doi:10.1111/j.1558-5646.2007.00159.x

LOSS AND RE-EVOLUTION OF COMPLEX LIFE CYCLES IN MARSUPIAL FROGS: DOES ANCESTRAL TRAIT RECONSTRUCTION MISLEAD?

John J. Wiens,[1,2] Caitlin A. Kuczynski,[1] William E. Duellman,[3] and Tod W. Reeder[4]

[1]*Department of Ecology and Evolution, Stony Brook University, Stony Brook, New York 11794-5245*

[2]*E-mail: wiensj@life.bio.sunysb.edu*

[3]*Natural History Museum and Biodiversity Research Center, University of Kansas, Lawrence, Kansas 66045-7561*

[4]*Department of Biology, San Diego State University, San Diego, California 92182-4614*

Received September 26, 2006
Accepted March 27, 2007

Using phylogeny-based methods to identify evolutionary transitions has become an integral part of evolutionary biology. Here, we demonstrate the potential for these methods to give statistically well-supported but misleading inferences about character evolution. We also show how inferences of character evolution can be informed using GIS-based methods to reconstruct ancestral environmental regimes. We reconstruct a phylogeny for marsupial frogs (Hemiphractidae) using nuclear and mitochondrial DNA sequences and estimate patterns of life-history evolution across the resulting tree. We find that *Gastrotheca* species with complex life cycles (i.e., egg, tadpole, and adult stages) are phylogenetically nested among species and genera with direct development (i.e., egg and adult stages only). Assuming a single rate for gains and losses in likelihood reconstructions, there is strong statistical support for the hypothesis that the tadpole stage was lost early in the phylogeny but reappeared within *Gastrotheca*. Assuming different rates of gain and loss, the model with significantly higher statistical support, the tadpole stage seems to have been lost multiple times but never regained. Given that both hypotheses cannot be correct, at least one reconstruction model must be giving well-supported but misleading results. Several lines of evidence (including GIS-based reconstructions of the ancestral climatic regime) suggest that the former hypothesis is correct, and that the tadpole stage has evolved from direct development within *Gastrotheca*, the only known case of such a reversal in frogs.

KEY WORDS: **Amphibians, ancestral reconstruction, development, life history, phylogeny.**

In recent years, phylogeny-based studies of character evolution have become an integral part of the evolutionary biology (e.g., Futuyma 2005). For example, it is virtually impossible to determine the number or direction of evolutionary changes for a given character within a group without considering phylogeny (e.g., Donoghue 1989; Brooks and McLennan 1991). Phylogenetic methods for reconstructing character evolution have become increasingly sophisticated (e.g., Schluter et al. 1997; Mooers and Schluter 1999; Pagel 1999; Huelsenbeck et al. 2003; Pagel et al. 2004). Nevertheless, the possibility remains that these methods can be misled with strong statistical support (for a laboratory study of bacteriophage see Oakley and Cunningham 2000). Identifying such cases is an important initial step in improving these methods. Unfortunately, misleading results may be difficult to identify in most natural systems, where taxa may have evolved over tens of millions of years and the true ancestral condition can only be inferred indirectly (including studies of fossils, but see Polly 2001; Webster and Purvis 2002; Finarelli and Flynn 2006). However,

the failure of a method may be clearly indicated if two approaches each show strong statistical support for discordant conclusions based on the same data, given the assumption that only one answer can be correct. In this study, we document such a case involving the gain and loss of complex life cycles in hemiphractid frogs.

In anuran amphibians (frogs and toads), a major evolutionary transition that seemingly permitted the vertebrate domination of terrestrial environments appears to have played out again and again (Duellman and Trueb 1986). The typical anuran life cycle is complex (or biphasic), with an aquatic tadpole stage that is very different in morphology, ecology, and behavior from the adult (Duellman and Trueb 1986; Pough et al. 2004). The tadpole stage is then followed by a dramatic metamorphosis, after which adults typically are semiaquatic or terrestrial. Nevertheless, adults of these species must return to water to breed and deposit eggs. Many other anurans have direct development, and thus lack a free-living aquatic larval stage. In these species, eggs typically are laid in moist terrestrial environments, and there is no need to use water to breed as in biphasic species. At least 11 families of anurans contain species with both complex and simple life cycles, a pattern suggesting repeated origins of direct development (Pough et al. 2004). Because of this extensive natural replication, anurans offer a promising system for studying this critical transition. Yet, few studies have addressed the ecological and evolutionary factors that drive changes from a biphasic life cycle to direct development using a comparative phylogenetic framework.

The presence of some direct-developing species within a clade does not necessarily mean that transitions between complex and simple life cycles have only occurred in one direction. Some phylogenetic studies (e.g., Duellman and Hillis 1987; Duellman et al. 1988) have suggested the intriguing possibility that the free-living tadpole stage evolved from direct-developing ancestors in one clade of frogs, the Hemiphractidae (previously hemiphractine hylids, containing the genera *Cryptobatrachus*, *Flectonotus*, *Gastrotheca*, *Hemiphractus*, and *Stefania*; Wiens et al. 2005). Reappearance of the larval stage is somewhat counterintuitive (especially given the success of direct-developing tetrapods in general), but a recent study found that the aquatic larval stage seemingly was lost and then reappeared in another group of amphibians, plethodontid salamanders (Chippindale et al. 2004). Re-evolution of the aquatic larval stage would be considerably more surprising in anurans than salamanders. Despite some important differences, larval salamanders are similar to adults in many aspects of their biology, including overall morphology and diet (Duellman and Trueb 1986; Pough et al. 2004). In contrast, larval anurans (tadpoles) typically differ from the adults in overall morphology (tailed and limbless vs. limbed and tailless), diet (herbivorous vs. carnivorous), and feeding morphology (beaks and filters vs. projectile tongues). So far, hemiphractids offer the only proposed case in which the tadpole stage has re-evolved from direct-developing ancestors, among > 5000 species of anurans. However, this putative reversal has yet to be addressed with a rigorous phylogenetic analysis. Although the re-evolution of complex traits has traditionally been considered unlikely (i.e., Dollo's law; Dollo 1893; Simpson 1953), a growing literature suggests that the reappearance of complex traits is theoretically plausible (e.g., Van Valen 1979; Marshall et al. 1994) and may have occurred in a variety of organisms and features (e.g., wings in stick insects: Whiting et al. 2003; shell coiling in gastropods: Collin and Cipriano 2003; compound eyes in crustaceans: Oakley 2003; digits in lizards: Kohlsdorf and Wagner 2006). Previous papers have addressed the possible re-evolution of feeding larvae from direct-developing ancestors in echinoderms (e.g., Wray 1996; Hart et al. 1997), but analyses of these data using maximum likelihood found only equivocal support for this pattern (Cunningham 1999).

Hemiphractid frogs consist of five genera and 83 currently recognized species from Central and South America (AmphibiaWeb 2006; but see Frost et al. 2006 for an alternate taxonomy). In all hemiphractids, eggs are carried on the female's dorsum for some or all of development (Duellman and Maness 1980; Wassersug and Duellman 1984). In *Cryptobatrachus, Hemiphractus,* and *Stefania*, eggs are exposed on the back and hatch directly into froglets. In *Flectonotus,* eggs are partially enclosed in an open pouch, and hatch into relatively advanced tadpoles that do not feed. In all *Gastrotheca*, eggs are fully enclosed in a pouch. However, in some *Gastrotheca* species the eggs hatch directly into froglets, whereas in others eggs hatch into tadpoles that are deposited in ponds and other bodies of water. In hemiphractids with direct development, the embryos may retain some morphological features characteristic of free-living tadpoles, but the retained features vary among direct-developing lineages (Wassersug and Duellman 1984). Hemiphractids may be the only direct-developing anuran lineages that retain larval-like features within the egg (Duellman and Trueb 1986).

Previous phylogenetic studies, based on allozymes or immunological distance data, suggested that species of *Gastrotheca* with free-living tadpoles are relatively derived within the genus (e.g., Duellman and Hillis 1987; Duellman et al. 1988). These results raise the possibility that the tadpole stage re-evolved from direct-developing ancestors. However, previous studies did not include rigorous analyses of trait evolution nor did they resolve the phylogeny of hemiphractid genera or that of most *Gastrotheca* species. A morphological study (Mendelson et al. 2000) suggested that *Hemiphractus* is nested within *Gastrotheca*, but this result may have been caused by misleading phylogenetic signals associated with hyperossification in the skull (Wiens et al. 2005).

In this study, we reconstruct phylogenetic relationships among hemiphractid frogs using nuclear and mitochondrial DNA sequences. We then use this phylogeny to reconstruct the evolution

of life-history modes within hemiphractids. We find that biphasic *Gastrotheca* are nested deep within direct-developing lineages. However, the reconstructed patterns of character evolution differ strikingly depending on the trait reconstruction methods used. We also explore, possibly for the first time, the relationship between climatic variables and evolutionary changes in life-history variables using GIS-based methods.

