Causes of variation in sexual allometry: a case study with the mating signals and genitalia of Enchenopa treehoppers (Hemiptera Membracidae)

R.L. Rodríguez & N. Al-Wathiqi

a Behavioral and Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin-Milwaukee, Lapham Hall, 3209 N. Maryland Ave, Milwaukee, WI, 53201, USA

b Biology Department, Tufts University, Barnum Hall, 163 Packard Ave, Medford, MA, 02155, USA

Available online: 21 Mar 2012

To cite this article: R.L. Rodríguez & N. Al-Wathiqi (2012): Causes of variation in sexual allometry: a case study with the mating signals and genitalia of Enchenopa treehoppers (Hemiptera Membracidae), Ethology Ecology & Evolution, 24:2, 187-197

To link to this article: http://dx.doi.org/10.1080/03949370.2011.645884

PLEASE SCROLL DOWN FOR ARTICLE
Causes of variation in sexual allometry: a case study with the mating signals and genitalia of *Enchenopa* treehoppers (Hemiptera Membracidae)

R.L. RODRÍGUEZ 1,3 and N. AL-WATHIQUI 2

1 Behavioral and Molecular Ecology Group, Department of Biological Sciences, University of Wisconsin–Milwaukee, Lapham Hall, 3209 N. Maryland Ave., Milwaukee, WI 53201, USA
2 Biology Department, Tufts University, Barnum Hall, 163 Packard Ave., Medford, MA 02155, USA

Received 31 March 2011, accepted 4 November 2011

Sexual traits show a great range of variation in static allometry, with some mating displays and weapons showing hyperallometry (steep increase in trait size with body size), others showing isometry (even scaling on body size), and others showing hypoallometry (shallow scaling on body size). We test two hypotheses that attempt to explain variation in sexual allometry, with a comparison of the allometry of behavioral (advertisement signals) and morphological (body and genitalia) traits. Our study species was a member of the *Enchenopa binotata* species complex of treehoppers (Hemiptera Membracidae), a group with sexually selected vibrational advertisement signals and naturally selected genitalia. Using advertisement signal traits, we find weak support for the hypothesis that the form of selection (stabilizing or directional) can account for variation sexual allometries. Using genitalia traits, we find support for the hypothesis that the naturally selected functions of genitalia (as opposed to their sexually selected functions) can account for the widespread pattern of hypoallometry in animal genitalia. We compare the relationship between the steepness and dispersion of signal and genitalia allometries, and discuss implications for their potential to be selected as indicators of body size.

KEY WORDS: genital, indicator trait, mating signal, phytophagous insect.

INTRODUCTION

Sexual traits show astonishing variation in how they scale relative to body size, i.e., in their static allometry. Some mating displays and weapons conform to the traditional expectation of steep scaling on body size, with larger individuals bearing...
disproportionately large traits (hyperallometry or positive allometry; KODRISC-BROWN et al. 2006). However, sexual traits also often scale in direct proportion to body size (isometry), or their scaling may be shallow, so that larger individuals bear disproportionately small traits (hypoallometry or negative allometry; CUERO & MØLLER 2001; BONDURIANSKY 2007). Indeed, entire classes of sexual trait, such as primary and secondary genitalia, are predominantly hypoallometric (EBERHARD et al. 1998; EBERHARD 2009). This variation is not predicted by current theory, and accounting for it will be crucial for understanding the evolution and expression of sexual traits (BONDURIANSKY 2007; EBERHARD 2009).

Here we test two hypotheses that seek to explain variation in the allometry of sexual traits. The first hypothesis posits that allometry is influenced by the form of selection. Specifically, stabilizing selection should favor hypoallometry, since it reduces variation in the trait relative to body size; by contrast, directional selection may favor allometries ranging from hyperallometry to hypoallometry depending on whether the strength of selection varies with body size (HOUSE & SIMMONS 2003; EBERHARD et al. 2009). We test this hypothesis with a suite of traits describing variation in the male advertisement signals of our study species (see below). Although we lack data on size-dependency of the strength of selection, we have detailed information about the form of selection on signals (see below).

