



Original Article

Macroevolutionary Patterns of Sexual Size Dimorphism Among African Tree Frogs (Family: Hyperoliidae)

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Abstract

Sexual size dimorphism (SSD) is shaped by multiple selective forces that drive the evolution of sex-specific body size, resulting in male or female-biased SSD. Stronger selection on one sex can result in an allometric body size scaling relationship consistent with Rensch's rule or its converse. Anurans (frogs and toads) generally display female-biased SSD, but there is variation across clades and the mechanisms driving the evolution of SSD remain poorly understood. We investigated these topics in a diverse family of African treefrogs (Hyperoliidae). Hyperoliids display traits considered rare among amphibians, including sexual dichromatism and protogynous sex change. Using phylogenetic comparative methods, we tested if adult ecology, sexual dichromatism, and sex change were predictors of body size or SSD. We also tested whether hyperoliids displayed allometric interspecific body size scaling relationships. We found a majority of hyperoliid taxa display female-biased SSD, but that adult ecology and sexual dichromatism are poor predictors of sex-specific body size and SSD. Regardless of the groupings analyzed (partitioned by clades or traits), we found support for isometric body size scaling. However, we found that sex change is a significant predictor of SSD variation. Species in the *Hyperolius viridiflavus* complex, which putatively display this trait, show a significant reduction in SSD and are frequently sexually monomorphic in size. Although protogynous sex change needs to be validated for several of these species, we tentatively propose this trait is a novel mechanism influencing anuran body size evolution. Beyond this association, additional factors that shape the evolution of anuran body size and SSD remain elusive.

Keywords: Body size, allometry, Rensch's rule, anurans, sexual dichromatism, protogyny

Sexual dimorphism in animal body size is a pervasive phenomenon thought to result from differences in selective pressures on the sexes within species (Darwin 1874; Shine 1989; Andersson 1994; Fairbairn 1997; Blanckenhorn 2005). Sexual size dimorphism (SSD) is regarded as a consequence of the adaptation of the sexes to their

reproductive roles and associated differences in ecology (Fairbairn et al. 2007). SSD can manifest as female-biased SSD (in which females are the larger sex) or male-biased SSD (males are the larger sex). By contrast, sexual size monomorphism (SSM) describes the absence of body size dimorphism between the sexes. Body size

affects reproductive success through different pathways in males and females. Fecundity selection favors larger females because they produce more offspring or have greater energy storage for reproduction (Roff 1993; Andersson 1994; Head 1995; Fairbairn 1997; Pincheira-Donoso and Hunt 2015). Larger male size can provide an advantage in intrasexual combat or in female-choice and is generally considered to be under strong sexual selection (Andersson 1994; Fairbairn 1997; Fairbairn et al. 2007). In addition to these widely accepted mechanisms, SSD may also be the consequence of intersexual niche partitioning, in which differences in size reduce ecological competition between the sexes (Shine 1989). In any species, the presence or absence of SSD is a consequence of the direction and strength of selection on each sex relative to constraints imposed by the shared genomes of the sexes. Consequently, a major focus has been understanding which mechanisms shape macroevolutionary patterns of SSD across different taxonomic levels and animal groups (Cox et al. 2003, 2007; Blanckenhorn et al. 2007; Lindenfors et al. 2007; Székely et al. 2007; Kupfer 2009; De Lisle and Rowe 2013; Han and Fu 2013).

One macroevolutionary pattern of SSD is known as Rensch's rule, which states that across species the degree of SSD increases with increasing body size when males are larger than females, but decreases with body size when females are the larger sex (Rensch 1950, 1960) (Figure 1). In other words, as species become overall larger in size, increases in adult male size are disproportionately larger relative to adult female size. This interspecific allometric scaling relationship is indicative of a greater evolutionary divergence in male body size that is attributed to stronger sexual selection in

males (Fairbairn and Preziosi 1994; Abouheif and Fairbairn 1997; Dale et al. 2007). A regression of log male size on log female size can be used to examine such interspecific scaling relationships, and in this context Rensch's rule is consistent with a slope (β) of greater than 1 (Figure 1, Lines A–C; Fairbairn and Preziosi 1994). The consequences of Rensch's rule depend primarily on whether the system displays male-biased SSD, female-biased SSD, or some combination of both. For systems with male-biased SSD, this interspecific allometry produces a “runaway” pattern where the difference between the larger male size and smaller female size widens as species grow in size, resulting in increasingly higher values of male-biased SSD in larger species (Figure 1, Line A). For systems with female-biased SSD there is instead a “catchup” pattern as species increase in size, in which the difference between the smaller male size and larger female size continually decreases (Figure 1, Line C). This results in decreasing values of female-biased SSD in larger species. For some groups displaying Rensch's rule, this allometric size-scaling can cause a shift from female-biased SSD in smaller species to male-biased SSD in larger species (Figure 1, Line B). The converse of Rensch's rule states that as species become larger in size, increases in adult female size are disproportionately larger relative to adult male size. This type of allometry produces a slope of less than 1 in a regression of log male size on log female size (Figure 1, Lines D–F). This particular interspecific allometry produces a “runaway” pattern with increasingly larger females in systems with female-biased SSD, a “catchup” pattern for female size in systems with male-biased SSD, and can result in a switch from male-biased SSD in smaller species to female-biased SSD in larger species (Figure 1, Lines D,