Materials and Methods

TAXON SAMPLING

We obtained DNA sequence data from 46 of 83 recognized hemiphractid species (AmphibiaWeb 2006), including all genera. With the exception of *Gastrotheca*, all genera are invariant in their overall life-history mode and appear to be monophyletic (see below). There is considerable life-history variation within *Gastrotheca,* and we included 34 of 51 described species, including all species groups recognized by Duellman et al. (1988). The possible effects of incomplete taxon sampling on our analyses of life-history evolution are addressed in the section on ancestral reconstructions below.

Wiens et al. (2005; their fig. 5) found strong support for placing hemiphractids in a clade including leptodactylids of the subfamilies Ceratophryinae (*Ceratophrys, Lepidobatrachus*), Eleutherodactylinae (*Eleutherodactylus, Ischnocnema, Phrynopus*), and Telmatobiinae (*Telmatobius*). Representatives of all of these clades were included as outgroups, along with one hylid (*Pseudacris regilla*).

MOLECULAR DATA

DNA sequence data were obtained from five gene regions, three mitochondrial and two nuclear. These included the mitochondrial ribosomal small subunit (12S; 1040 bp; also including adjacent tRNA-Phe and tRNA-sVal), ribosomal large subunit (16S; 605 bp), and NADH dehydrogenase subunit 1 (ND1; 820 bp; including adjacent tRNA genes), and the nuclear proopiomelanocortin A gene (POMC, 500 bp) and recombination activating gene 1 (RAG-1, 1407 bp). Some additional sequences of 12S, 16S, and RAG-1 were obtained from Darst and Cannatella (2004) and Faivovich et al. (2005) for taxa for which we lacked tissue samples. DNA was extracted from frozen and ethanol preserved tissues using standard methods and was amplified using the polymerase chain reaction (PCR). Primer sequences are listed in Table 1. Most PCR products were purified and sequenced directly using a Beckman CEQ (Beckman-Coulter, Fullerton, CA), ABI 377 (Applied Biosystems, Foster City, CA), or ABI 3130 automated sequencer (Applied Biosystems), whereas some were cloned prior to sequencing. Sequences were edited primarily using Se-Al version 2.0. Voucher specimens are listed in online Supplementary Appendix S1, and GenBank numbers are given in online Supplementary Appendix S2.

DNA ALIGNMENT AND PHYLOGENETIC ANALYSIS

Alignments were performed for each dataset using CLUSTAL X.1.81 (Thompson et al. 1994), using methods described by Wiens et al. (2005). Regions of gene sequences that differed in their alignment under different gap-opening penalties (12.5, 15, and 17.5) were considered to be ambiguously aligned and were excluded. Apart from the gap opening penalty, default parameters were used (gap extension = 6.666; delay divergent sequences = 30%; transition:transversion = 50%). After the initial CLUSTAL alignment, some final adjustments were made manually.

Phylogenetic analyses were performed using both Bayesian and parsimony methods and using both separate and combined analyses of the gene sequences. However, we consider the best estimate of phylogeny to come from Bayesian analysis of the combined data, given that the simple model assumed by uniformly weighted parsimony does not fit the data (see below), and given the potential for long branch attraction among many of the highly divergent lineages included in this study. The combined analysis was generally preferred given that it is based on the largest sample size of characters.

The best-fitting model for each gene was identified using hierarchical likelihood ratio tests implemented in MrModeltest version 2.0 (Nylander 2004). Bayesian analyses were conducted to determine if partitions within these genes were also supported (e.g., Brandley et al. 2005; Wiens et al. 2005). The harmonic mean of the log-likelihoods of the post burn-in trees for the Bayesian analyses with and without partitions within each gene were compared using the Bayes factor (following Nylander et al. 2004). The first, second, and third codon positions were treated as separate partitions for each protein-coding gene. Stems and loops were treated as separate partitions for the 12S and 16S genes, with the location of these secondary structures hypothesized based on comparisons with models for *Pseudacris regilla* (for 12S) and *Rana catesbiana* (for 16S), from the European ribosomal RNA database (http://oberon.fvms.ugent.be:8080/rRNA/). Placement of stems and loops appears to be highly conserved across anurans (>93% identical for species in different hyloid families for 12S; Wiens et al. 2005). The set of transfer RNAs adjacent to each gene was treated as a separate partition. These comparisons showed that partitioning within each gene significantly improved the likelihood of each dataset (results not shown), a pattern seen in many other studies (e.g., Brandley et al. 2005; Wiens et al. 2005). Different partitions were unlinked.

Bayesian analyses were performed using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Two replicate searches were performed on each dataset, each using four chains and default priors. Analyses used 3.0×10^6 generations each, sampling every 1000 generations. Trees generated prior to achieving stationarity were discarded as burn-in, and stationarity was identified based on (1) plots of log likelihoods over time, (2) similarity in topologies,

Table 1. Primers used for DNA amplification and sequencing. (F) = forward primer; (R) = reverse primer.

Primer	Sequence (5′–3′)	Source
12S		
T-Phe-Frog (F)	ATAGCRCTGAARAYGCTRAGATG	Wiens et al. (2005)
T-Val-Frog (R)	TGTAAGCGARAGGCTTTKGTTAAGCT	Wiens et al. (2005)
12S-Frog R1 (R)	TCRATTRYAGGACAGGCTCCTCTAG	Wiens et al. (2005)
12S-HEMI-F (F)	ACAAACTRGGATTAGATACCCYACTAT	This study
12S-HEMI-R (R)	GTATACTTACCATGTTACGACAATCCT	This study
12S-HEMI-F2 (F)	CAYAAAGGTTYGGTCCYAGCCTT	This study
ND1		
HEMI1-F (F)	TCAGGGTAYCCYAGTGGTGC	This study
HEMI1-R (R)	AATGGGGCTCGRTTAGTTTCAG	This study
HEMI2-R (R)	CAATTARTGCRTATTTAGARTTTGA	This study
16S-Frog (F)	TTACCCTRGGGATAACAGCGCAA	Wiens et al. (2005)
ND1-F5 (F)	TTACGACCTCGATGTTGGATCAG	This study
16S		
16S-AR (F)	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
16S-BR (R)	CCGGTCTGAACTCAGATCSCGT	Palumbi et al. (1991)
16sBR-H (R)	GGTTTGAACTCAGATCATGT	This study
POMC		
POMC-1 (F)	GAATGTATYAAAGMMTGCAAGATGGWCCT	Wiens et al. (2005)
POMC-5 (F)	GGARCACTTYCGATGGGGYAAACC	Wiens et al. (2005)
POMC-5R (R)	GGTTTRCCCCATCGRAAGTGYTCC	Wiens et al. (2005)
POMC-7 (R)	TGGCATTTTTGAAAAGAGTCAT	Smith et al. (2005)
RAG-1		
RS1F (F)	TGCAGTCAGTAYCAYAARATGTAC	Ventakesh et al. (2001)
RS6R (R)	TGCTATRARNGGGCTCAAGATGG	Chippindale et al. (2004)
R1-GFF (F)	GAGAAGTCTACAAAAAVGGCAAAG	Faivovich et al. (2005)
R1-GFR (R)	GAAGCGCCTGAACAGTTTATTAC	Faivovich et al. (2005)
Amp-RAG-1 F (F)	AGCTGCAGYCARTACCAYAARATGTA	San Mauro et al. (2004)
Hemi-RAG1-R (R)	CTCTGCAGCATTTCCAATGTCAC	This study

branch support (posterior probabilities, Pp), and log likelihoods between trees from each replicate, and (3) average standard deviation of split frequencies between runs. The phylogeny and branch lengths were estimated from the majority-rule consensus of the pooled post burn-in trees from the two replicates. Clades with Pp $\geq$ 0.95 were considered to be strongly supported (Wilcox et al. 2002; Alfaro et al. 2003; Erixon et al. 2003; Huelsenbeck and Rannala 2004).

The shortest parsimony trees for each dataset were found using a heuristic search with 500 random-taxon-addition sequence replicates. Support for individual branches was evaluated using nonparametric bootstrapping (Felsenstein 1985), with 200 bootstrap pseudoreplicates and 10 random-taxon-addition sequence replicates per bootstrap pseudoreplicate. Bootstrap values $\geq$70% were considered to be strongly supported, following Hillis and Bull (1993, but see their extensive caveats). All parsimony searches used PAUP* 4.0b10 (Swofford 2002).

