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Correlated and decoupled evolution of adult and larval body size in frogs

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The majority of animal species have complex life cycles, in which larval stages may have very different morphologies and ecologies relative to adults. Anurans (frogs) provide a particularly striking example. However, the extent to which larval and adult morphologies (e.g. body size) are correlated among species has not been broadly tested in any major group. Recent studies have suggested that larval and adult morphology are evolutionarily decoupled in frogs, but focused within families and did not compare the evolution of body sizes. Here, we test for correlated evolution of adult and larval body size across 542 species from 42 families, including most families with a tadpole stage. We find strong phylogenetic signal in larval and adult body sizes, and find that both traits are significantly and positively related across frogs. However, this relationship varies dramatically among clades, from strongly positive to weakly negative. Furthermore, rates of evolution for both variables are largely decoupled among clades. Thus, some clades have high rates of adult body-size evolution but low rates in tadpole body size (and vice versa). Overall, we show for the first time that body sizes are generally related between adult and larval stages across a major group, even as evolutionary rates of larval and adult size are largely decoupled among species and clades.

1. Introduction

Complex life cycles (CLCs) are those in which larval and adult stages are distinct in their morphology, ecology, behaviour, and/or physiology [1–3]. Anuran amphibians (frogs) provide a striking example of these differences. In anurans, most species have tadpoles: larval forms that are generally aquatic, have a complex morphological system used to feed largely on plants and/or detritus, and which are (prior to metamorphosis) limbless with robust tails [4]. By contrast, adult frogs are typically land-dwelling and carnivorous, with a body plan characterized by powerful hindlimbs and no external tail [4].

CLCs may seem unusual, but they are actually the most common life-history strategy among animals. For example, CLCs dominate in the most species-rich insect orders (Coleoptera, Diptera, Hymenoptera, Lepidoptera; [5]). CLCs are estimated to be present in approximately 90% of arthropod species overall (with arthropods encompassing approx. 80% of animals [6]) and in the majority of species in many other species-rich phyla, such as Annelida, Bryozoa, Cnidaria, Mollusca, and Platyhelminthes (reviewed in [6]). Thus, understanding the evolution and consequences of CLCs is broadly important across animals.

A key question about the evolution of CLCs is the extent to which evolution in one life stage impacts the others. For example, the widespread occurrence and persistence of CLC may be explained by the 'adaptive decoupling hypothesis': CLCs can be adaptive through a process of 'decoupling' such that evolution in one life stage does not affect the fitness of other stages [1,3,7]. Adaptive decoupling is thought to lead to 'mosaic evolution', in which traits from different stages evolve independently of each other [1,8]. However, the

support for the adaptive decoupling hypothesis is somewhat mixed (recent review in [3]), with some studies of individual species showing decoupling among life stages [7,9,10] and others showing carryover effects across stages [11,12].

One way to address the extent of coupling between life stages is to take a macroevolutionary approach, and test for covariation and correlated evolution in adult and larval morphology among species. Relatively few studies have taken this approach. Nevertheless, two important macroevolutionary studies recently addressed this hypothesis in frogs. Wollenberg-Valero *et al.* [13] examined many morphological traits in both larval and adult stages among 113 species of mantellid frogs. They found that rates of change in these variables were uncorrelated among species, with higher rates of change in the tadpole stage. However, they did find that shifts in phenotypic optima for adult and larval morphology occurred at the same nodes in the phylogeny significantly more often than expected by chance. Sherratt *et al.* [8] examined 166 species of Australian frogs from two families (Hylidae, Myobatrachidae). They characterized species' morphologies using multivariate geometric morphometrics and found different patterns of evolution between life stages, including greater phylogenetic signal in adult than larval morphology. Neither study focused primarily on traits that were directly comparable between life stages (e.g. body size), and so they did not test to what extent the same trait covaried between larvae and adults among species across the tree. In a classic study, Werner [14] investigated relationships between size at metamorphosis and adult size among 68 North American frog species in three families, finding significant, positive relationships in hylids and ranids but not bufonids. However, that study did not incorporate phylogenetic methods. A more recent study [15] found that tadpole size predicted adult size among nine species of *Hyperolius*. To our knowledge, no studies have looked broadly across frogs to test for correlated or decoupled evolution between adult and larval stages. Moreover, such broad-scale tests seem to be lacking in other organisms with CLCs. Nevertheless, these previous studies in frogs identified at least three key macroevolutionary questions: (i) are morphological variables from adults and larvae positively related among species, and does this relationship vary among clades? (ii) Do adult and larval morphology show similar levels of phylogenetic signal? (iii) Do they evolve at similar rates?

Here, we test for correlated evolution between adult and larval stages across frogs. We focus specifically on body size. Body size is a crucial trait in general, and is broadly comparable between life stages. Furthermore, much of the important theory about the evolution of CLCs has focused on body size [2,14,16,17]. Anurans also show intriguing variation among species in their relationships between adult and larval body size. For example, adults are approximately 10 times larger than tadpoles in the bufonid toad, *Rhinella* (*Bufo) schneideri* [18], whereas the giant tadpole of the hylid frog *Pseudis paradoxa* is roughly four times the size of the adult [19].

In this study, we analyse data on matched adult and larval body sizes for 542 species spanning 42 families. We use phylogenetic methods to address the following questions: (i) are larval body sizes and adult body sizes (and the ratio between them) phylogenetically conserved? (ii) Do adult and larval body sizes covary across anuran phylogeny? (iii) Do different clades show different patterns of covariation between adult and larval body size? (iv) Are rates of change in body size among species correlated between the adult and larval stages? We would consider significant, positive relationships between adult and larval body sizes among species to reject the adaptive decoupling hypothesis at the macroevolutionary scale (but not necessarily at the microevolutionary scale). By contrast, weak or variable relationships would potentially support mosaic evolution of adult and larval morphology, as would different levels of phylogenetic signal and uncorrelated rates between adult and larval stages.

2. Material and methods

(a) Morphological and phylogenetic data

We searched the literature for descriptions and measurements of adults and tadpoles, focusing on species included in time-calibrated phylogenies based on sequence data (e.g. [20]). We used information compiled in AmphibiaWeb [18], but we also searched for data on individual species using Google Scholar and Web of Science. For each species, we recorded maximum tadpole total length (TL; anterior tip of snout to posterior tip of tail, in mm), and mean adult female snout-vent length (SVL). However, for simplicity, we refer to these as tadpole size and adult size. We also quantified the ratio between tadpole and adult size for each species (TA ratio: TL/SVL). We focused on female size only given that it may be more directly relevant to offspring size than male size. Moreover, by focusing only on adult size in one sex (e.g. instead of averaging values between sexes), we avoid potential problems associated with variation in sexual-size dimorphism across anuran species [21]. SVL is tightly related to body mass in frogs [22], and is a standard measure of their body size [4,14,18,21].

