

ALLOMETRY AND PHENOTYPIC VARIATION IN THE MORPHOMETRICS OF *PUFFINUS* SHEARWATERS

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SUMMARY

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It has been shown previously, using coefficients of variation (CV), that bill measurements are significantly more variable than those of the wing or foot among 11 sexually size dimorphic and seven monomorphic *Puffinus* shearwater species. Patterns of phenotypic variation were unaffected by sexual dimorphism. However, morphologic variation can be attributable to allometry, because CV is affected by the slope of the regression of trait size on an indicator of body size, as well as by the dispersion of points around the regression line [the coefficient of residual variation (CRV)]. The effect of allometric variation on the previously reported patterns of trait variability in CVs is assessed, using tarsus length as an indicator of overall body size. It is clear that the pattern of variation in the CV is repeated in the CRV. Also, that pattern of variation is not found in allometric regression slopes, and variation in allometric regression slopes does not explain the high CVs reported for bill measurements (depth especially). We conclude that bill measurements are more variable than other measured traits, and that such variability is not a result of a “design feature” explained by allometry. This strengthens the previous suggestion of a biologic significance to the high variability in *Puffinus* bill depth. In addition, we show the importance of incorporating a test for allometry before offering possible biologic explanations for morphologic variation.

Key words: Allometric regression, coefficient of (residual) variation, morphologic variation, *Puffinus* shearwaters, testing for allometry

INTRODUCTION

The shearwater genus *Puffinus* contains approximately 20 species that vary in such aspects as ecology, geographic range, interspecific size, migratory habit, level of subspeciation, timing of breeding and the climatic zone, hemisphere and habitat in which they breed (Warham 1990). Such variability lends the genus to a study of size variation in seabirds.

Patterns of trait variation (bill depth at base and nares, bill length, wing length, tarsus length, mid-toe length) in the genus *Puffinus* were investigated by Bull *et al.* (2005) in terms of sexual, geographic and specific variation. Widespread species exhibited geographic variation in morphology. Species for which a significant sex difference was found exhibited low levels of sexual size dimorphism, expressed only in the bill depth dimensions: males' bills were deeper. No significant interaction was found between sex and population, indicating that no geographic variation is evident in the magnitude of sexual size dimorphism.

Bull *et al.* (2004) investigated the variability of the traits using coefficients of variation (CVs). High CVs were reported for the bill measurements, which were significantly more variable than measurements of the wing or foot; furthermore, bill depth dimensions exhibited the greatest amount of phenotypic variation. There was no difference in the CVs of dimorphic traits between sexes, and the patterns of CV variation over all traits were similar in dimorphic and monomorphic species. Bull *et al.* (2004) proposed that the observed variability among the traits was attributable to differences in the strength of natural selection, with the traits

under strong stabilizing selection (e.g. wing, tarsus and mid-toe) exhibiting reduced variability, because optimum dimensions are being selected for.

However, variation in body part measurements can be attributable to allometry. Gould (1966) defines allometry as “differences in proportions correlated with changes in the absolute magnitude of the total organism.” Other authors have stated that the use of the CV as a measure of the total amount of variation in the size of a morphologic trait is not completely appropriate (Eberhard *et al.* 1998, Cuervo & Møller 2001). The CV is influenced by two different factors: the slope of the regression line when regressing trait size on an indicator of body size, and the dispersion of points around the regression line (Eberhard *et al.* 1998, Cuervo & Møller 2001).

The aim of the present paper is to determine if the patterns of trait variability in CVs reported for *Puffinus* shearwaters in Bull *et al.* (2004) are explained by allometric variation. In particular, the relative importance of the effect on CVs is investigated for the slopes of allometric regression lines and for the dispersion of points around those regression lines.

METHODS

Data collection

Morphometric measurements were taken from 2689 museum specimens (prepared study skins) of 18 *Puffinus* species held in major museum ornithology collections (see Acknowledgements). The information on specimen labels regarding sex, date and origin of collection was recorded. Juvenile and immature specimens were not knowingly

included in the data set, but it is possible that some measured birds may not have been adults, but had attained adult plumage patterns.

