



Original Article

# How acoustic signals scale with individual body size: common trends across diverse taxa

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We use allometric analysis to explore how acoustic signals scale on individual body size and to test hypotheses about the factors shaping relationships between signals and body size. Across case studies spanning birds, crickets, tree crickets, and tree frogs, we find that most signal traits had low coefficients of variation, shallow allometric scalings, and little dispersion around the allometric function. We relate variation in these measures to the shape of mate preferences and the level of condition dependence of signal traits. We find 3 major patterns: 1) signal traits associated with closed mate preferences had lower coefficients of variation and shallower allometries than signal traits with open preferences, 2) signal traits with higher levels of condition dependence had higher coefficients of variation and steeper allometries, and 3) the relationship between condition dependence and allometry varied with preference shape. We find no difference in coefficient of variation or allometry between advertisement and aggressive acoustic signals. Together, our findings suggest 2 main conclusions: 1) most acoustic signals do not appear to have been selected to function as indicators of body size and 2) an interplay between the form of selection and body size–related cost/benefit relationships of trait expression has great potential to explain variation in sexual allometries.

**Key words:** communication, mating signal, scaling relationships, static allometry.

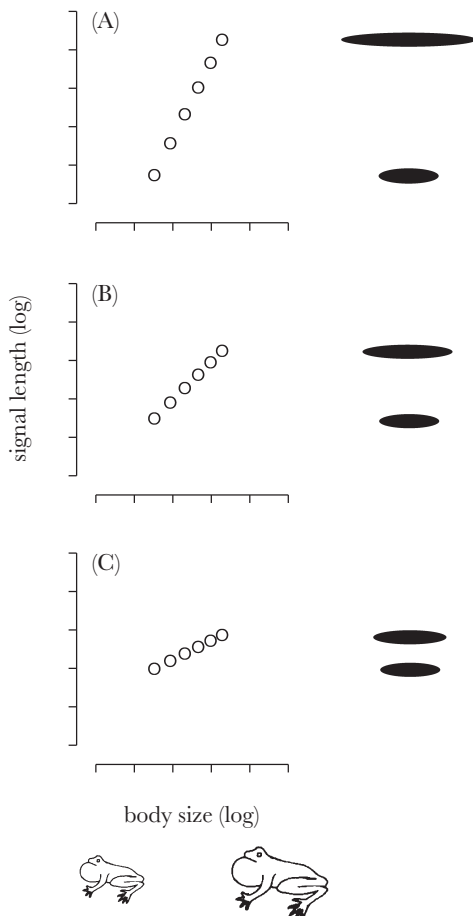
## INTRODUCTION

Biologists are often interested in how an animal's size influences its behavior and why. From questions of how to modulate aggression (Maynard Smith 1982) to deciding whether to attend to the courtship of suitors and how to find them (Andersson 1994; Gerhardt and Huber 2002; Greenfield 2002), and from the challenge of broadcasting signals unto the environment (Bennet-Clark 1998) to the consequences of falling down (Haldane 1926; Hooper 2012), body size has a central role in how biologists think about the evolution of behavior. But most research on the relationship between behavior and individual body size has been conducted with correlation analyses (Gerhardt and Huber 2002; Greenfield 2002; McLean et al. 2012) rather than with allometric analysis,

which offers a framework to test hypotheses about the relationship between trait size and body size in terms of sources of selection and constraints (Huxley 1932; Eberhard and Gutiérrez 1991; Emlen and Nijhout 2000; Gould 2002; Frankino et al. 2005; Eberhard et al. 2009; Egset et al. 2011, 2012). Allometric analysis has been used in comparative studies of the relationship between behavior and body size (Ryan and Brenowitz 1985; Cocroft and De Luca 2006; Dial et al. 2008; Hoskin et al. 2009; Gillooly and Ophir 2010; Martin et al. 2011), corresponding to evolutionary allometry (evaluated across taxa). But studies of static allometry (evaluated across adults within species) for behavior are rare (Cocroft and De Luca 2006; Rodríguez and Al-Wathiqui 2012), even though information about how signal features scale with individual body size is key for many hypotheses about the evolution of communication (Andersson 1994; Gerhardt and Huber 2002; Greenfield 2002; Searcy and Nowicki 2005; Bradbury and Vehrencamp 2011). Here, we focus on the static allometry of acoustic sexual signals.

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Behaviors do not have sizes like body parts do, but allometric analysis is ideal for describing how behavior varies with the size of the individual producing it. The main advantage offered by allometric analysis comes from its use of the slope ( $b$ ) of log–log regressions to describe trait scaling on body size. Traits with  $b \approx 1$  scale in proportion to body size and are said to exhibit isometry. Traits with  $b > 1$  have steep, exaggerated scalings, being disproportionately large in large individuals and disproportionately small in small individuals, and exhibit positive allometry (also termed hyperallometry). Traits with  $b < 1$  scale shallowly with body size, being disproportionately large in small individuals and disproportionately small in large individuals, and show negative allometry (hypoallometry). Thus, signals showing isometry directly reflect the size of the individuals producing them; signals with positive allometry offer a clearer indication of the size of the individual producing them than body size itself; and signals with negative allometry reflect body size poorly (Figure 1). An additional advantage of using allometric analysis for the study of acoustic signals is that log–log measures of the steepness of these relationships better approximate how receivers perceive variation in stimuli—that is, as per Weber’s Law (Stevens



**Figure 1**

Cartoon showing different patterns of static allometry for an acoustic signal. Signal traits (here, signal length) may vary in how they scale on individual body size: (A) Steep or positive allometry ( $b = 2$ ). (B) Proportional scaling or isometry ( $b = 1$ ). (C) Shallow or negative allometry ( $b = 0.5$ ). Frog cartoons show the relative size of the smallest and largest individual in each panel. Black ovals show the relative length of the shortest and longest signals in each panel.