The final estimate of phylogeny was based on a combined analysis of all five genes. However, each gene was analyzed separately prior to combination, to detect clades that are both strongly supported and incongruent between different separate gene datasets (Wiens 1998). Such conflicts may indicate incongruence between gene and species histories or other systematic errors. We also combined the nuclear genes and compared the results to those from the combined mitochondrial data. No strongly supported conflicts were found between the separate or combined genes using parsimony or Bayesian methods.

Some taxa lacked data for one or more genes, including many that proved difficult to sequence or amplify for a given gene despite repeated attempts. Recent simulations (e.g., Wiens 2003; Phillipe et al. 2004) and analyses of empirical data (e.g., Driskell et al. 2004; Phillipe et al. 2004; Wiens et al. 2005) suggest that such "incomplete taxa" can be accurately placed in phylogenetic analyses regardless of the number of missing data cells that they bear. However, the position of *Cryptobatrachus* was highly unstable (and this uncertainty obscured other generic-level relationships), likely because only fast-evolving mitochondrial data were available for this taxon. We performed analyses both including and excluding this taxon, and the main results that are presented exclude *Cryptobatrachus*.

PHYLOGENETIC ANALYSIS OF LIFE-HISTORY EVOLUTION

We compiled data on the general life-history mode of each sampled species from several literature sources, including Duellman and Maness (1980), Duellman and Hillis (1987), Duellman et al. (1988), and the Global Amphibian Assessment (IUCN et al. 2004). We focused primarily on whether species had an aquatic larval stage (regardless of length) or direct development (no aquatic larval stage outside the egg). The life-history patterns for two recently described species (*Gastrotheca atympana, G. zeugocystis*) are unknown, and these taxa were excluded from the analyses of life-history evolution. In general, there was a broad agreement among different literature sources regarding the life-history modes of individual species.

To reconstruct the general pattern of life-history evolution, we first mapped the presence of direct development or a larval stage on the combined-data phylogeny using parsimony and likelihood methods. Parsimony reconstructions were performed using MacClade, version 4.0 (Maddison and Maddison 2000), assuming equal rates for gains and losses of direct development. Maximum-likelihood reconstructions were performed using the model of Lewis (2001) as implemented in Mesquite version 1.05 (Maddison and Maddison 2004). Branch lengths for likelihood analyses were estimated from the Bayesian analysis of the combined nuclear and mitochondrial data (lengths for each branch averaged from among the post burn-in trees); we believe this general approach of using molecular branch length estimates is standard for most phylogenetic comparative studies. However, analyses also were performed using equal and ultrametric branch lengths (the latter generated using Mesquite). Initially, we assumed a single rate for losses and gains of the aquatic larval stage. The best estimate of the character state at each node was determined using the likelihood ratio test. If the log likelihoods of two states differed by 2.0 or more units, the state with the lower likelihood was rejected, and the alternate state was considered the best estimate for that branch with strong statistical support (following Pagel 1999). If the difference in log likelihoods was smaller (i.e., < 2.0), the reconstruction was considered ambiguous.

Next, we considered a model in which the transition rates to and from direct development (0 to 1 and 1 to 0) were treated as independent parameters. We then tested whether this two-rate model had a significantly better fit to the data than a one-rate model (i.e., same rate for 0 to 1 and 1 to 0 transitions), using a likelihood ratio test. Given that the two models are nested and differ by one free parameter, we assessed significance using a Chi-square test with one degree of freedom (Mooers and Schluter 1999; Pagel 1999).

Our initial results suggested that the aquatic larval stage reappeared from an ancestor with direct development within *Gastrotheca*. We examined the robustness of this result with respect to uncertainty in the phylogeny using Bayesian ancestral state reconstruction (e.g., Huelsenbeck et al. 2003), using MrBayes version 3.1.2. Based on the parsimony and likelihood reconstructions, a critical question is whether direct development was the condition in the ancestor of *Gastrotheca*. Although there is some uncertainty regarding the intergeneric relationships and relationships within *Gastrotheca*, the monophyly of the genus is strongly supported by separate and combined analyses of the mitochondrial and nuclear genes. Therefore, we constrained the monophyly of *Gastrotheca* and determined the posterior probabilities of the ancestral states at this node. To do this, we added life-history mode as a binary character to the combined data matrix, estimated the phylogeny, and reconstructed the ancestral state for *Gastrotheca* using Lewis' (2001) model of character evolution and the "inferanc" option in MrBayes. Adding this character does affect some weakly supported relationships in the tree. To have comparable results among methods, we performed maximum-likelihood reconstructions on these same trees, and used them as the primary trees for interpreting the results.

We also tested the sensitivity of the results to a range of different rates of gains and losses of direct development, following Oakley and Cunningham (2002). Using Mesquite, we examined several different ratios of gain and loss rate (i.e., rate of gains of 1 vs. rates of loss of 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001, and 0.0000001) and calculated the likelihood assuming that direct development is present in the common ancestor of *Gastrotheca* and assuming that the biphasic life cycle is present, under each combination of rates. The presence of direct development at this node would suggest that the biphasic life cycle re-evolved within *Gastrotheca*, whereas the presence of the biphasic life cycle would suggest that direct development evolved multiple times among hemiphractid genera without reversing. An absolute difference of 2.0 or greater in the log likelihoods for each state indicates potentially significant support for either evolutionary scenario. We also examined the consequences of decreasing the rate of gains relative to the rate of losses of direct development, using the same increments.

Given that we have sampled only about 50% of described hemiphractid species in our phylogeny, we also performed two experiments to address the possible impacts of incomplete taxon sampling on our likelihood reconstructions, again using Mesquite. First, we added unsampled species to the genera in which life-history mode is invariant (such that their uncertain phylogenetic placement should have limited consequence for the ancestral reconstruction). Second, we added all unsampled species, including species of *Gastrotheca*. For the first analysis, we added taxa to represent all unsampled species of *Cryptobatrachus, Flectonotus, Hemiphractus,* and *Stefania* (20 species total) in the Bayesian phylogram. Each species within a genus was initially given a branch length equal to the mean of all the branch lengths (internal

and terminal) within the crown group clade of that genus and assigned a life-history character state. After the species were added to a genus, the relationships within the genus were collapsed into a polytomy and then randomly resolved, using 10 different replicates (note that branch lengths also were randomized by Mesquite). Although this approach randomized the reconstructed relationships for the sampled species, note that all taxa had the same character state within a given genus (based on available life-history data summarized in the Global Amphibian Assessment; IUCN et al. 2004). Thus, relationships within these genera should have relatively little impact on the reconstructions. Relationships within *Gastrotheca* remained constant for these analyses. Reconstructions of life history were then performed on all 10 replicates using both the one-rate and two-rate models. For the second analysis, we also added 11 species of *Gastrotheca* for which life-history data were available on the Global Amphibian Assessment (IUCN et al. 2004); the life-history mode is unknown in the remaining *Gastrotheca* species. These 11 species were added to the phylogeny of *Gastrotheca* and were given the mean branch length for the sampled species (as above), and then relationships within *Gastrotheca* were collapsed and randomly resolved 10 times (note that the polytomies in the other genera were randomly resolved as well) and ancestral states were again reconstructed under both likelihood models on each replicate. Although this second analysis might be considered preferable in that it includes all the relevant unsampled species, it is also problematic in that the reconstructed relationships within *Gastrotheca* are lost, and these relationships are very important to the reconstruction given that both life-history modes occur in *Gastrotheca*. We describe the results of both analyses. Parsimony reconstructions were also performed.

Correlations of Life-History Changes with Climatic Distribution

Previous studies of hemiphractids raise the possibility that differences in life-history mode may be correlated with the climatic distribution of taxa (i.e., *Gastrotheca* with tadpoles occur at relatively high elevations; Duellman and Maness 1980; Wassersug and Duellman 1984). We obtained climatic data for each sampled species, and then tested for a relationship between the climatic distribution of species and changes in life-history mode.

We obtained locality data for each species from literature records and then determined the values for 19 climatic variables at each locality. For most localities, no coordinates were given, and localities were georeferenced using an online gazetteer (Global Gazetteer, Version 2.1; http://www.fallingrain.com/world/). We obtained from 1 to 15 georeferenced localities per species (mean = 3.3). Although sample sizes were small in many cases, many hemiphractid species have highly restricted geographic ranges and limited elevational distributions (mean < 1000 m), suggesting relatively narrow climatic distributions. We obtained climatic data for each locality for 19 climatic variables using the WORLDCLIM dataset at 2.5-sec resolution (Hijmans et al. 2004, 2005), using the BIOCLIMav extension (Beta 1.1; A. Moussalli, Cooperative Research Centre for Tropical Rainforest Ecology and Management, Brisbane, Australia) in ArcView GIS 3.3. We averaged climatic variables across localities within a species to estimate a mean value for each climatic variable for each species. We acknowledge that this characterization of climatic distribution is relatively crude, but should capture the dramatic differences in climate experienced by different hemiphractid species (i.e., hemiphractids collectively occur from tropical rainforests near sea level to above treeline at >4000 m in the Andes).

