Sexual size dimorphism, reproductive ecology and antipredator coloration in poison frogs: Rensch's rule revisited

Dimorfismo sexual en tamaño, ecología reproductiva y coloración antidepredatoria en ranas venenosas: re-evaluando la regla de Rensch

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A short informative title: Rensch’s rule in poison frogs

Teaser text:

Rensch’s rule (RR) states that sexual size dimorphism (SSD) is larger in species with male-biased SSD and smaller in species with female-biased SSD. In amphibians, assessments of RR have found no consensus in favour or against its validity. Using poison frogs (Dendrobatoidea), a group with diverse habitats, reproductive ecology and antipredator coloration, we found that general patterns of SSD do not follow RR, but the type of tadpole-deposition site used shapes SSD in a way opposite to RR, and aposematism, likely through decreased predation pressure, favours the evolution of larger body sizes and less pronounced (or absent) SSD.

Abstract

Body size differences are the most commonly studied form of sexual dimorphism. The extent of sexual size dimorphism (SSD) varies between species and in function of e.g. ecological factors and phylogenetic constraints. Rensch's rule (RR) states that SSD is larger in species with male-biased SSD and smaller in species with female-biased SSD. RR assessments in amphibians, which tend to have female-biased SSD, have found no consensus in favour or against its validity. Using poison frogs, a group with diverse reproductive ecology and antipredator coloration, we test how the rates of evolution of male and female body size vary over evolutionary time, and investigate whether SSD follows RR and is influenced by antipredator coloration and tadpole-deposition site types. We show that SSD in poison frogs does not follow RR in general, but the tadpole-deposition site type used shapes SSD in a way opposite to RR, likely because fecundity selection favours larger females and clutches. Aposematism, likely through decreased predation pressure on both sexes, favours the evolution of larger body sizes and less pronounced (or absent) SSD. We underscore the importance of integrating ecologically relevant traits into SSD studies to better understand the selective pressures underlying sex size differences in nature.

Keywords : anurans, ecogeographical rule, fecundity selection, parental care, tadpole-deposition sites

Resumen

Las diferencias en tamaño son la forma más estudiada de dimorfismo sexual. El grado de SSD varía entre especies y en función de, por ejemplo, factores ecológicos y restricciones filogenéticas. La regla de Rensch (RR) establece que el SSD es mayor en especies con SSD sesgado hacia los machos y menor en especies con SSD sesgado hacia las hembras. Las evaluaciones de la RR en anfibios, un taxón que suele presentar un SSD sesgado hacia las hembras, no han encontrado consenso a favor o en contra de su validez. Utilizando ranas venenosas, un grupo con hábitats, ecología reproductiva y coloración antipredatoria diversos, analizamos cómo varían las tasas de evolución del tamaño corporal de machos y hembras a lo largo del tiempo evolutivo, e investigamos si el SSD sigue la RR y está influenciado por sus tipos de coloración antipredatoria y el lugar de deposición de los renacuajos. Demostramos que los patrones generales de SSD en ranas venenosas no siguen la RR, pero que el tipo de lugar de deposición de los renacuajos determina un SSD de forma opuesta a la RR, probablemente porque la selección por fecundidad favorece a las hembras más grandes y las puestas de mayor tamaño. Subrayamos la importancia de estudiar la evolución del SSD a la luz de rasgos ecológicamente relevantes para comprender mejor las presiones selectivas que determinan las diferencias de tamaño entre los sexos.

Palabras clave : *cuidado parental, sitios de deposición de renacuajos, anuros, hábitat, selección por fecundidad*

Introduction

In multiple species across diverse taxa, males differ from females in more than their genetic makeup and reproductive capabilities (Mori et al. 2022). Differences in behaviour, coloration, shape and life history have intrigued naturalists for centuries. For instance, in many species of birds and fish, males and females have striking differences in their plumage and nuptial coloration, respectively (Badyaev & Hill 2003; Kodric-Brown 1998; Maan & Sefc 2013). Likewise, males, but not females, display distinct cranial ornaments in ungulates (Lincoln 1992), coloured dewlaps in lizards (Losos 2009), and vocal sacs in anurans (Elias-Costa & Faivovich 2025). However, differences in size between the two sexes are the most widespread and commonly studied case of sexual dimorphism (Clutton-Brock & Harvey 1977; Fairbairn et al. 2007; Caron & Pie 2024; Slavenko et al 2024; Bakonyi & Seres 2025).

Male birds and mammals frequently exhibit male-biased sexual size dimorphism (SSD), commonly interpreted as the outcome of strong (intra) sexual selection favouring larger males through increased male–male competition for access to mates and other resources (Andersson 1994; Fairbairn et al. 2007). In ectotherms, in contrast, females are generally larger than males, which has been commonly attributed to strong fecundity selection (Shine 1989; Pincheira-Donoso & Hunt 2017). In these organisms, larger females typically produce more or larger eggs, leading to a positive relationship between female body size and reproductive output that favours the evolution of female-biased SSD (Shine 1979; Andersson 1994; Blanckenhorn 2005). However, the extent of such differences in size is not constant, and several hypotheses have been put forward to explain the variation in SSD across species. For example, differences in the intensity of sexual selection among lineages: strong male–male competition may favour larger males in territorial or polygynous species but not necessarily in monogamous species or those exhibiting scramble and lek-like systems (Emlen & Oring 1977; Wells 2007). Ecological factors such as habitat use and dietary specialization may also contribute to SSD by promoting niche divergence between males and females and reducing intersexual competition for resources (Shine 1989; Agha et al. 2018). Furthermore, phylogenetic constraints and evolutionary history can influence SSD patterns, producing clade-specific trajectories in body size evolution (Abouheif & Fairbairn 1997; Fairbairn et al. 2007; Agha et al. 2018).