2000; Bradbury and Vehrencamp 2011)—than measures derived from correlation analysis.

Allometric analysis offers novel ways to test basic yet contrasting expectations about behavior. Behavioral traits such as acoustic signals are expressed flexibly and in specific contexts and times, as opposed to morphological traits (e.g., ornaments), which are carried permanently or at least for the duration of the mating season (West-Eberhard 2003; Zuk et al. 2014). Thus, investment trade-offs (Simmons and Emlen 2006; Emlen 2008) will often be simultaneous for body and a morphological ornament but temporally uncoupled for body and a behavioral signal, allowing for investment in body size followed by flexible investment in the signal. Individuals of different sizes may thus be better able to express exaggerated behavioral signals than exaggerated morphological ornaments. If so, acoustic signal traits should exhibit shallow allometries. We ask whether this is the case and discuss implications for the potential of acoustic signals to serve as indicators of body size. The background for this question is the common expectation that acoustic sexual signals will reflect or be constrained by the body size of the individuals producing them (Andersson 1994; Gerhardt and Huber 2002; Greenfield 2002; Searcy and Nowicki 2005; Bradbury and Vehrencamp 2011; McLean et al. 2012).

We also use our exploration of acoustic signal allometry to address an important problem in evolutionary biology: the observation that sexual traits exhibit a large and puzzling amount of variation in their allometries. Ornaments and weapons vary in allometry from very steep ( $b \gg 1$ ) to proportional ( $b \approx 1$ ) to shallow ( $b < 1$ ) (Cuervo and Møller 2001; Bonduriansky 2007; Schulte-Hostedde et al. 2011), with some spectacular ornaments having shallow allometries and other seemingly modest ornaments having unexpectedly steep allometries (Cuervo and Møller 2001). Why should sexually selected traits vary so much in their allometry?

We assess the role of 3 major factors that may help explain variation in the allometric scaling of sexual traits (summarized in Table 1): 1) the form of selection; 2) the level of condition dependence, as a proxy for body size–related variation in the strength of selection favoring trait increase; and 3) the nature of trait functions. These factors are predicted to influence allometries and coefficients of variation (CVs). We, therefore, assess their influence on both of these variables, and we relate differences in CVs to the steepness of allometric functions ( $b$ ) and to the dispersion around the allometric function (i.e., the residual variation in CVs after accounting for  $b$ ) (Figure 2).

### Form of selection and condition dependence

These 2 factors are part of a broad hypothesis proposed as an explanation for variation in the static allometry of sexual traits. This hypothesis posits an interplay between the form of selection and whether body size influences the net benefits of trait increase (Bonduriansky 2007; Eberhard et al. 2009). Here, we use proxies for the form of selection and for body size–related differences in the net benefits of trait increase to derive predictions for this hypothesis, as follows.

As a proxy for the form of selection, we use the shape of mate preferences associated with signal traits. We characterize mate preferences as either “closed” or “open,” with the former favoring intermediate signal trait values and the latter favoring extreme trait values. Closed preferences that favor a trait value corresponding to the population mean should exert stabilizing sexual selection; closed preferences favoring values other than the population mean, and open preferences, should exert directional selection (Gerhardt 1994; Ritchie 1996;

**Table 1**

**Hypothesized causes for differences in trait variability and the predictions that they make for 3 descriptors of trait variability: the CV, the allometric slope ( $b$ ), and the dispersion around the allometric function**

| Cause for differences in trait variability               | Descriptor of trait variability          |   |  |
|--|--|---|--|
|  | CV                                       | $b$   | Dispersion                               |
| Mate preference shape (open vs. closed)                  | Greater with open preferences            | Variable with open preferences <sup>a</sup> , lower with closed preferences | ?  |
| Level of condition dependence                            | Greater with higher condition dependence | Greater with higher condition dependence for traits with open preferences   | Greater with higher condition dependence |
| Nature of signal function (aggressive vs. advertisement) | Greater for aggressive signals           | Greater for aggressive signals  | ?  |

<sup>a</sup>According to the level of condition dependence.

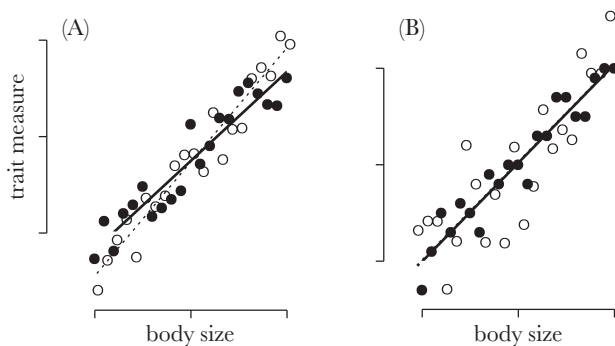
**Figure 2**

Diagram illustrating allometric analysis of differences in the CV in terms of the steepness and dispersion of allometric functions. (A) The data in open symbols have a higher CV than the data in closed symbols because of a steeper allometric function. (B) The data in open symbols have a higher CV than the data in closed symbols because of greater dispersion around the same allometric function. Figure drawn after Eberhard et al. (1998).

Rodríguez et al. 2006). Closed preferences favoring mean population values should select for lower CVs than open preferences, as closed preferences disfavor deviation from preferred values in any direction rather than only one (Rodríguez et al. 2006). Similarly, closed preferences should select for shallow allometries (Rodríguez and Al-Wathiqui 2012; cf. Eberhard et al. 2009). By contrast, the effect of directional selection (here, open preferences) on allometry depends on whether the net benefit of trait increase (i.e., the strength of selection favoring trait increase) varies with body size. If this net benefit is even across body sizes, the result should be isometry; however, if the net benefit is greater for larger males, the result should be steep allometry; conversely, if the net benefit is greater for smaller males, shallow allometry should result (Bonduriansky 2007; Eberhard et al. 2009).