We tested whether evolutionary changes in life-history mode were associated with significant changes in climatic distribution. Given that climatic variables are quantitative and continuous and reproductive mode is binary, we followed the general approach of McPeek (1995). We estimated the change in each climatic variable along each branch of the phylogeny and tested whether there was a greater evolutionary change in climatic distribution on branches in which life history changed versus those on which it remained the same, using a nonparametric Mann–Whitney U test in Statview. We focused on two climatic variables, annual mean temperature (Bio1) and annual mean precipitation (Bio12), which are obvious descriptors of the general climatic distribution of a species (especially in tropical latitudes with limited seasonality). We inferred the estimated change along each branch for each variable using the linear generalized least squares method of Martins and Hansen (1997), as implemented in COMPARE, version 4.6b (Martins 2004). Evolutionary changes in reproductive mode were inferred using likelihood as described above. Analyses were run separately using changes in reproductive mode inferred from the one-rate and two-rate analyses (reconstructions from the one-rate model are similar to those from parsimony, and some ambiguous branches in the one-rate likelihood analysis were resolved to match the parsimony reconstructions). We also performed nonphylogenetic analyses to test for difference in the climatic distribution of species with direct development versus a biphasic life cycle.

Some readers may be uncomfortable with the idea of analyzing the climatic distribution of species on a phylogeny. However, there is growing evidence that general climatic niches can be conserved over evolutionary time scales (e.g., Ricklefs and Latham 1992; Peterson et al 1999; review in Wiens and Graham 2005), perhaps even for hundreds of millions of years in some amphibians (e.g., tropical vs. temperate distribution; Wiens and Graham 2005). Statistical analyses in a related group of frogs (Hylidae) have found significant phylogenetic conservatism in climatic distribution variables in clades that are tens of millions of years old (Smith et al. 2005; Wiens et al. 2006). Furthermore, given that we are primarily focusing on how changes in climatic distribution are related to changes in life history, conservatism in climatic traits

Table 2. Summary of genes used in phylogenetic analyses.

Gene	Total characters	Variable characters	Parsimony-informative characters	Likelihood model
Mitochondrial genes				
12S	1040 (924 included)	531	403	GTR+I+Γ
ND1	820 (818 included)	513	443	GTR+I+Γ
16S	602 (515 included)	233	175	GTR+I+Γ
Nuclear genes				
POMC	500	213	139	GTR+Γ
RAG-1	1407	424	252	GTR+I+Γ

over long time scales is not required (i.e., we are actually assuming that climatic distribution will change among species, not stay the same).

Results

PHYLOGENY

Basic properties of the five genes are listed in Table 2. The combined nuclear and combined molecular data both show strong support for monophyly of Hemiphractidae (Fig. 1). We find no support for the recent suggestion that Hemiphractidae is polyphyletic and should be split into multiple families (Frost et al. 2006); their hypothesis of polyphyly may be an artifact of long-branch attraction in parsimony analyses of a dataset dominated by fast-evolving mtDNA data and with limited taxon sampling of hemiphractids.

There is relatively strong support for the relationships among hemiphractid genera, which comes largely from the slow-evolving nuclear genes. Although the results shown exclude *Cryptobatrachus*, analyses including this taxon show weak support for placing it as the sister group of *Stefania*. Duellman et al. (1988) recognized four species groups within *Gastrotheca* (*marsupiata, nicefori, ovifera,* and *plumbea*), and considered the *ovifera* group to be the sister taxon of the other three groups based on immunological distance data. Our results from the combined DNA sequence data are broadly concordant with this arrangement. However, we find that the *ovifera* group is paraphyletic and should not be recognized; although most species do form a clade, *G. fissipes* is the sister group to all other *Gastrotheca* and *G. ovifera* is more closely related to the other three species groups. All species of the former *ovifera* group appear to have direct development.

All remaining *Gastrotheca* form a strongly supported clade that includes all *Gastrotheca* with aquatic larvae. Most species fall into one of two clades, which are generally concordant with the *marsupiata* and *plumbea* groups of Duellman et al. (1988), with the *plumbea* group here including *G. monticola, G. litonedis, G. orophylax, G. plumbea, G. riobambae, G. dunni, G. argenteovirens, G. trachyceps, G. aureomaculata,* and *G. ruizi* and the *marsupiata* group including *G. zeugocystis, G. griswoldi, G. peruana, G. pseustes, G. stictopleura, G. atympana, G. excubitor, G. ochoai, G. marsupiata, G. gracilis, G. christiani,* and *G. chrysosticta* (Fig. 1). However, we find that *G. nicefori* (*nicefori* group) is nested within the *plumbea* group and so we do not recognize the *nicefori* group. We also find that *G. psychrophila* belongs to the *marsupiata* group (not the *plumbea* group) and that *G. galeata* (previously in the *marsupiata* group) is weakly placed outside both species groups.

CHARACTER EVOLUTION

Maximum-likelihood reconstruction assuming a single rate for both gains and losses of life-history mode (Fig. 1) strongly suggests that direct development was acquired early in the phylogenetic history of hemiphractids (in the sister group to *Flectonotus*) and that the tadpole stage re-evolved within *Gastrotheca* ($-\ln L = 26.1759$). These reconstructions also imply that direct development then evolved repeatedly within the *marsupiata* and *plumbea* groups of *Gastrotheca*. There also is very strong support for this pattern using Bayesian ancestral reconstruction, which takes into account uncertainty in the phylogeny and branch lengths (and also assumes one rate for both gains and losses). The posterior probability of direct development being the ancestral state for the ancestor of *Gastrotheca* is 0.999. A similar pattern is also found in parsimony analyses (not shown), and when likelihood analyses under the one-rate model are conducted using equal branch lengths or ultrametric branch lengths (results not shown).

A very different pattern is supported when gains and losses of direct development are allowed to evolve under different rates in the maximum-likelihood analysis ($-\ln L = 21.5631$). Under this model, there is no reappearance of the tadpole stage (Fig. 1). Instead, there has been repeated origins of direct development among hemiphractid genera and within *Gastrotheca*. Importantly, the two-rate model has a significantly better fit to the data than the one-rate model based on a likelihood ratio test (test statistic $= 9.2258$; df $= 1$; $P < 0.005$). However, when equal branch lengths are used, the reconstructions from this model unambiguously support the general pattern found under the one-rate model

