ORIGINAL ARTICLE

DID EGG-LAYING BOAS BREAK DOLLO'S LAW? PHYLOGENETIC EVIDENCE FOR REVERSAL TO OVIPARITY IN SAND BOAS (*ERYX*: BOIDAE)

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Re-evolution of lost complex morphological characters has been proposed for several characters, including insect wings, limbs, eyes in snakes, and digits in lizards, among others. There has also been much interest in whether the transition from oviparity to viviparity is reversible, particularly in squamate reptiles where the transition to viviparity has occurred more times than in any other lineage. Here, we present a phylogenetic analysis of boid snakes based on a concatenated multigene study of all genera of erycines, New and Old World boines, plus other groups thought to be closely related with boines such as monotypic species *Calabaria* and *Casarea*. We reconstruct ancestral parity mode on this phylogeny and present statistical evidence that oviparity reevolved in a species of Old World sand boa in the genus *Eryx* nearly 60 million years after the initial boid transition to viviparity. Remarkably, like other viviparous boas hatchlings of oviparous *Eryx* lack an egg-tooth providing independent evidence that oviparity is a derived state in these species.

KEY WORDS: Dollo's law, oviparity, parity mode, reversal, snake phylogeny, viviparity.

There is a growing interest in whether the loss of complex characters is evolutionarily reversible. Reevolution has been suggested for eyes in ostracods (Dingle 2003; Oakley 2003; Hunt 2007) and snakes (Laurent 1983; Coates and Rutta 2000), ocelli in cave crickets (Desutter-Grandcolas 1993), wings in water spiders (Anderson 1997), fig wasps (Whiting and Whiting 2004), and stick insects (Whiting et al. 2003), digits in lizards (Kohlsdorf and Wagner 2006; Brandley et al. 2008), developmental stages in salamanders (Chippindale et al. 2004; Mueller et al. 2004) and frogs (Wiens et al. 2007), teeth in lynx (Kurtén 1963), and thigh muscles in birds (Raikow 1975), among others (West-Ebehard 2003). These apparent violations of Dollo's law (Dollo 1893, 1922; Gould 1970) have shifted the debate on reversals away from whether they are theoretically possible, to what the evolutionary and developmental mechanisms of reversion may be (Marshall et al. 1994; Porter and Crandall 2003; Collin and Miglietta 2008). For example, how can a long lost structure be reevolved if the underlying developmental program for the structure is lost, and

how long can these networks survive unused in the absence of stabilizing selection (Bull and Charnov 1985)? These developmental objections to reversibility are bolstered by the recent finding that commonly used phylogenetic tests of irreversibility suffer from serious flaws, calling into question many previous findings of irreversibility (Goldberg and Igic 2008).

The evolutionary transition from oviparity (egg-laying) to viviparity (live-birth) has occurred numerous times in vertebrates, particularly in squamate reptiles. Indeed, the number of independent origins of viviparity from oviparity has occurred more times in squamates than in all other vertebrate lineages combined (Blackburn 1982, 1985; Shine 1985). Although this transition is generally considered to be irreversible (Neill 1964; Fitch 1970; Guillette et al. 1980; Blackburn 1982), there has been much debate recently over whether it is actually so, because it seems to be at least theoretically possible for reversion to egg-laying to occur (Lee and Shine 1998). The largest and most detailed analysis on the evolution of viviparity in reptiles thus far identified

five possible reversals (Lee and Shine 1998). Although these reversals were weakly supported, i.e., they could be "unreversed" by one or two additional parsimony steps, it was suggestive that reversals may have occurred. Similarly, other recent studies of parity evolution (deFraipont et al. 1996; Benabib et al. 1997; deFraipont et al. 1999; Schulte et al. 2000; Surget-Groba et al. 2006) have been inconclusive about possible reversals because alternate scenarios requiring no reversals were equally parsimonious, were a single step away from the next most parsimonious reconstruction that required no reversals, or were based on poorly resolved phylogenies (Méndez de la Cruz et al. 1998) and inappropriate tests of irreversible evolution (Goldberg and Igic 2008). Thus, none of the possible reversals to oviparity identified to date are strongly supported by statistical evidence, and there is little data that convincingly demonstrates viviparity is, in fact, reversible.