As a proxy for body size-related variation in the net benefit of trait increase, we use the level of condition dependence of signal traits. Our rationale is that traits linked to individual condition should be costly to express, especially so for smaller individuals for whom expression at a given trait level would be a relatively larger investment (cf. Petrie 1988; Bonduriansky 2007; Shingleton et al. 2007, 2008; Emlen et al. 2012; Voituron et al. 2012). Consequently, exaggeration of condition-dependent traits should bring higher net benefits to larger individuals. For the same reasons, costlier, more highly condition-dependent traits should be more variable (Møller and Pomiankowski 1995; Rowe and Houle 1996). We, therefore, use the level of condition dependence of signal traits to approximate potential body size-related variation in the net benefit of trait increase.

The above considerations lead to the following predictions (summarized in Table 1): 1) Signal traits associated with closed mate preferences should have lower CVs and shallower allometries than signal traits associated with open preferences (NB: In our case studies, all closed preferences favored mean population values; Supplementary Appendix). A study with insect vibrational signals found support for this prediction: signal traits with closed preferences had lower CVs and shallow, low-dispersion allometries (Rodríguez and Al-Wathiqui 2012). 2) Signal traits with higher condition dependence should have higher CVs and steeper allometries. 3) The relationship between condition dependence and the steepness of allometries should vary between signal traits with closed and open preferences.

### Nature of signal function

We compare aggressive signals (used solely in escalated male–male contests) with advertisement signals (used in mate attraction and male–male contests) (Wells 1977). This comparison offers a complementary way of assessing how acoustic signals relate to body size. It also allows us to test another hypothesis that seeks to explain variation in the allometry of sexual traits. This hypothesis is based on a contrast between the source of costs limiting the expression of aggressive and advertisement signals. Exaggeration of aggressive signals is in many cases limited by receiver-dependent costs, whereby individuals that signal beyond their capacity run the risk of injury if they are forced to back up an aggressive display with actual force (Searcy and Nowicki 2005; Eberhard 2009; Bradbury and Vehrencamp 2011; Anderson et al. 2012). Advertisement signals are unlikely to involve such risks. If body size is important in determining the outcome and risk of injury in aggressive interactions, then the net benefit of exaggeration for aggressive signals should be greater for larger individuals but is less likely to vary with body size for advertisement signals. This hypothesis, therefore, predicts steeper allometries (and higher CVs) for aggressive signals than for advertisement signals (Table 1). On the other hand, the role of individual body size in aggressive signaling may be more complex. For example, larger males may win low-level signaling contests but not physical fights (Reichert and Gerhardt 2011). Thus, the question of how the aggressive and advertisement signals scale on individual body size is of high interest.

Our tests required the following information and estimates for acoustic signal traits: the CV, the allometric slope ( $b$ ) and dispersion around the allometric function, the level of condition dependence, the shape of the corresponding mate preference, and whether the trait described variation in advertisement or aggressive signals. We are unaware of any study that has gathered such information, and

we, therefore, used data from our own work. We assembled a sample of 68 acoustic advertisement signal traits drawn from 11 species in 6 genera spanning birds, crickets, tree crickets, and tree frogs (Supplementary Appendix). The data set also included 48 traits comparing acoustic advertisement and aggressive signals, drawn from 3 species in 2 tree frog genera (Supplementary Appendix).

## METHODS

We estimated  $b$  with log–log ordinary least squares (OLS) regression on body size (Supplementary Appendix). There has been debate over this use of OLS regression due to the concern that it may bias  $b$  downward by ignoring error in the  $x$  axis (Green 1999). However, there is evidence that OLS regression does not underestimate  $b$  (Al-Wathiqui and Rodríguez 2011). Furthermore, there are problems with the preferred alternative method, reduced major axis (RMA) regression. RMA regression confounds variation in  $b$  with variation in dispersion around the allometric function; that is, it confounds scaling with dispersion (Figure 2). This is because  $b$  estimates obtained with RMA regression correspond to the ratio of the standard deviations in  $x$  and  $y$  (Eberhard et al. 1999; Voje and Hansen 2013; Voje et al. 2014). Additionally, RMA regression biases  $b$  toward 1 due to the variance-homogenizing effect of log–log transformations. We thus favor OLS regression for studies of allometry (and see further arguments for this conclusion in Eberhard et al. 1999; Voje and Hansen 2013; Cassidy et al. 2014; Voje et al. 2014).

To estimate the level of condition dependence for each signal trait, we first estimated condition for each individual, and then, we related variation in condition to variation in signal traits. We estimated individual condition with the residuals of an OLS regression of mass on body size (Figure 3A). This measure views condition as resources acquired and carried on the body, such as muscle and fat reserves (Hunt et al. 2004). There are alternative methods to estimate individual condition, such as using indicators of the health of cellular processes (Hill 2011), or experimentally manipulating condition with diet treatments (Kotiaho 1999; Tomkins et al. 2004). Our choice of estimate reflects a balance of feasibility and likely biological relevance. For a study such as ours, the measure based on residuals best permits comparison across case studies, whereas the other alternatives would

be impractical. Further, the mating systems of the species included in our sample feature multihour signaling over at least several days if not longer, where long-term endurance and energy reserves on the body are likely to be highly relevant for reproductive success (e.g., Gerhardt et al. 1987; Wells et al. 1995; Bertram et al. 1996; Höbel 2000). We, thus, consider that an estimate of condition reflecting resources carried on the body is well suited to our purposes. We are aware of critiques of the residuals metric (Green 2001; Peig and Green 2009, 2010). However, its use to estimate condition has been validated (Schulte-Hostedde et al. 2005).