We used only the maximum tadpole size for each species, to account for variability due to ontogeny, plasticity, and other factors. For example, each individual tadpole initially increases in size after hatching, but may then decrease in TL immediately prior to metamorphosis as their tail is absorbed [23]. If several maximum lengths were recorded from different literature sources, we used the maximum of these maxima. We also recorded the Gosner [23] stage, if it was reported. To reflect the size prior to metamorphosis, we excluded measurements from individuals above Gosner stage 42, the stage at which tail atrophy begins. At the same time, we included only measurements from stage 32 (i.e. when toe indentation begins) or after, to reflect body size close to metamorphosis (e.g. to avoid individuals that were small simply because they were young). When the developmental stage was reported using an alternative staging system, we used a translation table [24] to assign a Gosner stage. When the developmental stage was not reported, we estimated Gosner stage using the descriptions and figures (when possible). However, we lacked data on Gosner stage for 128 species.

It is possible that our results could be influenced by including too broad of a range of developmental stages (i.e. individuals might have short TL because they were too young or had begun tail recession at later stages). Therefore, we performed a limited set of analyses that included only the 276 species with data from Gosner stages 35–39 (instead of 32–42). Species with unknown Gosner stages were also excluded. Results were very similar to those including all species (see below).

Overall, our sampling spanned 42 of 53 anuran families, with data for 542 species. Many families were excluded because all or most species had direct development (i.e. Brachycephalidae, Brevicipitidae, Ceuthomantidae, Craugastoridae, Eleutherodactylidae, Hemiphractidae, Strabomantidae; [18,25,26]). Species with direct development lack a free-living larval stage and so could not be included [25,26]. Other excluded families lacked adequate descriptions of larvae. However, most excluded families were not species

rich [18], and included only one small genus (Allophrynidae, Microxalidae, Odontobatrachidae), or two (Sooglossidae). Overall, our sample of species within each family was strongly related to the number of described species in each family ($r^2 = 0.80$, $p < 0.0001$; data in table 1), using richness data from AmphibiaWeb [18].

We initially used the time-calibrated anuran phylogeny from Pyron & Wiens [20]. This tree is fully resolved, is based on sequence data for all species, and includes all the species sampled here for adult and tadpole morphological data. We show this tree in full in electronic supplementary material, figure S1 (it is too large for the main text). To address the robustness of the results to alternative trees, we also conducted secondary analyses using a more recent phylogeny [27], as described below. However, this phylogeny [27] was unresolved in several families (based on a consensus tree), even for some species that were resolved in the initial tree [20]. Therefore, we primarily used this initial tree [20]. Both trees included all 542 species with morphological data (and include thousands of species in total).

The species-level data are provided in electronic supplementary material, dataset S1, along with literature sources. The phylogeny is provided in electronic supplementary material, dataset S2 (and electronic supplementary material, figure S1). The number of sampled species and the means and ranges of size metrics among species for each family are summarized in table 1.

Tadpole TL incorporates both the tail and the rest of the body. It is possible to use tadpole SVL instead of TL. However, we were interested in the overall size of tadpoles, including the tail. Moreover, data on tadpole SVL were available for fewer species than TL. Therefore, we used a measurement (TL) that allowed us to maximize species sampling. Nevertheless, we did obtain data on maximum tadpole SVL for a set of 151 species (electronic supplementary material, dataset S3) that were also in our main dataset (electronic supplementary material, dataset S1), using the same selection criteria and sources listed in electronic supplementary material, dataset S1. Using the main tree, phylogenetic regression, and ln-transformed variables (see below), we found that: (i) tadpole SVL (dependent variable) and TL (independent) were strongly related ($r^2 = 0.7799$; $p < 0.0001$), (ii) tadpole SVL (dependent) and adult SVL (independent) were significantly but weakly related ($r^2 = 0.1383$; $p < 0.0001$), similar to the relationship between tadpole TL and adult SVL among all 542 species ($r^2 = 0.1692$, $p < 0.0001$), and (iii) tadpole TL (dependent) and adult SVL (independent) showed a relationship for these 151 species ($r^2 = 0.1510$; $p < 0.0001$) similar to that for all 542 species. These analyses strongly suggest that tadpole TL and SVL are closely related overall, and that using tadpole SVL instead of TL would not overturn our main conclusions.

(b) Statistical analyses

All statistical analyses were conducted using R v. 3.5.3 [28]. We first evaluated the strength of the phylogenetic signal for tadpole size, adult size, and TA ratio. We also evaluated the best-fitting likelihood model for the evolution of total tadpole TL, adult SVL, and TA ratio with the function 'fitContinuous' in the R package *geiger* v. 2.0.6.2 [29]. We compared the fit of Brownian motion (BM), estimated lambda (EL) [30], Ornstein–Uhlenbeck (OU), and white noise (non-phylogenetic) models. The best-fitting model was considered to be the one with the lowest value of the sample-size corrected Akaike information criterion (AICc) [31].

To determine whether larval and adult body sizes were significantly related, we first tested the relationship between tadpole and adult body size among all 542 species.

We used phylogenetic generalized least squares regression (PGLS) [32] to account for the statistical non-independence of species due to phylogeny. PGLS was performed using the R package *caper* v. 1.0.1 [33]. We initially used adult size as the independent variable and tadpole size as the dependent variable, based on the assumption that the size of tadpoles is ultimately more strongly constrained by the size of adult females than vice versa. However, we do not have strong evidence to support this assumption and our main question is actually agnostic about this assumption (i.e. we simply want to know if adult and larval body sizes are related at all, not which determines the other). Therefore, we performed a set of analyses that switched the independent versus dependent variables. These analyses gave similar results overall to our main analyses (electronic supplementary material, appendix S1), and we do not discuss them further. We also tested for relationships between tadpole size and TA ratio and adult size and TA ratio. We then performed the same PGLS analyses separately for each family that included five or more species. We used five since smaller sample sizes seemed unlikely to yield significant relationships. We found no families with less than 10 species had significant relationships (table 2), showing that five was not overly conservative.

Prior to these analyses, a Shapiro–Wilk test was performed to address whether each trait was normally distributed, under the null hypothesis that the distribution was normal [34]. Normality was initially rejected for all traits, but was achieved after ln-transformation ($p > 0.05$ for all traits). Ln-transformed data were used for all analyses.

We also tested if rates of body-size evolution of adults and larvae were related across families. We focused again on the 18 families having 5 or more species, which together encompass 491 species (90.5% of all 542 species sampled). For each family, we obtained the maximum-likelihood estimate of rate (σ^2) for tadpole size, adult size, and TA ratio. We do not report units for this rate estimate, following standard practice. We estimated σ^2 using the BM model with the 'fitContinuous' function in *geiger* [29]. Using estimates of σ^2 from the BM model is a standard approach for quantifying and comparing phenotypic rates among clades (e.g. [35]). Log-transformed variables are recommended for this approach [35].

We acknowledge that we could have used rates from other models, and tested the fit of each clade to each model. However, other models (besides BM) require estimating additional parameters, and so rate estimates from different models may not be fully comparable. Furthermore, simulations and subsampling analyses show that the ability to distinguish different models is highly contingent on the number of species (total or sampled) within a clade, and model selection can be biased for smaller clades [36]. For example, in simulations of 13 taxa, the white noise model is consistently selected when the true model is OU, and white noise is incorrectly chosen over the true BM model 50% of the time [36]. Therefore, we applied the same model (BM) to all clades, and obtained comparable rate estimates across clades and variables. Finally, for all three variables, the best-fitting model across all sampled species is the EL model with very high signal (similar to the BM model, in which $\lambda = 1.0$).