Although most studies on the evolution of viviparity focus on lizards, snakes have independently evolved viviparity more than 30 times, including at least 14 times in colubrids (Greene 1997) and 13 times in vipers (Lynch 2009). Reversions to oviparity may even have occurred in vipers, which have many closely related species that differ in parity mode (Greene 1997). Intriguingly, although the 41 species of Old and New World boas (Boidae) are generally thought to be exclusively viviparous (Greene 1997), there are at least two species of recently described Old World sand boas (*Eryx*), the Arabian sand boa (*E. jayakari*) and the Saharan sand boa (*E. muelleri*), that are oviparous (Fig. 1) (Staub and Emberton 2002). The placement of these oviparous species within an otherwise exclusively viviparous clade is extremely suggestive that reversions to oviparity may have occurred.

In this article, we conduct a phylogenetic analysis of boid snakes based on a concatenation of nuclear loci and mitochon-drial genes for 41 species of boid snakes, including all genera of erycines, New and Old World boines, plus other groups thought to be closely related with boines such as the Ungaliophi-idae, and the monotypic Bolyerid *Casarea*. Using this phylogeny, we reconstruct parity mode using phylogenetic models that account for the errors discovered by Goldberg and Igic (2008), and present strong statistical evidence that *E. jayakari* reevolved oviparity from viviparity; this conclusion is well supported by parsimony- and likelihood-based character reconstructions. These results suggest that reversals to oviparity can occur and suggest that parity reversals may be more common than previously, thought.

Materials and Methods
PHYLOGENETIC ANALYSIS

A robust phylogeny of snakes is vital for understanding the evolution of parity mode in this group. Recently, several molecular phylogenetic studies of snakes have been published that have used different genes, but have partially overlapping taxon sampling. To further resolve uncertain relationships within boids and generate a tree for character reconstructions, we combined sequences from sixteen nuclear loci, *c-mos, NT3, BDNF, RAG1, ODC* (Noonan and Chippindale 2006a), *AHR, FSHR, GPR37, MKL1, NGFB, PNN, PTGER4, PTPN12, SLC30A1, TRAF6*, and *ZEB2* (Wiens et al. 2008), with five mitochondrial loci, *CytB, 12s, tRNA^{val}, 16s*, and *NADH4* (Slowinski and Lawson 2002; Vidal and Hedges 2002; Wilcox et al. 2002; Noonan and Chippindale 2006a) from 41 in-group and three out-group species into a single concatenated dataset (Table 1). Alignments were the same as in the original investigations, and in the case of the *12s, tRNA^{val}*, and *16s*, data were based on a structural model of RNA folding (Slowinski and
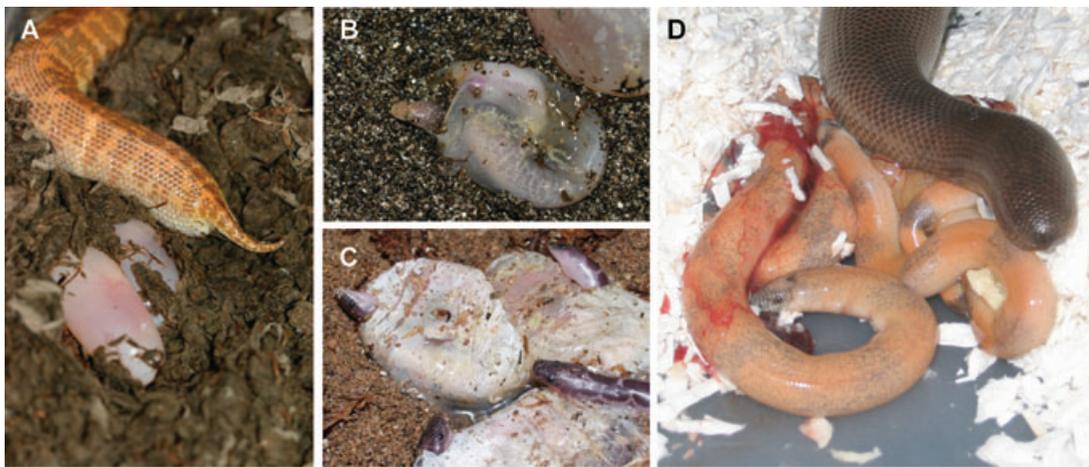