Species sample sizes varied because of specimen availability in the collections. Linear measurements of bills, wings and feet were taken, because these represent three distinct body regions and are considered principal targets of natural selection in birds (Zink & Remsen 1986). The traits measured were bill length (BL), bill depth at base (BDB), bill depth at nares (BDN), wing length (WL) (maximum flattened chord), tarsus length (TL) and mid-toe length (MT). Preparatory methods of the specimens dictated which traits could be measured; in some cases not all of the measurements could be taken from each specimen. All measurements were taken by LSB. Wing length was measured to the nearest 0.5 mm using a steel rule with an end stop, and bill, tarsus and mid-toe to the nearest 0.01 mm using digital Vernier callipers. Where appropriate, specimens were measured on the right hand side, to eliminate variability resulting from fluctuating asymmetry (Cuervo & Møller 1999). For each trait, each bird was measured three times, not consecutively, and the average was used in statistical analyses.

Shrinkage of museum specimens can amount to as much as 4% of the length of living body components (Kinsky & Harper 1968, Winker 1993). Measurements of tail, bill and tarsus lengths are likely to be less prone to shrinkage than are measurements of wings (Winker 1993). Consequently, shrinkage should not be ignored if the results of museum-based studies are applied directly to living birds, such as for sexing guidelines. However, the sole use of museum specimens here (and no combination with measurements from studies on live birds), coupled with no direct application of the results to living birds, has avoided issues such as measurement bias.

Statistical analysis

Following criteria adopted by Cuervo and Møller (1999, 2001), sexually size dimorphic traits were considered to qualify as secondary sexual characters if a sex difference in their size of at least 5% was observed. From a total of 18 *Puffinus* species, 11 were found to be sexually dimorphic in at least one of the morphometrics taken, and seven were sexually monomorphic (Bull *et al.* 2004). A list of the mean size, standard deviation and CV for each morphometric character of each species is given in Bull *et al.* (2004, Appendices 1 and 2).

Tarsus length was chosen as an indicator of overall body size (Cuervo & Møller 2001, Bull *et al.* 2004). Ordinary least-squares estimation of a general linear model fitted to \log_e -transformed data was used to investigate the allometric relationships between various morphologic traits. For each logged response trait, logged tarsus length was used as a covariate and sex as a factor, with an interaction initially allowed between sex and tarsus length. Of the 90 models (18 species \times five traits) initially fitted, sex-varying slopes were required in fewer than 10%. Therefore models without the sex-varying slope were re-fitted, but sex was retained as a factor (i.e. one slope parameter was fitted per species for each trait).

An obvious dimensionless measure of the dispersion of observed points around a regression line is what we shall term the coefficient of residual variation (CRV), motivated by the usual coefficient of variation statistic (e.g. Bull *et al.* 2004). The CRV is the standard error of the estimate [SEE (the square root of the residual mean square error from the regression line)] divided by the mean of the regression response variable. CRVs are therefore directly

comparable and should hence be preferred to the SEE measure used, for example, by Eberhard *et al.* (1998) and Cuervo and Møller (2001). We note that CRVs are part of the standard output produced by SAS/STAT software (version 8.02, 1999: SAS Institute, Cary, N.C., USA), but they are labelled as CVs (e.g. see output from SAS PROC ANOVA, PROC GLM and PROC REG).

In Bull *et al.* (2004), CVs were estimated for regions of the body by amalgamating over individual traits. Such an approach is not pursued here, because following regression on tarsus length, the bill is the only body region for which there is more than a single trait measured. Further, Bull *et al.* (2004) demonstrated that, with regard to *Puffinus* species, an assumption of phylogenetic independence was justified, and there was no difference between inference based on standard statistical approaches and that based on randomization tests.

To extend the analysis of trait variability presented in Bull *et al.* (2004), we focus on the following questions, addressed using allometric regression slopes and CRVs:

1. Is there any difference between particular traits in (a) regression slope and (b) CRV?
2. In species with some dimorphic traits, are the (a) regression slope and (b) CRV greater in sexually dimorphic traits than in other traits? This question is motivated by predictions that strong stabilizing selection is associated with decreased phenotypic variation, and phenotypic variation in sexually selected traits is higher than in non-sexual characters (Fitzpatrick 1997; Cuervo & Møller 1999, 2001; Bull *et al.* 2004).
3. Do sexually monomorphic and sexually dimorphic species differ in the patterns of phenotypic variation across traits, as measured by (a) regression slopes and (b) CRVs?