In spite of much interest in whether signal variation is associated with body size (reviews: GERHARDT & HUBER 2002; GREENFIELD 2002), research has lacked an allometric framework. We suggest that an allometric approach will bring new insights on the evolution of behavior and allometry. It is important to recognize that, unlike morphological traits, behavioral traits do not have a size – except perhaps with reference to signal amplitude, but we did not measure this trait because of its variability with distance from the source, and because it is highly plastic in our study species (see below). We are interested in an allometric approach to the study of behavior and signal evolution because it allows assessing the causes of variation in trait variability. For example, it allows partitioning variation in the coefficient of variation (CV) between the steepness of the allometric function and its dispersion (EBERHARD et al. 1998; CUERO & MØLLER 2001). This permits asking novel questions about signal evolution. For example, signal traits under stabilizing selection have lower CVs than traits under directional selection (RODRÍGUEZ et al. 2006), and we can ask whether these differences in CV evolve through the steepness or the dispersion of the allometric function (see below).

The second hypothesis we test deals with widespread hypoallometry in animal genitalia (EBERHARD et al. 1998; EBERHARD 2009). This pattern may be due either to the naturally selected functions of genitalia as sperm transfer devices, or to their sexually selected functions in sperm competition and cryptic female choice (EBERHARD 1985, 2009, 2010; HOUSE & SIMMONS 2003; HOSKEN & STOCKLEY 2004). We test the hypothesis that the naturally selected functions of genitalia account for their hypoallometry. We take advantage of evidence indicating that male genitalia in our study group evolve only under natural selection (see below) to test the prediction that they will also exhibit hypoallometry. By contrast, if sexually selected functions account for hypoallometry, we should find that genitalia allometry in our study species is at least as steep as that of body traits.

The above hypotheses are mainly concerned with variation in the steepness of the allometric function, but we were also interested in the dispersion around it. Both parameters bear on the potential for sexual traits to serve as indicators of body size and condition (HUNT et al. 2004; HILL 2011). Both shallow scaling and high dispersion can limit this potential; e.g., genitalia, with their predominantly shallow allometries,
are unlikely to be selected as body size indicators (EBERHARD et al. 1998, 2009). Here we explore the relationship between the steepness of trait allometry and the dispersion around the allometric function.

The evolutionary history of our study species, a member of the Enchenopa binotata complex (Hemiptera Membracidae), greatly facilitated our goals. These insects have rapidly diverging vibrational advertisement signals that evolve under sexual selection arising from well-characterized mate preferences (RODRÍGUEZ et al. 2004, 2006; COCROFT et al. 2010). Comparison with a competing source of selection – natural selection arising from transmission along plant substrates – indicates that sexual selection is stronger (MCNETT & COCROFT 2008; SULLIVAN-BECKERS & COCROFT 2010). By contrast, male genitalia in the complex appear to evolve only under natural selection: females are monogamous; males cannot force copulations (WOOD 1993; SULLIVAN-BECKERS & COCROFT 2010); and – as predicted by sexual selection theory from female monogamy (EBERHARD 1985, 2010) – there has been no recognizable divergence in male genitalia among the members of the complex, in spite of rapid divergence in ecology and behavior (PRATT & WOOD 1993; WOOD 1993; COCROFT et al. 2008, 2010).

METHODS

We used the member of the E. binotata complex that lives on Ptelea trifoliata (Rutaceae) host plants in Missouri, USA. Like most members of the complex, this species awaits formal description, and we refer to it by the name of its host plant, thus: E. binotata 'Ptelea'. We assessed the allometry of n = 7 signal traits, 3 body traits, and 7 genitalia traits (Fig. 1). Details on laser vibrometry for recording of vibrational signals and on signal analysis are provided in RODRÍGUEZ et al. (2008). Details on the body and genitalia measurements are provided in AL-WATHIQUI & RODRÍGUEZ (2011) and RODRÍGUEZ & AL-WATHIQUI (2011, 2012).

Data were derived from a prior study on genetic variation and plasticity which involved rearing 19 full-sib families on native and novel host plants (RODRÍGUEZ et al. 2008; RODRÍGUEZ & AL-WATHIQUI 2011, 2012). Here we use the data for individuals reared on the native host, which is the relevant environment for the evolution of allometry. However, we note that developing on native vs novel host plants had little or no effect on overall phenotypes and allometry (RODRÍGUEZ et al. 2008; RODRÍGUEZ & AL-WATHIQUI 2011, 2012). We obtained overall estimates of trait allometry by pooling data across the 19 families. Sample sizes varied among traits because we were not always able to obtain all measurements for all individuals. Mean sample size was n = 161 males (range = 149–170).