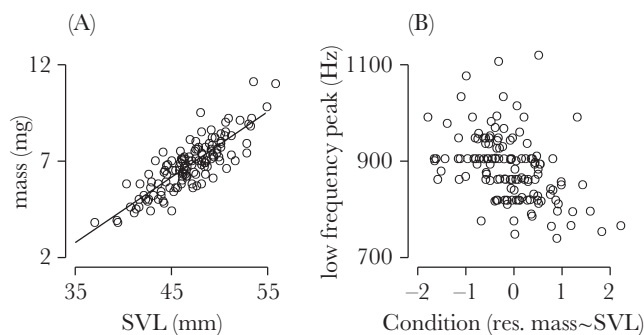
After calculating individual condition, we obtained the correlation between individual condition and signal trait values for each signal trait (Figure 3B) with Pearson's product–moment coefficient of correlation ( $r$ ). That  $r$  value was the level of condition dependence of each signal trait.

## Statistical analysis

We conducted all tests in JMP v. 7.0.1 (SAS Institute, Cary, NC). We used data from 2 to 11 signal traits for each species, with 1–4 species in each of the 6 genera (case studies). The sample of individual males measured for each species ranged from 19 to 363, with a mean of 90 and a median of 54.

Most case studies included either a single species per genus or species that were not very closely related to the others in the genus (with 2 exceptions: the *Gryllus* case study consists of a clade of 3 closely related species and the 2 *Hyla versicolor* lineages; Supplementary Appendix). Further, the case studies span a broad diversity of animal groups (Supplementary Appendix). Thus, rather than accounting for phylogenetic nonindependence, our main concern with the data set was to account for the use of multiple traits measured from the same individual for each species, which introduces the risk that the traits generate nonindependent data points for our analysis. Against this concern, we note that our analyses deal with the relationship between each signal trait and body size and condition, and these relationships are likely to vary among signal traits within species (see below). Also, correlations between signal traits were predominantly low: the absolute value of Pearson's  $r$  between signal traits ranged from 0.002 to 0.96 (mean = 0.27, median = 0.21), with 75% of the correlations being <0.36; that is, most correlations between signal traits were of weak effect size (Nakagawa and Cuthill 2007). It would thus seem that nonindependent data points are not a serious problem in our data set. Nevertheless, we caution about the risk for pseudoreplication in our analyses and the need for further work to assess how robust our findings are. To help deal with this potential problem, we included species as a random term (restricted maximum likelihood method) in all tests; the 95% confidence intervals for this term always overlapped zero and we do not report it below, but we retained it in all models. We initially included a term coding for whether signal traits represented temporal or spectral features (e.g., signal duration vs. dominant frequency, respectively) and its interactions with the other term. These terms were always nonsignificant ( $P \geq 0.071$ ), and we removed them from the final models.

For acoustic signals, negative values for  $b$  may indicate inverse relationships with body size, rather than merely shallow scaling; for example, signal frequency decreases with body size. Consequently, we used  $|b|$  in our analyses. However, we used signed values for condition dependence because they are informative: our estimate of individual condition is independent of body size (see above), so that correlations between signal traits and individual condition ask whether relatively heavy or light males produce higher or lower signal trait values across body sizes, which can influence allometry.



**Figure 3**

Calculation of the level of condition dependence of a signal trait. (A) Regression of mass on body size used to obtain the residuals that indicate individual condition. Individuals above the line are in better condition (being heavy for their size) than individuals below the line (which are light for their size). (B) Correlation between a signal trait and individual condition. The  $r$  value of the correlation describes the level of condition dependence for the signal trait. In this case,  $r = -0.44$ . Data are for *Hyla cinerea* green tree frogs, one of our case studies (Supplementary Appendix).

### Form of selection and condition dependence: advertisement signals

The CV and  $|b|$  were dependent variables in separate tests. Models included mate preference shape, the level of condition dependence, and their interaction as fixed effects (Table 2). For the test with dispersion around the allometric function as the dependent variable, we used the above model with  $|b|$  added as a covariate to test for residual variation in CVs after accounting for  $|b|$  (Table 2). To relate  $|b|$  to the dispersion around the allometric function (Figure 2), we used a model with the residuals of the regression of the CV on  $|b|$  as the dependent variable, and  $|b|$ , preference shape, and their interaction as fixed effects (Table 3).

### Nature of signal function: advertisement versus aggressive signals

The CV and  $|b|$  were dependent variables in separate tests. Models included condition dependence, signal type, and their interaction as fixed effects (Table 4). For dispersion around the allometric function as the dependent variable, we used the above model with  $|b|$  added as a covariate to test for residual variation in CVs (Table 4). To compare advertisement and aggressive signals in terms of the relationship between the steepness and the dispersion of allometric functions (Figure 2), we used a model with the residuals of the regression of the CV on  $|b|$  as the dependent variable, and  $|b|$ , signal type, and their interaction as fixed effects (Table 5).