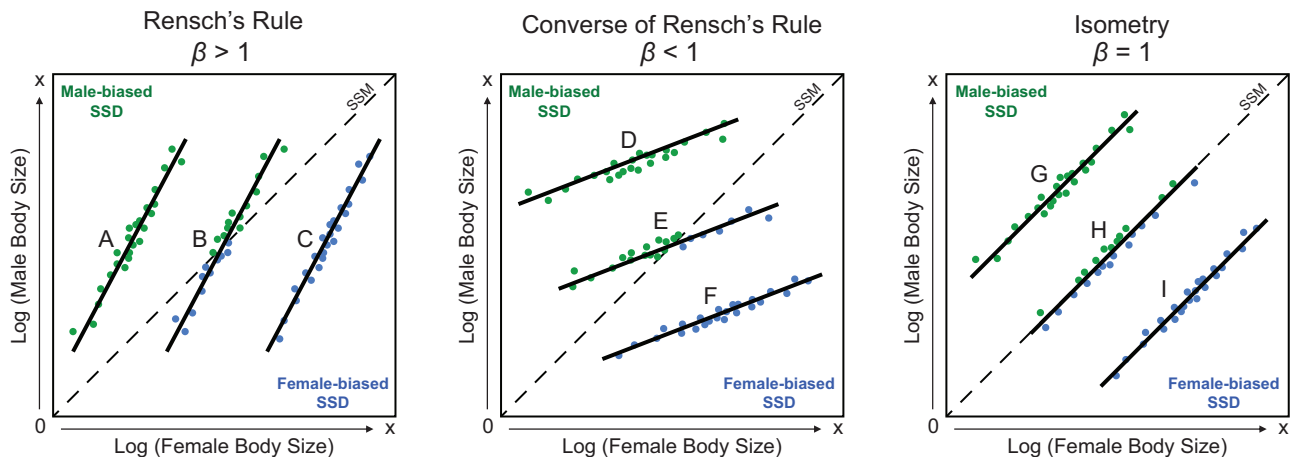


Figure 1. A stylized representation of interspecific scaling relationships of male and female body size following a pattern of Rensch's rule, the converse of Rensch's rule, and isometry. In all plots, log female body size is on the x-axis and log male size appears on the y-axis. Within each plot there are 3 hypothetical regression lines and associated species data points. The data points are color-coded (in the online version), with green representing species displaying male-biased SSD and blue representing female-biased SSD. The slope of the regression line is indicative of the type of body size scaling relationship (Rensch's rule, converse of Rensch's rule, isometry), regardless of whether the system displays male or female-biased SSD. The dotted line in the background represents isometry (slope = 1) and sexual size monomorphism (e.g., male and female body sizes are equal). In this graphical arrangement (e.g., females on the x-axis), Rensch's rule is represented by a regression line slope (β) > 1. Regression line A represents Rensch's rule in a system with male-biased SSD and demonstrates that as species increase in size the degree of male-biased SSD also increases (a “runaway” pattern for male size). Regression line B represents Rensch's rule in a system with female-biased SSD and shows that as species increase in size, the degree of female-biased SSD decreases (a “catchup” pattern for male size). In some cases, this particular allometry (β > 1) can cause a shift from female-biased SSD (in smaller species) to male-biased SSD (in larger species), and is represented by regression line C. The converse of Rensch's rule is represented by β < 1. Regression line D represents the converse of Rensch's rule in a system with male-biased SSD and demonstrates that as species increase in size the degree of male-biased SSD decreases (a “catchup” pattern for female size). Regression line E represents Rensch's rule in a system with female-biased SSD and illustrates that as species increase in size the degree of female-biased SSD increases (a “runaway” pattern for female size). This allometry (β < 1) can also cause a shift from male-biased SSD (in smaller species) to female-biased SSD (in larger species), which is represented by regression line F. Isometry occurs when the proportional relationship of male and female size is preserved regardless of whether species become larger or smaller, and is represented by β = 1. For any interspecific size changes, the SSD values remain constant. This is true for systems displaying male-biased SSD (regression line G), female-biased SSD (regression line I), or sexual size monomorphism (regression line H).

F, and E, respectively). Finally, isometry occurs when the proportional relationship of male and female size is preserved regardless of whether species become larger or smaller, and is represented by a slope of 1. As isometric scaling does not change the relative size of males versus females, SSD values remain constant regardless of whether the system displays male-biased SSD, female-biased SSD, or SSM (Figure 1, Lines G, I, and H, respectively). A majority of taxa displaying male-biased SSD support Rensch's rule, especially mammals and birds (Abouheif and Fairbairn 1997; Dale et al. 2007; Lindenfors et al. 2007; Székely et al. 2007). However, the trend is less clear for taxa displaying female-biased SSD, and studies of these systems have identified scaling patterns consistent with Rensch's rule, isometry, and the converse of Rensch's rule (Head 1995; Abouheif and Fairbairn 1997; Webb and Freckleton 2007; Han and Fu 2013; De Lisle and Rowe 2013) (Figure 1).

The order Anura (frogs and toads) exhibits a large range of body sizes and variation in SSD, yet a vast majority of anuran species display female-biased SSD (~90% of 700 species surveyed; Shine 1979; Han and Fu 2013) (Figure 2). The most common explanation for female-biased SSD in anurans is stronger selection on female body size attributed to the fecundity advantage (Shine 1988). There is a strong correlation between increases in female size and increases in egg size and/or clutch size across anurans (Salthe and Duellman 1973; Crump 1974; Lang 1995; Prado and Haddad 2005; Hartmann et al. 2010; Han and Fu 2013; Nali et al. 2014; Monroe et al. 2015). Despite this demonstrated relationship, fecundity traits are poor predictors of the degree of female-biased SSD in anurans (Monroe et al. 2015; Liao et al. 2017) and fecundity selection may only drive SSD evolution in smaller species (Nali et al. 2014). Several alternative hypotheses have been proposed to explain female-biased SSD that invoke selection for smaller male body sizes. These include energetic constraints on males resulting from reproductive behaviors (Woolbright 1983), higher predation rates on males resulting in younger and smaller individuals in the population (Wells 1977; Monnet and Cherry 2002), and the enhanced vagility of smaller male sizes (Ghiselin 1974). Male-biased SSD occurs less frequently in anurans, but when present it is typically attributed to territorial behavior and intrasexual combat (Trivers 1972; Wells 1977; Shine 1979). However, male combat is a poor predictor of male-biased SSD, and other traits such as male parental care may better predict patterns of male-biased SSD (Han and Fu 2013). Given the general lack of consensus on factors explaining SSD in anurans, alternative approaches have been taken to explore the evolution of body size, such as testing Rensch's rule. Meta-analyses of anurans have

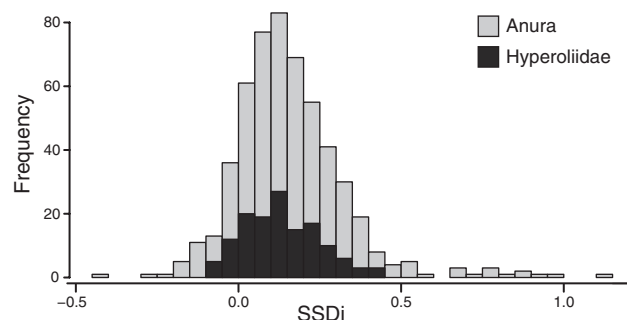


Figure 2. Frequency distribution of SSDi values for Hyperoliidae (dark gray) superimposed on the distribution of SSDi values for other anurans (light gray). The SSDi values for Anura are based on 534 species from Han and Fu (2013).

typically found an isometric scaling relationship between the body sizes of the sexes, as opposed to allometry (De Lisle and Rowe 2013; Han and Fu 2013; Nali et al. 2014). However, isometry is not ubiquitous across anurans and some families display allometric scaling relationships that are consistent with Rensch's rule or its converse, demonstrating clade-specific variation (Han and Fu 2013; De Lisle and Rowe 2013). It is important to consider that the most comprehensive analysis of SSD in anurans is based on very limited sampling (~850 of ~7,000 species, 20 of 54 families, 12% of anuran species diversity; De Lisle and Rowe 2013; AmphibiaWeb 2020), and that SSD remains unexplored for most (>6000) anuran species. It therefore seems premature to generalize these findings to explain the evolution of SSD across all anurans. We propose that newly generated data from densely sampled taxa are required to improve our understanding of this complex topic.