An influential macroevolutionary pattern proposed to explain interspecific variation in SSD is the Rensch's rule, which describes a systematic scaling relationship between male and female body size across species (Rensch 1950). According to the Rensch's rule, species with male-biased size dimorphism have increased SSD (hyperallometry), whereas this is low (hypoallometry) in species where size dimorphism is biased towards females (Abouheif & Fairbairn 1997). The validity of this rule has been investigated across a wide range of animal taxa with mixed support. The predicted allometric pattern has been documented in several groups with male-biased SSD, including birds, mammals and arthropods (Fairbairn 1997; Blanckenhorn et al. 2007; Webb & Freckleton 2007). These studies support the hypothesis that stronger sexual selection on males may drive the evolution of size differences between the sexes. However, numerous studies with reptiles and insects have failed to detect consistent support for the Rensch's rule (Abouheif & Fairbairn 1997; Guillermo-Ferreira et al. 2014; Burbrink & Futterman 2019). These contrasting results indicate that Rensch's rule is not a universal macroevolutionary pattern and that the scaling of SSD may depend on lineage-specific selective pressures and evolutionary trajectories.

Therefore, for a more comprehensive understanding of SSD and the Rensch’s rule, more studies are needed involving taxa with diverse life histories and ecological contexts (Toyama 2025).

In amphibians, females are often larger than males and this has been mostly explained by fecundity selection (Monnet & Cherry 2002; Kupfer 2007). Body size differences appear to be more pronounced in species with aquatic than terrestrial reproduction because of the stronger relationship between female body size and clutch size in the former (SIlva et al. 2020). In addition to fecundity selection, sex-specific differences in mortality can influence the evolution of SSD in anurans: higher mortality in one sex (males) may lead to alterations of age structure and growth trajectories, potentially limiting the evolution of a larger body size through reduced longevity and growth opportunities (Halliday & Verrell 1988; Monnet & Cherry 2002; Vargas-Salinas 2006). In particular, differential survival associated with reproductive behaviour, such as prolonged male calling activity or mate searching, may generate sex-specific selective pressures that contribute to interspecific variation in SSD among frog species (Shine 1979; Halliday & Tejedo 1995; Wells 2007). Examinations of Rensch’s rule in amphibians are scant in comparison to other taxa, and the available evidence suggests that anurans generally do not conform to the predicted allometric patterns even after accounting for phylogenetic effects (Monnet & Cherry 2002; Liao et al. 2013; Zhang & Lu 2013; Nali et al. 2014). However, studies focusing on a single group representing a wide array of microhabitats, clutch sizes, offspring development sites and social behaviours, and involving species with both female-biased and male-biased size dimorphism are scarce despite their often paradigm-shifting nature. An example of such a group is the superfamily Dendrobatoidea (Grant et al. 2006; Frost 2026), commonly known as poison frogs, a species-rich clade of Neotropical frogs with contrasting antipredator strategies and breeding microhabitat preferences. This ecological diversity provides a unique system for examining the evolutionary scaling of body size between the sexes and its correlates.

Poison frogs comprise 355 species belonging to two families (Dendrobatidae and Aromobatidae; Grant et al. 2006; Frost 2026) with terrestrial reproduction and elaborate social and parental behaviours (Summers & Tumulty 2014; Carvajal-Castro et al. 2021; Vargas-Salinas & Rojas 2024). Most species exhibit some degree of territoriality, with males displaying aggressive behaviours towards intruders spanning from increased call rate to intense physical contests (Pröhl 2005; Betancourth-Cundar et al. 2024). Moreover, poison frog species range from cryptic, weakly defended taxa to highly conspicuous and chemically protected lineages (i.e., aposematic) (Summers & Clough 2001; Santos et al., 2003; Rojas 2017; Rojas & Vargas-Salinas 2024). Aposematism reduces predation risk by signaling unprofitability to visually oriented predators, thereby reshaping the adaptive landscape (Poulton 1890; Ruxton et al., 2018). In fact, comparative analyses indicate that, in comparison to cryptic lineages, aposematic lineages experience higher diversification rates (Santos et al. 2014), have higher metabolic rates (Santos et al. 2011) and more complex parental care behaviours (Carvajal-Castro et al. 2021). Therefore, by reducing predation-related constraints, aposematism may also influence SSD patterns in poison frogs. Given that fecundity selection typically favours larger females in anurans, whereas male-male competition and territoriality often select for larger males, shifts in the balance between these selective forces may alter the intraspecific and interspecific allometric scaling. If aposematism enhances disproportionately the survival chances of males, aposematic species should show (1) a stronger increase in male body size relative to female body size across species than cryptic species; and (2) scaling patterns consistent with Rensch's rule. Conversely, if fecundity selection remains dominant despite relaxed predation pressure on males, aposematic and non-aposematic species should not differ in their allometric slopes or depart from Rensch's rule. Thus, aposematic and cryptic poison frog species provide a natural experiment to test whether relaxed predator selection modulates the macroevolutionary scaling of sexual size dimorphism.