Statistical analysis

The steepness of allometric scaling is described by the slope (b) of log-log regressions of trait size on body size. Thus, b > 1 corresponds to hyperallometry; b ≈ 1 corresponds to isometry; and b < 1 corresponds to hypoallometry. In addition to this framework, we also compare the relative magnitude of b among traits (EBERHARD et al. 2009). This approach focuses attention on effect sizes (i.e., the relative magnitude of b; NAKAGAWA & CUTHILL 2007), and it allows using different trait types as controls for one another. Thus, we explore and compare the allometry of behavior, body and genitalia traits.

Methods for assessing b in studies of allometry are controversial, and there are no clear guidelines (SOKAL & ROHLF 1995). Compare, e.g., the different recommendations offered by SOKAL & ROHLF (1995); FULLER (2006); WARTON et al. (2006); BONDURIANSKY (2007); and SMITH (2009). The main concern about OLS (Ordinary Least Squares) regression arises from the expectation that b estimates may be biased downwards by measurement error in x (MCARDLE 1988;
Fig. 1. — Traits used to study allometry in *E. binotata* ‘Ptelea’. Each number indicates a different trait. Body: (1) we used body length (i.e., pronotum length) as the x-axis to describe all allometric scalings; (2) “face” length; (3) length of the left femur III; (4) width of the left tibia II. Genitalia: (5)–(8) pertain to the aedeagus; (9) and (10) pertain to the style; (11) length of the subgenital plate. Terminology follows PRATT & WOOD (1993). Signals: (12) a signal bout produced by a male. Each signal is composed of a whine (14) followed by pulses (15), both consisting of a pure tone that slightly drops in frequency from beginning to end. We measured the following signal traits: signals/bout, i.e., number of signals in (12); signal interval (13); whine length (14); number of pulses/signal; pulse rate (= pulses/length of trait 15); pulse length (16); and frequency at the highest-amplitude section at the end of the whine (= 10 cycles/length of trait 17). Figure modified from RODRÍGUEZ & AL-WATHIQUI (2011, 2012).

SOKAL & ROHLF 1995; GREEN 1999). However, alternative model II regressions confound steepness with dispersion (SOKAL & ROHLF 1995; EBERHARD et al. 1999). Further, measurement error in x does not by itself invalidate OLS regression (FULLER 2006; WARTON et al. 2006). OLS slopes are lower than model II regression slopes by definition (EBERHARD et al. 1999; WARTON et al. 2006; SMITH 2009), but it follows that the latter may at least sometimes be overestimates – e.g., when the relative magnitude of measurement error in x and y leads to the expectation that OLS regression will perform better (MCARDLE 1988; BONDURANSKY 2007). In a separate study on *E. binotata* ‘Ptelea’ we show that OLS regression does not underestimate *b*, with specific reference to the effect of changes in the magnitude of the measurement error in x (AL-WATHIQUI & RODRÍGUEZ 2011). We therefore use OLS slopes as accurate descriptors of allometry. Further, we note that our focus on the relative magnitude of *b* means that even if estimates were biased our comparison of relative magnitudes is not compromised (EBERHARD et al. 1999, 2009).

We estimated *b* with OLS regressions on log-log transformed data, using body length (i.e., pronotum length) as the x-axis (Fig. 1). Body size is sexually dimorphic in the *E. binotata* complex, with females being larger than males. This likely represents fecundity selection on females (cf. FAIRBAIRN 2007), and thus our body measurements represent naturally selected traits. A concern here is whether our results may be an artefact of using the pronotum as body length; e.g., if the pronotum were hyperallometric, our estimates of *b* might be biased downwards. We assessed the potential for this problem by repeating our analyses with femur length (Fig. 1) as the x-axis. This showed that the pronotum was not hyperallometric (*b* = 0.73), so that our estimates are unlikely to be biased. Further, analyses with the femur or the pronotum as the x-axis yielded the same patterns (not shown), indicating that our results are robust to the body trait used as body size.

