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Geographic variation in morphology of Northern Cardinals: possible application of Bergmann's Rule?

Variación geográfica en la morfología de los Cardenales Norteños: ¿posible aplicación de la regla de Bergmann?

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ABSTRACT. Geographic variation in morphological characteristics of a species can be influenced by environmental conditions. Bergmann's rule states that endotherms inhabiting colder climates at higher latitudes are predicted to have larger body sizes. However, application of Bergmann's rule to songbirds has yielded mixed results. Our study examines whether geographic variation in morphology conforms to Bergmann's rule in the Northern Cardinal (*Cardinalis cardinalis*), a resident songbird with a broad distribution extending from Central America to Canada. Measures of body size (mass, wing chord, tarsus length) and feathers that could possibly serve as ornaments (tail, crest length) were compared in populations of cardinals near the northern (Ohio, New York; USA) and southern (Mississippi, Florida; USA) extremes of the species' North American distribution. Cardinal populations in Florida and Mississippi had significantly smaller body size than populations in Ohio and New York. Southern birds weighed significantly less than northern birds and had significantly shorter tarsus and wing chord lengths. Cardinal populations from higher and lower latitudes were clearly distinguished by a primary discriminant function comprising a linear combination of mass, wing chord length, and tarsus length. Northern and southern populations were not as clearly distinguished by tail or crest feather length. Our findings suggest that Bergmann's rule could apply to the Northern Cardinal. Given the broad distribution of this species, it is plausible that larger body sizes could be necessary to conserve heat, avoid starvation, or some combination of both factors in populations of cardinals at higher latitudes and that these adaptations are less necessary closer to the equator.

RESUMEN. La variación geográfica de las características morfológicas de una especie puede estar influenciada por las condiciones ambientales. La regla de Bergmann establece que los endotermos que habitan en climas más fríos y en latitudes más altas tienen, previsiblemente, un mayor tamaño corporal. Sin embargo, la aplicación de la regla de Bergmann a los pájaros cantores ha arrojado resultados contradictorios. Nuestro estudio examina si la variación geográfica de la morfología se ajusta a la regla de Bergmann en el Cardenal Norteño (*Cardinalis cardinalis*), un pájaro cantor residente con una amplia distribución que se extiende desde Centroamérica hasta Canadá. Se compararon medidas de tamaño corporal (masa, cuerda alar, longitud del tarso) y plumas que podrían servir de adorno (cola, longitud de la cresta) en poblaciones de cardenales cercanas a los extremos norte (Ohio, Nueva York; EE.UU.) y sur (Mississippi, Florida; EE.UU.) de la distribución norteamericana de la especie. Las poblaciones de cardenales de Florida y Misisipi presentaron un tamaño corporal significativamente menor que las poblaciones de Ohio y Nueva York. Las aves del sur pesaron significativamente menos que las del norte y tenían longitudes de tarso y cuerda alar significativamente más cortas. Las poblaciones de cardenales de las latitudes más altas y más bajas se distinguieron claramente por una función discriminante primaria que comprende una combinación lineal de masa, longitud de la cuerda del ala y longitud del tarso. Las poblaciones del norte y del sur no se distinguieron tan claramente por la longitud de las plumas de la cola o de la cresta. Nuestros resultados sugieren que la regla de Bergmann podría aplicarse al Cardenal Norteño. Dada la amplia distribución de esta especie, es plausible que los tamaños corporales más grandes puedan ser necesarios para conservar el calor, evitar la inanición o alguna combinación de ambos factores en las poblaciones de cardenales de latitudes más altas y que estas adaptaciones sean menos necesarias más cerca del ecuador.

Key Words: *Bergmann's rule; Cardinalis cardinalis; geographic variation; morphology; Northern Cardinal*

INTRODUCTION

Geographic variation in morphological characteristics occurs in species that inhabit a broad distribution. In some taxa, such variation in morphology is driven by variation in environmental conditions, such as temperature. Bergmann's rule states that endothermic species inhabiting colder climates at higher latitudes are larger in body size than endotherms living in warmer climates at lower latitudes (Bergmann 1847). This ecogeographical pattern is later modified by Rensch (1938) and James (1970) to emphasize intraspecific variation between populations that occur across a distribution.

Results of studies investigating the application of Bergmann's rule and body size measures are mixed (homeotherms, reviewed by Meiri and Dayan 2003; poikilotherms, reviewed by Vinarski 2014). Although the majority of examined mammal and avian species appear to conform to the pattern (reviewed in Ashton et al. 2000, Ashton 2002, Meiri and Dayan 2003), several do not (birds: e.g., Blem 1981, Freeman 2017; mammals: e.g., Ralls and

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Harvey 1985, Baumgardner and Kennedy 1993, Sargis et al. 2018). This pattern appears to have interspecific and intraspecific application, especially in birds (Ashton 2002, Salewski and Watt 2017).

Additional meta-analyses of bird studies examining geographic variation in body size suggest that sedentary (resident) species are more likely to follow Bergmann's rule than migratory species (reviewed in Meiri and Dayan 2003, but see Ashton 2002 for a slightly different conclusion). Among North American passerines, multiple resident species occupying a broad distribution exhibit larger sizes at higher latitudes (e.g., Blue Jays, *Cyanocitta cristata*, James 1970; Song Sparrows, *Melospiza melodia*, Smith 1998), while some do not (e.g., Horned Lark, *Eremophila alpestris*, Niles 1973). This warrants further investigation in additional non-migratory species whose distribution encompasses drastic variation in climate.

One possibility for conflicting results in studies examining Bergmann's rule in birds could be discrepancies in what body measures are used as proxies for "size" with some studies examining mass to draw conclusions (e.g., Meiri and Dayan 2003) and others using combinations of wing and leg measures as variables (e.g., Bull 2006, Fan et al. 2019, Lee et al. 2021). Further, most studies assessing Bergmann's rule in birds rely on examinations of museum specimens, which can limit the measures one can take because of the permanent position of prepared specimens, gradual deterioration of specimen quality, or a specimen having an incomplete collection record (e.g., the collector did not record the mass of the specimen). A novel study using multiple body measures of live birds as a proxy for body size, including mass, wing, leg, and feather measures, will be more informative in determining whether geographic intraspecific variation in body size exists between populations.

