

An investigation into the effects of allometric variation on patterns of trait variability in shearwater (*Puffinus*) species

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INTRODUCTION

The shearwater genus *Puffinus* contains approximately 20 species which 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 in the above parameters lends the genus to a study of size variation in seabirds.

Patterns of trait (bill depth at base and nares, bill length, wing length, tarsus length, mid-toe length) variation 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 difference was found between the sexes 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 there is no geographic variation 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 those 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 due to differences in the strength of natural selection, with those traits under strong stabilizing selection (e.g. wing, tarsus and mid-toe) exhibiting reduced variability, as optimum dimensions are being selected for.

However, variation in body part measurements can be due 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 morphological 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 this 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 study skins of 18 *Puffinus* species held in major museum ornithological collections (see Acknowledgements). Juvenile and immature specimens were not included in the data set. Species sample sizes varied due to specimen availability in the collections. Linear measurements of bills, wings and feet were taken as 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 study skins dictated which traits could be measured; in some cases not all of the above 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 due to fluctuating asymmetry (Cuervo & Møller 1999). For each trait, each bird was measured three times, not consecutively, and the average used in statistical analyses.

Statistical analysis

Following criteria adopted by Cuervo and Møller (1999, 2001), sexually size dimorphic traits were considered to qualify as secondary sexual characters if there was a sex difference in their size of at least 5%. 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

morphological 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. Hence the most general model for each trait in effect fitted separate regression lines for males and females. Of the 90 models (18 species \times 5 traits) initially fitted, sex-varying slopes were required in less than 10%. Therefore models without the sex-varying slope were re-fitted but sex was retained as a simple factor, thus in effect fitting parallel regression lines for males and females (i.e. one slope 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, but they are labeled as CVs (e.g., see output from SAS PROC ANOVA, PROC GLM and PROC REG, SAS Institute Inc. 1999).

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, since 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 regards to *Puffinus* species, an assumption of phylogenetic independence was justified and there was no difference between inference based on standard statistical approaches and 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, is 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, so requires a multivariate test of difference, to allow for correlations between traits. Wilks' lambda from a one-way MANOVA 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 (Figure 1a), but phenotypic CRVs were markedly different, with bill depth measurements exhibiting the greatest amount of variation (Figure 2a). The allometric slopes and CRVs for each species-trait combination are listed in the Appendix, 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), while 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).

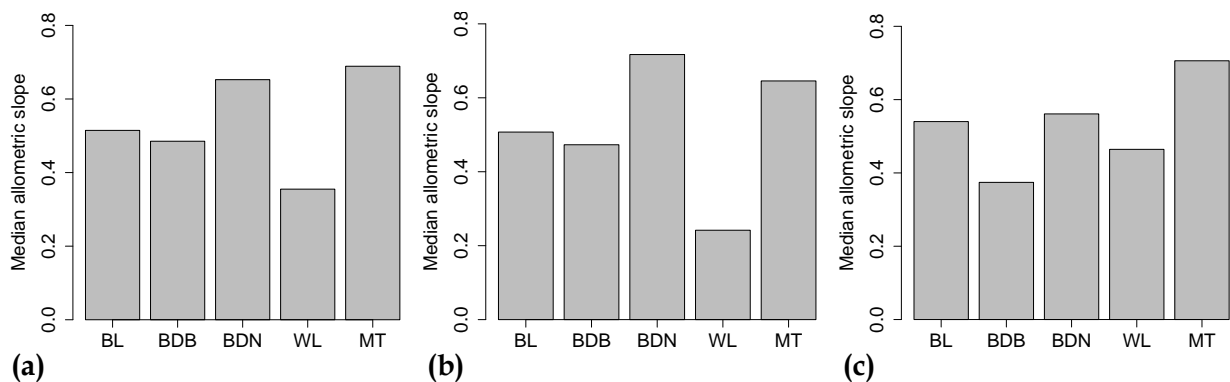


Figure 1. Median allometric regression slopes for the logged size of morphological characters after regression on logged tarsus length in (a) 18 *Puffinus* species, consisting of (b) 11 sexually dimorphic *Puffinus* species, and (c) seven sexually monomorphic *Puffinus* species. BL=bill length; BDB=bill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

