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Adult age confounds estimates of static allometric slopes in a vertebrate

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In many animal groups, the size of male genitalia scales shallowly with individual body size. This widespread pattern appears to admit some exceptions. For instance, steep allometries have been reported for vertebrate genitalia. This exception, however, may be due to a confounding effect arising from the continued growth of some structures during adulthood in vertebrates. Consider the possibility that genitalia continue to grow in adults while body size does not. If so, taking measurements from adults of different ages could yield steeper allometries than would be obtained from measurements of adults of the same age. We used vervet monkeys to test this hypothesis. We found that all body parts continued to grow in adult vervet monkeys, with sexual traits (including genitalia) showing faster growth rates. Traits with faster growth rates over adult ages had steeper allometries. And accounting for variation in adult age yielded shallower allometries, bringing vervet monkey genitalia in line with the predominant pattern observed in other animal groups. These results suggest that steep allometric slope estimates reported for other vertebrates may be due in part to mixing of adult ages, and reinforces one of the most consistent patterns yet detected in the study of static allometry.

KEY WORDS: genitalia, ontogeny, penis, primate, scaling relationships.

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INTRODUCTION

Sexual traits such as ornaments and weapons vary a great deal in how they scale on body size. The scaling of trait size with adult body size – static allometry – is described with the slope (b) of log–log regressions (HUXLEY 1932). Traits that scale in proportion to body size have allometric slopes of $b \approx 1$ and are said to show isometry. Traits that are disproportionately large in large individuals have $b > 1$ and exhibit positive allometry or hyperallometry. And traits that are disproportionately large in small individuals and disproprotionately small in large individuals have $b < 1$ and show negative allometry or hypoallometry. Allometric research shows that sexual traits show allometries that range from very steep ($b \gg 1$) to very shallow ($b \ll 1$) (EBERHARD et al. 1998; CUERVO & MØLLER 2001; BONDURIANSKY 2007; EBERHARD 2009; SCHULTE-HOSTEDDE et al. 2011). Against this backdrop of variation, a clear pattern is found in animal male genitalia: across various animal groups, genitalia predominantly have shallow allometries (EBERHARD et al. 1998; EBERHARD 2009). But there are exceptions even to this pattern, with vertebrate genitalia showing unusually steep allometries (reviewed in EBERHARD 2009).

This variation challenges students of allometry to answer three different questions: (i) What explains the huge range of variation in the allometry of sexual traits? (ii) What explains the predominantly shallow allometries exhibited by genitalia? (iii) What explains exceptions to the latter, as seen for instance in vertebrates?

Here we address the third question. We test the hypothesis that steep allometries in vertebrate genitalia are due to a confounding factor that biases allometric slope estimates upwards. Animals such as vertebrates may continue to grow after reaching sexual maturity – as opposed to most arthropods, for instance, that do not grow after the adult molt. In such cases, researchers may be at risk of mixing individuals of different adult or near-adult ages. This could confound static allometry with ontogenetic allometry and overestimate allometric slopes; for example, if genitalia reach full size at an intermediate adult age, they might appear to have a steeper allometry when assessed among adults of different ages than when assessed among adults of the same age. Indeed, in at least some of the studies on vertebrates in which adult age classes can be told apart, genitalic allometric slopes calculated for older males are shallower than slopes calculated for juveniles and adults combined (EBERHARD 2009).

If variation in adult age is a confounding factor in allometric studies, it would be because structures continue to grow in adults, and because different structures grow at different rates; for example, sexual traits may grow faster in adults than non-sexual traits. The first condition alone could force apparent isometry if trait scaling is assessed across adult ages. And both conditions together could force apparent positive allometry. Thus, the "age confound" hypothesis makes the following predictions: (i) there should be a positive relationship between growth rates and allometric slope estimates and (ii) allometric slope estimates calculated without taking variation in adult age into account should be steeper than estimates that account for such variation.

We tested this hypothesis with a study on morphological traits in adult male vervet monkeys, *Chlorocebus aethiops* (Primates Cercopithecidae). We first confirmed that the conditions required for adult age to confound allometry obtained in the vervet monkeys, and then we tested the two predictions. We found evidence that variation in adult age has a confounding effect on allometry, resulting in overestimation of the steepness of allometric slopes. Once adult age is taken into account, vervet monkey genitalia have a shallow allometry, matching the predominant pattern observed across other animal groups.