An important advantage of using clade-level estimates is that we can capture morphological evolution that occurs deep within each clade, not just at the species level. Furthermore, using clade-level estimates does not require or assume that all species in a clade share the same rate (e.g. a clade having strongly accelerated rates within a subclade will have a higher clade-level rate than one that does not, all else being equal).

For the family Dicroglossidae, the species *Limnonectes finchi* and *L. ingeri* were estimated to have split only 384 000 years ago (possibly because of an artefact in the mitochondrial data for these species, such as introgression). This relatively short branch seemed to strongly impact the estimated rate for adult body size ($\sigma^2 = 0.1040$) for the entire family, yielding a rate two orders of magnitude higher than the next highest family level rate. Therefore, we excluded *L. finchi* from our rate estimate for this family (we selected *L. finchi* based on alphabetical order). After excluding this species, the rate estimate for this family was only 0.0051,

Table 1. Summary data for anuran families sampled in this study. Values for each family are the mean and range of values among species. Ranges are only given for families with multiple species sampled. Species-level data are given in full in electronic supplementary material, dataset S1.

family	sampled	total richness	tadpole size	adult size	TA ratio
Alsodidae	5	23	39.34	51.57	0.77
			23.2–87	48–57.6	0.45–1.74
Alytidae	4	11	55.25	45.31	1.40
			35–76	33.4–56.5	0.62–2.28
Arthroleptidae	2	153	40.45	48.10	0.88
			30.9–50	31.2–65	0.77–0.99
Ascaphidae	2	2	61.00	44.50	1.37
			58–64	43.5–45.5	1.27–1.47
Batrachylidae	4	13	38.25	39.75	0.94
			28–57	32.5–50	0.85–1.14
Bombinatoridae	1	10	52.00	44.60	1.17
Bufonidae	49	618	25.09	73.17	0.41
			12.2–56	17.6–250	0.14–0.97
Calyptocephalellidae	1	5	107.40	76.00	1.41
Centrolenidae	7	160	31.10	26.57	1.17
			9.8–39	22.6–32	0.42–1.53
Ceratophryidae	3	12	69.17	87.33	0.90
			56–81.5	55–107.5	0.56–1.48
Conrauidae	1	6	45.00	245.00	0.18
Cycloramphidae	2	36	28.75	49.50	0.65
			26.5–31	32–67	0.46–0.83
Dendrobatidae	19	320	29.83	22.68	1.33
			13.9–50.2	16.4–34.5	0.82–2.02
Dicroglossidae	16	214	35.43	77.70	0.56
			15–71.88	27–152.5	0.22–1.37
Heleophrynidae	3	6	68.33	51.17	1.33
			60–85	46.5–55	1.17–1.55
Hemisotidae	1	9	55.00	43.00	1.28
Hylidae	147	998	46.51	50.62	1.01
			15.2–160	14.75–115	0.29–3.05
Hylodidae	2	47	58.09	36.55	1.63
			50.1–66.1	28.1–45	1.47–1.78
Hyperoliidae	19	232	49.67	35.01	1.42
			14.0–130	20.7–68.5	0.68–2.16
Leiopelmatidae	2	4	20.59	39.50	0.53
			20.4–20.8	35.5–43.5	0.48–0.57
Leptodactylidae	22	214	34.00	54.29	0.73
			8.67–83	14.9–153	0.24–1.44
Mantellidae	25	226	37.13	40.66	0.93
			20.1–106	20.5–74.5	0.51–1.77
Megophryidae	20	227	67.05	66.65	1.10
			33.2–117	27–131	0.38–1.99
Microhylidae	39	676	27.25	40.96	0.73
			12.1–51	21.5–118.11	0.24–1.38
Myobatrachidae	28	134	50.70	46.96	1.12
			13.2–90	18–100	0.48–2.04
Nasikabatrachidae	1	2	52.80	70.10	0.75
Nyctibatrachidae	1	39	39.04	71.00	0.55

(*Continued*.)

Table 1. (*Continued.*)

family	sampled	total richness	tadpole size	adult size	TA ratio
Odontophrynidae	10	52	50.80	57.19	0.95
			31–99	33.35–105	0.47–1.96
Pelobatidae	3	6	126.89	64.46	2.11
			70.67–180	53.89–77	0.92–3.34
Pelodytidae	1	5	38.33	42.50	0.90
Petropedetidae	3	13	30.27	53.03	0.58
			26.2–35	46.1–58	0.45–0.76
Phrynobatrachidae	2	94	26.50	29.00	0.91
			18–35	20.5–37.5	0.88–0.93
Pipidae	3	41	42.67	64.33	0.73
			21–70	35–100	0.37–1.21
Ptychadenidae	4	59	57.00	52.45	1.07
			41–95	41.5–67.3	0.79–1.41
Pyxicephalidae	13	87	47.20	58.42	1.05
			25–80	18–215	0.28–2.02
Ranidae	49	409	65.24	74.44	0.90
			27.7–162	36.15–155	0.41–2.07
Ranixalidae	2	18	22.40	35.31	0.63
			18.4–26.4	30.42–40.2	0.60–0.66
Rhacophoridae	12	426	36.65	62.26	0.64
			22.5–55	32–110	0.32–1.03
Rhinodermatidae	2	3	38.75	35.30	1.01
			16.5–61	28–42.6	0.59–1.43
Rhinophrynidae	1	1	45.00	74.00	0.61
Scaphiopodidae	6	7	50.37	56.75	0.93
			28–71	47.5–69.5	0.44–1.43
Telmatobiidae	5	62	87.15	68.12	1.33
			75.2–110	48.21–82	0.92–1.74

similar to the mean rate among families (0.0031). Some other species pairs were also associated with relatively short branches (and sometimes faster species-level rates, see below), including *Limnodynastes dumerilii* and *L. interioris* (Myobatrachidae), *Litoria freycineti* and *L. latopalmata* (Hylidae), *Cyclorana australis* and *C. novaehollandiae* (Hylidae), *Melanophryniscus klappenbachi* and *M. stelzneri*, and *Bufo boreas* and *B. nelsoni* (Bufonidae). However, we did not see the striking differences in rates seen for *Limnonectes*, and so we retained these species in the analyses.

We generated a reduced tree with the 18 families as terminal units for the PGLS analyses. Starting with the overall tree, we arbitrarily selected one tip from each family using the function 'keep.tip' from the R package *ape* v. 5.0 [37]. The resulting 18-species tree was used as the family-level phylogeny. The choice of species has no impact on the results, because all species have the same branch length when the family is reduced to a single species. The reduced tree is provided as electronic supplementary material, dataset S4. Rate estimates for each family are provided in electronic supplementary material, dataset S5.