Hyperoliidae is an African-endemic radiation that includes over 230 species distributed throughout sub-Saharan Africa, Madagascar, and the Seychelles Islands (AmphibiaWeb 2020). A majority of hyperoliids display a conserved treefrog morphology (e.g., *Afrixalus*, *Heterixalus*, *Hyperolius*), which consists of a slender body with relatively larger head and eyes, elongated limbs and digits, and enlarged toe pads (Schiotz 1967, 1999). However, the family also includes several terrestrial species (e.g., *Kassina*), which are characterized by a stout body, intermediate length limbs, and reduced webbing and toe pads. Hyperoliids exhibit moderate variation in body size, ranging from 17 to 65 mm (Schiotz 1967, 1999; Portik 2015), and territoriality with male combat occurs in many species (Telford 1985; Backwell and Passmore 1990; Rödel et al. 2006; Gilbert and Bell 2018; Portik et al. 2018). Limited observations of SSD indicate both male-biased and female-biased SSD occur in hyperoliids (Schiotz 1967, 1999; Rödel et al. 2006; Portik et al. 2018). However, previous studies of SSD have included less than 10 hyperoliids (De Lisle and Rowe 2013; Han and Fu 2013) and the overall patterns of SSD among hyperoliids remain unknown. The range in body size, variation in SSD, and differences in adult ecology make hyperoliids a suitable system for examining the evolution of body size, SSD, and scaling relationships.

In addition to these general characteristics, hyperoliids are unique in displaying 2 traits considered rare among anurans: sexual dichromatism and protogynous sex change. Sexual dichromatism is present in only ~2% of anuran species, and is a form of dimorphism in which the sexes differ in color (Schiotz 1967, 1999; Bell and Zamudio 2012; Bell et al. 2017b; Portik et al. 2019). Sexual dichromatism occurs in over 60 hyperoliids, and among closely related dichromatic species coloration tends to differ drastically among females but not males (Schiotz 1967, 1999; Amiet 2012; Portik et al. 2016a, 2019). The function of this trait in hyperoliids remains unresolved, but it is correlated with their rapid diversification and one possibility is that female color is an essential mate recognition signal (Portik et al. 2018, 2019). Interestingly, one assemblage of dichromatic hyperoliids displayed highly similar female body sizes but significantly different male body sizes (Portik et al. 2018). This finding suggests dichromatic species may experience greater selection on male body size, which is expected produce an allometric body size scaling relationship consistent with Rensch's rule. Protogynous sex change, in which adult (e.g., sexually mature) females transform into reproductively active males, has only been documented in 2 anuran species. Both are hyperoliids, and both belong to the *Hyperolius viridiflavus* species complex (Grafe and Linsenmair 1989; Wiczorek et al. 2000). Protogynous sex change might maximize lifetime reproductive success in highly seasonal environments where mortality

may be sex-dependent (Grafe and Linsenmair 1989). In these species, a breeding population can consist of primary males, females, and secondary males (e.g., females that have transformed into males). There are no significant differences in the body sizes of primary males and females, and as a result the secondary males are also comparable in size to primary males (Grafe and Linsenmair 1989). This finding suggests that protogynous sex change may be correlated with a reduction in SSD (e.g., a shift to SSM). The presence of sexual dichromatism and protogynous sex change in hyperoliids, therefore, offer an additional opportunity to study how these traits potentially influence the evolution of body size and SSD.

In this study, we investigate macroevolutionary patterns of body size evolution and SSD in hyperoliid frogs using phylogenetic comparative methods. We quantify sex-specific body sizes and SSD to address several hypotheses concerning the evolution of these traits. Given that a majority of anurans display female-biased SSD, we first test whether hyperoliids display primarily female-biased SSD or male-biased SSD. We then examine if particular traits are correlated with body size or SSD. Ideally, this would involve “classic” anuran traits such as male combat, clutch size, and egg size. However, few reproductive studies are available (but see Lampert and Linsenmair 2002; Rödel et al. 2006; Kouamé et al. 2015), and consequently, data for these traits are low-quality or lacking for a majority of hyperoliid species (Lawson et al. 2018). Instead, we test whether adult ecology (arboreal vs. terrestrial), sexual dichromatism, or protogynous sex change are predictors of sex-specific body sizes and SSD. To further explore the evolution of SSD, we take a statistical approach to classify species as sexually size dimorphic or monomorphic and examine the distribution of dimorphic and monomorphic species across groups. Finally, we test whether body size scaling relationships conform to Rensch’s rule, isometry, or the converse of Rensch’s rule. We assess scaling relationships at multiple taxonomic levels (family, subfamily, clade), and for groups displaying particular traits (sexual dichromatism, sex change). Although some of our analyses are exploratory in nature, we test several predictions based on previous findings. Specifically, we predict that sexually dichromatic species will display a scaling pattern consistent with Rensch’s rule. We also predict that protogynous sex change is correlated with reduced SSD, which is likely to manifest as SSM. Finally, we predict that species putatively capable of sex change (e.g., the *H. viridiflavus* complex) will display an isometric scaling relationship.

Methods

Molecular Phylogeny

We use a newly available time-calibrated phylogeny of Afrobatrachia (including Arthroleptidae, Brevicipitidae, Hemisotidae, and Hyperoliidae) produced by Portik et al. (2019) as a basis for our phylogenetic comparative methods. This phylogeny was created through a multi-step approach, which we briefly summarize here. A hyperoliid species tree of 140 lineages was constructed using 1047 exons obtained from transcriptome-based exon capture experiment (Portik et al. 2016b), and this species tree was used to constrain hyperoliid relationships in an expanded divergence dating analysis of Afrobatrachia based on a supermatrix of one mitochondrial (16S) and 5 nuclear loci (*FICD*, *KIAA2013*, *POMC*, *TYR*, and *RAG-1*) (Portik et al. 2019). The time-calibrated phylogeny contains 173 hyperoliid lineages, which were pruned to match the species representation in our morphological data set. We note that Hyperoliidae is in a state of taxonomic flux with recent studies recommending both the synonymy of species and splitting of species complexes (Rödel

et al. 2002, 2003, 2009, 2010; Wollenberg et al. 2007; Schick et al. 2010; Conradie et al. 2012, 2013, 2018; Dehling 2012; Channing et al. 2013; Greenbaum et al. 2013; Liedtke et al. 2014; Loader et al. 2015; Portik et al. 2016a; Barratt et al. 2017; Bell et al. 2017a). Therefore, we adopt the naming convention of Portik et al. (2019) to label taxa included in this study, where genetically and/or geographically distinct units within large species complexes are distinguished using integers (e.g., *Afrixalus dorsalis* 1, *Afrixalus dorsalis* 2).

We included 6 additional lineages of the *H. viridiflavus* complex represented in our morphological dataset that were not present in the phylogeny of Portik et al. (2019). These include *H. marmoratus verrucosus*, *H. m. argentovittis*, *H. viridiflavus ferniquei*, *H. v. goetzi*, *H. v. rubripes*, and *H. v. viridiflavus*. We obtained relevant 16S sequences from GenBank for these 6 lineages, which were generated by Wiczorek et al. (2000) in their mtDNA study of the *H. viridiflavus* species complex. We aligned these sequences with other 16S sequences from the *H. viridiflavus* complex available from Portik et al. (2019) using MAFFT with the automatic alignment algorithm selection option (Katoh and Standley 2013). We reconstructed a phylogeny for the *H. viridiflavus* group using a ML approach in RAxML v8 (Stamatakis 2014). We subsequently added the 6 missing tips to the time-calibrated Afrobatrachia phylogeny based on these phylogenetic results, using the “bind.tip” function in “phytools” package (Revell 2012) in R (R Core Team 2018).