To answer questions 1 and 2, differences between regression slopes and between CRVs of different traits were tested using the Wilcoxon signed rank test for paired data. The Wilcoxon test was used to allow for non-normality of the differences. Conclusions were unchanged, however, if paired *t*-tests were used instead. Sequential Bonferroni adjustment (Rice 1989) was used where necessary (for question 1) to control for multiple testing. Question 3 concerns the pattern of variation across five traits, and so requires a multivariate test of difference, to allow for correlations between traits. Wilks lambda from a one-way multivariate analysis of variance was used to test the null hypothesis of no difference between dimorphic and monomorphic species, in patterns of slopes and of CRVs over traits. All tests were two-tailed, other than for question 2, which has a specific directional (testable) implication.

RESULTS

In the genus *Puffinus*, allometric regression slopes were relatively similar, particularly for traits within the bill [Fig. 1(a)], but phenotypic CRVs were markedly different, with bill depth measurements exhibiting the greatest amount of variation [Fig. 2(a)]. The allometric slopes and CRVs for each species-trait combination are listed in Appendix 1, along with the *P* values from *F* tests of the fit of each of the estimated linear models. Among regression slopes, only WL differed significantly from any of the other traits (MT and BDN); and among CRVs, BL, BDB and BDN each differed significantly from all other CRVs, but WL and MT CRVs were not significantly different from each other (Table 1).

In species with some dimorphic traits, a significant difference was found between the CRVs of the dimorphic traits and the other traits ($P < 0.001$ for Wilcoxon signed-rank test), but not between the corresponding allometric slopes (Wilcoxon signed-rank test $P = 0.160$).

When considered separately as sexually monomorphic or dimorphic species, results change little. Allometric regression slopes were again relatively similar [Fig. 1(b,c)], but CRVs were markedly different, with bill depth measurements exhibiting the greatest amount of variation [Fig. 2(b,c)]. Furthermore, there was no significant difference in the patterns of phenotypic variation between monomorphic and dimorphic species (Wilks lambda for slopes: $P = 0.510$; Wilks lambda for CRVs: $P = 0.947$).

DISCUSSION

The results presented here demonstrate very clearly that the pattern of variation reported by Bull *et al.* (2004) in the CV is repeated in the CRV. Further, that pattern of variation is not repeated in allometric slopes, and variation in allometric slopes does not explain the high CVs reported for bills (depth especially) in Bull *et al.* (2004). Thus, we can confidently conclude that bill measurements (notably depths) are more variable than the other traits measured for this study, and that such variability is not a result of a “design feature” explained by allometry. These results serve to strengthen the suggestion made by Bull *et al.* (2004) with regard to there being a biologic significance to the high variability in *Puffinus* bill depth. In addition to confirming the hypotheses of Bull *et al.* (2004), the importance of incorporating a test for allometry when studying morphology variation has been demonstrated. Tests such

TABLE 1
Wilcoxon signed-rank tests on paired differences between slopes and coefficients of residual variation (CRV) from regressions of five logged traits on a logged indicator of body size (tarsus length) in 18 *Puffinus* species

Traits	Slope	CRV
	<i>P</i> value	<i>P</i> value
BL vs. BDB	0.865	<0.001 ^a
BL vs. BDN	0.246	<0.001 ^a
BL vs. WL	0.010	<0.001 ^a
BL vs. MT	0.054	<0.001 ^a
BDB vs. BDN	0.018	<0.001 ^a
BDB vs. WL	0.154	<0.001 ^a
BDB vs. MT	0.021	<0.001 ^a
BDN vs. WL	0.002 ^b	<0.001 ^a
BDN vs. MT	0.609	<0.001 ^a
WL vs. MT	<0.001 ^a	0.099

^a Significant difference at 1% after sequential Bonferroni adjustment.

^b Significant difference at 5% after sequential Bonferroni adjustment. (Slopes BL vs. WL, BDB vs. BDN and BDB vs. MT were not significantly different at a 5% level after sequential Bonferroni adjustment).

BL = bill length; BDB = bill depth at base; BDN = bill depth at nares; WL = wing length; MT = mid-toe length.