We then used PGLS to test for a relationship between rates of change in tadpole size and adult size among the 18 families. We also tested whether the rate of change in TA ratio was related to rates of change in tadpole and adult body size. We initially assumed that rates for tadpoles (dependent variable) were dependent on rates for adults (independent variable), and that rates for the TA were dependent on rates for adults and tadpoles (independent variables). We also performed analyses after reversing the choice of independent and dependent variables, and obtained very similar results (electronic supplementary material, appendix S1).

We also performed a limited set of analyses using rates estimated at the species level. We estimated rates for each extant species using the BM approach [38], as implemented in BayesTraits v. 3.0.2 [39]. The variable-rates model was run for 10^8 generations, sampling every 25 000 generations, with burn-in at 10^7 generations. The processing of output was completed online at http://www.evolution.reading.ac.uk/VarRates WebPP/. We used ln-transformed variables (adult size, tadpole size, TA ratio) and obtained the mean scalar for each species (i.e. the estimate of species-level rate [38]). We then performed analyses across all species using PGLS. We also repeated our analyses among the 18 families, using mean species-level rates within each clade. However, we did not repeat these time-consuming analyses across the alternative trees.

All analyses were conducted primarily using the tree of Pyron & Wiens [20]. For the alternative phylogeny [27], we conducted a set of analyses on 10 fully resolved trees that were evenly sampled from the posterior distribution of trees (the first tree from each of the 10 sets of 1000 trees). The 10 sampled trees are given in electronic supplementary material, dataset S6. Full results based on the alternative trees [27] are given in

Table 2. Fit of alternative evolutionary models to the data on tadpole and adult body size and the ratio between them. σ^2 = maximum-likelihood estimate of rate; LnL = likelihood of model; AICc = sample-size corrected Akaike information criterion for model; TA ratio = ratio between tadpole and adult body size. The best-fitting model for each variable is boldfaced. Species-level data are given in full in electronic supplementary material, dataset S1.

trait	model	σ^2	LnL	AICc
tadpole size	lambda ($\lambda = 0.9039$)	0.0020	−228.2197	**464.4841**
	Brownian motion	0.0050	−328.5487	661.1197
	Ornstein–Uhlenbeck	0.0080	−280.4174	556.8795
	white noise	0.0010	−387.6443	779.3110
adult size	lambda ($\lambda = 0.9466$)	0.0023	−222.2956	**450.6359**
	Brownian motion	0.0064	−398.2467	800.5158
	Ornstein–Uhlenbeck	0.0118	−317.1689	640.3825
	white noise	0.0010	−376.2024	756.4270
TA ratio	lambda ($\lambda = 0.8444$)	0.0001	474.2008	**−942.3571**
	Brownian motion	0.0004	330.5089	−656.9956
	Ornstein–Uhlenbeck	0.0008	411.8969	−817.7493
	white noise	0.0001	344.5165	−685.0108

electronic supplementary material, dataset S7 and are summarized in the last paragraph of the Results.

These trees ([20] versus [27]) are similar overall but differ primarily in the placement of some families sampled here. These differences include: (i) Nasikabatrachidae as sister to most other neobatrachians [20] or only ranoids [27], (ii) Conrauidae as sister to Petropedetidae + Pyxicephalidae [27] or a clade including Petropedetidae, Pyxicephalidae, Ranixalidae, Dicroglossidae, Ranidae, Mantellidae, and Rhacophoridae [20], (iii) Dendrobatidae as sister to Bufonidae [20] or other sampled hyloids [27], and (iv) Odontophrynidae as sister to Bufonidae [27] or in a clade including Alsodidae, Ceratophryidae, Cycloramphidae, Hylodidae, Rhinodermatidae, and Telmatobiidae [20]. These trees also showed some differences within families, and in their divergence dates.

3. Results

Data for each family are summarized in table 1. The EL model had the best fit for tadpole size, adult size, and TA ratio among the four tested models (table 2). There was strong phylogenetic signal in tadpole size ($\lambda = 0.904$), adult size ($\lambda = 0.947$), and TA ratio ($\lambda = 0.844$). The overall rate of body-size evolution was similar between adults and tadpoles (table 2), but higher for adults (best-fitting EL model: adult $\sigma^2 = 0.23$, tadpole = 0.20; BM: adult = 0.64; tadpole = 0.50).

There was a significant, positive relationship between tadpole size and adult size among the 542 species, explaining 17% of the variation in tadpole size among species (figure 1). Similarly, in 10 of the 18 families in which this relationship was tested separately there was a significant, positive relationship between tadpole size and adult size (table 3). Among these 10 families, adult size explained from 10% (Microhylidae) to 64% (Dicroglossidae) of the variation in larval size (table 3). Among the eight families without a significant relationship, the relationship was negative in Alsodidae, Scaphiopodidae, and Telmatobiidae (table 3). The overall relationship is almost identical when including only species with data from stages 35–39 ($r^2 = 0.1638$, $p < 0.0001$; $n = 276$).

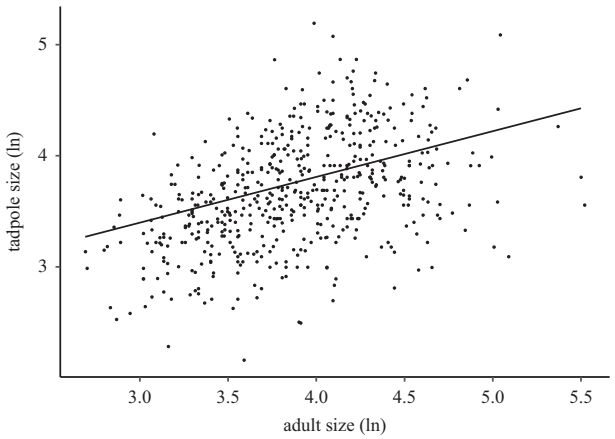

Figure 1. Relationship between tadpole size and adult size across 542 anuran species. Morphological variables (tadpole size, adult size) were ln-transformed. PGLS analysis of these data yields a significant positive relationship ($r^2 = 0.1692$, $p < 0.0001$). The raw data are shown. (Online version in colour.)

Rates of evolution in the 18 well-sampled clades are summarized in figure 2. There was no significant relationship between rates of body-size evolution in tadpoles and adults among these 18 families (figure 3*a*; $r^2 = 0.0157$, $p = 0.6199$). Among families, rates in tadpoles and adults can be very similar, or very different (figure 2; electronic supplementary material, dataset S4). For example, centrolenids have a relatively high rate of evolution in larval body size (0.0123) but a very low rate in adult body size (0.0001). Pyxicephalids have a relatively high rate for adult body size (0.0061) but a low rate for tadpole body size (0.0015). Bufonids and myobatrachids have relatively high rates for both adult and larval body size (Bufonidae: adult = 0.0073; larval = 0.0091; Myobatrachidae: adult = 0.0070; larval = 0.0079). Dendrobatids and scaphiopodids have relatively low rates for both (Dendrobatidae: adult = 0.0009; larval = 0.0024; Scaphiopodidae: adult = 0.0008; larval = 0.0023). Finally, there was a strong, positive relationship between rates of evolution in TA ratio and tadpole size (figure 3*b*; $r^2 = 0.6718$, $p < 0.0001$) but not rates of TA ratio and adult size (figure 3*c*; $r^2 = 0.0490$, $p = 0.3774$).

