

SHORT COMMUNICATION

Allometry and sexually dimorphic traits in male anurans

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*Department of Biology, Laurentian University, Sudbury, ON, Canada***Keywords:**

American toad;
bull frog;
green frog;
morphology;
sexual dimorphism;
sexual selection.

Abstract

Allometry of secondary sexual traits has been the subject of recent debate, and the generality of positive allometry and its association with sexual selection have been recently questioned. Whereas some studies suggest an almost universal positive allometry for traits under sexual selection and isometry or a negative allometry for traits not under such pressure, other studies argue that this pattern results from the study of exaggerated (ornamental) traits. To answer the call for an examination of the allometry of less-exaggerated sexually selected traits, we have examined morphological data from 14 sexually dimorphic traits and six monomorphic traits from three anuran species. Although we found evidence of positive allometry in male secondary sexual traits of several species and populations, not all nonsexual traits were isometric or exhibited negative allometry. Furthermore, our results indicate that larger traits in the populations that we studied were not associated with greater allometric slopes. Therefore, our study is in line with the contention suggesting no specific kind of allometric pattern for sexual and nonsexual characters, and we can only advocate for further investigation of trait allometry and sexual selection to understand the complexity underlying the evolution of allometry in sexual traits.

Introduction

Sexual selection is a powerful force influencing the evolution of morphological traits (Andersson, 1994). It has been proposed that secondary sexual traits such as enlarged traits used as ornaments for attracting mates or as weapons for male–male competition exhibit positive allometry – that is, large individuals have disproportionately larger traits relative to small individuals (e.g. Green, 1992; Petrie, 1992; Kodric-Brown *et al.*, 2006). This pattern of allometry is thought to be the result of intense directional selection whereby secondary sexual traits are expected to enhance mating success (either by attracting mates or fending off rivals). Individuals (usually males) that are large have thus more resources to allocate to increasing trait size, which leads to large males having disproportionately larger traits than smaller males

(Green, 1992). Alternatively, because an increased trait size is directly advantageous for mating success, there is a stronger emphasis on trait growth than on an increase in body size (Bonduriansky & Day, 2003).

Recently, the generality of positive allometry and its association with sexual selection have been questioned. Bonduriansky & Day (2003) concluded that positive allometry should only evolve when the combined effects of sexual and viability selection on trait size and body size result in increased relative fitness for larger individuals. Bonduriansky (2007) argued that the sexually selected traits that have been empirically examined and whose results have led to the conclusions regarding positive allometry are not an unbiased sample. In other words, researchers have focussed on the most exaggerated or ‘bizarre’ traits to investigate allometric relationships, and thus the positive allometry found in published studies reflects allometry of highly exaggerated traits, not necessarily of traits under sexual selection in general (Bonduriansky, 2007). To empirically assess the association between sexual selection and allometry requires the unbiased selection of traits, and a comparison of these

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sexually selected traits to allometric slopes derived from nonsexual traits (Bonduriansky, 2007). For example, Cuervo & Møller (2009) examined dimorphic feather ornaments in 67 species of birds, using both sexual and nonsexual traits, and concluded that no specific allometric pattern was associated with sexually selected feather ornaments.

Anurans (frogs and toads) are an excellent group of animals to test some of the ideas presented by Bonduriansky (2007). Although anurans generally lack exaggerated ornaments, they do have some traits that are sexually dimorphic and related to mating success (Monnet & Cherry, 2002). For example, the forelimbs and thumb pads of many male anurans present a seasonal enlargement that plays a role in grasping the female during oviposition (amplexus) (e.g. Lee, 2001). The tympanum is also larger in males and may play a role in male–male competition because it is particularly sensitive to sound frequencies produced by other male anurans (Hetherington, 1994), thus allowing individuals to determine the location of male rivals (Bosch & Marquez, 2000). Perhaps most importantly, the tympanum may be involved in sound transmission of calling sounds, and males with large tympana may be able to broadcast more powerful calls than males with small tympana (Purgue, 1997).

Here, we examine morphological data from sexually dimorphic traits from some frog species to answer Bonduriansky's (2007) call for an examination of the allometry of less-exaggerated, sexually selected traits. Where possible, we compare the allometric slopes of sexual and nonsexual traits, and test the prediction that positive allometry is enhanced in secondary sexual traits.