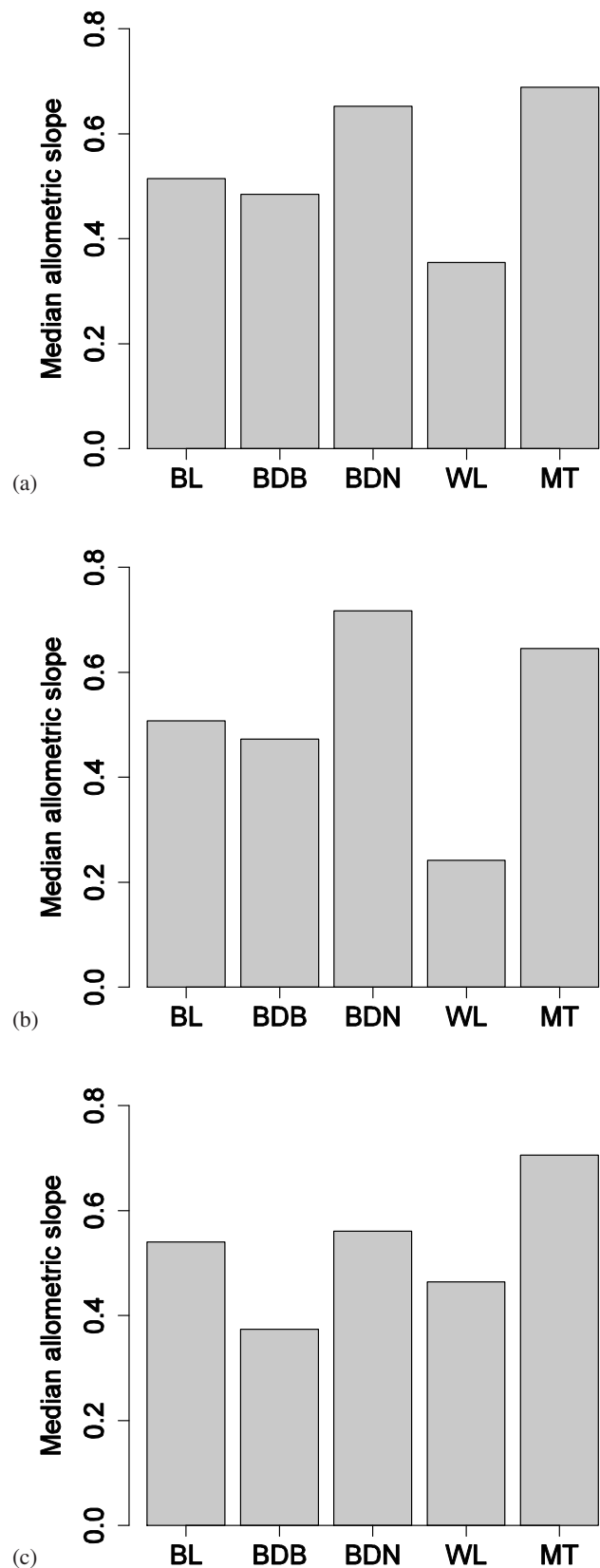


Fig. 1. Median allometric regression slopes for the logged size of morphologic characters after regression on logged tarsus length in (a) 18 *Puffinus* species, consisting of (b) 11 sexually dimorphic *Puffinus* species, and (c) seven sexually monomorphic *Puffinus* species. BL = bill length; BDB = bill depth at base; BDN = bill depth at nares; WL = wing length; MT = mid-toe length.