We also performed analyses comparing species-level rates across all 542 frog species (rates in electronic supplementary material, dataset S7). We found a significant and positive but very weak relationship between adult and larval rates including all species ($r^2 = 0.0210$, $p = 0.0007$), and after excluding two *Limnonectes* species (*L. finchi*, *L. ingeri*) with short branch lengths and exceptionally fast rates for adult size (figure 4; $r^2 = 0.0307$, $p < 0.0001$). In contrast to the clade-level results, there were similarly strong relationships between tadpole size and TA ratio ($r^2 = 0.3688$, $p < 0.0001$) and adult size and TA ratio ($r^2 = 0.3884$, $p < 0.0001$). When we analysed relationships for the 18 clades using mean species-level rates, results were similar to those using clade-level rate estimates, with weak relationships between tadpole and adult rates ($r^2 = 0.0036$, $p = 0.8195$) and stronger relationships between tadpole rates and TA ratio ($r^2 = 0.5556$, $p = 0.0004$) than adult rates and TA ratio ($r^2 = 0.2558$, $p = 0.0323$). These latter results suggest that the differences between species-level and clade-level results may be more related to differences in species sampling rather than different methodologies. We confirmed that there was no strong relationship between adult and larval rates when analyzing

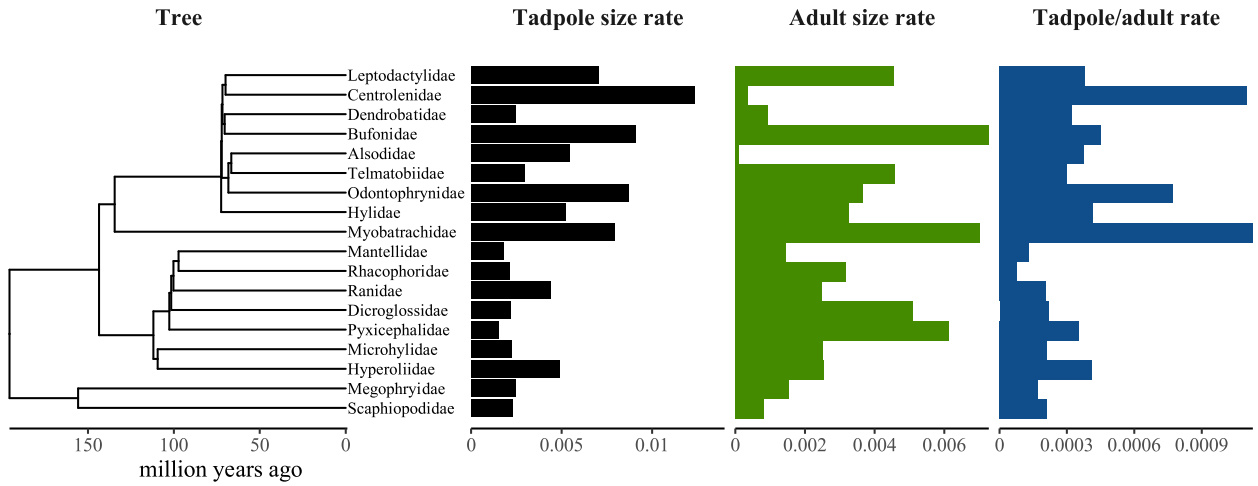

Figure 2. Phylogeny of 18 anuran families and their estimated rates of evolution for tadpole body size, adult body size, and the ratio of tadpole to adult body size. Rates of evolution are based on maximum-likelihood estimates of σ^2.

Table 3. Relationships between tadpole and adult size within anuran families. Only the 18 families with 5 or more species sampled are included. Boldfaced rows indicate families with significant relationships. Results are based on phylogenetic regression (PGLS).

family	coefficient	r^2	*p*-value
Alsodidae	−1.6275	0.0393	0.7492
Bufonidae	**0.3947**	**0.3453**	**<0.0001**
Centrolenidae	1.2897	0.0923	0.5077
Dendrobatidae	0.2952	0.0336	0.4523
Dicroglossidae	**0.1777**	**0.6421**	**0.0002**
Hylidae	**0.3048**	**0.1054**	**<0.0001**
Hyperoliidae	**1.0002**	**0.4983**	**0.0007**
Leptodactylidae	**0.6016**	**0.4991**	**0.0002**
Mantellidae	**0.6326**	**0.3255**	**0.0029**
Megophryidae	0.2854	0.0772	0.2357
Microhylidae	**0.2985**	**0.1037**	**0.0456**
Myobatrachidae	**0.8847**	**0.5585**	**<0.0001**
Odontophrynidae	0.2970	0.0547	0.5155
Pyxicephalidae	0.1570	0.0997	0.2932
Ranidae	**0.5801**	**0.2343**	**0.0004**
Rhacophoridae	**0.4212**	**0.3977**	**0.0280**
Scaphiopodidae	−0.1051	0.0040	0.9062
Telmatobiidae	−0.2040	0.0858	0.6324

only species with data from larval stages 35–39 ($r^2 = 0.0291$, $p = 0.0046$; $n = 274$: excluding *L. finchi* and *L. ingeri*).

The overall results were broadly similar using 10 alternative [27] trees (electronic supplementary material, dataset S8). Again, there was strong phylogenetic signal in tadpole size (mean $\lambda = 0.881$; range = 0.864–0.899), adult size (mean $\lambda = 0.920$; range = 0.888–0.938), and TA ratio (mean $\lambda = 0.827$; range = 0.812–0.849). Tadpole and adult size were significantly and positively related across the phylogeny (mean $r^2 = 0.2136$; range = 0.1798–0.3730; mean $p < 0.0001$, range ≤ 0.0001–0.0360). The relationships between rates of body-size evolution in tadpoles and adults among the 18 families with sufficient sampling were somewhat variable among trees, but were non-significant in 7 of 10 trees (mean $r^2 = 0.2590$, range ≤ 0.0001–0.8060; mean $p = 0.2031$; range ≤ 0.0001–0.9917). Across trees, there was a strong, positive relationship between rates of evolution in TA ratio and tadpole size (mean $r^2 = 0.5670$; range = 0.3107–0.8059, mean $p = 0.0053$; range ≤ 0.0001–0.0162) but not rates of TA ratio and adult size (mean $r^2 = 0.0108$, range ≤ 0.0001–0.0408; mean $p = 0.7397$, range = 0.4217–0.9993).

