

Tests of ecogeographical relationships in a non-native species: what rules avian morphology?

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Abstract The capacity of non-native species to undergo rapid adaptive change provides opportunities to research contemporary evolution through natural experiments. This capacity is particularly true when considering ecogeographical rules, to which non-native species have been shown to conform within relatively short periods of time. Ecogeographical rules explain predictable spatial patterns of morphology, physiology, life history and behaviour. We tested whether Australian populations of non-native starling, *Sturnus vulgaris*, introduced to the country approximately 150 years ago, exhibited predicted environmental clines in body size, appendage size and heart size (Bergmann's, Allen's and Hesse's rules, respectively). Adult starlings ($n = 411$) were collected from 28 localities from across eastern Australia from 2011 to 2012. Linear models were constructed to examine the relationships between morphology and local environment. Patterns of variation in body mass and bill surface area were consistent with Bergmann's and Allen's rules, respectively (small body size and

larger bill size in warmer climates), with maximum summer temperature being a strongly weighted predictor of both variables. In the only intraspecific test of Hesse's rule in birds to date, we found no evidence to support the idea that relative heart size will be larger in individuals which live in colder climates. Our study does provide evidence that maximum temperature is a strong driver of morphological adaptation for starlings in Australia. The changes in morphology presented here demonstrate the potential for avian species to make rapid adaptive changes in relation to a changing climate to ameliorate the effects of heat stress.

Keywords European starling · Invasive · Exotic · Alien · Tarsus length

Introduction

The invasion of non-native species poses a significant threat to global biodiversity (Clavero and Garcia-Berthou 2005). One reason for this is that non-native species can undergo rapid evolution and adaptation in novel environments, increasing their ability to establish and expand their range (Gilchrist et al. 2001; Phillips et al. 2006). Such species therefore provide opportunities to research natural experiments in contemporary evolution and adaptation (Lomolino et al. 2006). This is particularly true when considering ecogeographical rules, to which non-native species have been shown to conform to within relatively short periods of time (Gilchrist et al. 2001).

Ecogeographical rules explain predictable spatial patterns of morphology, physiology, life history and behaviour, at different levels of biological organisation, including intraspecific, interspecific and assemblage (Gaston et al. 2008). These patterns are believed to reflect local

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adaptation to the environment across species' ranges. Understanding ecogeographical rules provides insight into how species may respond on an evolutionary timescale to environmental change (Diamond 1984; Thomas et al. 2004; Gardner et al. 2011). Two commonly tested ecogeographical rules that relate to clinal variation in morphology within and across species' ranges are Bergmann's and Allen's (Bergmann 1847; Allen 1877).

Bergmann's rule predicts that individuals in colder climates will have a larger body size than those in warmer climates (Bergmann 1847). Bergmann's proposed mechanism for driving such a pattern was based on energy expenditure and thermoregulation, such that large individuals would be favoured in colder climates because a lower mass to surface area ratio would require less energy to regulate body temperature. The original postulation of Bergmann's rule only predicted size variation between species; however, it was later revised to include intraspecific adaptation (Rensch 1938). Although the mechanism that drives Bergmann's rule is contested (Blackburn et al. 1999), the general pattern of Bergmann's rule holds true, both intra- and interspecifically, for many endotherms (Ashton et al. 2000; Ashton 2002; Meiri and Dayan 2003). In particular, birds show clear evidence of Bergmann's rule, with conformity to the rule being found both at the inter- and intraspecific level (Millien et al. 2006; Olson et al. 2009).

Allen's rule predicts that endotherms living in hotter climates will have longer appendages (limbs, ears, bill, tails etc.) than those in colder climates (Allen 1877), and correspondingly a larger surface area to volume ratio, in order to allow for shedding of excess heat loads more efficiently. A number of studies have supported birds' conformity to Allen's rule at both the intra- and interspecific level, for bill surface area and exposed leg elements (Snow 1954; Laiolo and Rolando 2001; Nudds and Oswald 2007; Symonds and Tattersall 2010; VanderWerf 2012; Greenberg et al. 2012). Recent experimental evidence shows a relationship between the thermal environment during development and the appendage length of mice, *Mus musculus*, and Japanese quail, *Coturnix japonica* (Burness et al. 2013; Serrat 2013), suggesting an ontogenetic as well as heritable component to temperature-related variation in morphology. While the relative role of the mechanism(s) driving Allen's rule remains unclear, mounting evidence suggests that thermal environment and appendage length are linked.