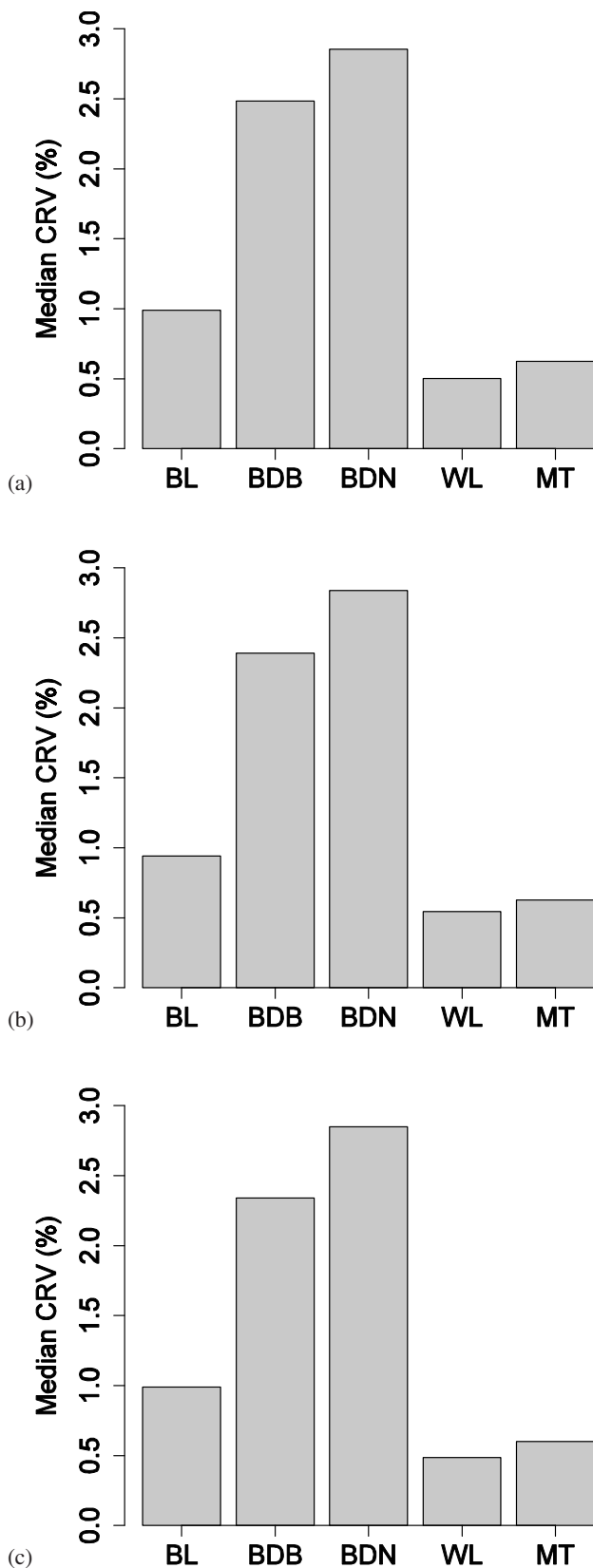


Fig. 2. Median coefficients of residual variation for the logged size of morphologic characters after regression on logged tarsus length in (a) 18 *Puffinus* species, consisting of (b) 11 sexually dimorphic *Puffinus* species, and (c) seven sexually monomorphic *Puffinus* species. BL = bill length; BDB = bill depth at base; BDN = bill depth at nares; WL = wing length; MT = mid-toe length.

as those presented here should be incorporated into initial statistical analyses to determine if trait variability is the result of allometry, before other possible biologic explanations are offered.

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APPENDIX 1
Morphologic characters in 18 *Puffinus* species