The Northern Cardinal (*Cardinalis cardinalis*; hereafter, cardinal) is a good candidate for intraspecific comparisons of morphological variation. Cardinals are a common resident bird in the United States with a generalist diet, a long breeding season (6+ months), and a broad distribution extending from Central America to southern Canada (Halkin et al. 2021). The climate of the lower extreme of this species' range is closer to tropical conditions than the climate of the northern extreme, which routinely experiences cold temperatures and significant snowfall every winter. Cardinal behavior, communication, and physiology have all been studied extensively (reviewed in Halkin et al. 2021), but comparative studies of morphological variation across the species' range are needed. Here, we investigate whether Bergmann's rule applies to this species by examining whether populations of live, wild cardinals at higher latitudes and colder climates (Ohio, New York) have larger body sizes than those inhabiting lower latitudes and warmer climates (Mississippi, Florida).

METHODS

Field techniques

Four non-captive populations of cardinals across the eastern continental United States Research were assessed in this study. Locations included: Davie, Florida (Tree Tops Park; 26°4'13.932" N; 80°16'31.248" W, data collected in 2020 by RA, JMJ, MS), Hattiesburg, Mississippi (Lake Thoreau Environmental Center; 31°20'55.821" N; 89°25'1.228" W, data collected in 2007–2011 by JMJ, MSD), Dayton, Ohio (Aullwood Audubon Center and Farm, 39°52'23.134" N; 84°16'30.032" W, data collected 1999–2002 by JMJ), and Oswego, New York (Rice Creek Field Station, 43°25'48" N; 76°32'58.995" W, data collected in 2019–2020 by DTB). Weather patterns (maximum average temperature, minimum average temperature, and precipitation; Cli-MATE, Midwestern Regional Climate Center) in all locations were typical for their respective regions in the years assessed, with the exception of 1999 in Dayton, Ohio, which experienced a drought (received 82% of normal precipitation amount, Cli-MATE, Midwestern Regional Climate Center). This anomaly in weather did not impact results and cardinals assessed during this year remained in the dataset. Data were collected year-round in some populations (Mississippi, Ohio), but not all (Florida, New York). Therefore, to eliminate a potential impact of seasonality on body mass, data analysis was restricted to measures taken between 1 February and 31 August (pre-breeding and breeding seasons) at all sites each year of the work.

Cardinals in all populations were captured in mist nets or Potter traps between 06:00–12:00. All birds received a USGS band plus a unique combination of three plastic color bands for field identification. Skeletal and feather measures were taken with the bird in a standard banding grip. Wing length (mm) was assessed using unflattened wing chord measurement, recording the length from the carpel joint to the end of the longest primary feather. Tarsus length (mm) of the tarsometatarsal bone was measured by folding a bird's foot 90 degrees to the tarsus and reading calipers placed from the top of the bend to the notch of the intertarsal joint. Body mass (g) in the Ohio, Mississippi, and New York populations was determined by placing a bird within a cloth holding bag, weighing the bird and the bag with a Pesola spring scale to the nearest 0.5 g, and then subtracting the mass of the bag to calculate mass of the bird. Mass of individuals in the Florida population was obtained by placing a bird wrapped in a nylon sock within a paper tube on a tared digital scale and recorded to 0.01 g. Even with the difference in mass measuring technique, Florida birds were lighter by several grams, a difference that is very unlikely to result from using a different type of scale (see Results).

Because it is uncertain how the crest and/or the tail function in Northern Cardinals (e.g., ornamentation, insulation, locomotion, etc.; Jawor et al. 2003), we included them in this analysis. Any variation (or the lack thereof) across populations could potentially provide some clarification of the full functions of these structures. Crest length (mm) was measured with a wing ruler placed on the skull of the bird behind the bird's crest. Feathers were not flattened and the length of the longest crest feather was recorded to the nearest mm. Tail length (mm) was measured with a wing ruler placed along the underside of the bird between the rectrices and the undertail coverts, until the ruler came to a stop at the bird's rump. Feathers were not flattened and the length of the longest rectrix was measured to the nearest mm. We acknowledge that having multiple researchers assessing multiple populations might result in subtle incongruencies with measures taken. However, one researcher (JMJ) in our study did measure both a northern population (Ohio) and a southern population (Mississippi), assuring that no differences in technique and accuracy existed between this northern-southern body size comparison. All individuals were released from their original point of capture following banding and data collection.

Statistical analyses

Analyses were conducted using the IBM SPSS Statistics package (version 28.0). Measurements of mass, wing chord length, tarsus length, crest length, and tail length taken from live cardinals from the following locations were analyzed: Ohio (N = 216; males (M) = 114, females (F) = 102), New York (N = 46; M = 31, F = 15), Mississippi (N = 124; M = 50, F = 74), and Florida (N = 46; M = 38, F = 8). These dependent measures were combined into a variable we will refer to as "body size" in a multiple analysis of variance. Significant effects of location on the combined dependent variable were followed by single analysis of variance on each variable component, which was in turn followed by Tukey's pairwise comparison post-hoc test.

The assumptions behind the multiple analysis of variance and discriminant analysis were checked with the following outcomes and actions: of 458 cases, four cases were filtered out as multivariate outliers and 22 cases were filtered out because they were missing one or more body feature measures. The data violated the assumption of multivariate normality, but both this and the following discriminant analysis were considered robust in the face of this violation, particularly with samples the size of those in this dataset (Tabachnik and Fidell 2013). When the linearity of relationship among all pairs of independent variables/predictors was assessed, only two pairs departed significantly from linearity (mass and tarsus length; mass and crest length). As a result, the analyses may have lost some statistical power. This would manifest as decreased ability to detect significant differences among the groups in the body measures in the case of multiple analysis of variance and decreased ability to detect significant contributions of each body measure to group discrimination in the case of discriminant analysis. The location groups have non-homogenous variances in the body feature measures. This was addressed in the multiple analysis of variance by using Pillai's trace rather than Wilk's lambda to evaluate significant effects of location on the combined dependent variable of body size. In the discriminant analysis, this required the use of separate covariance matrices rather than combined covariance matrices in creating the discriminant functions. The resulting discriminant functions also ran an inflated risk of misclassifying cases into location groups with larger variances. The dependent variables/predictors showed no evidence of multicollinearity. Multicollinearity was examined by checking tolerance and variance inflation factors as well as bivariate correlation coefficients. All tolerance values were well above 0.10, all variance inflation factors were no greater than 3.0, and the highest bivariate correlation coefficient was r = 0.58 (mass and wing chord length for the combined sexes). The group sizes were not equivalent, ranging from the mid-40's for New York and Florida-based observations to just over 200 observations from Ohio. Therefore, the discriminant analysis generated prior probabilities of group membership based on the actual sample sizes.