Figure 1. Examples of oviparous and viviparous *Eryx* species. (A) *Eryx jayakari* laying eggs. (B and C) *Eryx jayakari* and *Eryx muelleri* hatching. Note that hatchling *Eryx jayakari* lack and egg-tooth and force through the egg-shell with their heads and that the shell is very thin. (D) *Eryx johni* giving birth. Photos courtesy of Robert Stout (*Eryx jayakari* and *Eryx muelleri*) and Jason Hess (*Eryx johni*).

Table 1. Properties of genes used in the phylogenetic analyses.

Gene	Length (bp)	Variable characters	Informative characters	Taxa
Nuclear				
BDNF	668	166	53	20
CMOS	569	183	71	18
NT3	487	192	102	19
ODC	581	301	118	19
RAG1	1372	397	165	19
AHR	444	80	20	8
FSHR	753	74	56	10
GPR37	509	56	32	8
MKL1	927	125	35	8
NGFB	579	89	26	11
PNN	927	110	27	9
PTGER4	469	51	27	7
PTPN12	649	127	39	9
SLC30A1	522	68	20	10
TRAF6	633	75	18	9
ZEB2	882	66	14	9
Mitochondrial				
12S, tRNA^{VAL}, 16S	1520	712	536	11
CytB	1113	635	524	44
NADH4	813	429	262	20
Totals	14417	3936	2145	44

Lawson 2002; Vidal and Hedges 2002; Wilcox et al. 2002; Noonan and Chippindale 2006a; Wiens et al. 2008).

Sequence data for every gene were not available for all taxa. The combined dataset ranged in completeness from *Candoia bibroni* with only 270 (2%) characters sampled, to *Calabaria reinhardti* with 13,044 (99%) characters sampled, and a mean completeness of 4047 (30%) characters. This level of incompleteness is not unusual for multilocus analyses of higher-level phylogenies (Philippe et al. 2004; Rokas and Carroll 2006), and previous simulation and empirical studies have found that likeli-

hood and Bayesian analyses accurately reconstruct phylogenies with even large amounts of missing data (Wiens 2003; Driskell et al. 2004; Philippe et al. 2004; Wiens et al. 2005; Wiens 2006; Wiens and Moen 2008). In addition, although the mean completeness is only 30% of characters for most taxa, the major lineages of snakes important for our inference of parity reversal are sampled with significantly greater completeness. For example, at least one species from each major group is sampled with at least 78% completeness. Therefore, the relationships between the major lineages of boid snakes are based on large amounts of complete data, whereas intragroup relationships are based on smaller amounts of data (a "branch and leaf" model of character sampling). For example, *Eryx colubrinus* has ~83% completeness, whereas the remaining species of *Eryx* are 5–25% complete. Finally, although *E. muelleri* is not included in this study, its phylogenetic relationships to the other species of *Eryx* is not vital for our inference that a parity reversal occurred, although depending on its relationships to the other *Eryx* species more than one reversal may have occurred.

We used a combined maximum likelihood and Bayesian approach to infer phylogenetic relationships. Maximum likelihood partition selection for the phylogenetic analysis used RA×ML v2.2.3 (Stamatakis 2006) and increasingly complex data partitioning schemes. Following Castoe and Parkinson (2006), partition models ranged in complexity from a single partition that applied a single GTR + Γ substitution model across all sites and genes (1x model) to a partitioning scheme that applied a separate GTR + Γ substitution model to each codon position of each protein coding gene and each stem and loop of the rRNA genes (58×) model. Although RA×ML is primarily a phylogenetic inference program, it incorporates the option to apply different GTR models to user-specified partitions, thus, RA×ML can be used to determine an appropriate partitioning scheme before additional analyses. Based on ΔAIC comparisons, the 58× model was preferred (Table 2).

