative study of Australasian songbirds suggest that such structures likely also scale with the demands of climate. Shorebirds provide an excellent example, where bill size has dramatically increased with probing behavior (Barbosa and Moreno 1999). But such species can be observed with their bills tucked into insulating plumage when not in use, likely mitigating their thermoregulatory costs (Midtgård 1978).

For decades, the evolution of beak size in Galapagos finches has been an instructive model system for the study of adaptation, and findings using this system have largely highlighted the importance of trophic processes in the evolution of beak size (Grant et al. 1976; Grant and Grant 2002). However as island endemics, these species are only subject to the climates present in a narrow (if dynamic) geographic range (Grant and Boag 1980). We predict that if our study were repeated in that clade it would be difficult to find a similar effect of climate on beak size evolution; this contrast underscores the importance of broad comparative studies in evolutionary research. In contrast, our study of honeyeaters and allies across Australasia spans both tropical and temperate zones ~~as well as~~including a continental gradient in precipitation and an extended range of elevations up to above 4.5 km of elevation (Figure 4). ~~Our~~We interpret our findings to suggest that at greater spatial or temporal scales~~,~~ (Meliphagoidea originated 25-30 Mya in early Oligocene; Moyle et al. 2016, Marki et al. 2017), the beak's thermoregulatory role ~~explains~~may explain a more considerable amount of evolutionary change ~~(Figure 2).~~than previously thought. The relative importance of this mechanism versus foraging niche divergence in explaining beak evolution will be an exciting avenue of future research. Ultimately this finding highlights the diversity of selective pressures acting on species morphological traits

(Schluter et al. 1991) and the contrasting patterns they may produce at different spatial and temporal scales (Carroll et al. 2007).

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Figure Titles

Figure 1: Allometric plot of $\log_{10}$ beak surface area against $\log_{10}$ body ~~size~~mass, with body ~~size~~mass raised to the $^{2}/_{3}$ power to account for the dimensional difference between area and volume. Minimum winter temperatures, averaged across species ranges, are shown as the color of the points in the scatterplot, while taxonomic families are depicted with different symbols. Ordinary least squares linear models are shown to aid visualization of climate patterns relative to allometry relationships, with lines colored by family. Most species in regions with warm winters have large beaks for their body mass, whereas most species in regions with cold winters have small beaks for their body mass.

Figure 2: (A-B) Comparisons of beak surface area, as residuals of the relationship shown in Figure 1, to climate averages ~~and diet~~. (C-D) Effects of climate averages on four measures of beak size, shown as effect sizes (standardized β) with 95% confidence intervals. These effect sizes describe bivariate spatial PGLS models comparing trait values across species and including body ~~size~~mass as a covariate to account for allometry. Taxonomic families are represented by the symbols shown in the legend in the upper right.

Figure 3: Comparison of model support across spatial PGLS analyses. Bar plots show Akaike weights for models including combinations of predictor variables that reflect a priori hypotheses. Models that include minimum temperature have majority support in all analyses explaining beak surface area. Summer heat stress

(MaxTemp:~~MinPrecip~~Precip) models are a better fit as explanations of beak depth in Acanthizidae and Maluridae. However, these associations with summer heat stress were not statistically significant when winter minimum temperature was included in multivariate analyses (Table 1).

Figure 4: Geographic distribution of minimum winter temperature (a) and maximum summer temperature (b) from the Bioclim dataset (Hijmans et al. 2005). Beak surface area averaged across species present in 0.5° grid cells (as residuals of regression with body mass) for Meliphagidae (c), Acanthizidae (d) and Maluridae (e). Larger beaks are shown in red and smaller beaks are shown in blue. Cells with less than two species present were excluded (white).

Figure 5: At left, landmark (red) and sliding semi-landmark (magenta) positions used in scoring beak area and size. Semi-landmarks were set at equal intervals between landmarks. At right, effect sizes (standardized β) of spatial PGLS models comparing climate predictor variables to landmark-based measures of beak area and size; lines represent 95% confidence intervals.