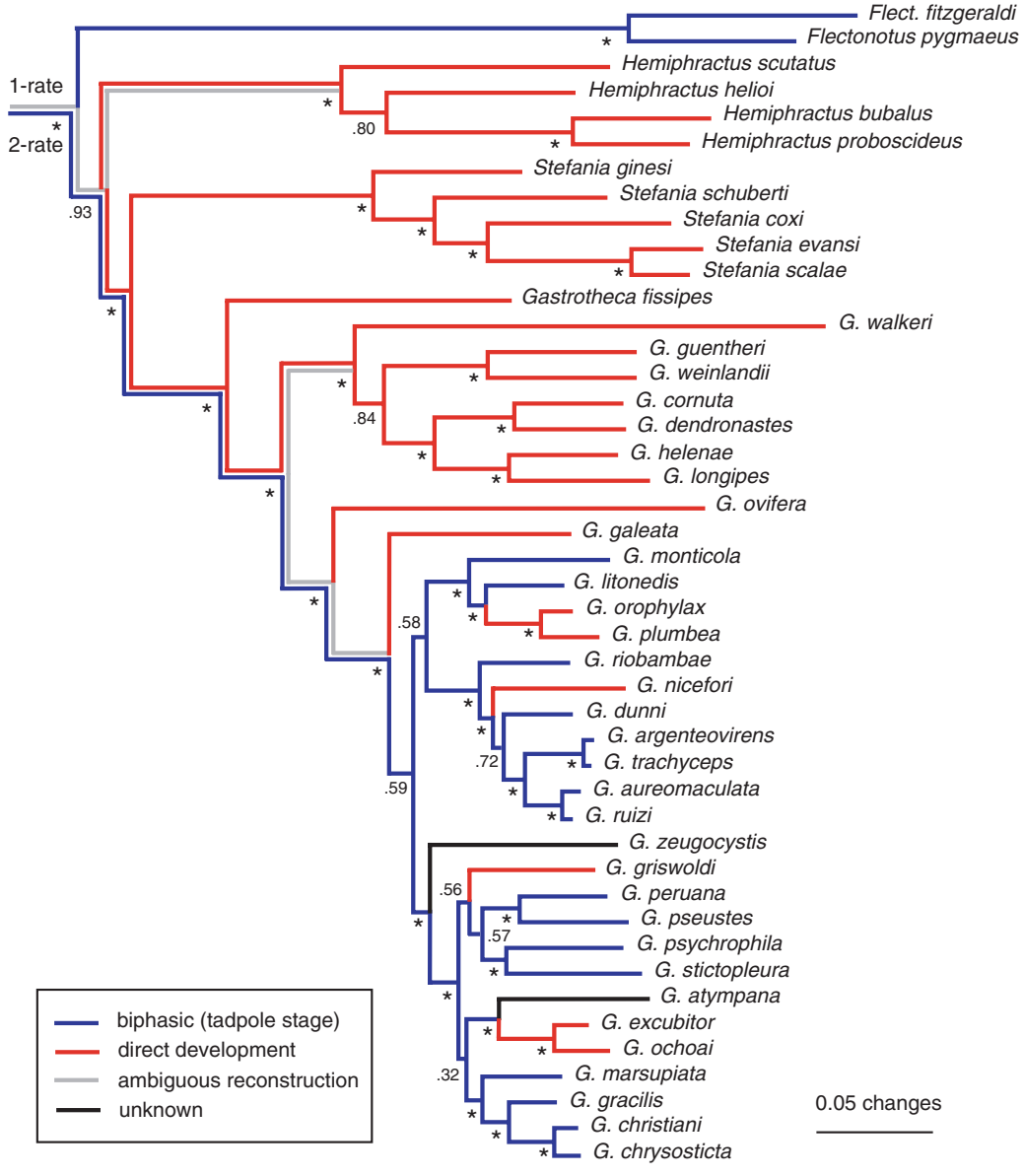

Figure 1. **Reconstructed phylogeny and life-history evolution in marsupial frogs (Hemiphractidae). Phylogeny is based on a combined, partitioned Bayesian analysis of two nuclear genes and three mitochondrial gene regions. Outgroup taxa are not shown. Asterisks indicate clades with posterior probabilities $\geq$ 0.95. Colored branches indicate the reconstructed ancestral state for life-history mode using maximum likelihood. Paired branches indicate that the reconstructions differ depending on whether a one-rate model (top branch) or two-rate model (bottom branch) is used (i.e., single rate for gains and losses of direct development vs. different rates for gain and loss). For unpaired branches, the same state is reconstructed under both models. For each branch and model, a reconstruction is only shown as unambiguous if it is significantly supported by a likelihood ratio test. Results shown were used in the Bayesian reconstruction of ancestral states (life-history mode included, *Gastrotheca* monophyly constrained) and *Cryptobatrachus* excluded, but results are similar in other analyses.**

(i.e., reversal of direct development), and reconstructions for the pivotal nodes are ambiguous when ultrametric branch lengths are used (results not shown).

Examining a range of relative rates of gains and losses of direct development shows significant support for the re-evolution of the biphasic life cycle in *Gastrotheca* even if the rate of gains is 10 times higher than the rate of losses (Fig. 2). However, if the rate of gains is 10,000 times higher or more, then the alternate hypothesis (multiple gains of direct development across hemiphractid genera and no reversal) is significantly supported. Indeed, under the two-rate model, the estimated rate of gains of direct development is 5.6382 and the rate of losses is 5.0505×10^{-6}. If the rate of

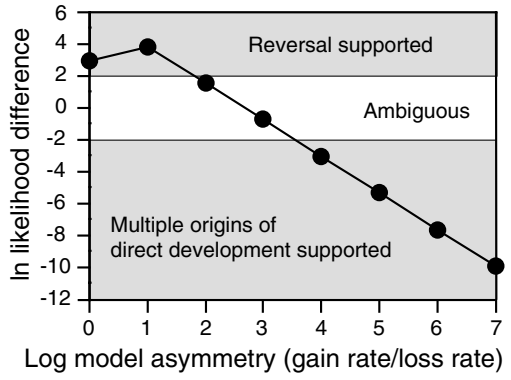

Figure 2. Likelihood sensitivity analysis for the reconstruction of life-history evolution in marsupial frogs (Hemiphractidae), focusing on the common ancestor of *Gastrotheca*. The presence of direct development at this node would suggest that the biphasic life cycle re-evolved in *Gastrotheca* from ancestors with direct development, whereas the presence of the biphasic life cycle would suggest that direct development evolved multiple times among hemiphractid genera without reversing. Model asymmetry refers to the rate of gain/rate of loss of direct development (e.g., rate of gains of 1 vs. rates of loss of 1, 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001, and 0.0000001). We calculated the likelihood assuming that direct development is present in the common ancestor of *Gastrotheca* and then assuming that the biphasic life cycle is present, under each combination of rates. An absolute difference of 2.0 or greater in the log likelihoods for each state under a given combination of rates indicates significant support for either evolutionary scenario. A similar analysis, but assuming higher rates of loss than gain, consistently favors the re-evolution of the biphasic life cycle in *Gastrotheca* (results not shown).

losses of direct development is higher than the rate of gains, then the reversal hypothesis is significantly supported across all values examined (results not shown).

When 20 unsampled species are randomly added to the genera *Cryptobatrachus, Flectonotus, Hemiphractus,* and *Stefania,* the reversal from direct development to the biphasic life cycle is unambiguously supported in all replicates under the one-rate model using maximum likelihood (also under parsimony). Under the two-rate model, this pattern is ambiguous in 9 of 10 replicates and the repeated origins are shown unambiguously in one replicate (but the likelihood values are close to those significantly supporting this pattern in the other nine replicates). When 31 unsampled species are added (including the 11 species of *Gastrotheca*), the pattern of reversal becomes ambiguous under the one-rate model, and the pattern of multiple gains (no reversal) is unambiguously supported in all 10 replicates (for parsimony, reversal is supported in seven replicates, multiple gains in two, and one is ambiguous). The differences between the results in the two analyses (20 vs. 31 added taxa) may be an artifact of the randomization of the phylogeny within *Gastrotheca*, given that many direct-developing lineages are no longer consistently placed at the base of this clade in the randomized trees as they are in the reconstructed phylogeny.

Analyzing the raw distributional and climatic data (without considering phylogeny), shows significant differences between the direct developing and biphasic hemiphractids ($P < 0.01$ based on Mann–Whitney U test for 43 species). On average, the 25 sampled species with direct development occur at lower elevations (mean = 1515.9 m), with higher annual mean temperatures (mean = 18.8°C) and precipitation (2422.0 mm), relative to the 18 sampled species with a free-living tadpole stage (mean elevation = 2279.1 m; mean temperature = 15.0°C; mean precipitation = 1346.0 mm). Phylogeny-based analyses suggest a marginally significant association between the evolution of direct development and shifts into climatic regimes with lower annual mean precipitation, when changes are reconstructed under the one-rate model ($P = 0.0418$). However, there is no relationship with mean temperature or elevation, or with any of these variables under the two-rate model ($P > 0.25$).

Discussion

The evolution of direct development is a critical transition in vertebrate history that has been replayed repeatedly, especially in anuran amphibians. There have been no hypothesized cases of this transition reversing in anurans, with the sole exception of hemiphractids (Duellman and Trueb 1986; Pough et al. 2004). Here, we test this hypothesis with a rigorous phylogenetic analysis. We find that the biphasic *Flectonotus* is at the base of the hemiphractid tree, and that the other biphasic hemiphractids (all montane *Gastrotheca*) are nested deep inside a clade of direct-developing species. Reconstructing the evolution of life-history modes on this tree suggests two possible explanations: (1) the free-living tadpole stage was lost and then re-appeared within *Gastrotheca*; (2) the free-living tadpole stage was retained in *Flectonotus* and some *Gastrotheca*, and lost (i.e., evolution of direct development) repeatedly in all other hemiphractid lineages. The first hypothesis is strongly supported by Bayesian, parsimony, and likelihood methods when assuming a single rate for gains and losses of direct development, whereas the second is strongly supported by maximum likelihood under the two-rate model. At least one of these ancestral reconstruction methods has been strongly misled, because both cannot be correct.

WHICH RECONSTRUCTION IS CORRECT?

The two-rate likelihood model has a significantly better fit to the data, seemingly making this the preferred model in this case (e.g., Pagel 1999; Mooers and Schluter 1999). However, several lines of evidence suggest that this is the analysis that has been misled.

Table 3. Reconstructed values of elevation and climatic variables for key branches having strongly divergent reconstructions for different life-history modes (Fig. 1), followed by ranges of mean values among species (and mean of mean values) for these variables for species with different life-history modes.