4. Discussion

Complex life cycles are widespread across animals, but whether morphological evolution is correlated between life stages among species has remained unclear [3]. Here, we undertake the broadest macroevolutionary test yet of whether body-size evolution is correlated or decoupled between life stages (i.e. the adaptive decoupling hypothesis), focusing on 542 species from across frog phylogeny. We show that tadpole size, adult size, and the ratio between them are all strongly conserved phylogenetically (table 2). We find an overall positive relationship between adult and larval body size among species. However, this relationship is relatively weak, with adult size explaining only 17% of the variance in larval size among species (figure 1). Our results demonstrate striking variability in the relationship between larval and adult size among 18 more well-sampled clades (table 3). In some families, this relationship is very strong, with adult size explaining 64% of the variance in tadpole size (Dicroglossidae), and approximately 50% in several others (Hyperoliidae, Leptodactylidae, Myobatrachidae). In other families, the relationship was very weak (six with $r^2 < 0.10$), and in three families, the relationship was even negative (but not strongly or significantly). We also show that rates of evolution in adult and larval body sizes within clades are largely uncorrelated among 18 well-sampled clades that together encompass 90.5% of the sampled species (figure 3). Some clades have high rates of body-size evolution in tadpoles but not adults, others have high rates in adults but not tadpoles, and some have both rates high or both low. Intriguingly, we find that evolutionary rates for the tadpole : adult size ratio are strongly related to tadpole size,

and not adult size among species in these 18 families (figure 3). This pattern suggests that variability in the size of tadpoles relative to adults is driven primarily by variation in tadpole size, not adult size. Results based on species-level rates across all sampled species also show only a very weak relationship between tadpole and adult rates (figure 4), but the relationships between rates for TA ratios and adult and larval sizes are similar in magnitude (suggesting that changes in both adult and larval body sizes contribute similarly to variation in TA ratio when all families are included). Overall, our results show that adult and larval body sizes are generally correlated across frogs (contrary to the adaptive decoupling hypothesis) but this relationship is highly variable among clades and rates of body-size evolution are largely decoupled between life stages.

(a) Comparison to other studies

Two recent, groundbreaking papers used phylogenetic methods to infer decoupling between the evolution of adult and larval morphology in frogs, including a study of Australian hylids and myobatrachids [8] and another on mantellids [13]. These studies included many morphological traits, but did not focus primarily on traits that were directly comparable between life stages. Here, we focused on body size, which can be compared (with the caveat that adult and larval body shapes are not identical). We found significant relationships between adult and larval body size in all three families examined in these two previous studies, with the relationship in myobatrachids being especially strong. This comparison suggests that adult and larval body sizes can be correlated, even when other aspects of morphology do not show similar rates or patterns of evolution. One potential explanation for these different patterns is that overall morphological evolution of adults and tadpoles may reflect divergent adaptations among species to diverse ecological conditions (e.g. microhabitat, diet), conditions that also differ strongly between life stages (e.g. arboreal versus terrestrial habitat for adults [40]; streams versus ponds for tadpoles [41]). By contrast, body sizes of adults and larvae may have more potential to impact each other.

These two previous studies [8,13] also compared levels of phylogenetic signal and rates of evolution between adult and larval morphology. Sherratt *et al*. [8] found lower phylogenetic signal in tadpole morphology than in adult morphology. We found that levels of phylogenetic signal were similar between adult and larval body size (adult $\lambda = 0.947$; tadpole $\lambda = 0.904$), but with tadpole signal slightly lower. Wollenberg-Valero *et al.* [13] found higher rates of morphological evolution in tadpoles than adults in mantellids. By contrast, we found higher rates in adults across all frogs (table 2). However, our data for mantellids also show higher rates for tadpoles than adults ($\sigma^2 = 0.0018$ versus 0.0014; electronic supplementary material, dataset S5). Interestingly, within the 18 well-sampled families, rates for tadpoles were also higher than for adults (mean tadpole rate among families = 0.0047; mean adult rate = 0.0032; electronic supplementary material, dataset S5). However, mean species-level rates across frogs were substantially higher in adults than tadpoles (adults = 12.2866; tadpoles = 3.4596; $n = 540$ species, excluding the two *Limnonectes* with exceptionally fast adult rates; electronic supplementary material, dataset S7). One explanation for the different patterns seen across frogs compared to those within families is that patterns across frogs reflect greater differences in adult size among families (relative to larval size). Large differences in adult size among families are not captured by rates within families. This pattern might also help explain why (in our results) rates for TA ratio are strongly related to rates for tadpole size but not adult size within families, whereas rates for TA ratio including all species seem more equally explained by rates in both adult and larval sizes. Overall, there are interesting similarities and differences between our results and those of previous studies [8,13] regarding rates and signal in adult and larval morphology, but it is important to remember that we focused on body size whereas these previous studies did not.

Werner [14] did focus on the relationship between adult and larval body size (using SVL), but not incorporating phylogenetic methods. He found significant relationships between adult and larval size (SVL) in hylids and ranids, but not bufonids. We found significant, positive relationships between adult and larval size in all three groups, with a stronger relationship in bufonids than hylids or ranids (table 3). One potential explanation for these differences is that our study included more species in all three families, as limited sampling can hide significant relationships (see below).

(b) Potential weaknesses

We acknowledge several potential weaknesses in our study, but none that should overturn our main conclusions. First, our sampling includes only a small fraction of all frog species. Incomplete sampling can strongly influence some types of macroevolutionary analyses, including the selection of models of phenotypic evolution (see Material and methods). However, simulations show that phylogenetic analyses of correlations between continuous variables can be robust to incomplete sampling, even when only 6% of the species in a tree are sampled [42]. Specifically, in these simulations, limited sampling (6%) did not lead to biased estimates of correlations and had no impact on type-1 error rates (i.e. false positives). Limited taxon sampling reduces statistical power [42], but our results are significant across frogs (and within most families; table 3), showing that limited power was not generally problematic. We further address potential biases associated with taxon sampling on trait relationships below.

Incomplete sampling might also influence estimated rates of body-size evolution. O'Meara *et al.* [35] showed that estimating phenotypic rates for clades with few species ($n = 4$) led to more error and more biased estimates (approx. 25%) relative to those with more species, but did not address how incomplete taxon sampling impacted rate estimates. However, simulations suggest that incomplete taxon sampling has little impact on phenotypic rate estimates and does not significantly bias them [36]. Furthermore, we found no relationship between the proportion of species sampled in a clade and the clade's estimated rate among the 18 focal clades (for rates for tadpole size, adult size, and TA ratio, $r^2 = 0.015$, 0.042, and 0.002, all $p > 0.40$, with proportional sampling within clades ranging from 0.028 to 0.857). Similarly, there was no significant impact of the number of species sampled on the rate estimates ($r^2 = 0.004$, 0.022, and 0.001, all $p > 0.50$, with species sampled ranging from 5 to 147). Overall, these results suggest that taxon sampling is not a dominant factor influencing rate estimates in our study.