The data were first analyzed with the data from both sexes combined, and then disaggregated by sex. However, we are cautious about generalizing the analysis for females to the population at large given the relatively small sample sizes (only eight observations from Florida and 15 from New York).

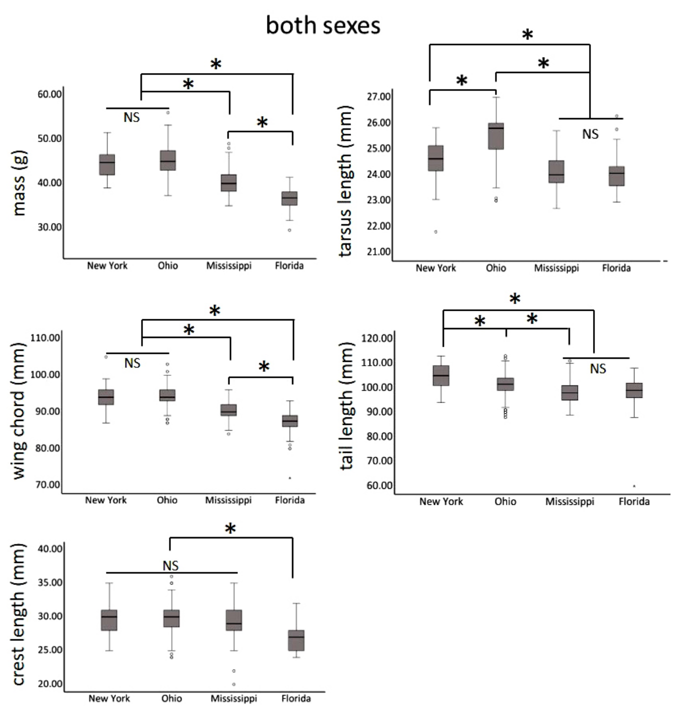
RESULTS

Analyses with sexes combined

Multiple analysis of variance

There was a significant effect of location on the combined body size variable (Pillai's trace = 1.007, $F_{(15, 1278)}$ = 43.07, P < 0.001, partial η^2 = 0.336). There were also significant effects of location on each of the five body measures; in descending order of effect size, mass ($F_{(3, 428)}$ = 155.02, P < 0.001, partial η^2 = 0.521), tarsus length ($F_{(3,428)}$ = 137.96, P < 0.001, partial η^2 = 0.492), wing chord length ($F_{(3,428)}$ = 115.00, P < 0.001, partial η^2 = 0.446), tail length ($F_{(3,428)}$ = 22.93, P < 0.001, partial η^2 = 0.138), and crest length ($F_{(3,428)}$ = 19.95, P < 0.001, partial η^2 = 0.123). Each of these significant effects was further explored with Tukey's post-hoc pairwise comparisons tests. Boxplots of the data from each location are shown in Figure 1.

Fig. 1. Boxplots for the five body measurements with sexes combined. Line in center of box median, bottom and top of box = 25th and 75th percentile, respectively, whiskers = minimum and maximum, open circles = outliers (defined as any observation more than 1.5 times larger than the interquartile range). The results of Tukey's pairwise comparison tests are shown above the boxes. NS = no significant difference between the groups, * = p < 0.001. Northern Cardinals (*Cardinalis cardinalis*) observed in New York and Ohio were significantly heavier than those observed in Mississippi and Florida, and also had significantly longer tarsus lengths, wing chords, and tails. Florida birds had significantly smaller crests than the other groups.



Northern Cardinals observed in Florida and Mississippi were significantly smaller than birds observed in Ohio and New York.

The southern birds weighed significantly less than the northern birds and also had significantly shorter tarsus and wing chord lengths than northern birds. Southern birds also had significantly shorter tails, though this difference was not as strong as the others. Finally, Florida birds were separated from the other groups by having significantly smaller crests.

Discriminant analysis

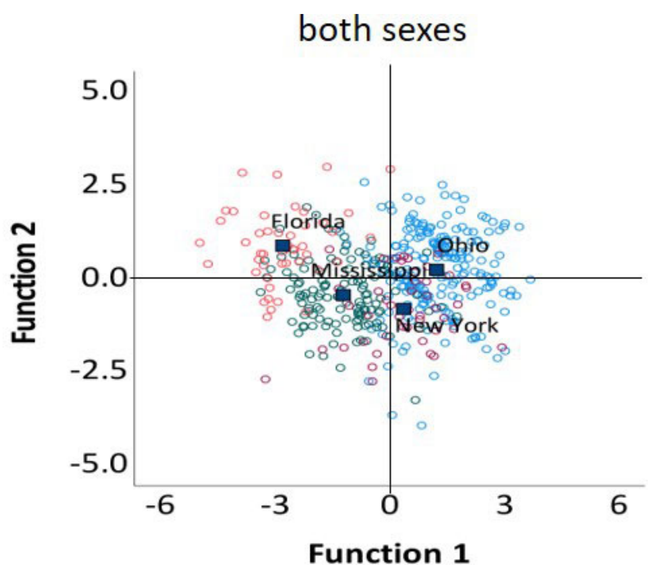
Based on the results of the multiple analysis of variance, mass, wing chord, and tarsus length were expected to make substantial contributions to predicting the location of residence of each bird. Based on hypotheses derived from Bergmann's rule, we further expected the location groups to form a south-to-north continuum along these measures, with southern U.S. birds having smaller body dimensions than northern U.S. birds.

Three discriminant functions accounted for the between-groups variance in the data. The first function accounted for 84.6% of the total variance (canonical correlation coefficient = 0.826), the second, 9.2% (canonical correlation coefficient = 0.434), and the third, 6.3% (canonical correlation coefficient = 0.370). Variables were retained in a discriminant function if both the standardized canonical discriminant function coefficient and the structure matrix coefficient relating that variable to the function were at least 0.30 in absolute value. Table 1 shows the standardized canonical discriminant function coefficients and the structure matrix coefficients for each function and variable.

Figure 2 shows the classification plot of each observation (as well as group centroids) along the axes formed by the first two discriminant functions. The first discriminant function comprised a linear combination of mass, tarsus length, and wing chord length. This function made a clear discrimination between birds observed in Florida and Mississippi (negative group centroid values along the first function axis) and birds observed in New York and Ohio (positive group centroid values along the first function axis). The second discriminant function comprised a linear combination of positive correlation with tarsus length and negative correlation with wing chord length. Based on the results of the multiple analysis of variance, this function may have discriminated between the two locations within the higher and lower latitudes, respectively. Specifically, it may have separated birds in Ohio (significantly longer tarsus length) from birds in New York, and birds in Florida (significantly shorter wing chord length) from birds in Mississippi. Finally, the third function (not shown in the classification plot) was rooted primarily in tail length, but could have also included a negative correlation with crest length. Tails and crests could have an ornamentation function in this species that is yet to be determined (Jawor et al. 2003); therefore, this function could reflect secondary discriminations based on ornamentation differences. This function's values at the group centroids suggested a separation of New York and Florida birds from Ohio and Mississippi birds. New York birds had significantly longer tails than any other group, while Florida birds had significantly smaller crests than any other group.