## RESULTS

Most acoustic signal traits had low CVs, shallow allometries, and low levels of condition dependence (Figure 4). But the range of

**Table 2**

**Variation in the CV, the absolute value of the allometric slope ( $|b|$ ), and the dispersion around the allometric function for acoustic advertisement signals**

|  | df (num, den) | <i>F</i> , <i>P</i>  |
|--|---------------|----------------------|
| Variation in CV                                    |               |                      |
| Preference shape                                   | 1, 28.92      | <b>18.00, 0.0002</b> |
| Condition dependence                               | 1, 29.15      | 2.93, 0.098          |
| Preference shape × condition dependence            | 1, 31.3       | <b>4.19, 0.049</b>   |
| Variation in $ b $                                 |               |                      |
| Preference shape                                   | 1, 27.96      | 2.53, 0.12           |
| Condition dependence                               | 1, 27.87      | 2.12, 0.16           |
| Preference shape × condition dependence            | 1, 29.12      | <b>13.00, 0.0011</b> |
| Variation in dispersion around allometric function |               |                      |
| Preference shape                                   | 1, 29.07      | <b>14.26, 0.0007</b> |
| Condition dependence                               | 1, 30.34      | <b>6.26, 0.018</b>   |
| Preference shape × condition dependence            | 1, 32.84      | 0.25, 0.62           |
| $ b ^a$  | 1, 28.26      | <b>8.68, 0.0064</b>  |

We tested the effect of the shape of mate preferences (open vs. closed) and of the level of condition dependence of signal traits. In the figure that corresponds to these tests (Figure 5), we distinguish between signal traits with preferences of open, closed, or unknown shape, but in the statistical tests, the cells for preference shape were left empty for signal traits with unknown preferences. This table shows the fixed effects of the statistical model, which also included a random term for species (see Statistical analysis). Significant effects in bold. df, degrees of freedom.

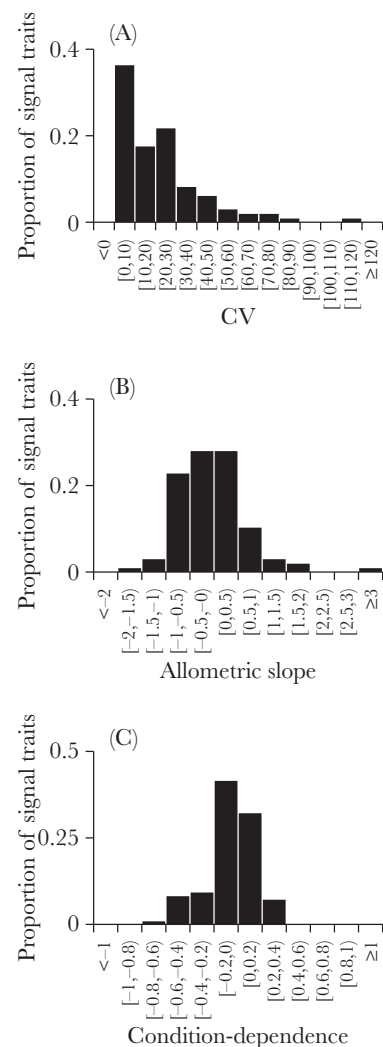
<sup>a</sup>We included  $|b|$  as a covariate in this model so that the test would reflect dispersion around the allometric function; we report its effect here for completeness.

variation in these features was broad, and in the analyses that follow, we tested hypotheses about the causes of such variation.

### Form of selection and condition dependence: advertisement signals

Signal traits associated with open mate preferences had higher CVs (Table 2 and Figure 5A). The effect of condition dependence varied with preference shape: only signal traits with open mate preferences increased in CV with condition dependence (significant preference shape × condition dependence interaction; Table 2) (Figure 5A). Signal traits with unknown mate preferences showed the same pattern as signal traits with open preferences (Figure 5A, gray symbols).

Allometric slopes ( $|b|$ ) were slightly steeper for signal traits associated with open mate preferences, but this difference was not



**Figure 4**

Overall patterns of variation in the CV, allometry, and condition dependence of acoustic signal traits. (A) Most signal traits had low CVs, but note that the range was quite broad. (B) Most signal traits had shallow allometric slopes ( $b$ ), but the range was also broad. This figure shows signed values of  $b$ , but in our analyses, we used absolute values ( $|b|$ ) (see text). (C) Most signal traits had low levels of condition dependence ( $|r| < 0.3$ ; cf. Nakagawa and Cuthill 2007). Histograms show pooled data across all species and advertisement and aggressive signals.

**Table 3**  
**Variation in the dispersion around the allometric function according to the steepness of allometric functions and the shape (open vs. closed) of mate preferences for advertisement signals**

|                               | df (num, den) | <i>F</i> , <i>P</i>  |
|-------------------------------|---------------|----------------------|
| $ b $                         | 1, 33.77      | 0.93, 0.34           |
| Preference shape              | 1, 29.5       | <b>20.15, 0.0001</b> |
| $ b  \times$ preference shape | 1, 32.54      | <b>5.49, 0.025</b>   |

This table shows the fixed effects of the statistical model, which also included a random term for species (see Statistical analysis). Significant effects in bold. df, degrees of freedom.

**Table 4**  
**Comparison of the CV, the absolute value of the allometric slope ( $|b|$ ), and the dispersion around the allometric function for acoustic advertisement and aggressive signals**

|  | df (num, den) | <i>F</i> , <i>P</i> |
|--|---------------|---------------------|
| Variation in CV                                    |               |                     |
| Signal type (advertisement or aggressive)          | 1, 41.1       | 0.03, 0.87          |
| Condition dependence                               | 1, 42.15      | 1.91, 0.18          |
| Signal type $\times$ condition dependence          | 1, 42.29      | 0.08, 0.77          |
| Variation in $ b $                                 |               |                     |
| Signal type (advertisement or aggressive)          | 1, 41.01      | 1.86, 0.18          |
| Condition dependence                               | 1, 41.78      | 0.46, 0.50          |
| Signal type $\times$ condition dependence          | 1, 41.89      | 1.73, 0.20          |
| Variation in dispersion around allometric function |               |                     |
| Condition dependence                               | 1, 41.88      | 2.54, 0.12          |
| Signal type  | 1, 40.75      | 0.03, 0.86          |
| Signal type $\times$ condition dependence          | 1, 41.97      | 0.01, 0.91          |
| $ b ^a$  | 1, 38.99      | 2.46, 0.12          |

We tested the effect of signal type (advertisement or aggressive) and of the level of condition dependence of signal traits. This table shows the fixed effects of the statistical model, which also included a random term for species (see Statistical analysis). df, degrees of freedom.