A third ecogeographical rule is Hesse's (1937), which predicts that species in colder climates will have larger hearts relative to body size than closely related species in warmer climates. Hesse et al. (1937) suggested that the greater metabolic work required to maintain body heat in cold environments would cause individuals to have

relatively larger heart mass and volume when compared to individuals living in hotter environments. This rule has received very little attention, although a recent study conducted by Müller et al. (2014) found that relative heart weight of two rodents, *Myodes glareolus* and *Apodemus flavicollis*, significantly increased with elevation. A small number of lab-based rodent and bird experimental acclimation studies have identified direct effects of ambient temperature on heart mass (Yahav et al. 1997; Hammond et al. 2001; Maldonado et al. 2009; Konarzewski and Diamond 2014; Müller et al. 2014). However, two similar experimental studies investigating six species of lark found no such effects (Williams and Tieleman 2000; Tieleman et al. 2003). Comparative interspecific evidence for Hesse's rule derives from Wiersma et al. (2007) who found heart mass to be significantly smaller in subtropical bird species compared to temperate species. The evidence to date suggests some support for Hesse's rule, potentially driven by intraspecific plastic responses to climate in addition to adaptive evolution. However, there have been no studies that investigate variation in heart mass with regards to latitudinal or climate within a species as a test of Hesse's rule.

Non-native species may experience strong selective pressures as they rapidly expand their geographic range in a new habitat and demonstrate the potential for rapid evolutionary change in response to a release from evolutionary constraints. As such, they shed light on the mechanism by which geographic clines can establish. For example, the house sparrow, *Passer domesticus*, in North America, introduced roughly a century ago, already conforms to both Bergmann's and Allen's rules, suggesting these clines can establish rapidly (Johnston and Selander 1964, 1973; Packard 1967; Baker 1980; Fleischer and Johnston 1982; Murphy 1985).

The common starling, *Sturnus vulgaris*, was introduced into Australia at several locations in the mid to late 1800s (Long 1981) and has since spread to encompass a large latitudinal range and environmental gradient across Australia's east coast. The starling is a successful non-native species throughout the world, having been introduced to three continents outside of its native range in Europe and west Asia (Lowe et al. 2000). The species therefore provides an ideal system in which to test rapid morphological adaptation and conformation to predictions based on ecogeographic rules. Here, we test whether non-native Australian populations of starlings show clinal patterns in morphology that are consistent with Bergmann's rule, Allen's rule and Hesse's rule. In relation to Bergmann's, Allen's and Hesse's rules, respectively, we predict that starlings will be larger, have shorter appendages and have larger hearts in colder climates than hotter climates.

Materials and methods

Sites and sampling

Adult starlings (180 females, 231 males) were collected from 28 localities (Fig. 1) across eastern Australia (maximum distance between sites 1745 km) between May 2011 and October 2012. A genetic study from 2009 suggested that starlings in eastern Australia make up a single genetic population (Rollins et al. 2009). Birds were collected either by trapping, or as carcasses that were collected from shooters or people who trapped and killed birds on their property. Carcasses were frozen on the day of collection for measurement at a later date. Global positioning system (GPS) co-ordinates were taken at the collection site of each individual. If birds collected from the public did not have

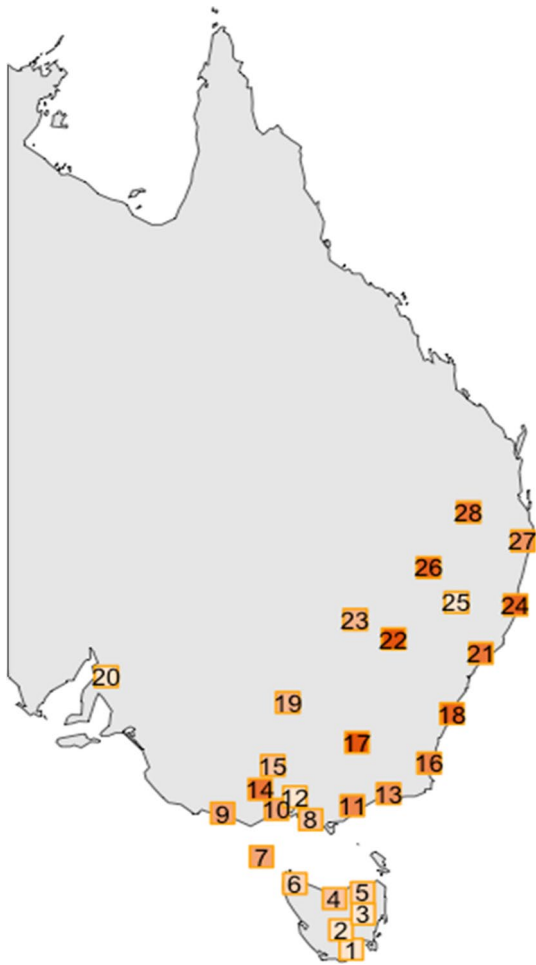


Fig. 1 Map of eastern Australia where the *squares* represent collection localities, with the *shade of colour* representing the number of samples collected. *n* ranged from 1 (light colour) to 31 (dark colour) samples. The *numbers* identify each location for reference against supplementary Table 1