The discriminant functions accurately classified 78.5% (341 out of 432) of cases. Observations from Ohio were correctly classified 91.7% of the time (198 out of 216 cases) and observations from Mississippi were correctly classified 76.6% of the time (95 out of 124 cases). Florida cases were classified with 67.4% accuracy (31 out of 46 cases). The analysis was least accurate in classifying observations from the relatively small New York sample, with an accuracy of only 32.6% (15 out of 46 cases). Misclassified cases were frequently misclassified into some other location in the same geographic region of the U.S. For example, of the New York misclassifications, 77% of cases were misclassified as coming from Ohio and the remaining 23% were misclassified as coming from Mississippi. Of the Florida misclassifications, 87% were misclassified into Mississippi and the rest (only two cases) were misclassified as coming from Ohio.

Fig. 2. Classification plot for both sexes combined. X axis = score on first discriminant function; Y axis = score on second discriminant function. Open blue circles = data from Ohio; open purple circles = data from New York; open green circles = data from Mississippi; open red circles = data from Florida, and filled blue squares = group centroids. Function 1 discriminates northern birds (generally, positive function scores) from southern birds (generally, negative function scores). Function 2 discriminates the two northern groups from each other (positive function scores for Ohio and negative function scores for New York) largely based on differences in tarsus length and the two southern groups from each other (positive scores for Florida and negative scores for Mississippi) largely based on difference in wing chord length.



Analyses split by sex
Multiple analysis of variance

There were significant effects of location on the combined body size variable among both males (Fig. 3) and females (Fig. 4) and the size of the effect was comparable (males: Pillai's trace = 1.084, $F_{(15, 681)} = 25.70$, $P < .001$, $\eta^2 = 0.361$; females: Pillai's trace = 0.95, $F_{(15, 579)} = 17.88$, $P < .001$, $\eta^2 = 0.317$). Within males, there were large to small significant effects of location on each of the five body measures: wing chord ($F_{(3, 229)} = 124.02$, $P < 0.001$, partial $\eta^2 = 0.619$), mass ($F_{(3,229)} = 109.96$, $P < 0.001$, partial $\eta^2 = 0.590$), tarsus length ($F_{(3,229)} = 59.97$, $P < 0.001$, partial $\eta^2 = 0.440$), crest ($F_{(3,229)} = 27.79$, $P < 0.001$, partial $\eta^2 = 0.267$), and tail length ($F_{(3,229)} = 15.26$, $P < 0.001$, partial $\eta^2 = 0.167$). Within females, there

Table 1. Standardized canonical discriminant function coefficients and structure matrix coefficients for each variable for all three discriminant functions derived from analyzing the data with the sexes combined. A variable was retained in a function if both coefficients exceeded 0.30 in absolute value. Function 1, which discriminated northern groups from southern groups, is a linear combination of mass, tarsus length, and wing chord length. Function 2 differentiated New York birds from Ohio birds based on differences in tarsus length and differentiated Mississippi birds from Florida birds based on differences in wing chord length. Finally, Function 3 differentiated Florida birds from the rest based on their significantly shorter tails.

	Function 1		Function 2		Function 3	
	Standardized coefficients	Structure matrix coefficients	Standardized coefficients	Structure matrix coefficients	Standardized coefficients	Structure matrix coefficients
Mass	0.566	0.706	-0.355	-0.186	0.169	0.272
Tarsus	0.405	0.622	0.938	0.771	-0.143	0.001
Wing chord	0.664	0.598	-0.566	-0.303	-0.131	0.353
Tail	-0.460	0.174	0.315	-0.076	1.099	0.772
Crest	0.139	0.227	-0.216	-0.294	-0.630	-0.241

were significant differences in tarsus length ($F_{(3,195)} = 90.04$, $P < 0.001$, partial $\eta^2 = 0.581$), mass ($F_{(3,195)} = 52.05$, $P < 0.001$, partial $\eta^2 = 0.445$), wing chord ($F_{(3,195)} = 51.82$, $P < 0.001$, partial $\eta^2 = 0.444$), and tail length ($F_{(3,195)} = 11.13$, $P < 0.001$, partial $\eta^2 = 0.146$). However, there were no significant differences in crest length ($F_{(3, 195)} = 2.43$, $P = 0.066$).

Fig. 3. Boxplots for the five body measurements, males only. See Figure 1 legend for details about symbols. Male Northern Cardinals (*Cardinalis cardinalis*) observed in New York and Ohio were significantly heavier than those observed in Mississippi and Florida and also had significantly longer tarsus lengths, wing chords, and tails. Florida birds had significantly smaller crests than the other groups.