<sup>a</sup>We included  $|b|$  as a covariate in this model so that the test would reflect dispersion around the allometric function; we report its effect here for completeness.

**Table 5**  
**Comparison of the relationship between the steepness and the dispersion of allometric functions for advertisement and aggressive signals**

|                          | df (num, den) | <i>F</i> , <i>P</i> |
|--------------------------|---------------|---------------------|
| $ b $                    | 1, 42.87      | 0.53, 0.47          |
| Signal type              | 1, 41.42      | 0.0004, 0.98        |
| $ b  \times$ signal type | 1, 41.89      | 2.02, 0.16          |

This table shows the fixed effects of the statistical model, which also included a random term for species (see Statistical analysis). df, degrees of freedom.

significant (Table 2 and Figure 5B).  $|b|$  increased with condition dependence for signal traits with open preferences but decreased for signal traits with closed preferences (significant preference shape  $\times$  condition dependence term in Table 2) (Figure 5B).

Dispersion around the allometric function was greater for signal traits with open preferences and increased with condition dependence (Table 2 and Figure 5C). This increase was not influenced by preference shape (nonsignificant preference shape  $\times$  condition dependence term in Table 2) (Figure 5C). Dispersion increased with  $|b|$  only for signal traits with open preferences (significant

terms for preference shape and  $|b| \times$  preference shape in Table 3) (Figure 5D).

### Nature of signal function: advertisement versus aggressive signals

We found no difference in CVs between advertisement and aggressive signals (Table 4 and Figure 6A). CVs increased with condition dependence (Figure 6A), but not significantly (Table 4).

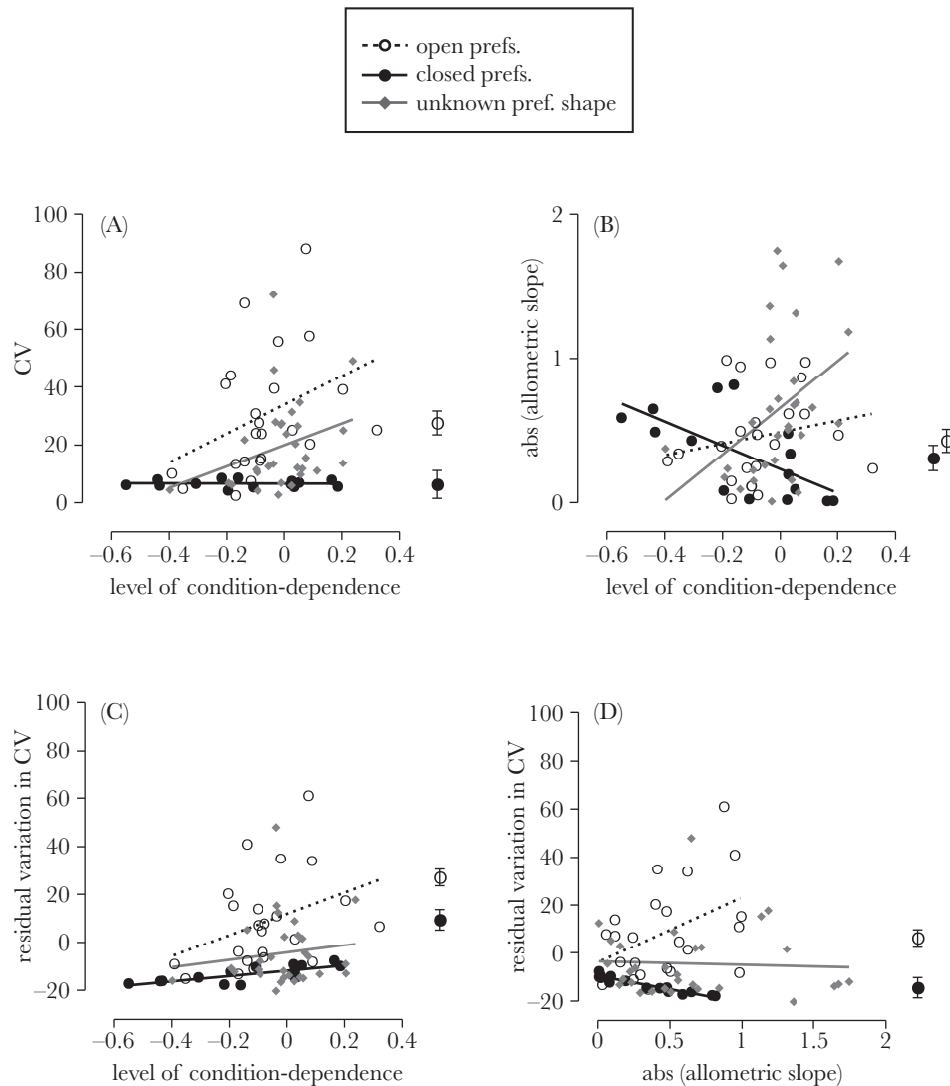
There was also no difference in  $|b|$  between advertisement and aggressive signals and no change in  $|b|$  with condition dependence (Table 4 and Figure 6B). This test may have been influenced by an outlier data point (the steepest  $b$  in our data set; Figure 6B). But the outcome was nearly identical when we removed the outlier: the main change was that the signal type  $\times$  condition dependence interaction went from nonsignificant (Table 4) to marginally significant ( $F_{1,40.85} = 3.44$ ;  $P = 0.071$ ), but note that there was no great difference between advertisement and aggressive signals in the relationship between  $|b|$  and condition dependence (Figure 6B).