Methods

Frogs and toads were collected for a variety of studies between 2004 and 2006. Morphological measurements were similarly taken for each of these projects, and these data were analysed with respect to allometry. Here, we assess allometric slopes of morphological traits from three populations of green frogs (*Rana clamitans*), a single population of bullfrogs (*Rana catesbeiana*) and a single population of American toads (*Bufo americanus*). In all cases, we restrict our analysis to males because our sample size of females was insufficient to assess allometric relationships.

Green frog sample 1

We sampled male green frogs from five ponds in and around the campus of Laurentian University, Sudbury, Ontario, early in the breeding season (late May to mid June) of 2004 (Schulte-Hostedde & Schank, 2009). Frogs were captured from dusk into the night (the active period for male green frogs) by hand using smelt nets. All males were calling when sampled.

Upon capture, green frogs were sexed (males are smaller than females with a tympanum at least as large as the eye (Zug *et al.*, 2001)), and a number of measurements taken. Snout–vent length (SVL; an estimator of body size) was measured using callipers (± 0.01 mm). Both the right and left side of the following traits were also measured – tympanum diameter, the length and maximum width of the forearm and nuptial thumb pad, and total thumb length. The vertical diameter of the tympanum was measured for all frogs. Forearm length was measured from the distal end of the radio-ulna (interface of the radio-ulna and the carpals) to the proximal end of the humerus (at the interface between humerus and clavicle and scapula). Thumb length was measured from the base of the thumb to the tip.

Green frog sample 2 and Bullfrog

Both species were collected by hand, using fishing nets at four different sites within a 100 km radius of Ottawa, Ontario. Sampling occurred between May and July 2006 usually from the late afternoon or at dusk until midnight. Upon capture, frogs were sexed based on colouration of the abdomen and the size of the tympanum (see above; Zug *et al.*, 2001) and SVL was measured. Several morphological measurements were also taken for the left and right sides of each frog, including the length of the hindlimb and the width of the front pad, a proxy for surface area. All morphological measurements were taken with a pair of digital calipers (± 0.01 mm).

Green frog sample 3

We sampled male green frogs from one population on the Collège Boréal campus, Sudbury, Ontario, in June 2005. Each individual was caught separately with disposable gloves, and several traits were measured for each individual using callipers (± 0.01 mm): SVL, the length of tibio-fibula (tibia), the length of foot that consists of the calcaneum to end of phalanges, the length of femur, the length of the forelimb that consists of the radio-ulna to tip of phalanges, the length of the thumb, the horizontal length of the tympanum and the vertical length of the tympanum. Each trait was measured and recorded three times on each side, and the mean of these three measurements was used in all analyses.

American toad

All of the individual toads were collected from the Ponderosa Wetlands in Sudbury Ontario in early May 2006. The wetland was sampled eight times during this period. The toads were identified by call with collection starting at 9:00 pm (dusk) and continuing until all visible amplexing pairs and all calling males had been captured by hand or by net, with the search effort lasting for 2.5–3 h each night. For each individual, SVL was measured

using callipers (± 0.01 mm) as well as forearm length, thumb length and tympanum diameter. Individuals were toe-clipped to avoid measuring the same individual more than once. We were unable to find any evidence that the tympanum of the American toad is sexually dimorphic, and thus in the interests of being conservative, we consider the tympanum as a monomorphic trait in this study.

Statistical analysis

Allometric slopes were determined for all measured traits for all anuran populations. \log_{10} -transformed measurements of these traits were plotted against the \log_{10} -transformed snout-vent measurements. Ordinary least squares (OLS) regression has been widely used for regression analysis, but has several assumptions associated with it that make OLS regression unsound for allometric investigations. OLS requires that the X (independent variable) and Y (dependent variable) are not interdependent and that there is no error in X , because X is set by the researcher, whereas reduced major axis (RMA) regression assumes some error in X (LaBarbera, 1989; Legendre & Legendre, 1998; Schulte-Hostedde *et al.*, 2005). Because the measurement of body length

was made with possible error, we used RMA (model II) regression to determine whether the slopes of the various measured rapid traits differed from isometry. For comparison, we also report OLS regression slope estimates. A Microsoft Excel spreadsheet written by D. Warton (available at <http://web.maths.unsw.edu.au/~dwardon/model2CI.xls>) was used to determine whether the RMA slopes were significantly different from isometry (slope = 1). All other statistical analyses were performed using STATISTICA 6.1 (StatSoft, Inc., Tulsa, OK, USA).