MATERIAL AND METHODS

We studied two vervet monkey subspecies: *Ch. a. sabaeus* at St. Kitts and Nevis ($n = 104$), and *Ch. a. pygerythrus* at South Africa ($n = 99$). For brevity, we indicate subspecies identity by site (St. Kitts and Nevis, South Africa). Data were taken by members of the International Vervet Research Consortium. Adult males were wild-caught following procedures described by GROBLER & TURNER (2010). Briefly, vervets were anesthetized while in traps, and measurements were taken from the anesthetized individuals. Vervets remained anesthetized for approx. 30 min, and were returned to the wild after data collection.

Researchers used measuring tape to measure 10 body traits (length of the body, head, upper arm, lower arm, upper leg [thigh], lower leg, sternal notch–pubic symphysis, and the girth of the chest, upper arm and thigh); and also three sexual traits (canine and penis length, and testes volume). Sample sizes vary between traits and subspecies because it was not always possible to take all measurements for all individuals. The measured vervets spanned three age categories of sexually mature adults, determined according to their patterns of dental eruption (CRAMER et al. 2013). These were age categories 6, 7 and 8 in International Vervet Research Consortium protocols, which correspond to sexually mature young adults, adults and older adults.

Estimating allometric slopes (b)

We estimated b with ordinary least squares (OLS) regression of trait size on a proxy for body size, with $\log_{10}$–$\log_{10}$ data. The proxy for body size (the x-axis in allometric functions) was the body measurement that we considered to have the best-defined landmarks, the length of the lower leg. We favored this measure over the whole-body measures (body length, sternal notch–pubic symphysis length) because we considered the latter to have less strictly defined landmarks. To estimate b for testes volume, we used the $\log_{10}$ of the cubic root of the volume measures. To prevent potential confounding effects due to among-measurer differences, we added measurer ID and its interaction with the x-axis as random covariates (restricted maximum likelihood method) to the standard least squares model that we used to estimate b.

There has been concern that OLS regression may bias estimates of b downwards by not taking into account measurement error in the x-axis (GREEN 1999; BONDURIANSKY 2007). However, two separate studies argue against this expectation and suggest that OLS regression does not bias b estimates downwards (AL-WATHIQUI & RODRÍGUEZ 2011; KILMER & RODRÍGUEZ unpublished). Further, the preferred alternative (reduced major axis (RMA) regression) confounds the steepness of allometric scaling with the amount of dispersion (EBERHARD et al. 1999; VOJE & HANSEN 2013; VOJE et al. 2014). Additionally, b estimates obtained with RMA regression may be biased towards 1 because they correspond to the ratio of the standard deviations of data whose variances have been homogenized by log transformation. Consequently, we favor OLS regression for allometric studies, emphasizing the importance of measurements based on well-defined landmarks.

Testing for the confounding effect of age

We first confirmed that structures continue to grow after sexual maturity in vervet monkeys, and that structures varied in growth rate. We described growth rates with the slope of OLS regressions of trait size ($\log_{10}$-transformed) on adult age (categories 6, 7 and 8). We compared growth rates between sexual and non-sexual traits with a statistical model that used the growth rate estimates (slopes) as the dependent variable, and included the following independent variables: trait type (sexual vs non-sexual), subspecies, and the trait type × subspecies interaction (model shown in Table 1).

We then tested the two predictions made by the age confound hypothesis. First, we assessed the relationship between variation in growth rates and b. We used a model with b estimates as the

Table 1.

Test for a difference in the growth rates of sexual and non-sexual traits in vervet monkeys, across adult age categories. Significant terms in boldface.

Factor	df num, denom	F, P
Trait type (sexual vs non-sexual)	1, 22	**49.8, < 0.0001**
Subspecies	1, 22	0.08, 0.78
Trait type × subspecies	1, 22	0.76, 0.39

Table 2.

Test for a relationship between growth rate across adult ages and estimates for the allometric slope (b) in vervet monkeys. Significant terms in boldface.