Trait Data

We obtained body size measurements in the form of snout-urostyle length (SUL) from 2771 preserved specimens representing 123 hyperoliid lineages from the following natural history collections: Burke Museum of Natural History and Culture, California Academy of Sciences, Cornell University Museum of Vertebrates, and the Museum of Vertebrate Zoology (Supplementary Appendix 1). Measurements were made with a Mitutoyo Series 500 Digimatic Caliper (Mitutoyo U.S.A., Illinois) and recorded to the nearest 0.1 millimeters. We obtained additional body size data from several published sources (Schlötz 1967, 1999; Nussbaum and Wu 1995; Channing 2001; Rödel et al. 2002, 2003, 2009, 2010; Glaw and Vences 2007; du Preez and Carruthers 2009; Harper et al. 2010; Amiet 2012; Conradie et al. 2012, 2013; Channing et al. 2013; Loader et al. 2015; Conradie et al. 2018) to either supplement the number of specimens included for a given species ($n = 9$ species) or provide data for species we could not access ($n = 26$ species) (Supplementary Appendix 1). The combination of empirical and published data resulted in a total of 142 lineages that have SUL data available for both sexes, of which 138 are represented in the revised phylogeny.

We classified species as sexually dichromatic or sexually monochromatic, following Portik et al. (2019). The 6 additional species of the *H. viridiflavus* complex were all considered to be sexually dichromatic (Schlötz 1999). Protogynous sex change was initially documented in 2 non-sister taxa in the *H. viridiflavus* super-species complex (including *H. viridiflavus ommatostictus* and *H. marmoratus taeniatatus*; Grafe and Linsenmair 1989), and never studied again. For the purpose of our analyses, we assumed this trait occurs throughout lineages currently or formerly classified as part of the *H. viridiflavus* complex, including all subspecies of *H. viridiflavus*, *H. parallelus*, and *H. marmoratus*. For our study, this included 14 lineages. We note that the clade containing these 3 species groups also contains several additional species (Figure 3), but given the uncertainty of this trait we provide a conservative estimate of its occurrence.



Figure 3. A time-calibrated phylogeny of Hyperoliidae displaying maximum likelihood reconstructions of SSDi, with darker fill branches showing slightly male-biased SSD and lighter fill showing pronounced female-biased SSD (purple and green, respectively, in the online version). The occurrence of sexual dichromatism is indicated by boxes at the tips, with black representing sexually dichromatic species and white representing sexually monochromatic species. Numbers on nodes refer to general shifts towards male-biased SSD/SSM (nodes 1, 3) and towards pronounced female-biased SSD (nodes 2, 4). The putative presence of protogynous sex change is denoted by species names that are followed by asterisks. Protogynous sex change was originally described in *Hyperolius viridiflavus ommatostictus* and *H. marmoratus taeniatus* (Grafe and Linssenmaier 1989).

We calculated a sexual size dimorphism index (SSDi) to quantify SSD across species using the equation $SSDi = [(larger\ sex / smaller\ sex) - 1]$, arbitrarily set negative if males are the larger sex and

positive if females are the larger sex (Lovich and Gibbons 1992). This SSDi has been widely used, is properly scaled around zero, and has high intuitive value because positive SSDi values indicate female-biased SSD and negative values indicate male-biased SSD (Lovich and Gibbons 1992). To calculate the SSDi, we used the mean body size of each sex when possible, but for some literature records that only provided body size ranges we used the range midpoint as an alternative.

When SSDi is calculated, a species is automatically classified as displaying male or female-biased SSD, even if the difference between male and female body size is minimal. In other words, a statistical distinction between SSD and monomorphism is not made based on this measure. To address this, we developed a new approach to statistically classify species as dimorphic or monomorphic, which relies on intraspecific variation in body size. For a given species with body size data available for multiple males or females, we calculated an SSDi for all possible male-female pairwise comparisons, and obtained the mean value. To distinguish this metric, we refer to it as the pairwise SSDi (vs. standard SSDi). We then performed a permutation test with 10 000 bootstrap replicates to evaluate the null hypothesis that male and female body sizes come from the same population (e.g., $SSDi = 0$). For each replicate, we randomly shuffled the labels of males and females, calculated all possible pairwise SSDi values, and obtained the mean SSDi value. The 10 000 simulated SSDi values of the permuted data represent the estimate of the sampling distribution under the null hypothesis. We then assessed whether the empirical mean pairwise SSDi was outside of the critical values of the simulated distribution (2.5% and 97.5%, representing a 5% significance level), and calculated the P -value for the permutation test following Phipson and Smyth (2010). In this context, a P -value < 0.05 allows rejection of the null hypothesis and classifies a species as displaying SSD. Alternatively, a nonsignificant P -value indicates there is not sufficient evidence to support the alternative hypothesis (e.g., male and female body sizes come from different populations, $SSDi \neq 0$). Here, we classify these species as displaying sexual size monomorphism, but acknowledge the failure to reject the null hypothesis could result from a true lack of body size difference between the sexes, or from artifacts such as insufficient sample sizes or high variation. We performed these analyses for all species with available data, which included 120 hyperoliid species. Using this method, the mean pairwise SSDi calculated for a given species takes intraspecific variation into account. Consequently, we were interested in determining how similar this value was to the standard SSDi calculated from an average male and female body size. To determine this, we simply calculated the absolute difference between the 2 SSDi measures for each of the 120 species. To perform all calculations of SSDi, pairwise SSDi, and permutation tests, we created a Python program (*SSDi-Calculator.py*), which is freely available from <https://github.com/dportik/SSDi-Calculator>.

Phylogenetic Comparative Analyses

To visualize the distribution of sex-specific body sizes and SSDi on the hyperoliid phylogeny, we performed maximum likelihood (ML) ancestral state reconstruction for continuous traits using the “contMap” function of the “phytools” package (Revell 2012) in R (R Core Team 2018). To visualize body sizes, we used log-transformed values of SUL.

We tested for phylogenetic signal in sex-specific body size and SSDi to assess if the evolution of these traits deviates from expectations based on Brownian motion (BM). We quantified the amount of phylogenetic signal using Blomberg’s K -statistic (Blomberg et al.

2003). For this metric, a K value close to 1 indicates trait evolution occurs according to a BM model. A K value of less than 1 indicates relatives are less similar than expected from BM, and a K value greater than 1 indicates relatives are more similar than expected. We used 1000 permutations in a randomization test to examine if the variance in the empirical data was significantly different than variance in randomized data sets with no phylogenetic signal. As a complement, we also examined phylogenetic signal using Pagel's λ , which is a scaling parameter used to transform the phylogeny such that it ensures the best fit to a BM model (Pagel 1999; Freckleton et al. 2002). If Pagel's λ is close to 1, the trait evolves according to BM, whereas values approaching 0 indicate the trait has evolved independently of the phylogeny (e.g., a lack of phylogenetic signal). We evaluated if the fitted value of λ was significantly different from $\lambda = 0$ using a likelihood-ratio test. We conducted both types of tests for phylogenetic signal using the `phylosig` function in the “phytools” package in R, using SSDi and log-transformed values of SUL.