There was also no difference in dispersion around the allometric function between advertisement and aggressive signals (Table 4 and Figure 6C). Dispersion increased with condition dependence (Figure 6C), but not significantly (Table 4). There was no clear relationship between the steepness and dispersion of allometric functions for advertisement or aggressive signals (Table 5 and Figure 6D). This result may have been influenced by the outlier; after removing it, the terms for  $|b|$  and for signal type remained nonsignificant, but the  $|b| \times$  signal type interaction became marginally significant ( $F_{1,40.37} = 3.19$ ;  $P = 0.082$ ). Thus, the relationship between the steepness and dispersion of allometric functions may differ between signal types, being positive for advertisement signals and negative for aggressive signals (dotted vs. gray lines in Figure 6D).

## DISCUSSION

We explored variation in acoustic signals with the framework of static allometry. Most signal traits had low CVs, shallow allometric slopes, and little dispersion around the allometric function (and see similar findings with insect vibrational signals in Cocroft and De Luca 2006; Rodríguez and Al-Wathiqui 2012). Thus, acoustic signals appear to allow for relatively even expression across body sizes. We suggest that this may be due to the potential for behavioral traits to show flexible, context-specific expression (cf. West-Eberhard 2003; Zuk et al. 2014). Remarkably, this pattern seems to hold in spite of some reason to expect limits to the flexibility of the expression of behavior because the structures used to perform behavior often have additional functions that should limit flexible expression but appear not to; for example, avian beaks are used for both feeding and signaling (Podos 2001). One implication of this finding is that even if signal traits are correlated with body size, they may not be useful indicators of body size because of their shallow allometries—they will at least offer no better indication than body size itself and often worse. There were some signal traits with  $b > 1$  that might provide a clearer indication of the signaler's body size and condition, especially those under directional selection (Figure 5B,C). However, the signal traits with steeper values for  $b$  also had higher dispersion around the allometric function (Figure 5D). Consequently, their reliability as indicators of body size remains limited (see also Rodríguez and Al-Wathiqui 2012).

Although most signal traits had low CVs and shallow allometries with little dispersion, the range of variation in these measures was