	Elevation (m)	Annual mean temperature (°C)	Annual mean precipitation (mm)
Gastrotheca + *Stefania*	993	21.1	2296
Gastrotheca	724	22.2	1764
Gastrotheca (excluding *G. fissipes*)	1064	20.7	1650
Extant biphasic species	1351–3396 (2492)	9.5–17.0 (14.0)	770–3424 (1325)
Extant direct developing species	6–3487 (1516)	8.1–25.2 (18.8)	608–5115 (2422)

First, the two-rate reconstruction suggests many independent gains of direct development on six phylogenetically adjacent branches (Fig. 1). Paradoxically, this reconstruction also shows unambiguously that the tadpole stage has been retained for long periods of time in the ancestral lineages leading to the extant biphasic *Gastrotheca*. Such a combination of a high rate of change and long-term stasis seems biologically unlikely (see also Kohlsdorf and Wagner 2006).

Second, the alternate hypothesis (reversal of direct development) is also more generally robust to different assumptions about branch lengths (e.g., equal, ultrametric), asymmetries in rates of gain and loss (Fig. 2), and the phylogenetically conservative addition of unsampled taxa. Thus, relatively minor changes to the analysis under the two-rate model will cause the results to converge on those of the one-rate model.

Third, analyses of the elevational and climatic distribution of life-history modes suggest that direct development is favored in hemiphractids that occur in lower elevation habitats. No biphasic hemiphractids occur in lower elevation habitats (none below 1000 m, and most above 2000 m), with the exception of *Flectonotus*, in which the tadpole stage is very short, tadpoles do not feed, and many typical larval features are reduced or absent (Wassersug and Duellman 1984). But the pattern suggested by the two-rate reconstruction implies that the tadpole stage was retained for millions or tens of millions of years under just these conditions (recent likelihood estimates for the age of hemiphractids range from 31.4 to 44.8 million years old; Wiens 2007). We also reconstructed ancestral values of elevation, temperature, and precipitation for the three branches on which reconstructions show significantly different results (*Stefania* + *Gastrotheca*; *Gastrotheca*; *Gastrotheca* exclusive of *G. fissipes*), using linear generalized least squares as described above. For all three branches, the reconstructed values of elevation and temperature are outside the range of mean species values for biphasic hemiphractids exclusive of *Flectonotus* (Table 3).

Finally, extant hemiphractid lineages that have a tadpole stage also have a pouch (absent in the direct-developing *Cryptobatrachus, Hemiphractus,* and *Stefania*). If the pouch is required for bearing tadpoles on the dorsum, then the two-rate reconstruction would require an unusual pattern of pouch evolution as well.

In summary, use of the "correct" model (i.e., the model with the highest likelihood) seemingly leads to an incorrect but statistically well-supported inference in this case, a finding that highlights the need for cautious interpretation and testing of the results of likelihood ancestral state reconstructions. Given the evidence favoring the results of the one-rate model, we cautiously add our analysis of hemiphractid frogs to the growing number of studies that show the loss and reappearance of seemingly complex and important morphological and ecological traits (e.g., wings, eyes, larvae, digits; Whiting et al. 2003; Oakley 2003; Chippindale et al. 2004; Kohlsdorf and Wagner 2006).

CAUSES OF METHOD FAILURE

What might cause likelihood ancestral reconstruction to give strongly supported but misleading results? Some insight into the potential causes of model discordance and failure in hemiphractids can be gleaned from comparison of our results with those of a recent study that inferred reappearance of the aquatic larval stage from direct-developing ancestors in plethodontid salamanders (Chippindale et al. 2004). In contrast to hemiphractids, the inference of reversal in plethodontids is relatively robust to different assumed rates of gains and losses. We hypothesize that the key difference is that in hemiphractids there clearly have been multiple origins of direct development among biphasic, high-montane *Gastrotheca* (under both models). This pattern suggests a high overall rate of gains of direct development, regardless of whether there are any reversals. In contrast, there are only two gains of direct development across all of plethodontids under the best-fitting likelihood model. Thus, there is little basis for postulating that rates of gain and loss are different in plethodontids, because both appear to be low. To test the hypothesis that the many unambiguous gains of direct development are responsible for the conflicting reconstructions in hemiphractids, we simply switched the life-history modes of select high-montane *Gastrotheca* from direct developing to biphasic (*G. excubitor, G. griswoldi, G. ochoai, G. litonedis,*

G. nicefori, G. orophylax). With these four unambiguous origins of direct development in *Gastrotheca* artificially removed, the results from the two-rate model converge on those of the one-rate model for the conflicting branches in Figure 1, and re-evolution of the biphasic life-history mode is strongly supported by likelihood under both models (results not shown).

We hypothesize that the two-rate model gives a misleading reconstruction because it assumes a uniformly high rate of gains of direct development across the tree, even though this high rate seems unlikely among the low-elevation hemiphractids. Such changes in rates across a phylogeny could be quite common, and the development of methods that can accommodate different rates of change across the tree may offer one way to ameliorate this problem (see also Mooers and Schluter 1999; Pagel 1999). Along these lines, Pagel's Discrete program now allows one to incorporate a parameter for heterogeneous rates of change across the tree (based on gamma from Yang 1994). Using Discrete, we found that adding a rate heterogeneity parameter when reconstructing the ancestral state for the ancestor of *Gastrotheca* has little impact on results for the one-rate model, but causes the results of the two-rate model to converge slightly toward the one-rate model (proportional likelihood of reconstructing direct development as present at this node goes from 0.98 to 0.63). However, addition of the gamma parameter does not significantly increase the likelihood for either the one- or two-rate models, based on likelihood ratio tests (one-rate: test statistic = 1.3356; $P > 0.20$; two-rate: test statistic = 0.1024; $P > 0.20$). Further exploration of approaches for incorporating rate heterogeneity in ancestral reconstructions should be a priority for future theoretical and empirical research. In an intriguing parallel, ignoring rate heterogeneity (among characters) may also cause likelihood methods for tree reconstruction methods to be consistently and strongly misled (e.g., Gaut and Lewis 1995).

Some readers may be concerned that our methodological results are simply an artifact of not including all hemiphractid species in our phylogeny. In theory, greater taxon sampling might cause the two models to converge on the same answer. We think the more important issues are that there are conditions in an empirical dataset in which the two models can give discordant answers with strong support, that the results from the significantly best-fitting model seem most likely to be incorrect in this case, and that (if our hypothesis about the causes of this pattern is correct) this same problem may occur in many other studies as well. We also note that our taxon sampling is neither trivial nor unusually poor (e.g., more species included in our study than in 89% of the studies in Table 1 of Mooers and Schluter 1999), given that we have sampled 46 hemiphractid species and > 50% of the described species diversity in this group. We also performed experiments to address the impact of adding the unsampled species, and found that adding taxa does not consistently favor either hypothesis.

EVOLUTION INSIDE THE EGG

Assuming that the biphasic life cycle does reappear, a critical question is whether the distinctive tadpole morphology actually re-evolves. The alternate hypothesis is that larval structures are retained in embryos inside the eggs of direct-developing species, rather than being evolutionarily lost and regained. Wassersug and Duellman (1984) studied the anatomy of larvae and embryos in 22 hemiphractid species, including those with biphasic life cycles and direct development. Mapping key larval features on our phylogeny for the 17 species sampled in both studies (and including *Cryptobatrachus* as the sister group to *Stefania*) shows different patterns for different larval features. For example, using parsimony, keratinized mouthparts are lost in the common ancestor of hemiphractids exclusive of *Flectonotus* and are regained independently in *Stefania* and in the ancestor of the biphasic *Gastrotheca* (but note that *Stefania* and basal *Gastrotheca* have direct development). Gill filters are lost in the ancestor of hemiphractids and regained in the ancestor of *Gastrotheca*. However, in both cases these putative reversals are ambiguous using one-rate maximum likelihood (but note that reconstructions without reversal imply some of the same improbable patterns of character retention discussed above). Using both parsimony and likelihood, the ventral velum and branchial food trap were both present in the ancestors of hemiphractids and *Gastrotheca*, but are reduced in many direct developers and entirely lost in *Hemiphractus* and *Stefania*. Overall, these observations suggest that some major features of the tadpole stage may have been evolutionarily lost and regained, whereas others are generally retained (although in reduced form) in species with direct development. However, there is considerable uncertainty in these reconstructions of tadpole structures within the egg.

CLIMATE AND LIFE-HISTORY EVOLUTION

What evolutionary and ecological forces drive transitions between direct development and complex life cycles? We addressed this question using GIS-derived climatic data, and our study may be one of the first to use these methods to test the environmental correlates of macroevolutionary character change. Nonphylogenetic analyses show that direct developers tend to occur in warm, wet, lowland tropical forests whereas most biphasic species occur in high-elevation habitats that are cooler and drier. However, phylogenetic analyses suggest that origins of direct development are associated with shifts to drier climatic regimes and not wetter climates.