We performed phylogenetic generalized least squares (PGLS) regression to examine potential correlations between several predictor variables (ecology, sexual dichromatism, and protogynous sex change) and several response variables (male SUL, female SUL, and SSDi). PGLS incorporates phylogenetic nonindependence into generalized linear models in the form of a phylogenetic variance-covariance matrix (Freckleton et al. 2002). We determined the most appropriate correlation structure for the residuals for each regression by comparing the fit of BM, BM plus the λ scaling parameter, and Ornstein-Uhlenbeck (OU) models using AIC scores.

Testing Rensch's Rule

For systems with female-biased SSD, Rensch's rule states that the magnitude of SSD decreases with increases in body size, and the converse of Rensch's rule states that the degree of SSD should increase with mean body size (Figure 1). These allometric scaling relationships are generally tested by determining if the regression slope between log-transformed male and female body size differs significantly from a value of one (Abouheif and Fairbairn 1997; Fairbairn 1997; Smith 1999). Model I regressions (including ordinary least squares, OLS) assume the values for the x -axis are known without error, and the OLS equation is not symmetrical, producing different predictions depending on which sex is defined as the independent variable (Warton et al. 2006; Smith 2009). Reduced major axis regression (RMA) is a Model II regression that is symmetric, meaning that a single line defines the bivariate relationship regardless of which variable is X and which is Y . Furthermore, it assumes that variables on both axes are measured with error (Warton et al. 2006; Smith 2009). We, therefore, chose to use RMA because our estimates of the body size for each sex contain error, and because the assignment of male or female size as X or Y is arbitrary. When female size is assigned to the x -axis and male size is assigned to the y -axis, allometry conforming to Rensch's rule is indicated by a slope greater than one, whereas the converse of Rensch's rule is supported when the slope is less than one (Figure 1). When the slope is not significantly different from one, male and female body size scale isometrically (Figure 1).

To account for shared evolutionary history, we performed a phylogenetic reduced major axis (pRMA) regression (Ives et al. 2007) using the `phyl.RMA` function in “phytools.” Hypothesis testing for a slope significantly different than 1 was calculated following Ives et al. (2007) and implemented using the `phyl.RMA` function. We performed pRMA regressions for multiple taxonomic levels (family, subfamily, clade), and for groups displaying particular traits (sexual dichromatism, protogynous sex change). Two subfamilies

are currently recognized within Hyperoliidae: Kassiniinae (~26 species), which is composed of both arboreal and terrestrial species, and Hyperoliinae (~206 species), which contains exclusively arboreal species (Figure 3). Variation in ecological and reproductive traits is greatest between these subfamilies, but important variation in arboreal species is also captured in 2 major clades within Hyperoliinae: one clade contains species of *Afraxalus*, *Heterixalus*, and *Tachycinemid* (~43 species, hereafter referred to as Clade A), and the other contains the hyperdiverse genus *Hyperolius* and 2 species-poor genera (*Cryptothylax*, *Morerella*) (~156 species, hereafter referred to as Clade B) (Figure 3). To disentangle any potential clade-specific patterns, we performed pRMA regressions for Hyperoliidae, Kassiniinae, Hyperoliinae Clade A, and Hyperoliinae Clade B. To investigate body size scaling as it relates to sexual dichromatism, we performed separate pRMA regressions for dichromatic and monochromatic species occurring in Hyperoliinae. To investigate scaling as it relates to protogynous sex change, we also performed a pRMA regression for species in the *H. viridiflavus* complex. To visualize these data, we created bivariate scatterplots from the natural-log transformed SUL of males and females for a given comparison (with female body sizes plotted on the x -axis) and plotted the relevant pRMA regression line.

Results

Body Size Patterns and SSD

We examined sex-specific body sizes and SSDi for 142 hyperoliid lineages (Supplementary Table 1; Supplementary Appendix 1). Across all species, the overall body size range of males (18.0–60.2 mm) was similar to females (18.8–62.6 mm), but the average female size (30.5 mm) was slightly larger than average male size (27.5 mm). Across Hyperoliidae, we found a mean SSDi of 0.116 and range of -0.092 to 0.372 (Figures 2 and 3; Supplementary Table 1). These SSDi values for hyperoliids are within the known SSDi range for other anurans (534 species, range = -0.43 to 1.12 , mean = 0.163 ; Han and Fu 2013) (Figure 2). The distribution of SSDi values differs between the subfamilies Kassiniinae (mean = 0.029 , range = -0.050 to 0.130) and Hyperoliinae (mean = 0.125 , range = -0.092 to 0.372). The greatest variation in SSDi occurs within the genus *Hyperolius* (Hyperoliinae Clade B), which contains the most exaggerated examples of male-biased SSD (-0.092 ; *H. jacobsoni*) and female-biased SSD (0.372 ; *H. bobirensis*) across the family (Figure 3; Supplementary Table 1).

Using a novel approach, we statistically classified species as displaying SSD or SSM. Of the 120 species with suitable data, we found 40 species display SSM (34%), 3 display male-biased SSD (2%), and 77 display female-biased SSD (64%) (Supplementary Table 1). These results indicate the family is strongly skewed towards female-biased SSD, that SSM occurs less frequently, and that male-biased SSD is rare. We found that the pairwise SSDi calculated was generally similar to the standard SSDi calculated from the average male and female body size (mean difference \pm SD = 0.003 ± 0.002). Given that our method accounts for intraspecific variation and allows for statistical classification of SSM versus SSD, we strongly recommend using it when suitable data are available. Taxa displaying SSM include 7 species of *Afraxalus*, 6 kassinoids (*Kassina*, *Paracassina*, *Phlyctimantis*), and 27 species of *Hyperolius*. Of the 27 *Hyperolius* species displaying SSM, 11 species (41%) belong to the *H. viridiflavus* complex, 6 (22%) belong to the *H. nasutus* complex, and the remaining 10 species are scattered across the phylogeny.

Phylogenetic Comparative Analyses

The ancestral state reconstruction of SSDi shows female-biased SSD values at the most recent common ancestor (MRCA) of Kassiniinae and Hyperoliinae (Figure 3). There are 2 general shifts towards SSM/male-biased SSD in Clade B: one in the *H. nasutus* species group (node 1, Figure 3) and another in a clade containing the *H. viridiflavus* complex (node 3, Figure 3). There are also 2 general shifts towards pronounced female-biased SSD in Clade B, one in a clade largely restricted to the Lower Guinea Forest Zone (node 2, Figure 3) and the other in the MRCA of a large assemblage distributed throughout sub-Saharan Africa (node 4, Figure 3). The reconstructions of log-transformed sex-specific body sizes highlight shifts towards overall larger sizes in Kassiniinae, *Tachycineta*, and *Cryptothylax*, and multiple shifts to smaller body sizes throughout *Afraxalus* and *Hyperolius* (Supplementary Figure 1).