Factor	df num, denom	F, P
Growth rate	1, 18	**9.39, 0.0067**
Trait type (sexual vs non-sexual)	1, 18	3.20, 0.09
Subspecies	1, 18	2.34, 0.14
Growth rate × trait type*	1, 18	0.03, 0.86
Growth rate × subspecies	1, 18	3.55, 0.076

*Removing this interaction did not alter the results: growth rate remained significant ($P = 0.0034$); trait type and the growth rate × subspecies interaction remained marginal ($P = 0.078$ and 0.066, respectively).

Table 3.

Comparison of allometric slope (b) estimates for vervet monkeys, obtained with three different statistical methods – (i) without accounting for age; (ii) with age as a covariate; (iii) with males of only one age category (see text for method details). Significant terms in boldface.

Factor	df num, denom	F, P
Model (w/o age, w/age, only one age)	2, 63	**7.40, 0.0013**
Trait type (sexual, non-sexual)	1, 63	0.46, 0.50
Subspecies	1, 63	0.75, 0.39
Model × trait type	1, 63	2.26, 0.11
Model × subspecies*	2, 63	1.42, 0.25

*Removing this interaction did not alter the results: model remained significant ($P = 0.0014$).

dependent variable, and the following independent variables: growth rate, trait type (sexual, non-sexual), subspecies, and interactions between growth rate and the other terms (model shown in Table 2). Second, we compared b estimates obtained with three methods: (i) without taking adult age into account (i.e., with the above basic b-estimation model); (ii) adding adult age and its interaction with the x-axis as covariates to the model; and (iii) as in (i) but with males of only one age category (we used the category of adult males, age 7, which was the most numerous). In this test, we used b estimates as the dependent variable, and the following independent variables: method (i, ii or iii); trait type (sexual, non-sexual), subspecies, and the method × subspecies and method × trait type interactions (model shown in Table 3).

We also evaluated the above methods for dealing with the age confound problem. We used a conservative criterion. The current understanding is that evolutionary change in allometric slopes is likely to be slow (VOJE & HANSEN 2013; VOJE et al. 2014). We would thus favor a method yielding the least amount of divergence in allometry between the two vervet subspecies. We note, however, that this favors the most conservative method, and not necessarily the most accurate. We thus compared the magnitude of subspecies differences in b detected by methods i, ii and iii.

Our data in the above analyses consist of allometric slopes and growth rates obtained from traits that are correlated with each other (i.e., a suite of different body parts), introducing the risk of spurious significance. But methods such as using principal component analysis (PCA) to generate uncorrelated traits would draw away from our focus on traits of interest, such as the penis. And methods to reduce the risk of spurious significance, such as the sequential Bonferroni correction (RICE 1989), compromise statistical power (NAKAGAWA 2004). We consider, however, that the likelihood of spurious results is minor in our tests. This is because we focus on trait scalings on body size, and traits are more likely to be independent of each other in such scaling relationships.

We conducted all analyses in JMP (v. 7.0.1) (SAS Institute, Cary, NC, USA).

RESULTS

We found that all body parts continued to grow in adults. For example, Fig. 1A shows the results for the lower leg and the penis. Note that the rate of growth was faster for the penis than for the lower leg, a fact that is more striking when trait sizes are plotted on a log scale (Fig. 1B). More generally, sexual traits had mean growth rates at least twice as high as those of non-sexual traits (Table 1; Fig. 2A). These results fulfill the requirements for variation in adult age to have a confounding effect on the study of vervet monkey allometry.

We then assessed the relationship between growth rates and estimates of allometric slopes (b). We found a significant and positive relationship between the growth rate of each trait and b estimates obtained without accounting for age (i.e., the "apparent" b) (Table 2; Fig. 2B). This relationship seemed to vary in steepness between subspecies (marginally significant growth rate × subspecies interaction in Table 2) (Fig. 2B).