We estimated the dispersion around the allometric function with the Standard Error of the Estimate (SEE), which is the square root of the residual Mean Square of the regressions...
(EBERHARD et al. 1998; ZAR 1999: 334–335), thus: \( \text{SEE} = \sqrt{\text{MS}_{\text{residual}}} \). The SEE is scale-dependent, but since log-log transformation reduces this problem, it yields similar results as scale-independent measures (EBERHARD et al. 1998).

We compared \( b \) and SEE across trait types with 1-way ANOVAs. There may be an important difference in how \( b \) should be interpreted for different trait types. For behavior, but not morphology, large negative \( b \) values may indicate steep negative scaling rather than shallow scaling; e.g., signal frequency may decrease markedly with body size (GERHARDT & HUBER 2002; GREENFIELD 2002). We thus used \( b \) and its absolute value (\(|b|\)) in comparisons involving signal traits. Data for signal trait allometries tended to show heteroscedasticity, but we had limited power to detect it with \( n = 17 \) traits. Consequently, we used Welch ANOVAs allowing for heteroscedasticity when visual inspection of the data suggested the possibility of heteroscedasticity (see below). Heteroscedasticity may also obscure patterns in tests across all trait types. Consequently, we used separate tests to compare signals vs body traits, genitalia vs body traits, and signals vs genitalia. This repeated testing may increase the risk of spurious significance (RICE 1989). However, corrections for repeated testing reduce statistical power (NAKAGAWA 2004), which is of special concern for us because of our relatively small sample size (i.e., \( n = 17 \) traits). We thus used the sequential Bonferroni correction (RICE 1989; e.g., the comparison of \( b \) for signals vs body, genitalia vs body, signals vs genitalia involved correcting for three tests), but we tempered it with consideration of the magnitude of the \( F \)-ratios. Our purpose was to avoid spurious results whilst emphasizing consideration of effect size and statistical power (NAKAGAWA & CUTHILL 2007).

We assessed the relationship between \( b \) and SEE with Pearson product-moment correlation coefficients (\( r \)). Because significance tests for these tests have low power with \( n = 17 \) traits, we focus on the magnitude of \( r \), which corresponds to the effect size (NAKAGAWA & CUTHILL 2007). We conducted all statistical analyses with JMP 7.0.1 (SAS Institute, NC).

**RESULTS**

There was substantial variation between traits in \( b \) and SEE, but we did not find any trait with hyperallometry (Fig. 2). Signals varied the most in \( b \) and SEE (Fig. 3), and one signal trait (signals/bout) had the steepest allometry of all the traits examined (\( b = 0.95 \); Figs 2, 3A). However, signals and genitalia tended to have shallower \( b \)s than body traits (Table 1; Fig. 3A). After the sequential Bonferroni correction, this difference remained significant only for genitalia vs body traits, but the large magnitude of the \( F \)-ratio for the difference for signal vs genitalia traits points to a biologically meaningful difference (Table 1). By contrast, signals and genitalia had similarly shallow \( b \)s (Table 1; Fig. 3A). We found a similar pattern for \(|b|\); signals and genitalia also had shallower \(|b|\)s than body traits (Table 1; Fig. 3B). After the sequential Bonferroni correction, this difference was significant only for genitalia vs body traits (Table 1). However, although the \( F \)-ratio for the difference for signal vs genitalia traits was smaller than for \( b \), it was also indicative of a biologically meaningful difference (Table 1). Signals and genitalia had similarly low \(|b|\)s (Table 1; Fig. 3B). In short, signal traits had variable but often shallow allometries, and genitalia had consistently shallow allometries.

Signals and genitalia had greater and more variable SEEs than body traits (Table 1; Fig. 3C). These comparisons are non-significant after the sequential Bonferroni correction, but their large \( F \)-ratios point to important differences (Table 1). Signals also had greater and more variable SEEs than genitalia, but this difference was non-significant (Table 1; Fig. 3C).