We found significant phylogenetic signal in the body sizes of each sex and for SSDi (Table 1). The evolution of female body size is consistent with a BM model based on both measures, and male body size is consistent with BM based on Pagel's λ but more similar than expected from BM based on Blomberg's K (Table 2). Overall, these values indicate that sex-specific body size tends to be more similar among closely related species versus distant relatives. Both Pagel's λ and Blomberg's K indicate phylogenetic signal is present for SSDi, but that SSDi values are less similar among relatives than expected from BM (Table 2). These results suggest that variation in SSDi occurs within clades as well as between clades, which can be seen visually in Figure 3.

We used PGLS to examine potential correlations between several predictor variables (ecology, sexual dichromatism, and protogynous sex change) and several response variables (male SUL, female SUL, and SSDi). For all comparisons, we found the BM plus the λ scaling parameter model provided the most appropriate correlation structure for the residuals (Table 2). We found that adult ecology, sexual dichromatism, and protogynous sex change were not correlated with male or female body sizes (Table 2). We did not find any correlation between adult ecology or sexual dichromatism and SSD. The distribution of SSDi values for dichromatic species (mean = 0.134, range = -0.074 to 0.347) is highly similar to that of monochromatic species (mean = 0.119, range = -0.092 to 0.372). However, we found a significant correlation between protogynous sex change and SSD (Table 2). Among lineages that potentially display protogynous sex change, the mean SSDi is close to sexual size monomorphism (0.018) and the range is considerably narrower (-0.074 to 0.165) than for other species of *Hyperolius* (mean = 0.156, range = -0.092 to 0.372).

Rensch's Rule

We conducted phylogenetic reduced major axis regressions of log-transformed body sizes to test whether slopes were significantly different from one, signifying allometric body size scaling relationships. Our pRMA regressions performed for all phylogenetic groups (Hyperoliidae, Kassiniinae, Hyperoliinae Clade A, Hyperoliinae Clade B) and trait-based groups (sexual dichromatism, protogynous sex change) resulted in slopes that were not significantly different

than one (Figure 4; Table 3). These results indicate body size scaling for all groupings conforms to a pattern of isometry (Figures 1 and 4).

Discussion

SSD is a consequence of differences in selective pressures on sex-specific body sizes. Among terrestrial vertebrates, anurans belong to a minority of taxonomic groups that primarily exhibit female-biased SSD (Shine 1979; Kupfer 2009; De Lisle and Rowe 2013; Han and Fu 2013). Several general mechanisms have been proposed to explain the overall evolution of SSD in anurans, which link fecundity selection to female-biased SSD and male combat to male-biased SSD (Trivers 1972; Wells 1977; Shine 1979; Shine 1988). However, these proposed mechanisms are not strongly supported in phylogenetic comparative studies (Han and Fu 2013; Monroe et al. 2015; Liao et al. 2017). For example, although many anuran species display a clear interspecific relationship between body size and fecundity in females (Salthe and Duellman 1973; Crump 1974; Lang 1995; Prado and Haddad 2005; Hartmann et al. 2010; Han and Fu 2013; Nali et al. 2014; Monroe et al. 2015), male body size describes variation in fecundity equally well for several anuran groups (Monroe et al. 2015). This indicates that selection for higher fecundity in females may often be accompanied by equivalent size changes in males that result from parallel selection (Emerson 1997; Byrne et al. 2002) or the shared genome (Fairbairn et al. 2007). Similarly, a recent study failed to find any correlation between male combat and male-biased SSD, but instead uncovered a potential link with male parental care (Han and Fu 2013). Due to a lack of suitable data, we were unable to explicitly test the fecundity and combat hypotheses using phylogenetic comparative methods. However, several observations in hyperoliids also question the proposed links between these traits and SSD. For example, despite the well-established female size-fecundity relationship in most anurans, a preliminary survey of hyperoliid species did not find a significant correlation between female body size and clutch size (Lawson et al. 2018). Using data from Lawson et al. (2018), we extended this analysis to fecundity (clutch size \times egg size), and failed to find a significant correlation between female body size and fecundity ($F_{(1,17)} = 3.432$, $P = 0.09$, $R^2 = 0.17$). Though this may prove to be an artifact of low-quality or sparse data, the current lack of a size-fecundity relationship in hyperoliids also blurs any potential link between fecundity traits and female-biased SSD. Similarly, territoriality and intrasexual combat have been observed in several hyperoliid species, yet these species mostly display female-biased SSD (Telford 1985; Backwell and Passmore 1990; Dyson and Passmore 1992; Portik et al. 2018). Likewise, male parental care has never been recorded in hyperoliids, and female parental care is only known to occur in a single species (Stevens 1971). Therefore, male combat and parental care are not likely to be influential factors driving SSD evolution in hyperoliids.

Mechanisms that drive sex-specific body size evolution, such as fecundity and male combat, are also expected to manifest as allometric body size scaling relationships. Patterns consistent with Rensch's rule indicate greater variation in male size, while the converse of Rensch's rule describes greater variation in female size (Abouheif and Fairbairn 1997; Fairbairn 1997) (Figure 1). Although a handful of anuran families support allometric scaling, most families display isometric scaling, which implies variation (and consequently selection) is not greater for male or female body size (De Lisle and Rowe 2013; Han and Fu 2013; Nali et al. 2014). Our results add to this pattern, as we also found a lack of support for allometric scaling (Figure 4, Table 3). The isometric relationship recovered for

Table 1. Measures of phylogenetic signal across traits

Trait	K	P -value	λ	P -value
Log Male SUL	1.14	<0.01	0.94	<0.01
Log Female SUL	1.02	<0.01	0.98	<0.01
SSDi	0.56	<0.01	0.81	<0.01

Table 2. Results of phylogenetic generalized least squares regressions for predictor and response variables

Predictor	Response	Correlation structure	AIC	df	α	λ	P-value
Adult ecology	Male SUL	Brownian	823.5	138	-	-	0.86
		Brownian + λ	811.8	138	-	0.92	0.68
		OU	825.4	138	0.015	-	0.78
Dichromatism	Male SUL	Brownian	822.4	138	-	-	0.28
		Brownian + λ	811.8	138	-	0.92	0.73
		OU	824.7	138	0.014	-	0.38
Sex change	Male SUL	Brownian	534.1	90	-	-	0.52
		Brownian + λ	518.3	90	-	0.80	0.52
		OU	528.7	90	0.063	-	0.48
Adult ecology	Female SUL	Brownian	851.4	138	-	-	0.67
		Brownian + λ	847.5	138	-	0.95	0.48
		OU	854.7	138	0.012	-	0.51
Dichromatism	Female SUL	Brownian	850.7	138	-	-	0.35
		Brownian + λ	847.8	138	-	0.96	0.61
		OU	854.6	138	0.010	-	0.46
Sex change	Female SUL	Brownian	547.9	90	-	-	0.62
		Brownian + λ	540.3	90	-	0.87	0.58
		OU	548.6	90	0.031	-	0.64
Adult ecology	SSDi	Brownian	-295.1	138	-	-	0.37
		Brownian + λ	-307.8	138	-	0.85	0.51
		OU	-303.9	138	0.046	-	0.91
Dichromatism	SSDi	Brownian	-294.5	138	-	-	0.70
		Brownian + λ	-307.6	138	-	0.85	0.63
		OU	-304.9	138	0.050	-	0.29
Sex change	SSDi	Brownian	-192.5	90	-	-	<0.01
		Brownian + λ	-198.2	90	-	0.85	<0.01
		OU	-194.0	90	0.041	-	<0.01