We then found that accounting for age lowered estimates of b for nearly all traits in both subspecies (Table 3; Fig. 3). This was the case comparing b estimates obtained without accounting for age (method i) vs estimates obtained with age as a covariate (method ii) ($F_{1,42} = 5.70$, $P = 0.022$) (Fig. 3). It was also the case in comparing b estimates obtained without accounting for age (method i) vs estimates obtained with males of only one age category (method iii) ($F_{1,42} = 13.53$, $P = 0.0007$) (Fig. 3). Notably, estimates of b for vervet monkey penes were lower when age was taken into account (methods ii or iii; Fig. 3), and matched the pattern observed across animal groups.

Finally, we compared the magnitude of subspecies differences in mean b. These differences were always non-significant (Table 4), but varied in effect size (NAKAGAWA & CUTHILL 2007) between methods. The method that returned the smallest differences was method (ii), which used age as a covariate when estimating allometric slopes (Table 4; Fig. 4).

DISCUSSION

Against the background of high variability of sexual allometries, shallow allometries for animal genitalia stand out as strikingly consistent – with the apparent
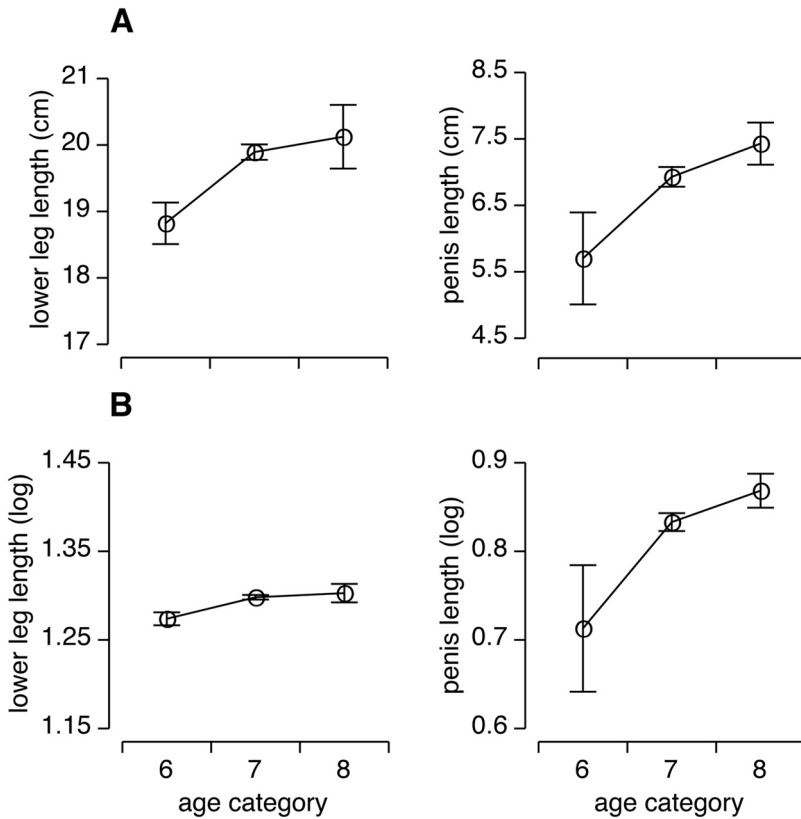
Fig. 1. — Example of trait differences in the rate of growth over adult ages in vervet monkeys. The categories are for sexually mature young adults, adults and older adults (numbers on the *x*-axes correspond to International Vervet Research Consortium protocols; see text). Note that the penis had a greater rate of growth than the lower leg. A: Growth in absolute units. Both *y*-axes span a range of 4 cm. B: Growth in relative ($\log_{10}$) units. Both *y*-axes span a range of 3 log units. Data from the South Africa subspecies.

exception of vertebrate genitalia (EBERHARD 2009). We tested the hypothesis that this exception is due to upward biasing of allometric slopes due to the confounding effect of adult age variation. We find strong support for this hypothesis. In vervet monkeys, all traits measured grew across adult stages; traits varied in growth rate, with sexual traits having the highest rates; there was a positive relationship between trait differences in growth rates and allometric slope estimates; and accounting for the effect of adult age lowered allometric slope estimates for nearly all traits, including the penis. These findings strongly suggest that mixing adult ages can introduce serious biases in allometric studies involving animals in which there is growth after reaching the adult stage.