GPS co-ordinates, co-ordinates of the nearest town or reference point were recorded from Google Maps. Collection date was assigned a Julian day number between 1 and 365, with the year beginning on 1 January. The Julian day was circularly transformed following Greenberg and Danner (2012) to create an oscillating date value that forces values at dates closer to one another to be more equivalent. This was done by first transforming the Julian day values into radians ($2\pi(\frac{\text{day}}{365})$) and then summing the sine and cosine value for each radian date. The subsequent cyclical date values represent a numeric variable with August corresponding to the minimum values and February corresponding to the maximum values.

Morphological measurements

Adult birds were identified by their plumage and only adult birds were included in this study. Frozen carcasses were defrosted and birds were sexed anatomically. Measurements included mass (± 0.1 g), tarsus length (from the notch at the intertarsal joint to the ankle, with the foot bent downwards), bill length (the top surface of the bill from the bill tip to the union with the base of the skull), bill width (transactional length from one side of the bill to the other at the posterior edge of the nostrils), bill height (transactional depth from the top surface of the bill to the bottom surface of the bill at the posterior edge of the nostrils). All length measurements were measured using vernier callipers (± 0.02 mm). Bill surface area was calculated following Greenberg et al. (2012), using the approximate formulae for the lateral surface of an elliptical cone ($(\frac{\text{Bill depth} + \text{Bill width}}{4}) \times \text{Bill length}$). Hearts were removed from the bird by cutting the aorta at the closest point to the body of the heart without removing any of the heart proper; the mass was then measured using scientific scales (± 0.001 g).

Body mass can fluctuate in response to short-term environmental factors, such as resource availability, and hence might not always reliably reflect body size. Consequently, we also tested whether a reliable measure of body condition, the scaled mass index (SMI), varies with environmental factors. Finding similar environmental relationships in mass and SMI would suggest that changes in body condition underlie changes in mass, rather than effects of climate. SMI was calculated following Peig and Green (2009), $\text{SMI}_i = m_i \left[\frac{L_0}{L_i} \right]^{b_{\text{SMA}}}$; where m = mass of individual i , L = structural length (e.g. skeletal measurement) for individual i , L_0 = population mean of structural length, and b_{SMA} = the slope of an SMA regression of $\log(\text{population mass}) = \log(\text{population length}) + \beta + \varepsilon$. SMI has been shown to be a good proximate measure for body condition when compared to other indices (Peig and Green 2010). The SMI parameters that required the ‘population’ statistic

were calculated with all samples being included as the ‘population’.

All measurements were made by a single investigator (A. P. A. C.). Measurements were repeated three times on 234 individuals with intraclass correlation coefficients [R package ICC (Wolak et al. 2012)] for each morphological feature as follows: tarsus = 0.984, bill length = 0.999, bill width = 0.976, bill height = 0.998. Mass was used as the measure for body size. When using a univariate measure mass is the best indicator of body size (Rising and Somers 1989; Freeman and Jackson 1990).

Geographic and climatic data

Geographic variables included latitude (degrees south), Euclidean distance (kilometres) to the nearest coastline, and Euclidean distance to the nearest known site of introduction. Euclidean distance to the nearest coastline acts as a geographic proxy for rainfall and temperature, with individuals further from the coast experiencing less rainfall and higher temperatures. ‘Latitude’ was derived from GPS co-ordinates (see above). Climatic variables were extracted for each sample locality from Bioclim data sets (Hijmans et al. 2005) using the raster package in R (Hijmans 2015). Bioclim data sets are interpolated raster files that represent average climatic values for the period 1950–2000. The Bioclim data sets used in this study included Maximum summer temperature (the mean maximum temperature in degrees Celsius of the warmest month), Minimum winter temperature (the mean minimum temperature of the coldest month; in degrees Celsius), and Dry season rainfall (the mean precipitation of the driest month; millimetres).

Statistical analysis

To test Bergmann’s, Allen’s and Hesse’s rules global models were created and a model-averaging approach was taken using corrected Akaike’s information criterion (AICc) (Burnham and Anderson 2002). While all-model comparison is sometimes advised against in regard to model inference (Burnham et al. 2011), it is necessary in

a model-averaging context to produce meaningful estimates, confidence intervals and predictor weights. All models were run as linear models, with response variables of body mass and SMI (Bergmann’s rule), bill surface area and tarsus length (Allen’s rule) and heart mass (Hesse’s rule). Sex was included as a predictor variable in all global models to control for sex-based variation in morphological measurements. Date was included as a predictor variable in all global models to control for seasonal variation in morphological measurements. Body mass was included as a predictor variable when testing Allen’s and Hesse’s rule to control for the effect of body size. Collinearity between geographic and climatic predictor variables required that separate models be created to test geographic predictors and climatic predictors independently. Latitude and Distance to Coast were predictors in the geographic models, whilst the climatic models included Maximum summer temperature, Minimum winter temperature, and Dry season rainfall. All model variables were normalised so that they had a mean of 0 and a SD of 1, to enable comparison of predictors. The sample sizes for models testing Bergmann’s, Allen’s, and Hesse’s rules and condition varied because only samples with records for all of the variables being included in the model were kept (some individuals were damaged during collection and could not be completely measured for all morphological traits).