Bonduriansky's (2007) assertion of bias in the selection traits implies that trait allometry will be correlated with relative trait size. As in Cuervo & Møller (2009), we estimated relative trait size by subtracting \log_{10} -transformed trait size from \log_{10} -transformed SVL. Where sample size (the number of traits measured) was adequate within a sampled population, we conducted a correlation between mean relative trait size and allometric slope (calculated via RMA regression).

Results

Ordinary least squares and RMA slope estimates were similar (see Table 1) although OLS slopes estimates were shallower than RMA slopes. The only series of slope

Table 1 Coefficient of determination (r^2), OLS slope, RMA slope, 95% confidence intervals (CI), F -value and probability that the RMA slope is significantly different from isometry (slope = 1) for morphological traits measured for males from three green frog populations, single bullfrog and American toad populations. In bold are statistically significant P -values. All OLS slopes were significantly different from 0 ($P < 0.05$).

	r^2	OLS slope	RMA slope	CI	F	P
Green Frog 1 ($n = 30$)						
Tympanum diameter*	0.667	1.001	1.225	0.982–1.529	3.53	0.071
Forelimb width*	0.676	1.092	1.328	1.067–1.652	7.13	0.012
Forelimb length*	0.400	0.694	1.098	0.817–1.476	0.41	0.527
Thumb pad width*	0.776	1.178	1.337	1.114–1.603	10.84	0.003
Thumb pad length*	0.479	0.909	1.314	0.997–1.731	4.11	0.052
Total thumb length*	0.416	0.831	1.288	0.962–1.724	3.14	0.087
Green Frog 2 ($n = 19$)						
R thumb pad width*	0.514	1.193	1.664	1.173–2.361	9.89	0.006
R leg length	0.378	0.605	0.983	0.663–1.457	0.01	0.93
Green Frog 3 ($n = 20$)						
R Tibia length	0.895	1.005	1.062	0.906–1.246	0.64	0.435
R Foot length	0.826	1.087	1.197	0.974–1.469	3.36	0.083
R Femur length	0.720	1.141	1.345	1.038–1.743	5.82	0.027
Forelimb length*	0.854	1.100	1.190	0.986–1.435	3.77	0.068
R Thumb*	0.712	1.157	1.370	1.054–1.782	6.42	0.021
R Vertical tympanum*	0.809	1.081	1.202	0.970–1.489	3.22	0.089
R Horizontal tympanum*	0.714	1.124	1.330	1.024–1.729	5.27	0.034
Bullfrog ($n = 12$)						
R thumb pad width*	0.889	1.595	1.691	1.340–2.234	27.3	< 0.001
R leg length	0.501	0.761	1.076	0.666–1.737	0.11	0.75
American Toad ($n = 33$)						
Thumb length*	0.365	0.802	1.328	1.000–1.763	4.17	0.049
Forelimb length*	0.565	0.789	0.873	0.646–1.180	0.82	0.371
Tympanum diameter	0.388	0.896	1.432	1.089–1.900	7.22	0.011

OLS, ordinary least squares; RMA, reduced major axis.

All traits marked * are sexually dimorphic (i.e. larger in males than females).

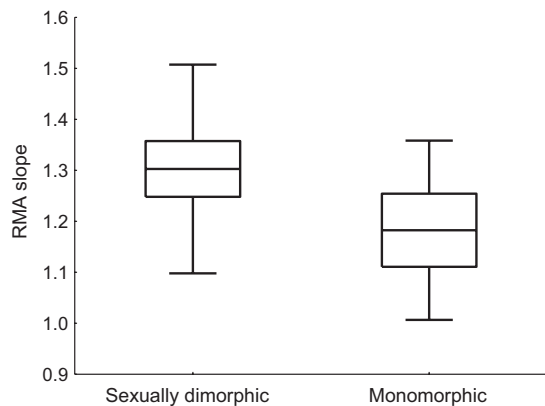


Fig. 1 Plot of mean, standard error (box) and standard deviation (whiskers) of RMA slopes of sexually dimorphic and monomorphic traits for all populations of anurans examined. There was no significant difference between the two groups of traits ($t_{18} = 1.25$, $P = 0.23$).