In this study, using poison frogs as a model system, (1) we test how the rates of evolution of male and female body size vary over evolutionary time. In addition, we investigate whether (2) SSD in poison frogs follows the Rensch's rule; (3) size variation in males and females is influenced by their type of antipredator coloration (cryptic, aposematic) and by the type of tadpole deposition site used (phytotelma, non-phytotelma); and (4) changes in female size and SSD over evolutionary time are associated with changes in clutch size (i.e., fecundity). Addressing these questions will further our understanding on how ecological traits and life-history strategies interact to shape the evolution of body size and SSD in vertebrates.

Materials and Methods

We obtained morphological, behavioural, and ecological information for poison frogs from Carvajal-Castro et al. (2021). Briefly, we collected the mean body size (snout–vent length, SVL) of males and females to calculate a SSD index (i.e., log Female body size / log Male body size). Additionally, for each species we obtained 1) the most common habitat in which animals live categorized as near-riparian / away from streams (i.e., terrestrial); 2) tadpole deposition sites classified as phytotelma / non-phytotelma; and 3) a score of warning coloration based on a system established by Santos et al. (2014) and Carvajal-Castro et al. (2021). Briefly, this system consists of adding up the chromatic contrasts of 11 non-overlapping body segments of the frogs, where a value of 1 was assigned to conspicuous colours and 0 to cryptic ones against a natural background (leaf litter). The final score yielded a continuous estimate of phenotypic visibility ranging from 0 (minimum contrast) to 11 (maximum contrast).

We used a time-calibrated phylogeny of poison frogs (Dendrobatoidea) for all comparative analyses (Jetz & Pyron 2018). First, we evaluated the phylogenetic signal for all variables to determine whether closely related species exhibited similar trait values due to shared ancestry (Pagel 1997a; Pagel 1999). To obtain the phylogenetic signal of the variables, we employed four different metrics: Pagel's λ (Pagel 1997), Blomberg's K (Blomberg et al. 2003), Abouheif's Cmean (Abouheif 1999), and Moran's I (Gittleman & Kot 1990), and compared and selected the best one using Akaike Information Criterion corrected (AICc). Models with the lowest AICc values were chosen as the best supported based on pairwise comparisons, such that ΔAICc values ≤ 2 (Burnham & Anderson 2001).

To test whether SSD in poison frogs follows Rensch's rule, we assessed the relationship between male and female body size using a phylogenetic reduction major axis (PRMA) regression (Revell 2012). For this approach, we used the function phyl.RMA in the *phytools* package (Revell 2012), with a method equal to "lambda" and adjusting this value based on phylogenetic signal results. The slope of the regression was compared against the null hypothesis of isometry (slope= 1). Under this framework, Rensch's rule is supported if the slope is significantly greater than 1; a slope lower than 1 indicates the opposite tendency to that of Rensch's rule (Fairbairn 1997; Abouheif &

Fairbairn 1997). To evaluate Rensch's rule across binary ecological and behavioural traits, species were first assigned to categories based on their ecological attributes, namely antipredator coloration (i.e., aposematic vs. non-aposematic), habitat (terrestrial vs. riparian), and type of tadpole-deposition site (phytotelmata vs. non-phytotelmata) and the PRMA test was run for each subgroup. Finally, we tested the correlation between mean clutch size and female body size with coloration as fixed effect using a phylogenetic generalized least square (PGLS) with the Caper package (Orme et al. 2013). This and all statistical analyses were done in R v4.4.3 (R Core Team 2025).

Because fecundity selection typically favours larger females, whereas territoriality and male-male competition often select for larger males, shifts in these lineage-specific selective pressures may decouple specific clades from ancestral allometric scaling. To address this, we incorporated an analysis of evolutionary regime shifts as a complementary approach. Rather than assuming that a single evolutionary rule explains the diversity in the whole dataset, detecting shifts in evolutionary optima allows us to identify whether discrete ecological or behavioural transitions have driven independent, localized changes in body size evolution. To detect evolutionary regime shifts in male body size, female body size, and SDD, we used the R package *l1ou* (Khabbazian et al. 2016). This analysis employs a lasso regression framework to fit and compare single- and multi-optimum Ornstein-Uhlenbeck (OU) models (Hansen 1997; Butler and King 2004), where shifts in trait values occur along the branches of a phylogeny directly from the data. We evaluated models with 0 to 20 shifts, ranking them using the phylogenetically corrected Bayesian information criterion. We also ran 1000 iterations to obtain 95% CI based on bootstrap values.