Species	BL			BDB			BDN			WL			MT							
	Sample (n)	Slope	CRV (%)	Fit P	Sample (n)	Slope	CRV (%)	Fit P	Sample (n)	Slope	CRV (%)	Fit P	Sample (n)	Slope	CRV (%)	Fit P				
Sexually dimorphic <i>Puffinus</i> species																				
<i>P. assimilis</i>	142	0.37 ^c	1.47	0.0001	121	0.74 ^c	2.76	0.0001	113	0.72 ^c	4.03	0.0001	141	0.52 ^c	0.67	0.0001	113	0.80 ^c	0.76	0.0001
<i>P. carneipes</i>	103	0.52 ^c	0.94	0.0001	44	0.51	1.68	0.0001	82	0.78 ^c	1.93	0.0001	71	0.03	0.43	0.9468	95	0.88 ^c	0.57	0.0001
<i>P. creatopus</i>	107	0.39 ^b	0.86	0.0001	23	0.47	1.89	0.0271	71	0.57 ^a	2.13	0.0108	73	0.14	0.43	0.4524	91	0.76 ^c	0.55	0.0001
<i>P. gavia</i>	108	0.79 ^c	1.05	0.0001	55	0.40	3.22	0.0224	72	1.16 ^a	3.97	0.0193	98	0.59 ^c	0.64	0.0001	75	0.63 ^c	0.63	0.0001
<i>P. gravis</i>	98	0.59 ^c	0.87	0.0001	45	0.89 ^a	2.39	0.0037	84	0.67 ^b	2.79	0.0021	86	0.42 ^a	0.83	0.0566	88	0.58 ^c	0.61	0.0001
<i>P. huttoni</i>	53	0.49 ^b	0.87	0.0001	39	0.41	2.00	0.0014	52	0.10	2.84	0.0082	50	0.14	0.39	0.2012	48	0.52 ^b	0.83	0.006
<i>P. lherminieri</i>	282	0.70 ^c	1.65	0.0001	145	0.99 ^c	3.07	0.0001	222	1.05 ^c	3.87	0.0001	241	0.50 ^c	0.60	0.0001	243	0.97 ^c	0.96	0.0001
<i>P. mauretanicus</i>	8	0.94	1.30	0.4411	7	0.12	2.58	0.9694	6	0.38	1.82	0.1661	6	0.17	0.21	0.0801	7	0.69 ^a	0.20	0.016
<i>P. nativitatis</i>	142	0.51 ^c	0.89	0.0001	68	0.68 ^a	2.28	0.0002	103	0.77 ^a	2.90	0.0001	126	0.37 ^c	0.44	0.0001	138	0.57 ^c	0.61	0.0001
<i>P. opisthomelas</i>	65	0.27 ^a	0.88	0.0001	26	0.29	1.91	0.0033	58	0.82 ^b	2.63	0.0001	51	0.24	0.55	0.1863	58	0.65 ^c	0.65	0.0001
<i>P. tenuirostris</i>	160	0.45 ^c	1.12	0.0001	109	0.03	2.62	0.0001	140	0.42 ^a	3.33	0.0001	143	0.06	0.94	0.2167	144	0.47 ^c	0.72	0.0001
Sexually monomorphic <i>Puffinus</i> species																				
<i>P. auricularis</i>	13	1.11 ^a	0.88	0.0372	6	-0.24	1.88	0.7268	11	-0.02	1.83	0.8942	13	0.63 ^a	0.38	0.0648	11	0.69	0.68	0.1655
<i>P. bulleri</i>	79	0.37 ^b	0.89	0.0023	28	0.02	1.55	0.0658	65	0.63 ^b	2.59	0.0017	70	0.34 ^b	0.51	0.0016	73	0.87 ^c	0.54	0.0001
<i>P. griseus</i>	194	0.35 ^c	1.01	0.0001	99	0.50 ^a	2.70	0.021	164	0.50 ^c	2.83	0.0001	162	0.18	0.90	0.0689	177	0.73 ^c	0.56	0.0001
<i>P. newelli</i>	51	0.35 ^a	0.97	0.001	11	0.25	2.92	0.9216	46	0.62	3.37	0.2036	52	0.66 ^c	0.61	0.0004	39	0.57 ^c	0.58	0.0001
<i>P. pacificus</i>	427	0.71 ^c	1.08	0.0001	195	0.87 ^c	2.05	0.0001	335	1.02 ^c	2.87	0.0001	292	0.59 ^c	0.47	0.0001	407	0.88 ^c	0.62	0.0001
<i>P. puffinus</i>	53	0.72 ^b	1.03	0.0269	39	0.76 ^a	2.63	0.0417	44	0.40	3.55	0.4204	53	0.27 ^a	0.34	0.0002	39	0.49 ^a	0.65	0.0001
<i>P. yelkouan</i>	34	0.64 ^b	1.13	0.0155	29	0.97 ^a	3.19	0.0446	27	3.43 ^c	3.40	0.0001	33	0.94 ^c	0.50	0.0002	27	0.85 ^c	0.62	0.0001

^a $P < 0.05$.
^b $P < 0.01$.
^c $P < 0.001$.

BL = bill length; BDB = bill depth at base; BDN = bill depth at nares; WL = wing length; MT = mid-toe length; Slope = allometric regression slope of logged trait size on logged tarsus length; CRV = coefficient of residual variation; Fit $P = P$ value from an F test of the linear model fit.