Phylogenetic correlation structures correspond to the following functions in the R package APE: Brownian, corBrownian; Brownian + λ , corPagel; OU, corMartins. For each relationship tested, the best AIC score of the 3-model set is indicated in bold. Models involving sex change as a predictor used a tree consisting of Hyperoliinae Clade B, as sex change is only known to occur in the genus *Hyperolius*.

hyperoliids indicates that change in the body size of one sex is generally accompanied by equivalent size change in the opposite sex. As described above, this could be the result of parallel sex-specific selection (e.g., related to fecundity) or the shared genome. Rensch's rule appears to be mainly supported in groups that display male-biased SSD (Abouheif and Fairbairn 1997; Lindenfors et al. 2007; Székely et al. 2007), and groups with female-biased SSD generally show mixed support for isometry or the converse of Rensch's rule (Head 1995; Abouheif and Fairbairn 1997; Webb and Freckleton 2007; De Lisle and Rowe 2013).

Beyond characterizing general patterns of SSD in hyperoliids, we explored whether variation in body size or SSD could be explained by various phenotypic traits. We focused on general anuran traits such as adult ecology, as well as clade-specific traits (including sexual dichromatism and protogynous sex change). In hyperoliids, terrestrial and arboreal species display variation in reproductive characteristics, including oviposition sites (Portik and Blackburn 2016). These reproductive trait combinations could be accompanied by different selective pressures on body size. Previously, it was proposed that arboreal oviposition may place upper size constraints and males and females (Portik et al. 2018), leading to a narrower range of adult body sizes in arboreal compared to terrestrially ovipositing hyperoliids. We found that adult ecology (e.g., arboreality, terrestriality) was unable to predict variation in male body size, female body size, or SSD in this family (Table 2). However, not all arboreal hyperoliids share the same reproductive characteristics. For example, these species use a variety of aquatic and arboreal sites for egg deposition, some of which are similar to terrestrial hyperoliids. It is possible that

oviposition site or other reproductive characteristics may be stronger predictors of sex-specific body sizes, and future analyses may help clarify these relationships.

Hyperoliids are unique among anurans in having a high prevalence of sexually dichromatic species (Bell and Zamudio 2012; Portik et al. 2019). Sexual dichromatism in hyperoliids may be involved in crypsis, sexual signaling, or intersexual niche partitioning, and is likely shaped by natural and sexual selection (Hayes 1997; Bell and Zamudio 2012; Bell et al. 2017b; Portik et al. 2019). Previous work demonstrated that a community of dichromatic hyperoliids showed greater variation in interspecific male body size versus female size (Portik et al. 2018), suggesting sexually dichromatic species might display a scaling pattern consistent with Rensch's rule. However, we found evidence for an isometric scaling relationship in both dichromatic and monochromatic species (Figure 4, Table 3). One possible explanation for these apparently contradictory results is that male body size is strongly partitioned at the community level and related to interspecific niche partitioning (Portik et al. 2018), but that this signal is eroded at the family level. We also did not find any significant differences in sex-specific body sizes or SSD values between dichromatic and monochromatic species (Table 2), and both groups display a similar SSDi average and range. Together, these results indicate that patterns of SSD are not distinct in sexually dichromatic lineages, and that sexual dichromatism does not have a consistent or predictable effect on body size evolution.

Protogynous sex change occurs when sexually mature females transform into reproductively active secondary males, and in anurans this phenomenon is only known to occur in the *H. viridiflavus*

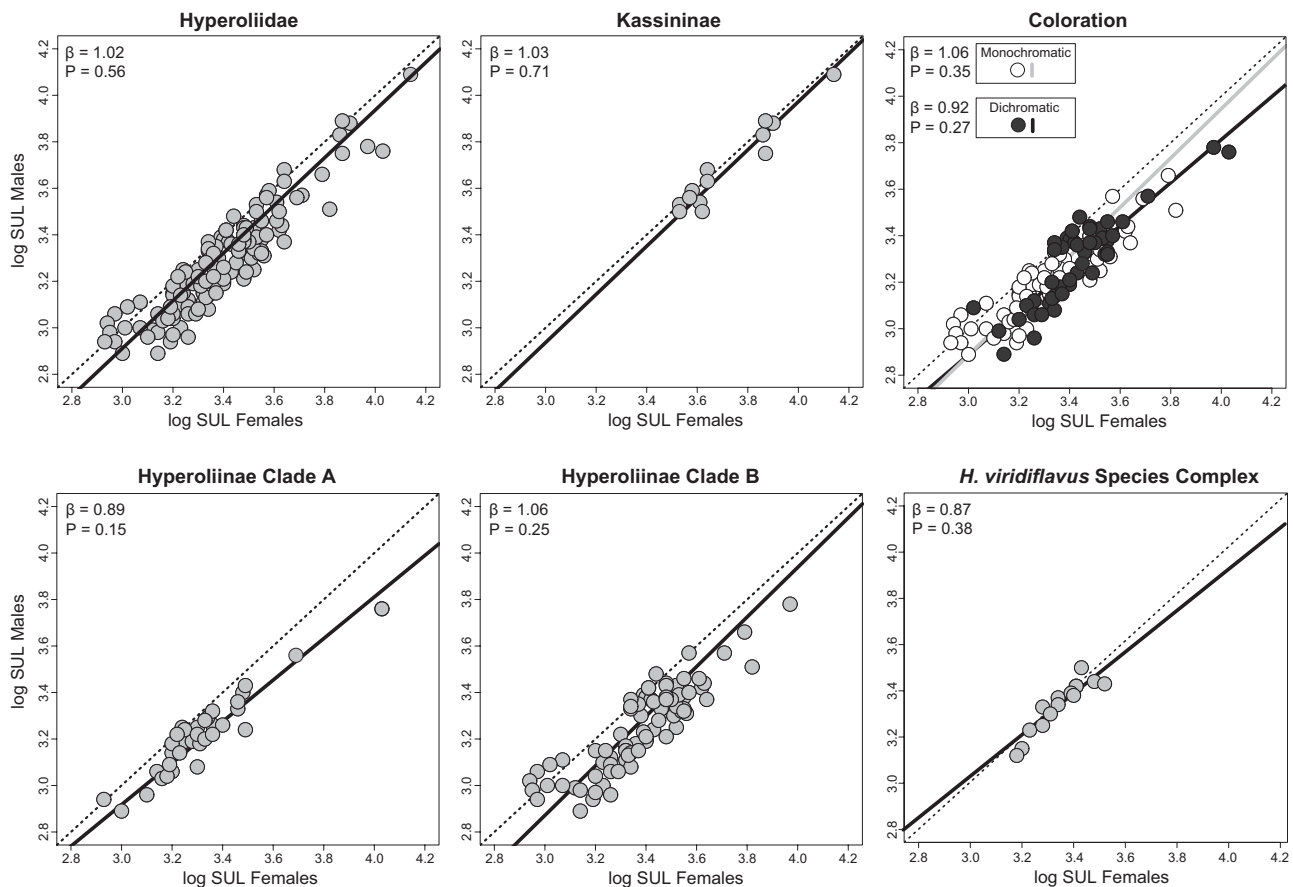


Figure 4. Plots of the phylogenetic reduced major axis (pRMA) regressions performed using log male and log female body size for various phylogenetic and trait-based groupings. All plots show pRMA regression lines, the slope (β), and corresponding P -value indicating if the slope is significantly different from one. In each plot, the dotted line has a slope of one and represents an isometric relationship of equally sized males and females (e.g., sexual size monomorphism). Areas above this dotted line represent male-biased SSD, and areas below represent female-biased SSD.