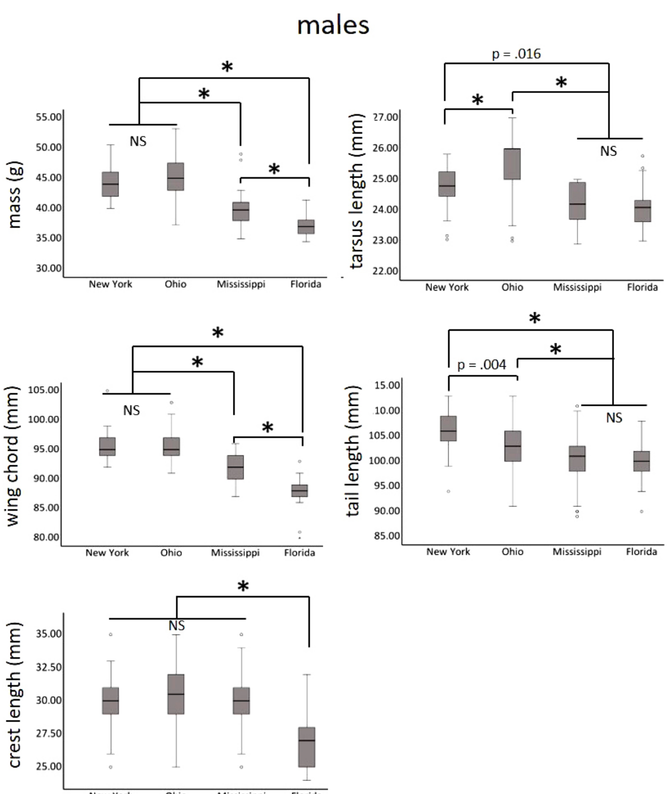
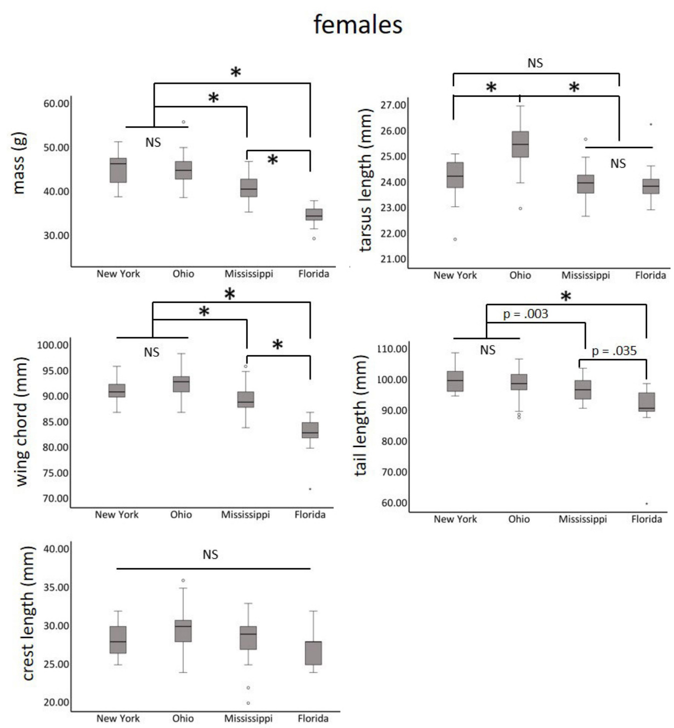


Fig. 4. Boxplots for the five body measurements, females only. See Figure 1 legend for details about symbols. Location differences in body measurements were generally weaker than those observed among male birds, although female Northern Cardinals (*Cardinalis cardinalis*) observed in New York and Ohio were significantly heavier than those in Mississippi and Florida and also had significantly longer wing chords and tails.



These results were further examined using Tukey's post hoc tests. The north-to-south discrimination was weaker among the females than the males, yet both sexes showed strong-to-moderate significant effects of location on mass, tarsus length, and wing chord length and a smaller, but still significant, effect of location on tail length. There was a sex difference in which measures were

Table 2. Standardized canonical discriminant function coefficients and structure matrix coefficients for each variable for all three discriminant functions derived from analyzing the data from males only. Function 1, which discriminated northern groups from southern groups, is a linear combination of mass, and wing chord length, with a possible contribution from tarsus length. Function 2 differentiated Mississippi birds from Florida birds based largely on differences in crest length. Function 2 may also further differentiate northern groups from southern groups based on the former's significantly longer tarsus and tail lengths. Finally, Function 3 differentiated New York birds from Ohio birds based on the shorter tails and longer tarsus lengths of New York birds.

	Function 1		Function 2		Function 3	
	Standardized coefficients	Structure matrix coefficients	Standardized coefficients	Structure matrix coefficients	Standardized coefficients	Structure matrix coefficients
Mass	0.497	0.680	0.283	0.359	-0.149	-0.041
Tarsus	0.279	0.466	0.368	0.481	0.760	0.656
Wing chord	0.743	0.720	-0.401	-0.094	-0.0319	-0.505
Tail	-0.355	0.185	0.762	0.333	-0.552	-0.648
crest	0.207	0.303	-0.714	-0.601	0.263	-0.086

most strongly affected by locations. In order of effect size, the three strongest effects within males were: wing chord length, mass, and tarsus length, whereas in females the order was: tarsus length, mass, and wing chord (i.e., wing chord and tarsus length measures switch position between the two sexes).

Discriminant analysis

For both sexes, there were three discriminant functions that accounted for significant amounts of the between-group variance in the data. As in the analysis that combined sexes, a variable was retained as a predictor in a function if both its standardized discriminant function coefficient and its structure matrix coefficient exceeded 0.30. The first function, which performed most of the discrimination for both sexes (88.4% of variance among the males and 84.2% among the females), primarily comprised a linear combination of wing chord, mass, and tarsus measures, although tarsus length played less of a role in discrimination among males than it did in discrimination among females. The discriminant analysis results are presented below separately for the two sexes.

Males

The classification plot for the males is shown at the top of Figure 5. The first discriminant function comprised a linear combination of mass and wing chord length, with a possible contribution from tarsus length (see Table 2 for the standardized canonical discriminant function coefficients and the structure matrix coefficients for each function and variable). Consistent with the results of the MANOVA, this function clearly separated birds from Florida and Mississippi (negative group centroid values) from birds in New York and Ohio (positive group centroid values). The second function, which accounted for only 6.7% of variance, was a linear combination of positive correlation with tarsus and tail length, but a negative correlation with crest length. This function may serve to separate Florida and Mississippi males based on the fact that Florida birds have significantly smaller crest lengths. The tarsus and tail components may reinforce the separation of the southern groups from the northern groups, as the latter have significantly larger measures of those features. Finally, the third function, which accounted for only 4.9% of the variance, may be separating New York males from Ohio males based on the fact that New York males have shorter tails and longer tarsus lengths than Ohio males.

Fig. 5. Classification plots for males (top) and females (bottom). See Figure 2 legend for details about symbols. Males: Function 1 discriminates northern birds from southern birds. Function 2 discriminates the two southern groups from each other largely based on differences in crest length but does not clearly discriminate the two northern groups from each other. Females: Function 1 makes a weaker northern versus southern discrimination than is true for the males, although Mississippi and Florida birds are clearly discriminated from Ohio birds. Function 2 appears to discriminate New York birds from Ohio birds based largely on differences in tarsus length, and to discriminate Florida birds from Mississippi birds based largely on differences in mass and wing chord length.