There was a positive relationship between \(|b|\) and SEE, and it was considerably stronger for signals (Fig. 4). This was not an artefact of an outlier signal trait (signals/bout; Fig. 1): excluding the outlier reduced the correlation from \( r = 0.90 \) to \( r = 0.63 \), but the latter value is still three times greater than correlations for body and genitalia (Fig. 4).
Fig. 2. — Examples of variation in trait allometry in *E. binotata* 'Ptelea'. Each column shows two examples per trait type. The x-axis is identical in all panels. The y-axis corresponds to a range of 0.2 log units in all panels except for the top right panel, where it is 6 times broader in order to accommodate this trait’s much larger dispersion around the allometric slope. For this trait, the change in elevation from the beginning to the end of the trend line corresponds to the y-axis in the other panels.

Fig. 3. — Allometry of male body, genitalia and signal traits in *E. binotata* 'Ptelea'. In each panel, each symbol corresponds to one trait. A: Allometric slopes (*b*). B: Absolute value of the allometric slopes (*|b|*). C: Dispersion around the allometric function (SEE).

**Allometry of behavior and the form-of-selection hypothesis**

We conducted a preliminary test of the hypothesis that the form of selection can explain variation in allometry. We based this test on prior work on *E. binotata*.
Table 1.
Comparison of the allometric slope ($b$ and $|b|$) and dispersion around the allometric function (SEE) between traits describing the advertisement signals, body, and genitalia of *Enchenopa binotata* ‘Ptelea’ treehoppers. We report results for 1-way ANOVA comparisons, or Welch ANOVAs when there was heteroscedasticity. We give uncorrected $P$-values, and indicate in boldface tests that remain significant after the sequential Bonferroni correction (performed across repeated tests with the same data; i.e., across each row).

| Allometric parameter | Trait type comparison | $b$ | | $|b|$ | |
|----------------------|----------------------|-----|-----|-----|
|                      |                      | Welch ANOVA | $F_{1, 8} = 12.81$, $P = 0.0072$ | Welch ANOVA | $F_{1, 6.89} = 0.92$, $P = 0.37$ |
|                      |                      | $F_{1, 6.75} = 6.63$, $P = 0.038$ | | |
|                      |                      | Welch ANOVA | $F_{1, 8} = 13.71$, $P = 0.006$ | Welch ANOVA | $F_{1, 7.39} = 0.19$, $P = 0.67$ |
|                      |                      | $F_{1, 4.71} = 3.40$, $P = 0.13$ | | |
|                      |                      | Welch ANOVA | $F_{1, 6.02} = 9.34$, $P = 0.022$ | Welch ANOVA | $F_{1, 6.32} = 7.12$, $P = 0.035$ |
|                      |                      | | | |

Fig. 4. — Relationship between the absolute value of the allometric slope ($|b|$) and the dispersion around the allometric function (SEE) in male body, genitalia and signals in *E. binotata* ‘Ptelea’. Each type of symbol corresponds to one trait type. The solid line indicates the relationship for all signal traits; the dotted line indicates this relationship after excluding the outlier point (signals/bout; see Fig. 1).

‘Ptelea’. In this species, there is stabilizing sexual selection on two signal traits and directional sexual selection on four other traits (Rodríguez et al. 2006; Sullivan-Beckers & Cocroft 2010). We were thus able to test the prediction that signal traits under stabilizing selection would have lower $|b|$'s than signal traits under directional selection. We found the predicted difference (Fig. 5A), but it was not significant (Welch ANOVA: $F_{1, 3.21} = 2.81$, $P = 0.19$). Signal traits under stabilizing selection also had (non-significantly) lower SEEs (Welch ANOVA: $F_{1, 3.67} = 1.81$, $P = 0.26$; Fig. 5B).
Fig. 5. — Allometry of signal traits subject to directional or stabilizing selection in *E. binotata* 'Ptelea'. A: Differences in the absolute value of the allometric slope (\(|b|\)). B: Differences in the dispersion around the allometric function (SEE).

Allometry of genitalia and the natural-selection hypothesis

We tested the hypothesis that the naturally selected functions of genitalia can account for their shallow allometry, as opposed to their sexually selected functions. We based this test on evidence suggesting that male genitalia in the *E. binotata* complex evolve only under natural selection (see Introduction). This hypothesis therefore predicts shallow bs for *E. binotata* 'Ptelea' male genitalia, both in terms of \(b < 1\), as well as of being shallower than for body traits measured in the same individuals. We found support for this prediction: *E. binotata* 'Ptelea' male genitalia had bs that ranged between −0.02 and 0.34, and which were significantly shallower than those of body traits (Table 1; Fig. 3A).