Our test should be replicated using other species with indeterminate growth before firm conclusions can be made. But our results already have two broad implications. First, research on the evolution of allometry requires accurate estimates of allometric slopes (EBERHARD et al. 1999; AL-WATHIQUI & RODRÍGUEZ 2011; KILMER & RODRÍGUEZ unpublished). Mixing adult ages can be an important source of
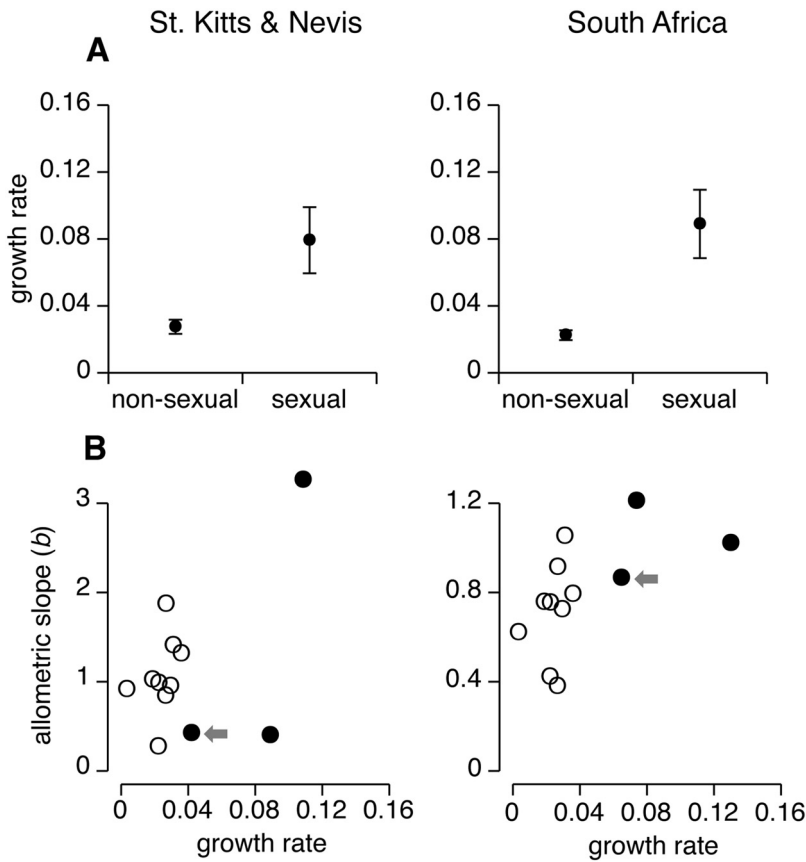
Fig. 2. — Differences in the rate of growth over adult ages in sexual and non-sexual traits in vervet monkeys, and relationship with estimates for allometric slopes (*b*). A: In both subspecies, sexual traits had higher growth rates over adult ages than non-sexual traits. Panels show mean growth rates ±1 SE. B: In both subspecies, traits with faster growth rates had steeper *b* estimates. Open symbols: non-sexual traits; closed symbols: sexual traits. Grey arrows indicate the penis.