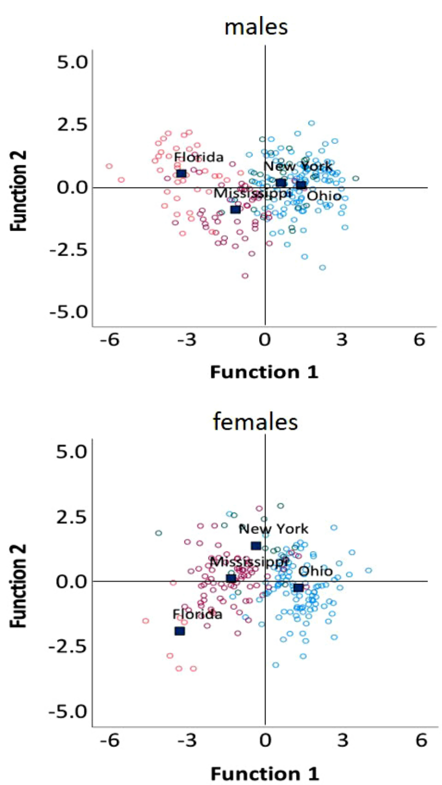


Table 3. Standardized canonical discriminant function coefficients and structure matrix coefficients for each variable for all three discriminant functions derived from analyzing the data from females only. Function 1, which discriminated northern groups from southern groups, is a linear combination of mass, tarsus length and wing chord length. Function 2 differentiated New York birds from Ohio birds based on differences in tarsus length and differentiated Mississippi birds from Florida birds based on differences in mass and wing chord length. Finally, Function 3 was the only function with strong contributions from tail and crest measures, which did not contribute much to discriminating among groups.

	Function 1		Function 2		Function 3	
	Standardized coefficients	Structure matrix coefficients	Standardized coefficients	Structure matrix coefficients	Standardized coefficients	Structure matrix coefficients
Mass	0.398	0.597	0.559	0.533	0.261	0.350
Tarsus	0.643	0.809	-0.777	-0.538	0.238	0.211
Wing chord	0.485	0.606	0.456	0.451	-0.816	-0.412
Tail	-.0210	0.0238	0.188	0.405	0.868	0.383
crest	-0.015	0.0125	-0.214	-0.010	-0.482	-0.393

The discriminant functions were 79.4% accurate in classification (185 out of 233 cases). As is true in the analysis in which the sexes were combined, New York observations were most likely to be misclassified, but the majority of those misclassifications (82%) were into the Ohio group.

Females

Although the MANOVA demonstrated no significant location group differences in crest length in females, crest length was included as a variable in the discriminant analysis. It appeared to contribute to the third discriminant function, which accounted for only 1.8% of the variance accounted for by the three retained functions.

The classification plot for the females is shown at the bottom of Figure 5. As was the case for males, the first discriminant function separated Florida and Mississippi females from New York and Ohio females, although the separation between the southern groups and the New York females was not as definitive as it was for males. Consistent with the results of the multiple analysis of variance, this function appeared to be a linear combination of mass, tarsus length, and wing chord length, with the southern birds scoring lower than the northern birds on these dimensions (see Table 3 for the standardized canonical discriminant function coefficients and the structure matrix coefficients for each function and variable). This function also separated Ohio birds from New York birds, probably based on the fact that Ohio females had significantly longer tarsus lengths than New York females.

The second function accounted for 14% of the variance, more than the second function accounts for among male birds. This function was a linear combination of a positive correlation with mass and wing chord length, but a negative correlation with tarsus length. Based on the function values at the group centroids, this function may have separated the southern groups from one another and the northern groups from one another. The northern groups may be discriminated by their significant difference from each other in tarsus length (Ohio longer than New York). The southern groups may be discriminated based on their significant differences in mass and wing chord length (Mississippi heavier and with longer wing chord than Florida).

The third function, accounting for only 3.8% of the variance, did not appear to contribute much to discriminating among the groups, but it was the only function with strong contributions from tail and crest measures, which are two body features that might have additional function as ornamentation. In the multiple analysis of variance, the location groups had somewhat ambiguous differences among them in tail length and no significant differences in crest length. Very subtle discriminations in these measures could exist, but the pattern of such discrimination was not entirely clear in these data.

The discriminant functions were 85.4% accurate (170 out of 199 cases) in classification. As was true in the analysis aggregated across the sexes, New York females were most likely to be misclassified; 60% of those misclassifications were into the Ohio group.

DISCUSSION

In summary, Northern Cardinals in southern U.S. populations at lower latitudes (Florida and Mississippi) are of significantly smaller body size than cardinals in northern populations and higher latitudes (Ohio and New York). Birds in northern U.S. populations weigh significantly more, have longer tarsi, and have longer wing chords than birds in southern U.S. populations. This general pattern held when the data are split by sex, though discrimination among location groups for females is weaker, presumably because of smaller sample sizes at some locations (e.g., Florida, $N = 8$). With regard to additional feather measures taken, northern U.S. cardinals also have significantly longer tails than southern U.S. birds, though the effect of location on this body measure is not as strong. Finally, shorter crest length seems to separate Florida birds from the other groups.

Given the broad distribution of populations we assess in this study, it is possible that not all are of the same Northern Cardinal subspecies, which could impact the patterns observed here. There are 18 recognized subspecies of Northern Cardinal currently separated by bill shape, crest feathers, and/or plumage coloration (Halkin et al. 2021). Complete descriptions of body size measures for each subspecies are lacking and descriptions of ranges are often confusing, as the geographic distribution of cardinals is gradually expanding. Yet, based on the current range descriptions, it is possible that the Florida population assessed in this study represents *Cardinalis cardinalis floridanus* (Sprunt 1954, Bent and Austin 1968), whereas the Mississippi, Ohio, and New York populations all represent *Cardinalis cardinalis*

cardinalis. It has been noted that *floridanus* is similar in appearance to *cardinalis*, but both sexes are smaller in size (Ridgeway 1901, Bent and Austin 1968), which is supported in our findings because cardinals in the Florida population in our study are smaller in body size than all other populations assessed. However, if the currently assessed Florida population is *floridanus*, it does not negate our findings that the body sizes in cardinals increases with latitude, as the remaining populations assessed (Mississippi, New York, Ohio) all represent populations of the same *cardinalis* subspecies and birds in New York and Ohio are significantly larger in size than those in Mississippi.

Multiple hypotheses could explain the geographic pattern in body size variation that Bergmann (1847) first describes. We first consider the heat-conservation hypothesis, as Bergmann (1847) proposes that larger organisms are able to better survive in colder climates at higher latitudes because they lose less heat because of larger body size, resulting in a reduction in surface-to-volume ratio. Further work suggests that Bergmann's rule may apply more to sedentary than migratory species (Meiri and Dayan 2003). Northern Cardinals are year-round residents and those in the northern part of their range routinely experience below-freezing average temperatures in the winter months and endure significant snowfall, while the populations in the southern climate experience much warmer winters where below-freezing temperatures and snow rarely (if ever) occur. Adaptations to conserve heat can be advantageous at the higher latitudes observed in this study. Studies with cardinals in Ohio found that metabolic rates in the non-breeding season exceeded metabolic rates during breeding season (Sgueo et al. 2012), supporting the importance of being able to buffer against lower temperatures.