DISCUSSION

We used the allometry of sexually selected advertisement signals and naturally selected genitalia in *E. binotata* 'Ptelea' treehoppers to test two hypotheses seeking to explain variation in sexual allometry. We characterized the first hypothesis in simplistic terms as stating that stabilizing selection will result in shallower allometry than directional selection. We tested it with a suite of traits describing variation in vibrational advertisement signals. We found that signal traits under stabilizing selection had shallower allometries than signal traits under directional selection, but the difference was not significant and the test statistic was not very large (\(F = 2.81\)). Thus, even taking power into account (with \(n = 6\) traits), support for this hypothesis is weak. However, we emphasize that the predictions we derived are overly simplistic: stabilizing selection should result in shallow allometries, but directional selection can result in a variety of allometries depending on whether its strength varies with body size (see Fig. 2 in EBERHARD et al. 2009). Thus, some of the shallow bs found for signal traits under directional selection may be consistent with the hypothesis, but we lack the data to make this assessment. We thus tentatively interpret our findings as supporting the hypothesis that the form of selection can explain variation in allometry.

We also used the allometry of advertisement signals to ask how selection shapes the CV. One prediction of sexual selection theory is that traits subject to stabilizing selection should have lower CVs than traits subject to directional selection, and this
prediction is met in the *E. binotata* complex (Rodríguez et al. 2006). Here we asked if this outcome has come about through the evolution of the steepness of allometry, or rather through the dispersion around the allometric function. We found weak evidence for both pathways, with a suggestion that the effect of steepness may be stronger.

The second hypothesis we tested focuses on the pattern of widespread hypoallometry in animal genitalia. We asked if this pattern may be due to the naturally selected functions of genitalia, as opposed to their sexually selected functions (House & Simmons 2003; Eberhard 2009). We find support for this hypothesis, since the naturally selected male genitalia of *E. binotata* 'Ptelea' fit the overall pattern of hypoallometry. We suggest that this may explain why the pattern of hypoallometry in genitalia is so widespread: genitalia may or may not be sexually selected, but they always have to fulfill their naturally selected functions.

We were also interested in relating variation in $b$ to variation in dispersion, with emphasis on the potential of traits as indicators of body size/condition. Signals and genitalia seem to have limited potential in this sense, but for different reasons: *E. binotata* 'Ptelea' male genitalia have shallow allometries, and are thus poor indicators of body size, as is the case across animals (Eberhard et al. 1998; Eberhard 2009). Signals, by contrast, varied markedly in $b$, but showed a strong relationship between $b$ and dispersion. Thus, traits that might be selected as body size indicators because of steep $b$s also had greater dispersion around the allometric function, which would make them unreliable as indicators.

Our sample of signal traits included the two most divergent adult traits among the members of the *E. binotata* complex (signal frequency and whine length; Fig. 1; Rodríguez et al. 2006; Crocroft et al. 2010). These two signal traits are under stabilizing sexual selection, and they showed some of the shallowest $b$s we measured ($b = 0.05$ and $−0.12$, respectively). Thus the allometry of advertisement signals leads to a similar problem as the allometry of animal genitalia: the challenge of explaining rapid divergence in traits that experience stabilizing selection and that scale poorly on body size.

We suggest that comparisons of behavioral and morphological traits may be very useful in testing hypotheses about sexual selection and the evolution of allometry. Some behavioral and morphological traits may evolve under similar forms of selection that nevertheless differ in their cause. For instance, some signal and genitalia traits may be subject to the same form of selection – e.g., stabilizing sexual selection due to mate choice – that may nevertheless vary considerably in how it comes to act on behavior or morphology – e.g., through very different sensory pathways for the perception of signals and genitalia. This combination of broad similarity and variety in detail may allow for robust tests of general hypotheses.

ACKNOWLEDGEMENTS

We thank Gerlinde Höbel and four anonymous reviewers for very helpful comments and discussion. This work was funded by NSF Grant IOS-0919962 to R.L. Rodríguez, and by UWM SURF awards to N. Al-Wathiqi and R.L. Rodríguez.

REFERENCES