We also tested if sampling impacted whether we obtained a negative or positive relationship between adult and larval

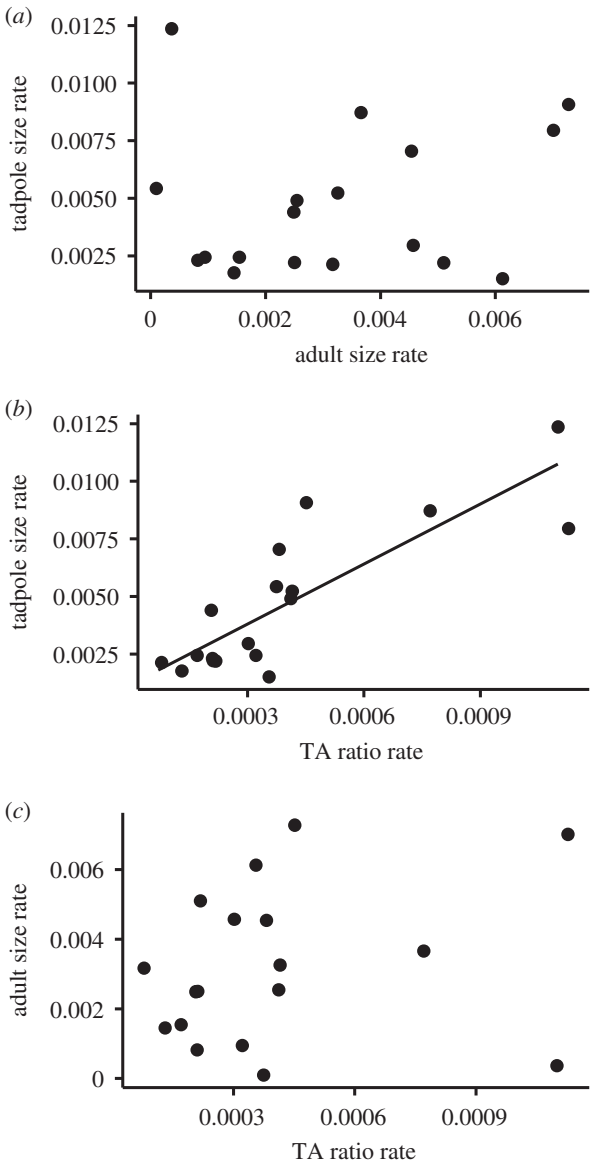

Figure 3. Relationships between estimated evolutionary rates (σ^2) among 18 frog families. Results are based on PGLS and are for: (*a*) tadpole size and adult size ($r^2 = 0.0157$, $p = 0.6199$), (*b*) tadpole size and TA ratio ($r^2 = 0.6718$, $p < 0.0001$), and (*c*) adult size and TA ratio ($r^2 = 0.0490$, $p = 0.3774$).

body sizes within each of the 18 family level clades (table 3). We found that the three groups with negative relationships had proportionally higher sampling than the 15 with positive relationships (means: positive = 0.103; negative = 0.385; $p = 0.0108$, unpaired *t*-test). Thus, the weak, negative relationships in some groups were not an artefact of limited sampling of species within these groups. Furthermore, there was no significant difference in the number of species sampled between these two groups of clades (means: positive = 31.67; negative = 5.33; $p = 0.2148$). Groups with negative relationships tended to be smaller in terms of their overall number of species, regardless of sampling (means: positive = 332.87; negative = 30.67; $p = 0.0646$). Thus, groups with negative relationships had both few species and few species sampled, but a significantly higher proportion of species sampled.

Finally, we emphasize that a macroevolutionary approach does not directly address if or how selection is similar or different at each life stage in each species. However, these broad-scale analyses highlight interesting patterns for future microevolutionary studies, such as the different rates in

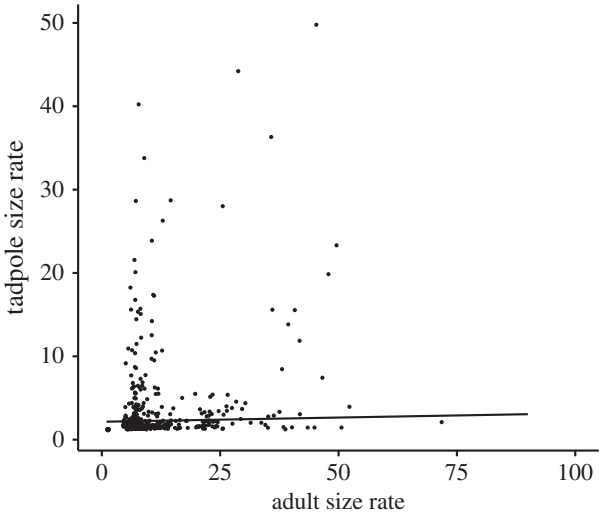

Figure 4. Relationship between rates of evolution for adult and larval body size based on species-level rate estimates. PGLS analysis ($r^2 = 0.0307$, $p < 0.0001$) is based on analysis of 540 species, after excluding two species of *Limnonectes* with exceptionally fast adult rates. Including these two species gives an even weaker relationship ($r^2 = 0.0210$, $p = 0.0007$). The raw data are shown.

different stages in different groups (figure 3) and the variation in relationships among clades (table 3).

5. Conclusion

In summary, we show that adult and larval body sizes are generally positively (but weakly) related among species across frogs. However, this relationship is highly variable among clades, with some showing strong relationships and others showing weak or even negative relationships (especially in smaller clades). We show that rates of evolution in tadpole and adult body sizes are uncorrelated among clades, and are only weakly related when all frog species are analysed simultaneously.

These results raise many questions for future research. Why do some clades show weak or negative relationships between adult and tadpole body size, and others show such strong positive relationships? What explains differences in rates of body-size evolution among clades, especially for tadpole body size? Finally, which of these patterns are replicated in the many other groups that have complex life cycles across the animal Tree of Life? For example, will patterns of evolution be very different in groups (like holometabolous insects [5]) in which there is little or no growth in the adult stage? Overall, our study shows that the hypothesis of adaptive decoupling and 'mosaic evolution' (i.e. divergent evolution between adults and larvae) itself shows a mosaic pattern among clades, with relationships between adult and larval size (and their rates of change) varying among subgroups within frogs. We anticipate that this clade-level variability in adaptive decoupling between life stages may extend beyond frogs.

Ethics. Our study did not include human subjects, live animals, or fieldwork.

Data accessibility. All data are available as electronic supplementary information.

Authors' contributions. T.X.P. and J.J.W. designed the study and wrote the paper. J.C.S.N. and A.J.N. collected data. T.X.P. performed the analyses.

Competing interests. We have no competing interests to declare.