confounding variation in such estimates. In animals that continue to develop some structures as adults, it may be easy to overestimate the steepness of the allometry of the late-developing structures. In such cases, it is important to take a fine-grained age estimate into account when estimating static allometric slopes (our method ii), or to use individuals of the same adult age (our method iii). Our assessment suggests that the former is preferable, but we offer some caveats. We used a conservative criterion that favored the method returning the least divergence in allometry. This seemed more defensible in view of evidence suggesting that evolution of trait allometries is likely to be slow (Voje & Hansen 2013; Voje et al. 2014), but it is not necessarily more accurate. Also, using only individuals of one age category (our method iii) reduces the sample of individuals included in the analysis. In our data set, the sample for each trait ranged from $n = 22$–99 vervets with method (ii) and from $n = 16$–75 with method (iii). This reduction in sample size may account for why method ii seems to perform better,
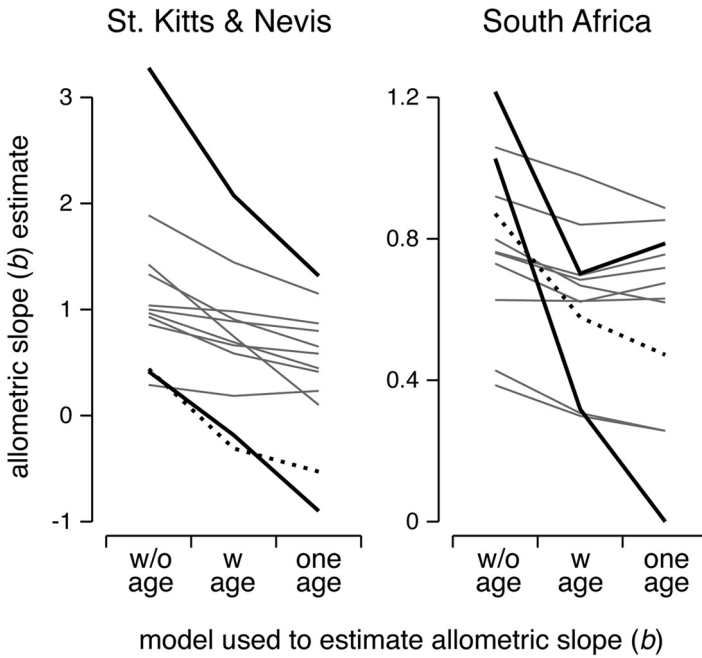
Fig. 3. — Comparison of vervet monkey allometric slopes (b) estimated with three different methods – (i) without accounting for age; (ii) with age as a covariate; (iii) with males of only one age category (see text for method details). In both subspecies, estimates of b obtained without taking age into account were predominantly higher. Grey lines: body traits; black lines: sexual traits; dotted black line: the penis.

Table 4.

Comparison of subspecies differences in mean allometric slopes (b) for vervet monkeys, as detected by three different statistical methods – (i) without accounting for age; (ii) with age as a covariate; (iii) with males of only one age category (see text for method details).

Factor	df num, denom	Method i F, P	Method ii F, P	Method iii F, P
Subspecies	1, 21	2.15, 0.16	0.32, 0.58	0.61, 0.44
Trait type	1, 21	1.21, 0.28	0.62, 0.44	3.52, 0.08

The subspecies × trait type interaction was non-significant with all three methods (in all cases, $P \geq 0.37$).

but with larger overall samples, method iii might yield more comparable results. Thus, broader testing with other species will be required to arrive at a more robust prescription for how to deal with the age confound problem.

The second implication of our findings is that reports of steep allometries for vertebrate genitalia may represent overestimates. If so, vertebrate genitalia may fit in with the widespread pattern of shallow genitalic allometries observed across a variety of animal groups (EBERHARD et al. 1998; EBERHARD 2009). If this result holds with
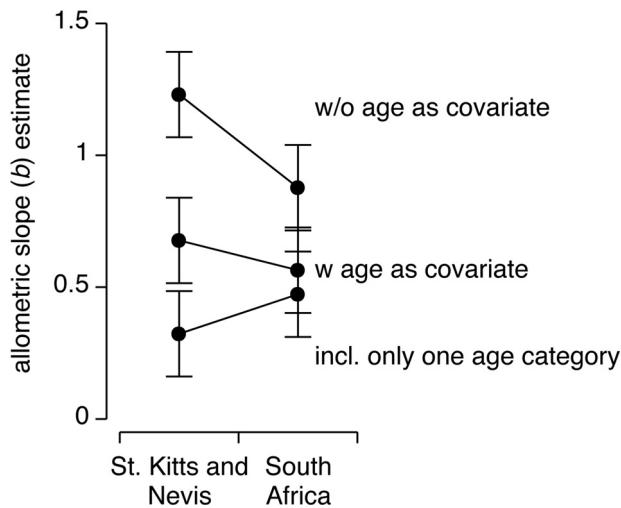
Fig. 4. — Comparison of the magnitude of subspecies differences in vervet monkey allometric slopes (*b*), as detected by three different statistical methods – (i) without accounting for age; (ii) with age as a covariate; (iii) with males of only one age category (see text for method details). The figure shows the least square means ± SE values for *b* for each subspecies, from the models in Table 4.

broader testing, it would reinforce one of the most striking and consistent patterns in the study of allometry to be uncovered to date.

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