estimates in which OLS slopes were clearly different from RMA slopes was in the American toad, where relatively low r values led to OLS slope estimates being < 1 when compared to RMA slopes that were significantly > 1 (Table 1). Among the three green frog populations and one population each of bullfrogs and American toads, we measured a total of 20 traits, 14 of which were dimorphic traits (Table 1) – that is, these traits are larger in males than females. All allometric slopes were significantly different from 0 ($P < 0.05$) and were in the positive direction. Of the six monomorphic traits, only two of six (33.3%) traits showed evidence of positive allometry. Among the dimorphic traits, seven of 14 (50%) dimorphic traits showed positive allometry.

A further seven traits (five dimorphic and two monomorphic) showed a nonsignificant trend ($0.1 < P < 0.05$) towards a positive allometric slope that was different from 1. Inclusion of these results into the above comparisons indicated that 12 of 14 dimorphic traits (85.7%) showed evidence of positive allometry, whereas four of six monomorphic traits (66.6%) showed evidence of positive allometry. Finally, we compared the mean of the allometric slopes of sexually dimorphic and monomorphic traits. No significant difference was detected for OLS slopes or RMA slopes [OLS slopes – $t_{18} = 1.13$, $P = 0.27$; RMA slopes – $t_{18} = 1.25$, $P = 0.23$ (Fig. 1)].

We did note homogeneity in the allometric slopes of secondary sexual traits among green frog populations. For example, in the two populations of green frogs for which thumb and tympanum measurements were available (green frogs 1 and 3), positive allometry was evident.

There was no evidence that larger traits had more positive allometric slopes. In the two populations for which an adequate number of traits were measured (Fig. 2), the allometric slopes of the traits are negatively

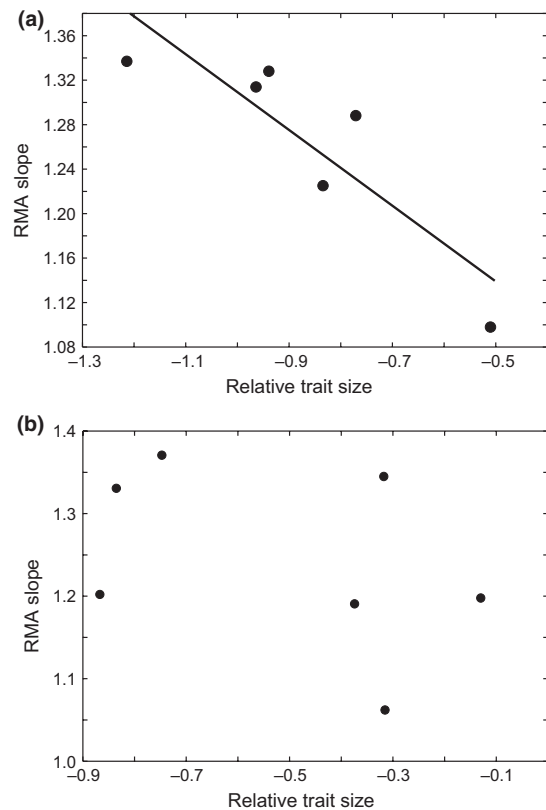


Fig. 2 Plot of relative trait size and allometric slope (RMA regression) for two populations of green frogs. In green frog sample 1 (a), the allometric slopes of the traits are negatively correlated with relative trait size ($r = -0.874$, $P = 0.023$, $n = 6$), whereas in green frog sample 3 (b), no relationship exists between allometric slope and relative trait size ($r = -0.427$, $P = 0.34$, $n = 7$).

correlated with relative trait size in 'Green frog sample 1' ($r = -0.874$, $P = 0.023$, $n = 6$), whereas in 'Green frog sample 3', no relationship exists between allometric slope and relative trait size ($r = -0.427$, $P = 0.34$, $n = 7$).