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References

1. Moran NA. 1994 Adaptation and constraint in the complex life cycles of animals. *Ann. Rev. Ecol. Syst.* **25**, 573–600. (doi:10.1146/annurev.es.25.110194.003041)
2. ten Brink H, de Roos AM, Dieckmann U. 2018 The evolutionary ecology of metamorphosis. *Am. Nat.* **193**, E116–E131. (doi:10.1086/701779)
3. Collet J, Fellous S. 2019 Do traits separated by metamorphosis evolve independently? Concepts and methods. *Proc. R. Soc. B* **286**, 20190445. (doi:10.1098/rspb.2019.0445)
4. Pough FH, Andrews RM, Crump ML, Savitzky AH, Wells KD, Brandley MC. 2016 *Herpetology*, 4th edn. Sunderland, MA: Sinauer Associates.
5. Grimaldi D, Engel MS. 2005 *Evolution of the insects*. Cambridge, UK: Cambridge University Press.
6. Jezkova T, Wiens JJ. 2017 What explains patterns of diversification and richness among animal phyla? *Am. Nat.* **189**, 201–212. (doi:10.1086/690194)
7. Phillips PC. 1998 Genetic constraints at the metamorphic boundary: morphological development in the wood frog, *Rana sylvatica*. *J. Evol. Biol.* **11**, 453–463. (doi:10.1007/s000360050099)
8. Sherratt E, Vidal-García M, Anstis M, Keogh JS. 2017 Adult frogs and tadpoles have different macroevolutionary patterns across the Australian continent. *Nat. Ecol. Evol.* **1**, 1385–1391. (doi:10.1038/s41559-017-0268-6)
9. Aguirre JD, Blows MW, Marshall DJ. 2014 The genetic covariance between life cycle stages separated by metamorphosis. *Proc. R. Soc. B* **281**, 20141091. (doi:10.1098/rspb.2014.1091)
10. Goedert D, Calsbeek R. 2019 Experimental evidence that metamorphosis alleviates genomic conflict. *Am. Nat.* **194**, 356–366. (doi:10.1086/704183)
11. Stoks R, Córdoba-Aguilar A. 2012 Evolutionary ecology of Odonata: a complex life cycle perspective. *Annu. Rev. Entomol.* **57**, 249–265. (doi:10.1146/annurev-ento-120710-100557)
12. Wilson ADM, Krause J. 2012 Personality and metamorphosis: is behavioral variation consistent across ontogenetic niche shifts? *Behav. Ecol.* **23**, 1316–1323. (doi:10.1093/beheco/ars123)
13. Wollenberg-Valero KC *et al.* 2017 Transcriptomic and macroevolutionary evidence for phenotypic uncoupling between frog life history phases. *Nat. Commun.* **8**, 15213. (doi:10.1038/ncomms15213)
14. Werner EE. 1986 Amphibian metamorphosis: growth rate, predation risk, and the optimal size at transformation. *Am. Nat.* **128**, 319–341. (doi:10.1086/284565)
15. Dehling JM, Sinsch U. 2019 Partitioning of morphospace in larval and adult reed frogs (Anura: Hyperoliidae: *Hyperolius*) of the Central African Albertine Rift. *Zool. Anz.* **280**, 65–77. (doi:10.1016/j.jcz.2019.04.003)
16. Wilbur HM, Collins JP. 1973 Ecological aspects of amphibian metamorphosis. *Science* **182**, 1305–1314. (doi:10.1126/science.182.4119.1305)
17. Rowe L, Ludwig D. 1991 Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology* **72**, 413–427. (doi:10.2307/2937184)
18. AmphibiaWeb. 2019 See https://amphibiaweb.org. University of California, Berkeley, CA, USA (accessed 29 July 2019).
19. Emerson SB. 1988 The giant tadpole of *Pseudis paradoxa*. *Biol. J. Linn. Soc.* **34**, 93–104. (doi:10.1111/j.1095-8312.1988.tb01951.x)
20. Pyron RA, Wiens JJ. 2013 Large-scale phylogenetic analyses reveal the causes of high tropical amphibian diversity. *Proc. R. Soc. B* **280**, 20131622. (doi:10.1098/rspb.2013.1622)
21. De Lisle SP, Rowe L. 2015 Independent evolution of the sexes promotes amphibian diversification. *Proc. R. Soc. B* **282**, 20142213. (doi:10.1098/rspb.2014.2213)
22. Santini L, Benítez-López A, Ficetola GF, Huijbregts MAJ. 2018 Length-mass allometries in amphibians. *Int. Zool.* **13**, 36–45. (doi:10.1111/1749-4877.12268)
23. Gosner KL. 1960 A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16**, 183–190.
24. McDiarmid RW, Altig RS. 1999 Research materials and techniques. In *Tadpoles: the biology of anuran larvae* (eds RW McDiarmid, R Altig), pp. 7–23. Chicago, IL: University of Chicago Press.
25. Callery EM, Fang H, Elinson RP. 2001 Frogs without polliwogs: evolution of anuran direct development. *Bioessays* **23**, 233–241. (doi:10.1002/1521-1878(200103)23:3<233::AID-BIES1033>3.0.CO;2-Q)
26. Gomez-Mestre I, Pyron RA, Wiens JJ. 2012 Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes in frogs. *Evolution* **66**, 3687–3700. (doi:10.1111/j.1558-5646.2012.01715.x)
27. Jetz W, Pyron RA. 2018 The interplay of past diversification and evolutionary isolation with present imperilment across the amphibian tree of life. *Nat. Ecol. Evol.* **21**, 850–858. (doi:10.1038/s41559-018-0515-5)
28. R Core Team. 2018 *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See https://www.R-project.org/.
29. Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, FitzJohn RG, Alfaro ME, Harmon LJ. 2014 geiger v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics* **15**, 2216–2218. (doi:10.1093/bioinformatics/btu181)
30. Pagel M. 1999 Inferring the historical patterns of biological evolution. *Nature* **401**, 877–884. (doi:10.1038/44766)
31. Burnham KP, Anderson DR. 2002 *Model selection and multi-model inference. A practical information-theoretic approach*. New York, NY: Springer.
32. Martins EP, Hansen TF. 1997 Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**, 646–667. (doi:10.1086/286013)
33. Orme D, Freckleton R, Thomas G, Petzoldt T, Fritz S, Isaac N, Pearse W. 2018 caper: comparative analyses of phylogenetics and evolution in R. R package version 1.0.1. Retrieved from http://CRAN.R-project.org/package=caper.
34. Shapiro SS, Wilk MB. 1965 An analysis of variance test for normality (complete samples). *Biometrika* **52**, 591–611. (doi:10.1093/biomet/52.3-4.591)
35. O'Meara BC, Ané C, Sanderson MJ, Wainwright PC. 2006 Testing for different rates of continuous trait evolution using likelihood. *Evolution* **60**, 922–933. (doi:10.1111/j.0014-3820.2006.tb01171.x)
36. Jezkova T, Wiens JJ. 2016 Rates of change in climatic niches in plant and animal populations are much slower than projected climate change. *Proc. R. Soc. B* **283**, 20162104. (doi:10.1098/rspb.2016.2104)
37. Paradis E, Schliep K. 2018 ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528. (doi:10.1093/bioinformatics/bty633)
38. Venditti C, Meade A, Pagel M. 2011 Multiple routes to mammalian diversity. *Nature* **479**, 393–396. (doi:10.1038/nature10516)
39. Pagel M, Meade A, Barker D. 2004 Bayesian estimation of ancestral character states on phylogenies. *Syst. Biol.* **53**, 673–684. (doi:10.1080/10635150490522232)
40. Moen DS, Morlon H, Wiens JJ. 2016 Testing convergence versus history: convergence dominates phenotypic evolution for over 150 million years in frogs. *Syst. Biol.* **65**, 146–160. (doi:10.1093/sysbio/syv073)
41. Altig R, Johnston GF. 1989 Guilds of anuran larvae: relationships among developmental modes, morphologies, and habitats. *Herpetol. Monogr.* **3**, 81–109. (doi:10.2307/1466987)
42. Ackerly DD. 2000 Taxon sampling, correlated evolution, and independent contrasts. *Evolution* **54**, 1480–1492. (doi:10.1111/j.0014-3820.2000.tb00694.x)