Table 3. Summary of phylogenetic reduced major axis regressions conducted on various groupings

Group	df	Slope	r^2	P -value
Hyperoliidae	95.1	1.02	0.76	0.56
Hyperoliinae Clade B	60.9	1.06	0.74	0.25
Hyperoliinae Clade A	24.8	0.89	0.8	0.15
Kassiniinae	9.5	1.03	0.89	0.71
Hyperoliinae: Dichromatism	35.1	0.92	0.77	0.27
Hyperoliinae: Monochromatism	51.6	1.06	0.74	0.35
<i>Hyperolius viridiflavus</i> complex	11.5	0.87	0.73	0.38

P -values indicate whether the RMA slope is significantly different from 1.0 ($P < 0.05$).

species complex (Grafe and Linsenmair 1989). In their study, Grafe and Linsenmair (1989) proposed protogyny could be advantageous in hyperoliids if differential male reproductive success is age or size-specific (e.g., the size-advantage hypothesis; Ghiselin 1969, 2006), or mortality is sex-dependent. This proposal was likely influenced by contemporary studies of protogyny in fish. Many of these fish species display a clear male size advantage, in which females transform into large males that monopolize matings in harem social systems (Warner 1988; Ross 1990; Lutnesky 1994; Avise and Mank 2008). In the context of hyperoliids, protogynous sex change in a species with female-biased SSD would result in secondary males (e.g., the transformed females) that are larger than primary males.

Grafe and Linsenmair (1989) suggested that these larger secondary males should have a competitive advantage over the primary males (through combat, call quality, or other attributes), and by extension that protogyny may be advantageous for species with female-biased SSD. However, we predicted that protogynous sex change would be correlated with a reduction in SSD. We derived this prediction from several key observations. First, in *H. viridiflavus ommatostictus* and *H. marmoratus taeniatus*, there are no significant differences in sex-specific body sizes (Grafe and Linsenmair 1989). Second, despite the presence of territoriality and combat in many hyperoliids, large-male advantage in mating success has not been documented in any species (Passmore and Telford 1983; Telford and Dyson 1988;

Dyson et al. 1998). Third, despite evidence that females of some hyperoliid species prefer lower frequency calls associated with larger males (Dyson and Passmore 1988; Jennions et al. 1995), this preference breaks down in bigger choruses (Bishop et al. 1995). Given there is no clear male size advantage in hyperoliids, protogynous sex change may not provide a strong benefit in the context of female-biased SSD. In fact, we believe that protogynous sex change could be disadvantageous for species with female-biased SSD. Hyperoliid females have a high ability to discriminate between conspecific and heterospecific calls (Telford and Passmore 1981), and in hyperoliids male body size is correlated with peak call frequency (Gilbert and Bell 2018). For species with pronounced female-biased SSD, if the larger secondary males produce calls outside the typical frequency range of smaller primary males, they may not be recognized as conspecifics. Therefore, based on these observations, we predicted that protogynous sex change would be associated with reduced female-biased SSD or sexual size monomorphism. Our results strongly support this hypothesis. We detected a significant relationship between protogynous sex change and SSD and found that sex change is associated with a significantly lower mean SSDi that approaches SSM (Table 2). Compared to all other groups, the *H. viridiflavus* complex also contains the highest proportion of species that display SSM. Similar to all other groupings tested, we found an isometric scaling relationship for the *H. viridiflavus* complex. The isometric relationship is not unique to the *H. viridiflavus* complex, but it is important in the context of sex change because as species change size, reduced SSD or SSM is maintained. The link between protogynous sex change and SSM is therefore supported conceptually by hyperoliid reproductive biology and empirically by our study. We acknowledge that our analyses of protogynous sex change rely on the assumption that all lineages of the *H. viridiflavus* complex display this trait, and that this requires validation. Regardless, based on all available evidence, we tentatively propose that protogynous sex change is a novel mechanism influencing the evolution of SSD within hyperoliids.

Conclusion

Anurans are a species-rich group that displays incredible variation in ecology, morphology, and life history. Consequently, anurans offer opportunities to study the relationship between phenotypic traits and the evolution of SSD. However, SSD has only been explored in a small fraction of anurans (~850 of ~7000 species), and the primary mechanisms driving macroevolutionary patterns of SSD remain unresolved. Here, we generated new body size data to study the evolution of SSD in ~140 species of hyperoliid frogs. We did not find evidence for allometric scaling relationships in hyperoliids, such as Rensch's rule or its converse. We also found that adult ecology (arboreality vs. terrestriality) and sexual dichromatism did not predict variation in body size or SSD. However, we found tentative support for an association between protogynous sex change and sexual size monomorphism in hyperoliids. Though this trait may be driving body size evolution in hyperoliids, protogynous sex change is rare among anurans and is unlikely to be a widespread mechanism. Moving forward, we emphasize that newly generated data from densely sampled taxa are required to improve our understanding of anuran SSD. Future studies of SSD should move beyond testing classical hypotheses (such as fecundity and male combat), and should ideally investigate a variety of traits (both general and clade-specific). We propose that reproductive traits, including oviposition site, may provide the strongest insights into the evolution of sex-specific body size and SSD in anurans.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

Supplementary Figure 1. Chronograms of family Hyperoliidae displaying maximum likelihood reconstructions of the log SUL of males (left) and females (right) for each species, with red representing smaller body size, yellow representing intermediate size, and blue representing larger body size.

Supplementary Table 1. Summary of specimen counts, body size measurements (in mm), sexual size dimorphism indices, and permutation test results for 142 hyperoliid species for which at least one male and female size measurement was available.

Supplementary Appendix 1. Empirical and published measurements of SUL and associated data (species, museum catalogue number, country, and sex) for all species included in this study.

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Data Availability

The Python program for performing all calculations of SSDi, pairwise SSDi, and permutation tests (*SSDi-Calculator.py*) is freely available from: <https://github.com/dportik/SSDi-Calculator>. The R script, tree file, and data files required to run all comparative phylogenetic analyses are deposited on DRYAD (doi:10.5061/dryad.t76hdx7z6) and on github: <https://github.com/dportik/frog-body-size-evolution>.

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