Using the R package MuMIn (Barton 2013), every permutation of predictor variables was run for each global model using the function dredge. The resulting model selection object was passed to a function from the same package, model.avg, which produced average model parameters from across all models. It also calculated variable importance for each predictor variable, which is the sum of the Akaike weights of each model in which the variable appeared. R^2 -values were taken from the global model of each test, to provide an indication of the predictive value of these models. The same subset of data was used in the geographical and climatic models (Table 1), making the AICc scores between geographic and climatic models for each of our tests comparable.

Table 1 Summary statistics for each of the response variables used in the analysis

Response variable	Test	No. samples (female/male)	Mean \pm SD	Coefficient of variation	Minimum	Maximum
Mass (g)	Bergmann’s rule	383 (165/218)	78.0 \pm 5.8	7.4	60.0	93.5
Scaled mass index	Bergmann’s rule	370 (159/211)	77.9 \pm 6.2	8.0	59.7	100.8
Bill surface area (mm ²)	Allen’s rule	346 (153/193)	260.4 \pm 17.6	6.8	204.1	325.1
Tarsus length (mm)	Allen’s rule	369 (158/211)	28.4 \pm 0.9	3.2	25.9	31.0
Heart mass (g)	Hesse’s rule	322 (147/175)	1.1 \pm 0.2	18.2	0.7	1.6

Table 2 Model-averaged output of linear models testing the relationship between environmental variables, geographic and climatic, and starling mass

Response variable $N = 383$	Predictor variable	Estimate	Lower CI 2.5 %	Upper CI 97.5 %	Z-value	Importance
Mass $R^2 = 0.215$ AICc = 1005.217	Intercept	-0.393	-0.530	-0.256	5.634	
	Sex ^a	<i>0.691</i>	<i>0.509</i>	<i>0.872</i>	7.457	1.000
	Latitude ^a	<i>0.115</i>	<i>0.027</i>	<i>0.232</i>	1.806	0.890
	Distance to coast ^a	-0.239	-0.347	-0.132	4.349	1.000
	Date	-0.026	-0.149	0.033	0.609	0.443
Mass $R^2 = 0.211$ AICc = 1004.410	Intercept	-0.385	-0.522	-0.248	5.521	
	Sex ^a	<i>0.676</i>	<i>0.495</i>	<i>0.858</i>	7.314	1.000
	Maximum temperature ^a	-0.304	-0.399	-0.209	6.276	1.000
	Minimum temperature	0.012	-0.062	0.134	0.352	0.319
	Dry season rainfall	0.004	-0.087	0.114	0.133	0.271
	Date	-0.010	-0.124	0.058	0.345	0.316

Estimates are based on scaled variables. For definition of variables see text. Geographic and climatic models were run on the same data set ($n = 383$ individuals) to enable comparison of corrected Akaike's information criterion (AICc) values

^a Variables with confidence intervals (CI) that do not include 0 (*italic*)

Results

Bergmann's rule

Australian starling populations showed trends in mass that were consistent with Bergmann's rule (Table 2; Fig. 2), with heavier individuals found at higher latitudes and in cooler environments. Body condition (SMI) did not show the same trend as mass in regards to latitude and/or mean maximum temperature, which increases our confidence that changes in mass reflect true changes in overall mass, rather than fluctuations in body condition (Table 3).

Distance to coast also showed a strong negative relationship with mass, with starlings further inland being lighter than those nearer the coast (Table 2). But here, body condition (SMI) also showed a strong negative relationship with the distance to the coast, suggesting birds were in worse condition the further they were from the coast, possibly indicating more challenging foraging conditions at the edge of their range.

The best climatic model for mass had a slightly lower (i.e. better) AICc value than the best geographic model ($\Delta\text{AICc} = 0.807$). The global geographic and climatic models explained 21.5 and 21.1 % of the variation in the data, respectively (Table 2). In contrast, the geographic or climatic models explained little of the variation in starling body condition (SMI) with 3.7 and 3.3 % explained by each model respectively (Table 3), although the negative relationship with date in both geographic and climatic models suggests birds were in better condition at the beginning of the breeding season (August) than at the beginning of the non-breeding season (February) (Table 3).