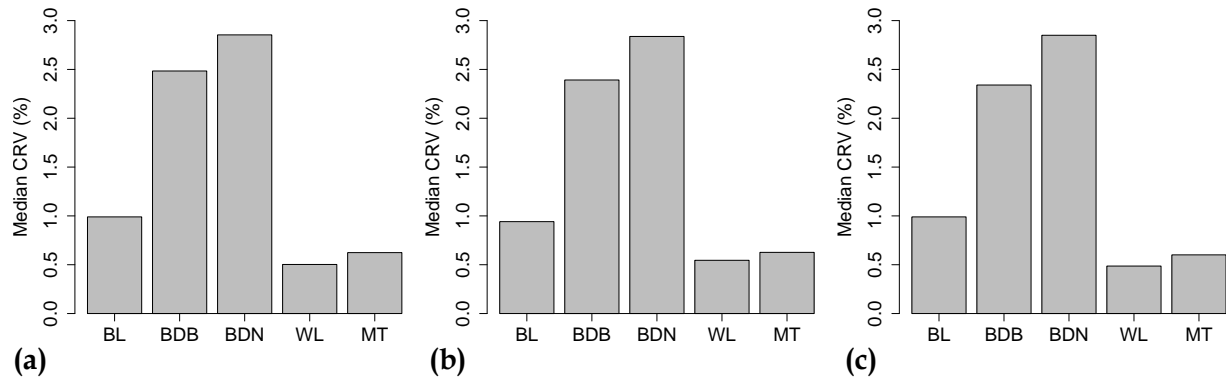


Figure 2. Median coefficients of residual variation for the logged size of morphological characters after regression on logged tarsus length in (a) 18 *Puffinus* species, consisting of (b) 11 sexually dimorphic *Puffinus* species, and (c) seven sexually monomorphic *Puffinus* species. BL=bill length;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

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. BL=bill length; BDB=bill depth at base; BDN=bill depth at nares; WL=wing length; MT=mid-toe length. **, * Significant difference at 1%, 5% after sequential Bonferroni adjustment. (For slopes, BL vs WL, BDB vs BDN and BDB vs MT were not significantly different at a 5% level, after sequential Bonferroni adjustment.)

| | Slope <i>P</i> values | CRV <i>P</i> values |
|---------------|--------------------------|------------------------|
| <i>Traits</i> | | |
| BL vs BDB | 0.865 | <0.001** |
| BL vs BDN | 0.246 | <0.001** |
| BL vs WL | 0.010 | <0.001** |
| BL vs MT | 0.054 | <0.001** |
| BDB vs BDN | 0.018 | <0.001** |
| BDB vs WL | 0.154 | <0.001** |
| BDB vs MT | 0.021 | <0.001** |
| BDN vs WL | 0.002* | <0.001** |
| BDN vs MT | 0.609 | <0.001** |
| WL vs MT | <0.001** | 0.099 |

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 (Figure 1b and 1c), but CRVs were markedly different, with bill depth measurements exhibiting the greatest amount of variation (Figure 2b and 2c). 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 regards to there being a biological 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 morphological variation has been demonstrated. Tests such as those presented here should be incorporated into initial statistical analyses in order to determine if trait variability is due to allometry before other possible biological explanations are offered.

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APPENDIX

Sample size (n), allometric regression slope of logged trait size on logged tarsus length, coefficient of residual variation (CRV, %), and P value from an F test of the linear model fit, for morphological characters in 11 sexually dimorphic *Puffinus* species (top) and seven sexually monomorphic *Puffinus* species (bottom). Slopes significantly different from 0 are indicated by * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$). BL=bill length; BDB=bill depth at base; BDN=bill depth at nares; WL=wing length; MT=mid-toe length.

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