An alternative hypothesis to explain geographic variation in body size could be the starvation resistance hypothesis, which proposes that larger body sizes are necessary to avoid starvation at higher latitudes where resource availability fluctuates seasonally (Boyce 1979, Calder 1984). This hypothesis may explain the body size patterns in Northern Cardinals, as having larger body sizes to prevent starvation would be advantageous at higher latitudes in the cardinal's range. Wild food resources (e.g., seeds, fruit, insects) are drastically reduced during the winter months in Ohio and New York, while resources remain plentiful in Mississippi and Florida because of a milder climate. The starvation resistance hypothesis has support in data from other species that follow Bergmann's rule. For instance, ant lions (*Myrmeleon immaculatus*) of larger body size and from higher latitudes survive more readily that those from lower latitudes when fed a common diet (Arnett and Gotelli 2003). There are ethical limitations to studying dietary restriction in vertebrate homeotherms. However, McNamara et al. (2016) model the effect of several ecological factors such as predation, starvation, and temperature on a hypothetical homeotherm. This work suggests that species experiencing long interruptions in foraging opportunities may benefit from large body size. Although cardinals are omnivores with no specialized dietary requirements, their foraging may be interrupted by extended periods of inclement weather, which may be more likely in northern latitudes. Therefore, larger body size might buffer caloric shortfall. It is also possible that the geographical pattern in body size demonstrated by cardinals could reflect a combination of application of both the heat conservation and starvation prevention hypotheses. If so, larger body sizes in cardinals at higher latitudes would allow individuals to survive harsh winters when both temperatures are low and/or calories are scarce.

Bergmann's rule is not the only plausible explanation for our findings. For instance, spatial variation in geographic range size and species richness are also related to body size (e.g., in birds: Cousins 1989; in mammals: Pagel et al. 1991). Our data may support Blackburn and Gaston's (1996) suggestion of an inter-relationship among body size, species richness, and geographic range size. Specifically, Northern Cardinals, with a large geographic range, are smaller in body size closer to the equator because lower latitudes tend to have greater species richness, and greater species richness implies more competition for resources. Additional work with species of broad geographic range is needed in this area.

Anthropogenic changes to the environment might also impact the morphology of Northern Cardinals. A recent application of Allen's rule (which predicts that endotherm appendages are smaller in size in cooler environments and larger in warmer environments) to cardinals demonstrates a complex relationship with bill size, environmental temperature, and housing density across multiple cardinal populations (Miller et al. 2018). Although the overall geographic pattern of bill size follows the prediction of Allen's rule (birds in warmer temperatures generally have larger bills), sex-specific variation and the impact of urbanization made interpretation of the findings difficult (Miller et al. 2018). Northern Cardinals are a generalist species that thrive in edge habitat resulting from urban development. All of the study sites within this study are located within 10 miles of a city, with the Florida location being most suburban-like. Given that this relatively urban population in Florida is smaller in body size than other populations assessed in this study (which are located farther away from city limits), the potential influence of urbanization (e.g., increased stress, higher predation, etc.) on body size cannot be ruled out and deserves greater attention. Relationships between anthropogenic factors and morphological characteristics of widely distributed species, such as the Northern Cardinal, will likely continue to change because of rapid climate change and expansion in urban development. Therefore, a traditional application of some biogeographical "rules" might not be entirely appropriate as some species are undergoing evolutionary change to adapt to a rapidly changing planet.

Although meta-analyses have come to the consensus that Bergmann's rule is a valid potential explanation of intraspecific variation in morphology in birds (Ashton 2002, Meiri and Dayan 2003) and large mammals (Ashton et al. 2000), Blackburn et al. (1999) propose that "James's rule" might be more appropriate when referring to geographic variation in body size within species. "James's rule" refers to work by James (1970) suggesting that intraspecific variation in body size across geographic gradients should be considered separately, as Bergmann's rule could have been formulated in reference to interspecific comparisons. Thus, by separating interspecific and intraspecific patterns of geographic variation in body size, more accurate assessments of the selective pressures driving such patterns would be better elucidated (Blackburn et al. 1999). Application of James's rule is emerging (e.g., Daufresne et al. 2009), with results from our study suggesting that this modification of

Bergmann's rule could be appropriate for the Northern Cardinal. Therefore, we encourage others to consider the possibility of its application in similar studies of species with broad distribution.

CONCLUSION

To conclude, our study found that Northern Cardinal populations at higher latitudes have larger body sizes than populations at lower latitudes in the United States. Given the broad distribution of Northern Cardinals, future work should examine morphological variation in the eastern and western extremes of the range, which would allow for a more comprehensive assessment of geographic variation of this species. Similar studies should be performed with additional resident species with broad distribution to determine if ecogeographical rules concerning body size and geographic relationships (e.g., Bergmann's, James's) have similar application.

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Data Availability:

The data that support the findings of this study are openly available in Open Science Framework at DOI 10.17605/OSF.IO/MUZ2R, registered from osf.io/yfs2v. Ethical approval for this research study was granted by the University of Southern Mississippi Animal Care and Use Committee 08021402 and 11092214, SUNY Oswego Animal Care and Use Committee 2018.05, Florida Atlantic University IACUC protocol A19-02, and University of Dayton Animal Care and Use Committee A3092-01.

LITERATURE CITED

Arnett, A. E., and N. J. Gotelli. 2003. Bergmann's rule in larval ant lions: testing the starvation resistance hypothesis. Ecological Entomology 28:645-650. https://doi.org/10.1111/j.1365-2311.2003.00554.x

Ashton, K. G. 2002. Patterns of within-species body size variation of birds: strong evidence of Bergmann's rule. Global Ecology and Biogeography 11:505-523. https://doi.org/10.1046/j.1466-822X.2002.00313.x

Ashton, K. G., M. C. Tracy, and A. de Queiroz. 2000. Is Bergmann's rule valid for mammals? American Naturalist 156:390-415. https://doi.org/10.1086/303400

Baumgardner, G. D., and M. L. Kennedy. 1993. Morphometric variation in Kangaroo-rats (Genus *Dipodomys*) and its relationship to selected abiotic variables. Journal of Mammalogy 74:69-85. https://doi.org/10.2307/1381906

Bent, A. C., and O. L. Austin. 1968. Life histories of North American cardinals, grosbeaks, towhees, finches, sparrows, and allies. Bulletin of the United States National Museum 1-1889. https://doi.org/10.5479/si.03629236.237.1

Bergmann, C. 1847. Ueber die Verhaltnisse der Warmeokonomie der Thierezuihrer Grosse. Gottinger Studien 3:595-708.