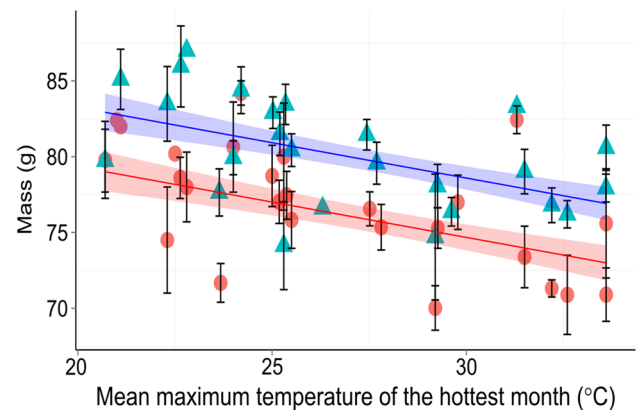


Fig. 2 Modelled relationship between starling mass and mean maximum temperature of the hottest month. The lines represent the fitted values for starling mass across the range of the mean maximum temperature of the hottest month, while all other predictor variables are held at the mean value. The shaded areas represent the 95 % confidence intervals for the fitted values. The lower line and circle points represent female starlings, and the upper line and triangle points represent males. Data points represent mean values (with SE bars) for starlings at each collection locality (colour figure online)

Allen's rule

Australian starling populations showed trends in bill surface area, but not tarsus length, which were consistent with Allen's rule. Mean maximum temperature and dry season rainfall of the driest month showed strong positive relationships with bill surface area, suggesting that birds living in hotter climates have larger bill surface areas than those in colder climates, and birds in wetter areas have larger

Table 3 Model-averaged output of linear models testing the relationship between environmental variables, geographic and climatic, and starling scale mass index (SMI)

Response variable <i>N</i> = 370	Predictor variable	Estimate	Lower CI 2.5 %	Upper CI 97.5 %	Z-value	Importance
SMI <i>R</i> ² = 0.037 AICc = 1044.600	Intercept	<0.000	−0.101	0.101	0.000	
	Sex	0.020	−0.049	0.154	0.484	0.376
	Latitude	−0.005	−0.143	0.107	0.147	0.294
	Distance to coast ^a	−0.098	−0.227	−0.013	1.438	0.813
	Date ^a	−0.122	−0.236	−0.032	1.956	0.912
SMI <i>R</i> ² = 0.033 AICc = 1047.000	Intercept	<0.000	−0.101	0.101	0.000	
	Sex	0.018	−0.051	0.152	0.466	0.367
	Maximum temperature	−0.022	−0.170	0.054	0.494	0.386
	Minimum temperature	0.032	−0.040	0.182	0.616	0.451
	Dry season rainfall	0.029	−0.044	0.178	0.576	0.429
	Date ^a	−0.125	−0.240	−0.034	1.985	0.916

Estimates are based on scaled variables. For definition of variables see text. Geographic and climatic models were run on the same data set (*n* = 370 individuals) to enable comparison of AICc values. For other abbreviations, see Table 2

^a Variables with CIs that do not include 0 (*italic*)

bills than those in dryer areas (Table 4; Fig. 3). We found no relationship between our geographic variables and bill surface area but the climatic model fit the data considerably better than the geographic model (Δ AICc = 19.273; Table 4). Both global models accounted for more than 30 % of variation in bill surface area.

By contrast, tarsus length showed a contra-Allen's rule pattern: a positive relationship with latitude and a negative relationship with mean maximum temperature, suggesting birds further from the equator and in colder environments have longer tarsi (Table 4). The best geographic model had a slightly lower AICc value than the best climatic model (Δ AICc = 1.048). The global geographic and climatic models explained 22.9 and 23.0 % of the variation in the data, respectively (Table 4).

Hesse's rule

Heart mass showed a strong positive relationship with mass, whereby larger individuals have larger hearts (Table 5). The only other effect we discovered was a positive relationship between heart mass and distance from coast. Otherwise, there was no evidence that any geographic or climatic variables influenced heart mass.

Discussion

Non-native species are useful for testing patterns of evolutionary change because the time they have had to adapt to the local environment is known. Patterns of variation in

mass and bill surface area in non-native common starlings in Australia were consistent with Bergmann's and Allen's rule, respectively. We found no evidence to support Hesse's hypothesis that relative heart size is larger in individuals which live in colder climates. These results suggest that starlings in Australia have responded to large-scale environmental pressures and within ~150 years show morphological variation across their geographical range in relation to environmental conditions. These results are consistent with similar studies that tested Bergmann's and Allen's rules on bird species (Millien et al. 2006) and particularly non-native birds (Johnston and Selander 1964).