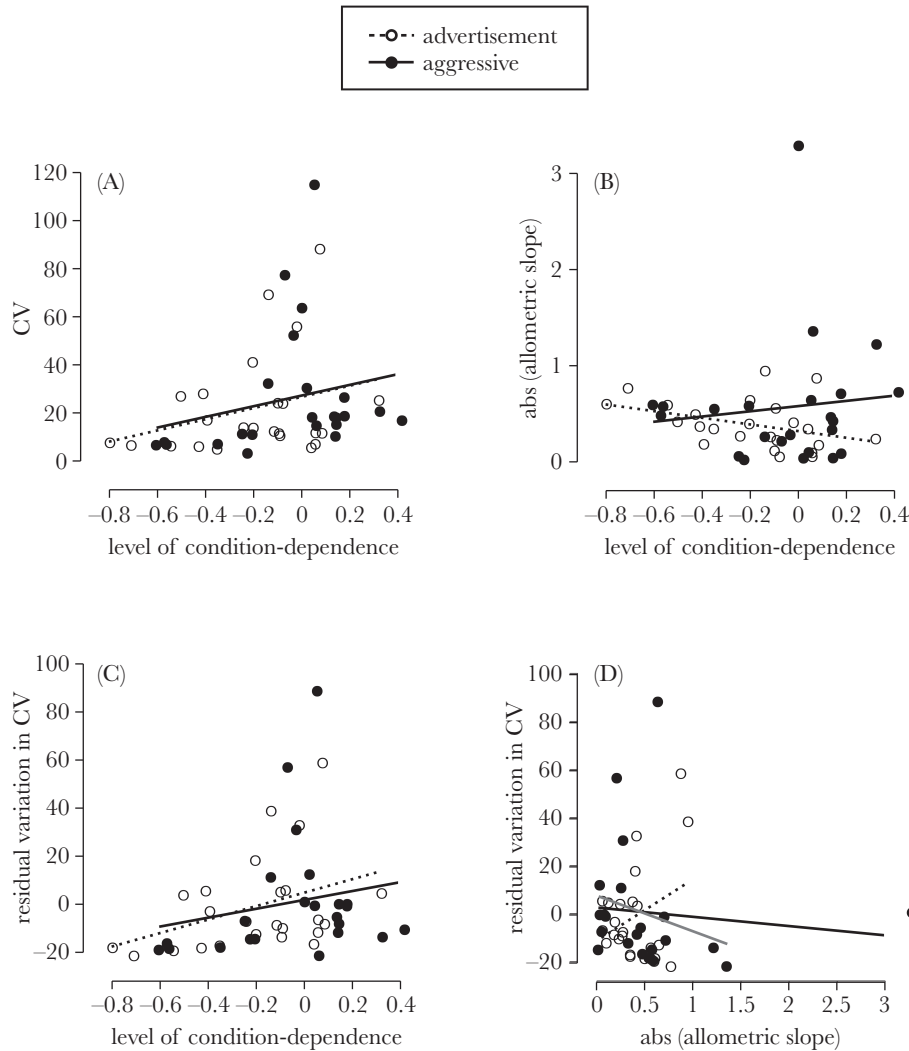
**Figure 5**

Causes of variation in the CV and allometry of acoustic advertisement signals. (A) Relationship between preference shape, level of condition dependence, and signal trait CVs. Signal traits with open preferences had higher CVs than traits with closed preferences. CVs increased with condition dependence for signal traits with open preferences. (B) Relationship between preference shape, condition dependence, and the steepness of allometric slopes ( $|b|$ ). Signal traits with open preferences had slightly steeper slopes, but this difference was not significant (Table 2). The relationship with condition dependence varied with preference shape: it was positive for signal traits with open preferences and negative for traits with closed preferences. (C) Relationship between preference shape, condition dependence, and the dispersion around the allometric function. For plotting, we used the residuals of a regression of signal trait CVs on  $|b|$  for the  $y$  axis (see text). Signal traits with open preferences had greater dispersion, and dispersion increased with condition dependence. (D) Relationship between the steepness of allometric slopes ( $|b|$ ) and the dispersion around the allometric function for signal traits associated with open and closed mate preferences. Dispersion increased with  $|b|$  for signal traits with open preferences but not for traits with closed preferences; dispersion was greater for signal traits with open preferences. Symbols next to each panel show least square means  $\pm 1$  SE obtained from the corresponding statistical models shown in Tables 2 and 3.

broad, and this allowed us to test hypotheses about such variation. These hypotheses involved 3 variables: the shape of mate preferences (as a proxy for the form of selection), the level of condition dependence (as a proxy for body size-related variation in the net benefit of trait increase), and the nature of signal functions.

Signal traits with open preferences had higher CVs than traits associated with closed mate preferences, especially for the more condition-dependent traits (Figure 5A). This result supports the prediction that traits under stabilizing selection should have lower CVs than traits under directional selection. The relationship between allometry and condition dependence varied between signal traits

with open and closed mate preferences: For signal traits with open preferences (but not with closed preferences), the steepness and dispersion of allometry increased with condition dependence (Figure 5B,C). These results help elucidate how differences in CVs arise: The effect of the form of selection is straightforward, but the effect of condition dependence can be complex and is influenced by the form of selection. This complexity may help explain why signal trait CVs show a continuous range of variation, rather than the bimodal distribution that might be predicted for traits under stabilizing versus directional selection (Reinhold 2009; cf. Gerhardt 1994; Gerhardt and Huber 2002).



**Figure 6**

Comparison of the CV and allometry of acoustic advertisement and aggressive signals. (A) Relationship between signal type, level of condition dependence, and signal trait CVs. (B) Relationship between signal type, level of condition dependence, and the steepness of the allometric slope ( $|b|$ ) of signal traits. The outlier data point ( $b = 3.29$ ) corresponds to the duty cycle of aggressive signals in *Dendropsophus ebraccatus* tree frogs (Supplementary Appendix); this trait was an outlier only for  $|b|$ ; its CV was 65.6, and its residual CV was 0.93. (C) Relationship between signal type, condition dependence, and the dispersion around the allometric function (visualized with the residuals of a regression of signal trait CVs on  $|b|$  as the  $y$  axis; see text). (D) Relationship between the steepness of allometric slopes ( $|b|$ ) and the dispersion around the allometric function. The relationship was positive for advertisement signals, but for aggressive signals, it was either weakly negative (including the outlier; black line) or more strongly negative (excluding the outlier; gray line).

Our results also address the evolutionary problem presented by the great range of variation in the allometry of sexual traits (Cuervo and Møller 2001; Bonduriansky 2007; Schulte-Hostedde et al. 2011). We find support for a hypothesis that may account for a broad range of variation in sexual allometries: Signal traits subject to stabilizing selection and with low levels of condition dependence appear to evolve shallower allometries than signal traits subject to directional selection, especially if the latter are highly condition dependent. Thus, the form of selection and the level of condition dependence may interact to generate diverse allometries. We emphasize that our test only approximates the full hypothesis, which involves an interaction between the form of selection and body size-related differences in the strength of selection favoring trait increase (Bonduriansky 2007; Eberhard et al. 2009). Future work should test this hypothesis more fully.

Finally, we found no difference in CVs or allometries between aggressive and advertisement signals. This result offers only a weak rejection of the hypothesis that traits with aggressive functions should be more variable and scale more steeply on body size (Eberhard 2002, 2009) because aggressive signals likely function to induce (rather than force) rivals to retreat, just as advertisement signals induce mates to approach. It would thus be interesting to expand tests for this hypothesis to include traits with more forceful functions used in interactions with higher risk of injury. Nevertheless, this finding offers insight into the evolution of acoustic signals, emphasizing the key suggestion that most acoustic signals (including aggressive signals) do not provide clear indications of body size to potential mates or to rivals.

Our survey may suffer from some potential limitations. First, although we find evidence that variation in allometry may be explained by the form of selection and the level of condition



dependence, most of the signal traits we measured had a relatively narrow range of variation in allometry. It will be interesting to expand tests across a greater range of variation in allometries, as well as across a broader sampling of taxonomic diversity. Broader tests will also help assess how robust our findings are against potential problems caused by data obtained from traits correlated with each other. Another issue is that, although our case studies encompassed a wide variety of signal traits, we did not include measures of signal intensity. This will require standardized measures across individuals and species to account for variation in signal intensity with distance from the source. Finally, for each case study, we used the most appropriate body size metric for which reliable measurements could be obtained (Supplementary Appendix). Thus, there were various proxies for body size in our analyses, and this diversity may hide patterns that might emerge with more standard measures. However, we do not expect that this introduced bias in our analyses, as our choices reflect the challenge that receivers face in nature, where direct assessment of body size is more difficult than assessment of traits that relate to it in some unknown way.

We hope that this article will generate enthusiasm for the use of allometric analysis in the study of behavior. This quantitative framework can provide fresh insights into the evolution of behavior, and using behavioral traits to test hypotheses about the evolution of allometry opens up novel possibilities for answering broad questions in evolutionary biology.

## SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.behco.oxfordjournals.org/>

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