Results

We obtained information on body size for 210 species of poison frogs (i.e., ~ 66% of species in Dendrobatoidea). Males were larger than females in 14 species (6.6%) while the opposite was true in 193 species (92.8%). In three species (1.4%) size was not significantly different between the sexes. Male and female body size showed a strong phylogenetic signal (λ= 0.86, N= 210 spp, and λ= 0.90, N= 209 spp, respectively), whereas SSD showed a lower phylogenetic signal (λ =0.22, N= 200 spp) (Fig.1). Regardless, in all cases the tendency was highly significant ($P <0.001$). Phylogenetic signal values and AIC values supporting these results are shown in Tables S1 and S2. Differences in sample size between the sexes across analyses stem from data unavailability for one sex or both. However, the same tendency was maintained when we strictly reduced the sample size to species with data for both sexes.

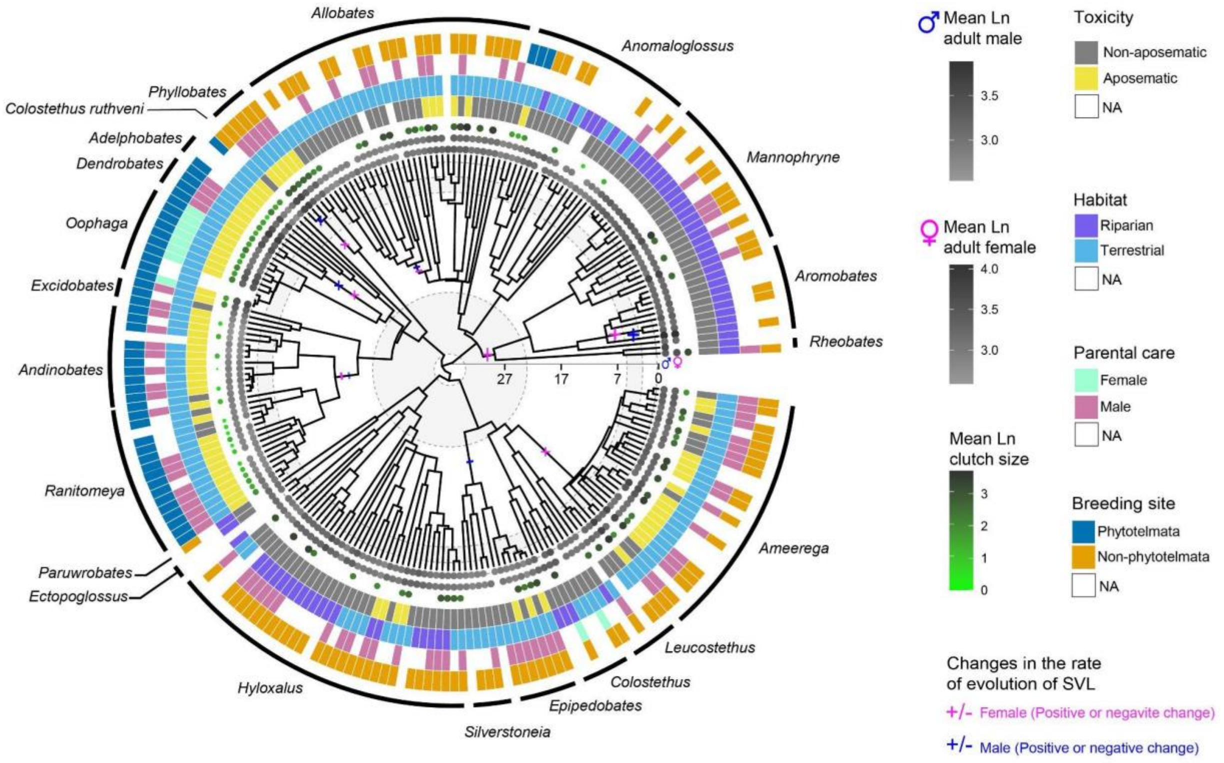

Figure 1. Phylogenetic hypothesis for 210 species of Dendrobatidae showing the distribution of body size (SVL), clutch size, and ecological traits across genera. Grayscale tip bars represent mean log-transformed adult male SVL (♂) and adult female SVL (♀), with darker shades indicating larger body sizes. Green bars denote mean log-transformed clutch size. Coloured columns to the right indicate categorical ecological variables: habitat type (purple = riparian, light blue = terrestrial), antipredator coloration (grey = non-aposematic, yellow = aposematic), parental care (aquamarine = male, dark pink = female), and tadpole deposition sites (dark blue = phytotelmata, orange = non-phytotelmata). Regime shifts in SVL are indicated on internal nodes: pink asterisks (♀) and blue asterisks (♂) denote significant rate shifts for females and males, respectively; "+" symbols indicate an increase in the regime rate of SVL and "−" symbols indicate a decrease in the regimen rate of SVL. The x-axis represents divergence time in millions of years (Ma).