As predicted by Bergmann's rule, starlings were larger in colder localities and at higher latitudes than in hotter environments (Bergmann 1847; Ashton et al. 2000). In the past, studies investigating Bergmann's rule have often used latitude as a proxy for temperature where temperature data were not available. This is problematic because latitude does not directly affect body size but is rather correlated with many factors that do (Hawkins and Felizola Diniz-Filho 2004; Yom-Tov and Geffen 2011). Our data are consistent with the interpretation that latitudinal trends are driven by the interaction of latitude with environmental variables such as maximum temperature. For starlings in Australia, the climatic models including mean maximum temperature were better fits for the data than the geographic models including latitude.

Body mass can fluctuate in response to short-term environmental factors, such as resource availability, and might not always reliably reflect structural body size, but rather body condition. However, in this study, mean maximum temperature was not a strong predictor of starling body

Table 4 Model-averaged output of linear models testing the relationship between environmental variables, geographic and climatic, and starling bill surface area (BSA) and tarsus length

Response variable $N = 346$ and 369	Predictor variable	Estimate	Lower CI 2.5 %	Upper CI 97.5 %	Z-value	Importance
BSA $R^2 = 0.307$ AICc = 863.293	Intercept	-4.569	-5.905	-3.234	6.705	
	Sex ^a	<i>0.342</i>	<i>0.247</i>	<i>0.437</i>	7.065	1.000
	Mass ^a	<i>0.059</i>	<i>0.041</i>	<i>0.076</i>	6.720	1.000
	Latitude	-0.010	-0.128	0.062	0.334	0.314
	Distance to coast	0.012	-0.061	0.134	0.356	0.322
	Date	-0.004	-0.105	0.075	0.166	0.274
BSA $R^2 = 0.358$ AICc = 844.020	Intercept	-4.622	-5.978	-3.266	6.681	
	Sex ^a	<i>0.345</i>	<i>0.253</i>	<i>0.437</i>	7.318	1.000
	Mass ^a	<i>0.059</i>	<i>0.042</i>	<i>0.077</i>	6.694	1.000
	Maximum temperature ^a	<i>0.114</i>	<i>0.026</i>	<i>0.234</i>	1.748	0.880
	Minimum temperature	-0.063	-0.196	0.007	1.03	0.669
	Dry season rainfall ^a	<i>0.239</i>	<i>0.137</i>	<i>0.342</i>	4.592	1.000
	Date	-0.026	-0.149	0.032	0.616	0.448
Tarsus $R^2 = 0.229$ AICc = 962.665	Intercept	<0.000	-0.091	0.091	0	
	Sex ^a	<i>0.183</i>	<i>0.086</i>	<i>0.280</i>	3.613	0.997
	Mass ^a	<i>0.325</i>	<i>0.222</i>	<i>0.427</i>	6.214	1.000
	Latitude ^a	<i>0.132</i>	<i>0.041</i>	<i>0.237</i>	2.284	0.947
	Distance to coast	<0.001	-0.113	0.111	0.008	0.272
	Date ^a	<i>0.093</i>	<i>0.018</i>	<i>0.201</i>	1.603	0.851
Tarsus $R^2 = 0.230$ AICc = 963.713	Intercept	<0.000	-0.091	0.091	0.000	
	Sex ^a	<i>0.185</i>	<i>0.088</i>	<i>0.283</i>	3.657	0.997
	Mass ^a	<i>0.322</i>	<i>0.218</i>	<i>0.426</i>	6.060	1.000
	Maximum temperature ^a	<i>-0.136</i>	<i>-0.248</i>	<i>-0.040</i>	2.223	0.944
	Minimum temperature	-0.019	-0.153	0.051	0.475	0.374
	Dry season rainfall	-0.016	-0.150	0.059	0.413	0.348
	Date ^a	<i>0.125</i>	<i>0.039</i>	<i>0.224</i>	2.300	0.949

Estimates are based on scaled variables. For definition of variables see text. Geographic and climatic models were run on the same data set ($n = 346$ and 369 individuals respectively, for the morphological response variables) to enable comparison of AICc values. For other abbreviations, see Table 2

^a Variables with CIs that do not include 0 (*italic*)

condition (SMI), which suggests that the observed temperature cline in body mass was not due to changes in condition, but rather represents a true change in body size. By contrast, the predictor variable Distance to coast held the same relationship with mass and body condition suggesting that changes in size when starlings move away from the coast may be due to changes in condition. This may be attributed to changes in resource availability and landscape productivity as the birds moves from wet, productive coastal habitat to dry, unproductive inland habitat (Morton et al. 2011). Date was the only other predictor variable that had a relationship with body condition; this is unsurprising given that changes in resource availability and life history throughout a year can significantly influence body condition. Our measures of mass and condition do not account for complex seasonal variation driven by climatic or geographic factors and specific dietary and reproductive needs,

and hence may include additional unexplained variation that may obscure broader patterns. Nevertheless, we believe that evidence of relationships between environmental variables and body condition at least allow the identification of cases, as with our relationship with distance from coast, where variation in size (mass) is likely due to differences in fluctuating condition. While the evidence suggests that starlings have the capacity to adapt to varied climatic conditions it is important to recognise the complex interactions that changes in body size can have on fitness. For instance, smaller body size has the associated cost of a decreased capacity to deal with thermal extremes (Peters 1983); thus, in a world of increased climatic extremes, smaller individuals may be at a disadvantage.