These results suggest an important dichotomy between those conditions in which a trait is most favorable versus those where a trait is most likely to change. Assuming that environmental conditions do drive changes in a given trait, then we might expect few changes under environmental conditions in which the trait is most favorable, and expect the most changes under conditions in which

the fitness of both traits are more nearly equal. For phylogeny-based methods, the relationship between variables may be determined primarily by evolutionary changes in these traits. Thus, the fact that one variable is favored under a given set of conditions may be completely obscured, because the methods identify the conditions in which the trait is changing the most rather than the conditions in which the trait is favored and being maintained without change. In hemiphractids, we see few changes at lower elevations (where direct development seemingly is favored), and many changes at higher elevations where both direct developing and biphasic species occur. Given the climatic data alone, it is not clear what drives transitions between direct development and biphasic lifestyles.

We speculate that competitive release might also be an important factor in the reacquisition of the free-living tadpole stage. In much of the range of the genus, *Gastrotheca* are the only larvae inhabiting high-elevation ponds, and the only relatively large tadpoles in other parts of their range (Wassersug and Duellman 1984). Wassersug and Duellman (1984) and Duellman et al. (2001) noted that pond-breeding *Gastrotheca* species do not occur in sympatry. Thus, reacquisition of aquatic larvae may have been favored by an open niche in high-elevation ponds. Many experimental studies suggest that larvae of pond-breeding anuran species compete (Alford 1999; Pough et al. 2004). The absence of other anuran larvae in high-elevation ponds may be explained by the inability of low-elevation clades to tolerate high-elevation climatic regimes, or the inability of most pond-breeding anuran lineages to colonize steep mountain slopes. Most anuran species on the Andean slopes are direct developers (e.g., *Eleutherodactylus*) or else have tadpoles that develop in fast-flowing streams (e.g., centrolenids, *Colostethus* [Dendrobatidae], some hylids; Duellman 1988). However, climate seems likely to play an important role in the distribution of direct development in anurans on a global scale, given that most cool temperate regions lack species with direct development (e.g., northern North America, Europe, northern Asia, southern South America; IUCN et al. 2004; AmphibiaWeb 2006). This remains an area in need of further study.

Conclusions

Our study addresses several general issues. First, we demonstrate that phylogenetic reconstructions of character evolution in natural systems can generate misleading results with strong statistical support (as previously demonstrated for phylogeny estimation; Felsenstein 1978). Such results do not call for the abandonment of phylogenetic comparative methods, but underscore the need for their continued testing, improvement, and appropriately cautious interpretation (e.g., Oakley and Cunningham 2000; Polly 2001; Webster and Purvis 2002; Finarelli and Flynn 2006). Second, we show how GIS-based environmental data can be integrated into studies of character evolution. These methods helped to identify the potentially misleading results, given that the tadpole stage appears more likely to have re-evolved than be retained for long periods under seemingly unfavorable environmental conditions. Third, our results suggest an important dichotomy between those selective regimes in which a trait is favored and those where it is most likely to change, a dichotomy that might also lead to erroneous conclusions in some cases. Fourth, our results further demonstrate that, contrary to some interpretations of Dollo's law, many important morphological and ecological traits can reappear after being evolutionarily lost (e.g., wings in stick insects; Whiting et al. 2003; shell coiling in gastropods; Collin and Cipriano 2003; compound eyes in crustaceans; Oakley 2003; digits in lizards; Kohlsdorf and Wagner 2006). Finally, as in plethodontid salamanders (Chippindale et al. 2004), we speculate that ecological release from competition may be an important factor in the reacquisition of complex life cycles.

ACKNOWLEDGMENTS

Many hemiphractid samples used in the present study were initially used by Duellman et al. (1988), and we thank the many individuals who helped WED and L. Maxson obtain samples for that earlier study, including D. C. Cannatella, S. Gorzula, W. R. Heyer, J. D. Lynch, R. W. McDiarmid, and C. W. Myers. S. B. Hedges kindly facilitated our access to these samples more recently. We thank the following individuals and institutions who helped in providing additional tissue samples for our study, including E. Lehr (Museum für Tierkunde Dresden), R. MacCulloch (Royal Ontario Museum), and E. Wild. For assistance in the laboratory we thank S. Bermann, J. Fetzner, V. Jurgens, C. Parkinson, R. Peter, S. Smith, and C. Yesmont. For helpful comments on the manuscript we thank D. Moen, S. Smith and other members of the Wiens lab group, T. Oakley, and two anonymous reviewers. Financial support was provided by grants from the U.S. National Science Foundation (DEB 0331747 and EF 0334923) to JJW.

LITERATURE CITED

Alfaro, M. E., S. Zoller, and F. Lutzoni. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. Mol. Biol. Evol. 20:255–266.

Alford, R. A. 1999. Ecology: resource use, competition, and predation. Pp. 240–278 *in* R. W. McDiarmid and R. Altig, eds. Tadpoles. The biology of anuran larvae. Univ. of Chicago Press, Chicago, IL.

AmphibiaWeb. 2006. Information on amphibian biology and conservation. [web application]. Berkeley, California: AmphibiaWeb. Available: http://amphibiaweb.org/. Accessed: June 11, 2006.

Brandley, M. C., A. Schmitz, and T. W. Reeder. 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of scincid lizards. Syst. Biol. 54:373–390.

Brooks, D. R., and D. A. McLennan. 1991. Phylogeny, ecology, and behavior: a research program in comparative biology. Univ. of Chicago Press, Chicago, IL.

Chippindale, P. T., R. M. Bonett, A. S. Baldwin, and J. J. Wiens. 2004. Phylogenetic evidence for a major reversal in life history evolution in plethodontid salamanders. Evolution 58:2809–2822.

Collin, R., and R. Cipriano. 2003. Dollo's law and the re-evolution of shell coiling. Proc. R. Soc. Lond. B. 270:2551–2555.

Cunningham, C. W. 1999. Some limitations of ancestral character-state reconstruction when testing evolutionary hypotheses. Syst. Biol. 48:665–674.

Darst, C. R., and D. C. Cannatella. 2004. Novel relationships among hyloid frogs inferred from 12S and 16S mitochondrial DNA sequences. Mol. Phylogenet. Evol. 31:462–475.

Dollo, L. 1893. Les lois de l'évolution. Bul. Soc. Belge Géol. Pal. Hydr. 7:164–166.

Donoghue, M. J. 1989. Phylogenies and the analysis of evolutionary sequences, with examples from seed plants. Evolution 43:1137–1156.

Driskell, A. C., C. Ané, J. G. Burleigh, M. M. McMahon, B. C. O'Meara, and M. J. Sanderson. 2004. Prospects for building the tree of life from large sequence databases. Science 306:1172–1174.

Duellman, W. E. 1988. Patterns of species diversity in anuran amphibians in the American tropics. Ann. Mo. Bot. Gard. 75:79–104.

———. 2001. Hylid frogs of Middle America. 2nd ed. Society for the Study of Amphibians and Reptiles, Lawrence, KS.

Duellman, W. E., and D. M. Hillis. 1987. Marsupial frogs (Anura: Hylidae: *Gastrotheca*) of the Ecuadorian Andes: resolution of taxonomic problems and phylogenetic relationship. Herpetologica 43:141–173.

Duellman, W. E., and S. J. Maness. 1980. The reproductive behavior of some hylid marsupial frogs. J. Herpetol. 14:213–222.

Duellman, W. E., and L. Trueb. 1986. Biology of amphibians. McGraw-Hill, New York.

Duellman, W. E., L. R. Maxson, and C. A. Jesiolowski. 1988. Evolution of marsupial frogs (Hylidae: Hemiphractinae): immunological evidence. Copeia 1988:527–543.

Duellman, W. E., E. Lehr, and C. Aguilar. 2001. A new species of marsupial frog (Anura: Hylidae: *Gastrotheca*) from the Cordillera Azul in Peru. Sci. Pap. Nat. Hist. Mus. Univ. Kansas 22:1–10.

Erixon, P., B. Svennblad, T. Britton, and B. Oxelman. 2003. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Syst. Biol. 52:665–673.

Faivovich, J., C. F. B. Haddad, P. C. A. Garcia, D. R. Frost, J. A. Campbell, and W. C. Wheeler. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bull. Am. Mus. Nat. Hist. 294:1–240.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27:401–410.

———. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.

Finarelli, J. A., and J. J. Flynn. 2006. Ancestral state reconstruction of body size in the Caniformia (Carnivora, Mammalia): the effects of incorporating data from the fossil record. Syst. Biol. 55:301–313.

Frost, D. R., T. Grant, J. Faivovich, R. Bain, A. Haas, C. F. B. Haddad, R. O. de Sá, S. C. Donnellan, C. J. Raxworthy, M. Wilkinson, et al. 2006. The amphibian tree of life. Bull. Am. Mus. Nat. Hist. 297:1–370.