Discussion

It has been argued that traits under sexual selection exhibit positive allometry whenever the benefit of an enlarged trait increases at a greater rate than the benefit of a large body (Green, 1992; Petrie, 1992; Kodric-Brown *et al.*, 2006) but Bonduriansky & Day (2003) suggested that positive allometry should only evolve when the combined effects of sexual and viability selection on traits size and body size results in increased relative fitness for larger individuals. Bonduriansky (2007) argued that the general observation of positive allometry among secondary sexual traits may result from a bias in the selection of traits for analysis. Large, exaggerated traits tend to be the subject of study, and thus the majority of evidence consistent with positive allometry of secondary sexual

traits is only applicable in this context. We have tried to answer this criticism by examining multiple secondary sexual traits in a group of species that generally lack such exaggerated traits. Indeed, although sexually dimorphic traits do exist among anurans, the species we examined do not have the types of extreme dimorphism evident in other groups (e.g. birds, mammals, insects) that Bonduriansky (2007) describes as being responsible for bias in allometric analyses. Our results indicate that larger traits in the populations that we studied were not associated with greater allometric slopes. Indeed, in one population of green frogs there was a negative association between relative trait size and allometric slope – thus, the increase in size was less pronounced in larger traits relative to smaller traits. The reason for this pattern is unclear, but it may be related to the fitness payoff associated with trait size. For example, the relative benefit of developing an enlarged trait may be tempered by the relative cost of that trait. Large and exaggerated traits may aid in the acquisition of mates, but the cost of maintaining a large trait may be high. Smaller, alternative traits that also enhance mating success but carry lesser costs may be a more economical alternative in terms of supporting trait development.

In our study, we found evidence of positive allometry in male secondary sexual traits of several species and populations of anurans. Most secondary traits exhibited positive allometry but not all nonsexual traits were isometric or exhibited negative allometry. Thus, we cannot conclude that positive allometry is the exclusive purview of sexual traits nor that nonsexual traits are exclusively isometric or negatively allometric. Indeed, the average allometric slope did not differ between sexually dimorphic and monomorphic traits. These results have two consequences. First, our data do support the observation that secondary sexual traits exhibit positive allometry with 12 of 14 traits showing at least a trend towards positive allometry. Second, our results uphold the complexity surrounding the issue of allometry of traits under sexual selection with three of six monomorphic traits showing evidence of positive allometry. Therefore, it appears that although most traits under sexual selection show positive allometry, not all traits exhibiting positive allometry are under sexual selection. The complexity underlying the evolution of allometry in sexual traits outlined by Bonduriansky & Day (2003) and Bonduriansky (2007) is thus upheld, and we can only conclude that further examination of this issue is warranted.

The cause of sexual size dimorphism in anurans and the implications for allometry have been debated (Monnet & Cherry, 2002; Sagvik & Uller, 2008) and two main hypotheses have been presented. In anurans, thyroid hormones controlling the metamorphosis are the same controlling hindlimb development, and it is possible that a prolonged exposure to these hormones in the late metamorphosing tadpoles could be responsible for positive allometry (Emerson, 1978). Additionally, most

of the variation in size dimorphism in anurans can be explained in terms of differences in the age structure between the sexes in breeding populations (Miaud *et al.*, 1999; Monnet & Cherry, 2002) whereby females and males reached sexual maturity at different age and size. Therefore, if sexual selection has an effect on size dimorphism in anurans, it is likely to be only a secondary one.

In conclusion, the results of this study indicate that further investigation of trait allometry and sexual selection in anurans is warranted. Our sample of species is relatively limited ($n = 3$), and they all belong to the Neobatrachia, suggesting that a broader analysis of trait allometry across anurans would be informative (e.g. Cuervo & Møller, 2009). In addition, to understand the evolution of trait allometry, it is important to understand the fitness consequences of trait size to determine the direction and magnitude of selection. An extensive study of reproductive success and survival of a marked population may be helpful in this regard.

Acknowledgments

This study has grown from the undergraduate theses of CS, SK, CM and the M.Sc. work of Val. St-Amour. We thank the various landowners for access to their property. Michel Laurin and an anonymous reviewer provided helpful comments on a previous version of this article. Funding for this project was received from the Natural Sciences and Engineering Council (NSERC) of Canada (ASH, DL), Laurentian University, and the Canadian Fund for Innovation/Ontario Innovation Trust (CFI/OIT to DL).

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Received 12 January 2011; revised 31 January 2011; accepted 3 February 2011