Bird bills are highly vascularised appendages that contribute to thermoregulation (Tattersall et al. 2009; Symonds and Tattersall 2010; Greenberg et al. 2012; Burness et al.

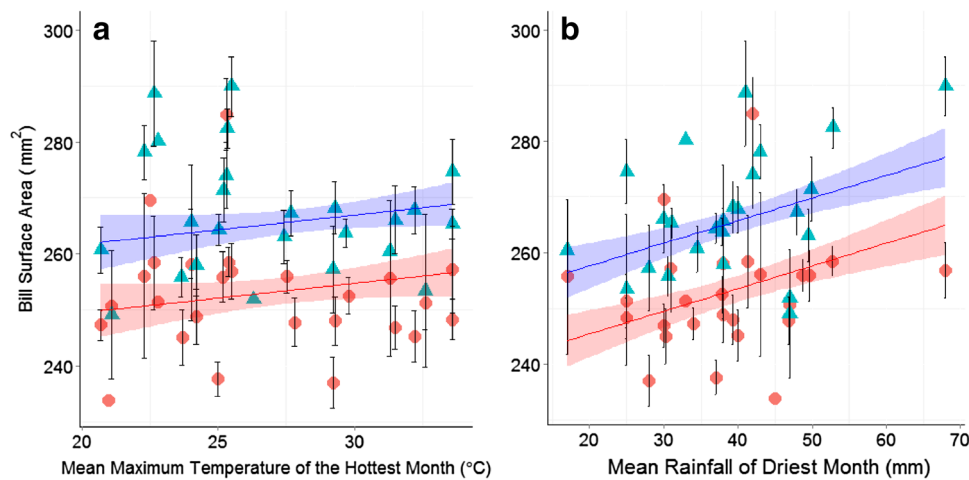


Fig. 3 Modelled relationships between starling bill surface area and **a** mean maximum temperature of the hottest month and **b** mean rainfall of the driest month. *Lines* represent the fitted values for starling bill surface area across the range of a single predictor variable, while all other predictor variables were held at their mean value.

The *shaded areas* represent the 95 % confidence intervals for the fitted line. The *lower line* and *circle points* represent females, and the *lower line* and *triangle points* represent males. The *points* represent the mean bill surface area (with SE) of female and male starlings at each collection locality (colour figure online)

Table 5 Model-averaged output of linear models testing the relationship between environmental variables, geographic and climatic, and starling heart mass

Response variable $n = 322$	Predictor variable	Estimate	Lower CI 95 %	Upper CI 95 %	Z-value	Importance
Heart mass $R^2 = 0.122$ AICc = 881.276	Intercept	<0.000	-0.104	0.104	0.000	
	Sex	-0.076	-0.223	0.004	1.083	0.690
	Mass ^a	<i>0.375</i>	<i>0.248</i>	<i>0.502</i>	5.774	1.000
	Latitude	0.005	-0.110	0.143	0.135	0.286
	Distance to coast ^a	<i>0.103</i>	<i>0.012</i>	<i>0.246</i>	1.394	0.801
	Date	-0.003	-0.118	0.095	0.108	0.268
Heart mass $R^2 = 0.121$ AICc = 883.696	Intercept	<0.000	-0.104	0.104	0.000	
	Sex	-0.061	-0.211	0.014	0.926	0.617
	Mass ^a	<i>0.355</i>	<i>0.233</i>	<i>0.476</i>	5.715	1.000
	Maximum temperature	0.049	-0.027	0.210	0.775	0.539
	Minimum temperature	0.017	-0.068	0.165	0.397	0.342
	Dry season rainfall	-0.030	-0.183	0.043	0.588	0.434
	Date	<0.001	-0.106	0.107	0.006	0.263

Estimates are based on scaled variables. For definition of variables see text. Geographic and climatic models were run on the same data set ($n = 322$ individuals) to enable comparison of AICc values. For abbreviations, see Table 2

^a Variables with CIs that do not include 0 (data in *italic*)