Blackburn, T. M., and K. J. Gaston. 1996. Spatial patterns in the body sizes of bird species in the New world. Oikos 77:436:446. https://doi.org/10.2307/3545933

Blackburn, T. M., K. J. Gaston, and N. Loder. 1999. Geographic gradients in body size: a clarification of Bergmann's rule. Diversity and Distributions 5:165-174. https://doi.org/10.1046/j.1472-4642.1999.00046.x

Blem, C. R. 1981. Geographic variation in mid-winter body composition of starlings. Condor 83:370-376. https://doi.org/10.2307/1367508

Boyce, M. S. 1979. Seasonality and patterns of natural selection for life histories. American Naturalist 114:569-583. https://doi.org/10.1086/283503

Bull, L. S. 2006. Geographical variation in the morphology of the Wedge-tailed Shear-water (*Puffinus pacificus*). Emu - Austral Ornithology 106:233-243. https://doi.org/10.1071/MU05050

Calder, III, W. A. 1984. Size, function and life history. Harvard University Press, Cambridge, Massachusetts, USA.

Cousins, S. H. 1989. Species richness and the energy theory. Nature 340:350-351. https://doi.org/10.1038/340350b0

Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Science 106:12788-12793. https://doi.org/10.1073/pnas.0902080106

Fan, L., C. Tianlong, Y. Xiong, G. Song, and F. Lei. 2019. Bergmann's rule and Allen's rule in two passerine birds in China. Avian Research 10:34. https://doi.org/10.1186/s40657-019-0172-7

Freeman, B. G. 2017. Little evidence for Bergmann's rule body size clines in passerines along tropical elevational gradients. Journal of Biogeography 44:502-510. https://doi.org/10.1111/jbi.12812

Halkin, S. L., D. P. Shustack, M. S. DeVries, J. M. Jawor, and S. U. Linville. 2021. Northern Cardinal (*Cardinalis cardinalis*), version 2.0. In P. G. Rodewald and B. K. Keeney, editors. Birds of the world. Cornell Lab of Ornithology, Ithaca, New York, USA. https://doi.org/10.2173/bow.norcar.02

James, F. C. 1970. Geographic size variation in birds and its relationship to climate. Ecology 51:365-390. https://doi.org/10.2307/1935374

Jawor, J. M., S. U. Linville, S. M. Beall, and R. Breitwisch. 2003. Assortative mating by multiple ornaments in Northern Cardinals (*Cardinalis cardinalis*). Behavioral Ecology 14:515-520. https://doi.org/10.1093/beheco/arg041

Lee, C., Y. Fu, C. Yeh, C. K. L. Yeung, H. Hung, C. Yao, P. L. Shaner, and S. Li. 2021. Morphological variations in a widely distributed Eastern Asian passerine cannot be consistently explained by ecogeographic rules. Ecology and Evolution 11:15249-15620. https://doi.org/10.1002/ece3.8208

McNamara, J. M., A. D. Higginson, and S. Verhulst. 2016. The influence of the starvation-predation trade-off on the relationship between ambient temperature and body size among endotherms. Journal of Biogeography 43:809-819. https://doi.org/10.1111/jbi.12695

Meiri, S., and T. Dayan. 2003. On the validity of Bergmann's rule. Journal of Biogeography 30:331-351. https://doi.org/10.1046/j.1365-2699.2003.00837.x

Miller, C. R., C. E. Latimer, and B. Zuckerberg. 2018. Bill size variation in Northern Cardinals associated with anthropogenic drivers across North America. Ecology and Evolution 8:4841-4851. https://doi.org/10.1002/ece3.4038

Niles, D. M. 1973. Adaptive variation in body size and skeletal proportions of Horned Larks of the Southwestern United States. Evolution 27:405-426. https://doi.org/10.1111/j.1558-5646.1973.tb00688.x

Pagel, M. D., R. M. May, and A. R. Collie. 1991. Ecological aspects of the geographical distribution and diversity of mammalian species. American Naturalist 137:791-815. https://doi.org/10.1086/285194

Ralls, K., and P. H. Harvey. 1985. Geographic variation in size and sexual dimorphism of North American weasels. Biological Journal of the Linnean Society 25:119-167. https://doi.org/10.1111/j.1095-8312.1985.tb00389.x

Rensch, B. 1938. Some problems of geographical variation and species formation. Proceedings of the Linnean Society of London 150:275-285. https://doi.org/10.1111/j.1095-8312.1938.tb00182k.x

Ridgeway, R. 1901. The birds of North and Middle America, Part 1. Bulletin of the U.S. National Museum 50, part 1.

Salewski, V., and C. Watt. 2017. Bergmann's rule: a biophysiological rule examined in birds. Oikos 126:161-172. https://doi.org/10.1111/oik.03698

Sargis, E. J., V. Millien, N. Woodman, and L. E. Olson. 2018. Rule reversal: ecogeographical patterns of body size variation in the common treeshrew (Mammalia, Scandentia). Ecology and Evolution 8:1634-1645. https://doi.org/10.1002/ece3.3682

Sgueo, C., M. E. Wells, D. E. Russell, and P. J. Schaeffer. 2012. Acclimatization of seasonal energetics in Northern Cardinals (*Cardinalis cardinalis*) through plasticity of metabolic rates and ceilings. Journal of Experimental Biology 215:2418-2424. https://doi.org/10.1242/jeb.061168

Smith, J. I. 1998. Allometric influence on phenotypic variation in the Song Sparrow (*Melospiza melodia*). Zoological Journal of the Linnean Society 122:427-454. https://doi.org/10.1111/j.1096-3642.1998.tb02158.x

Sprunt, Jr., A. 1954. Florida birdlife. Coward-McCann, New York, New York, USA.

Tabachnik, B. G. and L. S. Fidell. 2013. Using multivariate statistics. Sixth edition. Pearson Education, Inc., Boston, Massachusetts, USA.

Vinarski, M. V. 2014. On the applicability of Bergmann's rule to ectotherms: the state of the art. Biology Bulletin Reviews 4:232-242. https://doi.org/10.1134/S2079086414030098