Following the fitting and ranking of several Ornstein-Uhlenbeck (OU) and Brownian Motion (BM) models, our analysis revealed evidence for multiple evolutionary regime shifts in both male and female body sizes across the dendrobatid phylogeny. For males, the best-supported models (accounting for 42.2% of the pBIC weight) identified four strongly supported shifts in the evolutionary optima for body size. Those evolutionary shifts toward increased male body size occurred in a subclade of the genus *Dendrobates*, as well as in *Aromobates nocturnus*, *Oophaga histrionica*, *O. sylvatica*, *O. lehmani*, *Phyllobates bicolor*, and *P. terribilis*. Conversely, there were evolutionary shifts toward decreased male body size in the genus *Andinobates*, *Anomaloglossus*, *Epipedobates, Ranitomeya*, *Silverstoneia*, and a subclade of *Allobates*. For females, the analysis indicated greater lability in body size compared to males. The best-supported models (accounting for 41.3% of the pBIC weight) suggested ten evolutionary shifts across the phylogeny. Those evolutionary shifts toward increased female body size occurred in a subclade of *Aromobates, Rheobates*, *Manophryne*, *Phyllobates*, *Dendrobates* and *Ameerega*. There were evolutionary shifts toward decreased body size in *Andinobates* and *Ranitomeya*, as well as in a subclade of *Allobates* (Fig. 1).

After applying a phylogenetic reduced major axis regression (PRMA), we did not find support for Rensch's rule in poison frogs, as the scaling relationship between male and female body size did not differ from isometry (H_0: $\beta = 1$; $t = 1.24$, $P = 0.215$). Similar results were observed when we split up the species according to their habitat (Terrestrial: $t = 0.312$, $P = 0.755$; Riparian: $t = 0.660$, $P = 0.512$; Fig. 2A). Regarding antipredator coloration, there was a biologically relevant, marginally significant trend for SSD to be larger as female body size increases (i.e., opposite to Rensch's rule) in non-aposematic species (aposematic: $t = 0.209$, $P = 0.834$; non-aposematic: $t = 1.779$, $P = 0.078$; Fig. 2B). When we split the species according to their tadpole-deposition site, we found that the relationship between the body sizes of males and females of species using phytotelmata did not deviate from isometry ($t = 0.193$, $P = 0.847$; Fig. 2C). However, body size differences between the sexes in species which use tadpole-deposition sites other than phytotelmata were consistent with the inverse of Rensch's rule ($t = 2.146$, $P = 0.035$; Fig. 2A; Table 1). In other words, SSD increased with an increase in female size, which was larger than male size.