2013). A resurgence of interest in Allen's rule has focussed on the bird bill because its thermoregulatory function makes it a good candidate as an appendage under selection in relation to ambient temperature. A manipulative experiment using Japanese quail, *C. japonica*, suggested that intraspecific variation in bill attributes relating to thermoregulation is possible (Burness et al. 2013), although the relative roles that phenotypic plasticity and adaptation play in this variation are unclear. Our data are consistent with Allen's rule, supporting the idea that rapid adaptation, driven by a climatic pressure, is possible. Dry season

rainfall was also an important factor in the Allen's rule model, with birds in wetter environments having larger bills than those in drier areas. This contrasts with reports for North American song sparrows, *Melospiza melodia*, where bill size increased with aridity (Greenberg and Danner 2012), but conforms with trends observed in crimson rosellas, *Platycercus elegans*, in Australia (Campbell-Tennant et al. 2015). Although, the different ways in which these species use their bills for foraging makes it difficult to draw conclusions, the result for starlings may reflect the difficulties of shedding heat loads by evaporative heat loss in hot

and wet environments, selecting for larger bills for non-evaporative heat loss in such environments. Alternatively, differences may be driven or mediated by other selective pressures, for instance, longer bills may improve starling access to invertebrates in wet soil when probing during foraging (East and Pottinger 1975). It is important to note that sex explained a large amount of the variation in models that tested mass and bill surface area. This is unsurprising given starlings show a small amount of sexual dimorphism with females generally being smaller than males.

Contrary to Allen's rule, tarsus length in Australian starlings showed that individuals in hotter climates were more likely to have shorter legs than those in colder climates. Although leg elements have been shown to play an important role in heat transfer for starlings during flight (Ward et al. 1999), our results confirm a previous study which found no relationship between starling tarsus length and temperature (Blem 1974). While there is clear evidence that clines in tarsus length of some taxa follow Allen's rule (Laiolo and Rolando 2001; VanderWerf 2012), for other taxa the relationship is either not strong or even weakly opposite (Symonds and Tattersall 2010). This evidence, coupled with our results, suggests that the effect of temperature on tarsus length varies by species and that other factors have a contributing effect that may exceed that of temperature. For example, as starlings forage on the ground, leg length will be fundamental for foraging ecology. The evolutionary drivers are therefore unclear.

To our knowledge this is the first study to test Hesse's rule by looking at intraspecific variation in heart mass across a large ecogeographical cline. While previous work has found patterns of intraspecific variation in heart mass that were consistent with Hesse's rule (Yahav et al. 1997; Hammond et al. 2001; Maldonado et al. 2009; Konarzewski and Diamond 2014), the results here do not support Hesse's prediction. The studies that have tested the effects of climatic variation on heart mass have been predominantly experimental, and have identified a causal relationship between temperature change and heart mass (Yahav et al. 1997; Hammond et al. 2001; Maldonado et al. 2009; Konarzewski and Diamond 2014; Müller et al. 2014). Heart mass did show a relationship with distance to coast, with birds inland having bigger hearts than those on the coast, which could be due to inland habitats being more metabolically demanding because they are drier and hotter environments (Morton et al. 2011). We note the possibility that blood clots within the heart may add noise to the data, obscuring any underlying pattern; however, we find it unlikely that this contributed significantly within this relatively large data set.

Evidence suggests that northern and southern populations of starlings in Australia were established from two separate source populations (Jenkins 1977; Balmford 1978). If significant morphological variation existed

between these source populations it is possible that the variation seen along a latitudinal gradient was a historic signal of morphological variation. However, high levels of gene flow across the geographic region investigated (Rollins et al. 2009) suggest significant movement between northern and southern populations, likely making it difficult to maintain morphological differences based on source population. Also, patterns of morphological variation were most strongly related to maximum temperature which was not strictly related to latitude, or a north–south divide. For these reasons we do not believe that possible historic patterns of morphological variation from source populations are driving the patterns we see in Australian starlings.

Together, these results demonstrate the ability of non-native species to respond to new environments and undergo rapid morphological change. The anatomical changes documented here have occurred within approximately 40 starling generations. These changes demonstrate the potential for avian species to make rapid adaptive changes in relation to a changing climate to ameliorate the effects of heat stress. This is supported by evidence for changes in body size and bill size over the past century consistent with the effects of climatic warming (Gardner et al. 2009; Campbell-Tennant et al. 2015). Further controlled rearing experiments under standardised conditions are required to tease apart the environmental and genetic bases of these morphological adaptations. Further studies will allow documentation of how species might fare in a rapidly disrupted climatic environment.

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Author contribution statement A. P. A. C., M. R. E. S., K. L. B., P. C. and C. D. H. S. conceived and designed the study. A. P. A. C. collected the data. A. P. A. C. and M. R. E. S. analysed the data. A. P. A. C., M. R. E. S. and K. L. B., wrote the manuscript; all authors provided editorial advice.

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