Futuyma, D. J. 2005. Evolution. Sinauer Associates, Sunderland, MA.

Gaut, B. S., and P. O. Lewis. 1995. Success of maximum likelihood phylogeny inference in the four-taxon case. Mol. Biol. Evol. 12:152–162.

Hart, M. W., M. Byrne, and M. J. Smith. 1997. Molecular phylogenetic analysis of life-history evolution in asterinid starfish. Evolution 51:1848–1861.

Hijmans, R. J., S. E. Cameron, J. L. Parra, P. G. Jones, and A. Jarvis. 2004. The WorldClim interpolated global terrestrial climate surfaces. Version 1.3. Available at http://biogeo.berkeley.edu/.

———. 2005. Very high resolution interpolated climate surfaces for global land areas. Internat. J. Climatol. 25:1965–1978.

Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182–192.

Huelsenbeck, J. P., and B. Rannala. 2004. Frequentist properties of Bayesian posterior probabilities. Syst. Biol. 53:904–913.

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Huelsenbeck, J. P., R. Nielsen, and J. P. Bollback. 2003. Stochastic mapping of morphological characters. Syst. Biol. 52:131–158.

IUCN, Conservation International, and NatureServe. 2004. Global amphibian assessment. www.globalamphibians.org. Accessed on 15 October 2004.

Kohlsdorf, T., and G. P. Wagner. 2006. Evidence for the reversibility of digit loss: a phylogenetic study of limb evolution in the genus *Bachia* (Gymnophthalmidae: Squamata). Evolution 60:1896–1912.

Leviton, A., R. Gibbs, E. Heal, and C. Dawson. 1985. Standards in herpetology and ichthyology: Part 1. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. Copeia 1985:802–832.

Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. Syst. Biol. 50:913–925.

Maddison, D. R., and W. P. Maddison. 2000. MacClade 4.0. Sinauer Associates, Sunderland, MA.

———. 2004. Mesquite: a modular system for evolutionary analysis. Version 1.05 http://mesquiteproject.org.

Marshall, C. R., E. C. Raff, and R. A. Raff. 1994. Dollo's law and the death and resurrection of genes. Proc. Natl. Acad. Sci. USA 91:12283–12287.

Martins, E. P. 2004. COMPARE, version 4.6. Computer programs for the statistical analysis of comparative data. Distributed by the author at http://compare.bio.indiana.edu/. Department of Biology, Indiana Univ., Bloomington, IN.

Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. Am. Nat. 149:646–667.

McPeek, M. A. 1995. Testing hypotheses about evolutionary change on single branches of a phylogeny using evolutionary contrasts. Am. Nat. 145:686–703.

Mendelson, J. R. III, H. R. Da Silva, and A. M. Maglia. 2000. Phylogenetic relationships among marsupial frog genera (Anura: Hylidae: Hemiphractinae) based on evidence from morphology and natural history. Zool. J. Linn. Soc. 128:125–148.

Mooers, A. Ø., and D. Schluter. 1999. Reconstructing ancestor states with maximum likelihood: support for one- and two-rate models. Syst. Biol. 48:623–633.

Nylander, J. A. A. 2004. MrModeltest 2.0. Program distributed by the author. Evolutionary Biology Centre, Uppsala Univ. (http://www.ebc.uu.se/systzoo/staff/nylander.html).

Nylander, J. A. A., F. Ronquist, J. P. Huelsenbeck, and J. L. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. Syst. Biol. 53:47–67.

Oakley, T. H. 2003. On homology of arthropod compound eyes. Integr. Comp. Biol. 43:522–530.

Oakley, T. H., and C. W. Cunningham. 2000. Independent contrasts succeed where explicit ancestor reconstructions fail in a known bacteriophage phylogeny. Evolution 54:397–405.

———. 2002. The independent evolutionary origin of an arthropod compound eye. Proc. Natl. Acad. Sci. USA 99:1426–1430.

Pagel, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. Syst. Biol. 48:612–622.

Pagel, M., A. Meade, and D. Barker. 2004. Bayesian estimation of ancestral character states on phylogenies. Syst. Biol. 53:673–684.

Palumbi, S. R., A. Martin, S. Romano, W. O. MacMillan, L. Stice, and G. Grabowski. 1991. The simple fool's guide to PCR, version 2.0.

Department of Zoology and Kewalo Marine Laboratory, Univ. of Hawaii, Honolulu, Hawaii.

Peterson, A. T., J. Soberon, and V. Sanchez-Cordero. 1999. Conservatism of ecological niches in evolutionary time. Science 285:1265–1267.

Phillipe, H., E. A. Snell, E. Bapteste, P. Lopez, P. W. H. Holland, and D. Casane. 2004. Phylogenomics of eukaryotes: impact of missing data on large alignments. Mol. Biol. Evol. 21:1740–1752.

Polly, P. D. 2001. Paleontology and the comparative method: ancestral node reconstructions versus observed values. Am. Nat. 157:596–609.

Pough, F. H., R. M. Andrews, J. E. Cadle, M. L. Crump, A. H. Savitzky, and K. D. Wells. 2004. Herpetology. 3rd ed. Prentice-Hall, Upper Saddle River, NJ.

Ricklefs, R. E., and R. E. Latham. 1992. Intercontinental correlation of geographical ranges suggests stasis in ecological traits of relict genera of temperate perennial herbs. Am. Nat. 139:1305–1321.

San Mauro, D., D. J. Gower, O. V. Oomen, M. Wilkinson, and R. Zardoya. 2004. Phylogeny of caecilian amphibians (Gymnophiona) based on complete mitochondrial genomes and nuclear RAG1. Mol. Phylogenet. Evol. 33:413–427.

Schluter, D., T. Price, A. Ø. Mooers, and D. Ludwig. 1997. Likelihood of ancestor states in adaptive radiation. Evolution 51:1699–1711.

Simpson, G. G. 1953. The major features of evolution. Columbia Univ. Press, New York.

Smith, S. A., P. R. Stephens, and J. J. Wiens. 2005. Replicate patterns of species richness, historical biogeography, and phylogeny in Holarctic treefrogs. Evolution 59:2433–2450.

Swofford, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony*, version 4.0b10. Sinauer Associates, Sunderland, MA.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673–4680.

Van Valen, L. 1979. Switchback evolution and photosynthesis in angiosperms. Evol. Theory 4:143–146.

Ventakesh, B., M. V. Erdmann, and S. Brenner. 2001. Molecular synapomorphies resolve evolutionary relationships of extant jawed vertebrates. Proc. Natl. Acad. Sci. USA 98:11382–11387.

Wassersug, R. J., and W. E. Duellman. 1984. Oral structures and their development in egg-brooding hylid frog embryos and larvae: evolutionary and ecological implications. J. Morph. 182:1–37.

Webster, A. J., and A. Purvis. 2002. Testing the accuracy of methods for reconstructing ancestral states of continuous characters. Proc. R. Soc. Lond. B. 269:143–150.

Whiting, M. F., S. Bradler, and T. Maxwell. 2003. Loss and recovery of wings in stick insects. Nature 421:264–267.

Wiens, J. J. 1998. Combining data sets with different phylogenetic histories. Syst. Biol. 47:568–581.

———. 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Syst. Biol. 52:528–538.

———. 2007. Global patterns of species richness and diversification in amphibians. Am. Nat.: *In press*.

Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. Ann. Rev. Ecol. Syst. 36:519–539.

Wiens, J. J., J. W. Fetzner, C. L. Parkinson, and T. W. Reeder. 2005. Hylid frog phylogeny and sampling strategies for speciose clades. Syst. Biol. 54:719–748.

Wiens, J. J., C. H. Graham, D. S. Moen, S. A. Smith, and T. W. Reeder. 2006. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. Am. Nat. 168:579–596.

Wilcox, T. P., D. J. Zwickl, T. A. Heath, and D. M. Hillis. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25:361–371.

Wray, G. A. 1996. Parallel evolution of nonfeeding larvae in echinoids. Syst. Biol. 45:308–322.

Yang, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. J. Mol. Evol. 39:306–314.

Associate Editor: K. Crandall

Supplementary Material

The following supplementary material is available for this article:

Appendix S1. Voucher specimens. Standard institutional abbreviations follow Leviton et al. (1985), and nonstandard abbreviations are: ICNMNH = Instituto de Ciencias Naturales, Museo de Historia Natural, Universidad Nacional de Colombia; LM = Linda Maxson voucher specimen; MHNSM = Museo de Historia Natural, Universidad Nacional Mayor de San Marcos; MTD = Museum für Tierkunde Dresden; RWM = Roy W. McDiarmid field series.

Appendix S2. GenBank accession numbers. Unless otherwise indicated, numbers refer to sequences generated for this study.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1558-5646.2007.00159.x
(This link will take you to the article abstract.)

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.