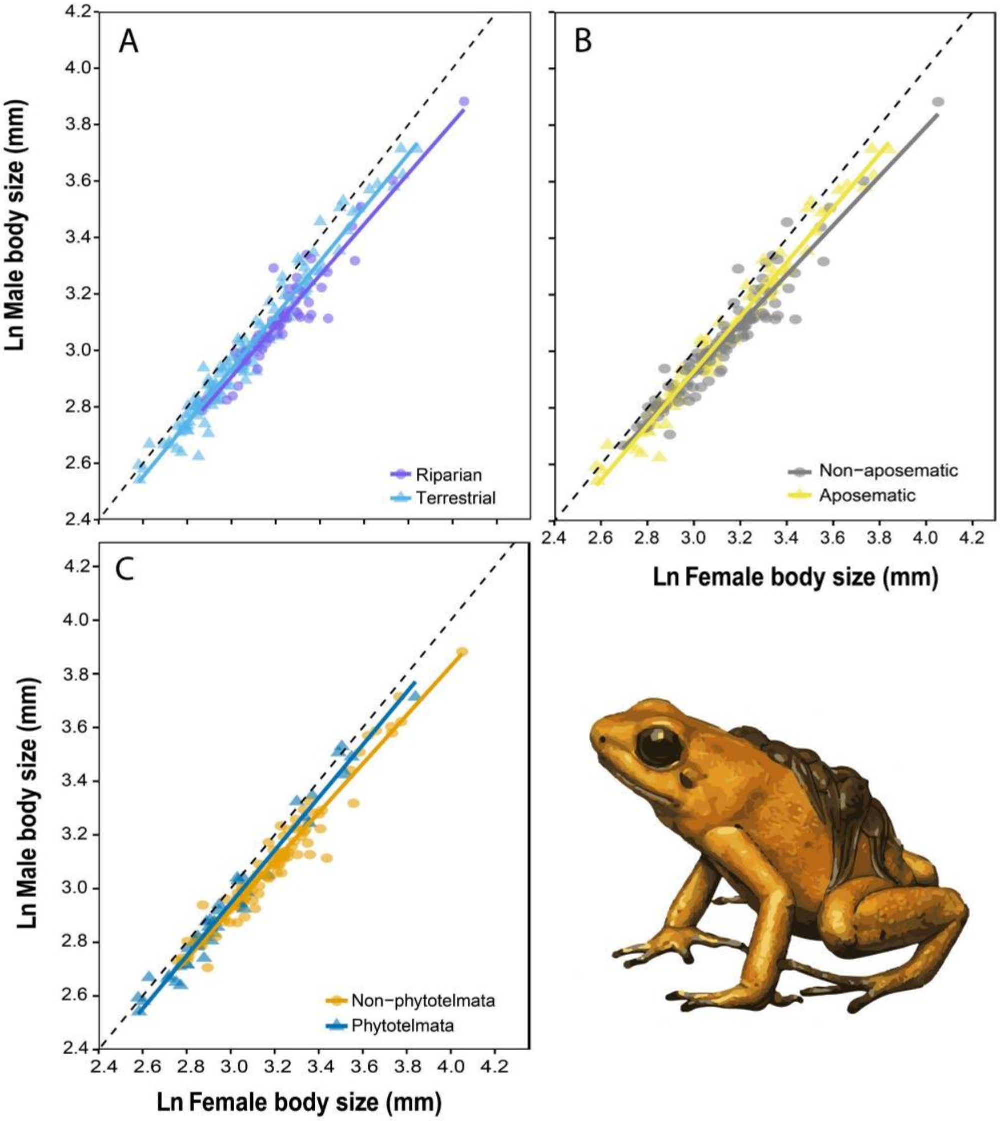

Figure 2. Allometric relationship between males and female body size in dendrobatid frogs in relation to (A) habitat (i.e., Riparian vs. Terrestrial); (B) antipredator coloration (i.e., aposematic vs. non-aposematic); and (C) tadpole-deposition sites (i.e., phytotelmata vs. non-phytotelmata). Dashed lines indicate isometry (slope = 1).

Our analysis also revealed a consistent positive relationship between female body size and reproductive output across all studied lineages (β =1.017, t = 4.20; P-value < 0.001, N = 100 spp).

Aposematic and non-aposematic species exhibited a similar increase in clutch size with increasing female body size, but we found a tendency for a higher increase in aposematic species, as indicated by a marginally significant interaction ($\beta = 0.878$, $t = 1.754$; P-value = 0.083, N = 100 spp) (Fig.3 and Table S2).

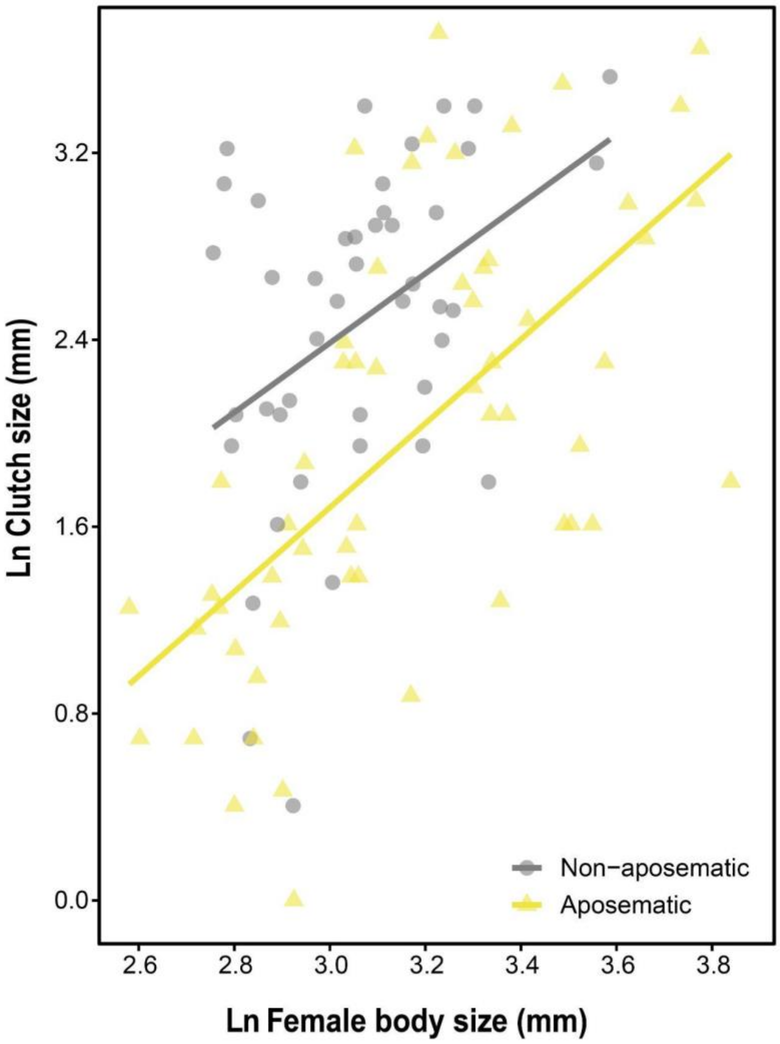

Figure 3. Relationship between female body size and clutch size (mean number of eggs per clutch) in dendrobatid frogs in relation to their antipredator coloration (Aposematic, Non-aposematic) based on a coloration score scale proposed by Santos et al. (2014) and Carvajal-Castro et al. (2021).

Table 1. Results of the phylogenetic reduced major axis (PRMA) regression analysis testing Rensch's rule in poison frog species and subgroups defined by antipredator defensive strategy, type of habitat, and tadpole-deposition sites. Note that the number of species (N) differs between analyses. The regression slope (β), the associated *P*-value and *t*-value indicate results of the test for the null hypothesis of isometry (β = 1).

Level of analysis		N	β	P-value	t value
Full (All species)		200	0.976	0.215	1.244
Antipredator coloration	Aposematic	73	0.995	0.834	0.209
	Non-aposematic	122	0.947	0.078	1.779
Habitat	Riparian	60	0.970	0.512	0.660
	Terrestrial	138	0.994	0.755	0.312
Tadpole deposition sites	Phytotelmata	44	0.994	0.847	0.193
	Non-phytotelmata	109	0.952	0.035	2.146

Discussion

Because of their highly diverse colour patterns and the elaborate ways in which they look after their offspring, poison frogs have slowly become model systems for the study of the function and evolution of antipredator coloration and parental care (Rojas & Vargas-Salinas 2024; Vargas-

Salinas & Rojas 2024). This has, in turn, revealed how natural and sexual selection interact to shape some of their most emblematic traits. Here, using phylogenetic comparative analyses, we investigate whether and how the presumed relaxed selection conferred by aposematic coloration alters the macroevolutionary scaling of sexual size dimorphism (SSD) in poison frog species, and estimated the rates of body size evolution of males and females over time, paying special attention to when and in which clades shifts in body size between the sexes occurred.

In agreement with previous studies in anurans (Liao et al. 2013; Dugo-Cota et al. 2022), our results do not support Rensch's rule at the family level, as the allometric relationship between male and female body size did not deviate significantly from isometry. Fecundity selection on females, which is particularly strong in ectotherms, has been proposed to counterbalance sexual selection favouring larger male size, resulting in a near-isometric relationship between the sexes that departs from the expectations of Rensch’s rule (Pincheira-Donoso & Treguenza 2011; Liao et al. 2013). In dendrobatids, this pattern may be further reinforced by aspects of their reproductive biology, i.e. fecundity advantages for larger females in both aposematic and non-aposematic species, as larger females produce on average larger clutches. This pattern has been commonly reported in anurans (Shine 1979; Han & Fu 2013). In contrast, sexual selection in many poison frog species appears to operate primarily through male behavioural and acoustic traits rather than through male body size. Males typically defend territories and attract females using advertisement calls; therefore, their reproductive success may depend on body size, but also on call features and chorus tenure, visual signalling, and territory quality rather than only on size-related male–male competition (Zimmermann 1990; Pröhl 2005; 2012; Forsman & Hagman 2006; Maan and Cummings 2009; Brown et al. 2010; Souza et al. 2021). Additionally, the widespread occurrence of paternal care behaviours, such as clutch attendance and tadpole transport (Ringler et al. 2013; Rojas & Pašukonis 2019; Carvajal-Castro et al. 2021; Schulte & Summers 2022), may further reduce the strength of directional selection for increased male size. Together, these factors may constrain the evolution of pronounced allometric divergence in body size between the sexes in this group, producing patterns of SSD closer to isometry and therefore inconsistent with Rensch's rule.

A closer look into the implications of these results taking into account differences in key ecological traits, reveals that the overall isometric pattern masks biologically meaningful variation tied to reproductive and antipredator ecology. One of the most compelling results to emerge from these subgroup analyses is the contrasting allometric pattern between species that deposit their tadpoles in phytotelmata versus those that do not. In species that use phytotelmata, which are typically small and resource-limited microhabitats (Schlippe Justicia et al. 2025), SSD was isometric. This might be the case because depositing tadpoles in such microhabitats commonly involves the transport of one or few tadpoles at a time and, in some cases, provisioning with trophic eggs, resulting in small clutch sizes and high per-offspring female investment (Summers and McKeon 2004; Carvajal-Castro et al., 2021). This high female investment, in turn, likely weakens selection on female body size and the related fecundity, thereby restricting the evolution of female-biased SSD and producing isometric scaling between the sexes. By contrast, species that use other tadpole deposition sites (i.e. non-phytotelmata), such as ponds or streams, show an SSD pattern inverse to Rensch's rule (i.e., SSD increases with female body size). This pattern could arise because of a strong selection on female body size and fecundity given that such microhabitats can sustain larger clutches and female parental investment per offspring is on average lower than in phytotelm-breeding species (Summers and McKeon 2004; Wells 2007). These ideas are consistent with (1) a recent comparative analysis (Silva et al. 2020) indicating that the oviposition site imposes restrictions on female size and reproductive output in frogs; and (2) our results, in which for a similar body size, females of non-aposematic species, many of them non-phytotelm-breeders, produce on average larger clutches than aposematic species, which tend to transport tadpoles to phytotelmata (Caldwell & Araújo 1998; Carvajal-Castro et al. 2021).

We found a tendency, though statistically marginal, for SSD in non-aposematic species to follow a pattern opposite to Rensch's rule. SSD in aposematic species, by contrast, did not differ from isometry. This divergence in SSD patterns may be explained by differences in the balance between natural and sexual selection mediated by coloration and associated life-history strategies. Aposematism is linked to reduction in predation risk and potentially relaxing constraints in aspects such as movement, mate searching, resource exploitation and parental care (Rojas et al. 2026), all of which have been demonstrated for poison frogs (Santos et al. 2014; Carvajal-Castro et al. 2021; Pašukonis et al. 2022). Under these conditions, both sexes may experience similar selective pressures on body size, leading to more isometric scaling. Additionally, aposematic coloration can also function as a sexual signal in mate choice (Maan and Cummings 2009; Rojas et al. 2018; Hsu et al., 2021), which may reduce the relative importance of body size as a target of sexual selection and further constrain divergence in SSD. By contrast, non-aposematic species are likely subject to stronger predation pressures limiting the evolution of larger body sizes; this is because larger cryptic prey are usually easier to detect by predators (Karpestam et al. 2014). Therefore, in non-aposematic species, fecundity selection on females may become a dominant force shaping SSD (but see Monroe et al. 2015 for cautions about considering the positive relationship between female body size and clutch size as enough evidence of fecundity selection driving the evolution of female-biased SSD in frogs). Although not statistically significant, our results suggest that the evolution of aposematism may modulate the relative strength of fecundity and sexual selection, thereby influencing the scaling relationship between male and female body size in dendrobatids.

In most species of poison frogs parental care is provided by males, although females may occasionally attend the clutches and shuttle and/or feed the tadpoles (Weygoldt 1987; Summers and Tumulty 2014). The number of species exhibiting primarily maternal care in our database was relatively small (up to 13 species), preventing the formation of a statistically robust category to be analysed independently without reducing the statistical power and reliability of our comparative analyses. Consequently, we did not explicitly test whether patterns of SSD differ between species with male versus female parental care. Despite this limitation, the caregiving sex has strong theoretical relevance for understanding the evolution of SSD and compliance with Rensch's rule in the studied system. This is because parental care is a major life-history trait that has repeatedly shaped reproductive strategies and macroevolutionary diversification across amphibians (Furness et al. 2015; Vági et al. 2020). Accordingly, differences in reproductive investment between the sexes influence the strength and direction of sexual selection, with the more investing sex expected to be more selective in mate choice and the less investing sex experiencing stronger intraspecific competition for reproductive opportunities (Trivers 1972). These intersexual differences can ultimately shape body size evolution through their effects on the interplay between sexual selection and fecundity selection. Comparative studies across anurans have shown that parental care is generally associated with reduced SSD, a pattern interpreted as evidence that the increased offspring survival resulting from parental care may relax fecundity selection on female body size (Shine 1979; Han and Fu 2013; Dugo-Cota et al. 2022). Current evidence does not allow for robust predictions on whether maternal care should promote stronger, weaker, or qualitatively different patterns of dimorphism than paternal care in poison frogs. However, empirical evidence in Caribbean *Eleutheroactylus* species (frogs with direct development) suggests that paternal care tends to reduce female-biased SSD by promoting larger male body size (Dugo-Cota et al. 2022). Clearly, determining how parental care influences body size evolution and patterns of SSD in anurans warrants further investigation.

Body size appears to have evolved more rapidly and experienced shifts more frequently in females than in males. Interestingly, shifts towards increased male body size tended to occur mostly in aposematic clades. Previous research has shown that small size is favoured by selection in cryptic species because larger prey are easier to detect by predators (Karpestam et al. 2014; Yu et al. 2024). In fact, both an association between conspicuousness and body size (Hagman & Forsman 2003), and the loss of conspicuousness with decreasing body size (Rudh 2013) have been previously reported in poison frogs, providing support to our findings. In both sexes, shifts towards smaller body size were found, among others, in clades which are predominantly aposematic, arboreal and which use phytotelmata in bromeliad axils as tadpole-deposition sites, such as *Ranitomeya* and *Andinobates*. These patterns are likely supported by the lower energetic costs of climbing incurred by smaller species (Ruxton & Wilkinson 2014) and the increased metabolic rates of aposematic versus cryptic species (Santos et al. 2011).

Despite the insights our study provides, we also acknowledge limitations mostly related to the assignment of continuous traits to binary categories. Classifying antipredator coloration as aposematic vs. non-aposematic may have reduced our ability to detect finer-scale patterns in the relationship between coloration, body size evolution, and patterns of SSD. Likewise, the classification of tadpole breeding sites and habitat as phytotelmata vs. non-phytotelmata, and riparian vs. terrestrial, respectively, may obscure variation within each category that could influence the observed allometric trajectories. Future studies that incorporate these traits as continuous variables within phylogenetic comparative frameworks could provide a more detailed understanding of the selective pressures shaping SSD in poison frogs. In addition, our dataset does not account for intraspecific variation in body sizes and SSD. A recent study with the dyeing poison frog (*Dendrobates tinctorius*) revealed high levels of variation in body sizes and SSD across populations, such that some populations displayed isometry, whereas others had remarkable female-biased SSD despite a positive cross-population correlation between female size and clutch size (Schlippe Justicia et al. 2024).

In conclusion, our findings show that the general patterns of SSD in poison frogs do not follow Rensch's rule. Instead, certain ecological traits, particularly the type of tadpole-deposition site used, shape SSD in a way opposite to Rensch's rule, most likely because fecundity selection favours larger females; species with larger females, and therefore larger clutches, are more likely to be constrained to use larger waterbodies for tadpole deposition than the size-limited phytotelmata. Aposematism, likely through decreased predation pressure on both sexes, favours the evolution of larger body sizes and less pronounced (or nearly absent) SSD. We highlight the importance of studying SSD and its evolution in light of ecologically relevant characteristics to get a better understanding of how different selective pressures interact to shape the size differences between the sexes observed today.

Data availability

Data used can be found here (https://figshare.com/s/338fc287ef792d432bb8) and the R script used for analyses can be found here (https://doi.org/10.6084/m9.figshare.32680452)

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