Bayesian phylogenetic analyses were performed with Mr-Bayes v3.0 (Huelsenbeck and Ronquist 2001) using the 58×

Table 2. Description of data partitioning used for model selection. The best model (AIC rank 1) is in bold. Various partitioning schemes (description) were evaluated using RA×ML, and the negative log likelihood of each model (−ln *L*) is shown, along with its ΔAIC score and rank. The number of parameters in the model is shown in PRMS.

Model	PRMS	Description	−ln L	ΔAIC	Rank
1x	10	Single GTR+Γ4 model extended to entire data set	−50,394.56	6717.84	6
2x	20	Independent GTR+Γ4 models for protein-coding, rRNA genes	−50,372.52	6693.76	5
21x	210	Independent GTR+Γ4 models for each gene	−48,351.67	3032.06	4
38x	380	Independent GTR+Γ4 models for 1st+2nd and 3rd codon positions; RNA genes	−46,850.31	369.34	3
56x	560	Independent GTR+Γ4 models for each codon position; RNA genes	−46,547.08	122.88	2
58x	**580**	**Independent GTR+Γ4 models for each codon position; stems and loops of RNA genes**	**−46,465.64**	**0.00**	**1**

partitioning scheme. Two independent Bayesian analyses were run for 10,000,000 generations each with two runs of four chains sampled every 1000 generations and a burnin of 9000 trees. Run progress was visually checked with TRACER v1.4 (Rambaut and Drummond 2007) by plotting the log-likelihoods of sampled generations, and the stability of parameter estimates (chain convergence) checked by ensuring that the standard deviation of split likelihood frequencies was below 0.01 whereas the potential scale reduction factor (PSRF) was close to 1.0 for all parameters. The Bayesian consensus tree was generated by randomly sampling 100 trees from the postburnin sample of 1000 trees.

CHARACTER EVOLUTION

Likelihood reconstructions of character evolution were performed with MESQUITE v2.01 (Maddison and Maddison 2009). The rates of forward (q_{01}) and reverse transitions (q_{10}) under an asymmetrical likelihood model with separate rates for forward and reverse transitions were estimated directly from the data (rMk2) and compared to an irreversible model (iMk2) that constrained the reverse rate to zero ($q_{10} = 0$). Because recent studies indicate that character-associated changes in diversification rate can lead to erroneous rejection of irreversible models (Goldberg and Igic 2008), we tested for character-associated diversification using the BiSSE module (Maddison et al. 2007) implemented in MESQUITE v2.01. For both Markov and BiSSE models, a stationary or "uninformative" prior was used to infer the state of the root node under reversible models, while irreversible models fixed the state of the root node at zero. The tree for the Mk2 model was the consensus derived from the Bayesian analysis transformed into ultrametric tree with an arbitrary root age of 100 using the penalized likelihood method implemented in R8s v1.7 (Sanderson 2003). For the BiSSE analysis, we accounted for uncertainty in the phylogeny and branch length estimates by summarizing parameters of the BiSSE model and testing irreversibility across the 100 randomly sampled trees used to generate the Bayesian consensus tree. Prior to BiSSE analysis, trees phylograms were transformed into ultrametric trees with an arbitrary root age of 100 using the penalized likelihood method implemented in R8s v1.7 (Sanderson 2003).

Results and Discussion

Although boas are generally considered to be all viviparous, the recent identification of two oviparous species of sand boas in the genus *Eryx* (Staub and Emberton 2002; Staub and Treacher 2007) implies that reversions to egg-laying may have occurred in these species. To test this possibility, we constructed a phylogeny of boid snakes based on data from several recent studies, including both nuclear genes and mitochondrial genes, and used maximum-likelihood methods (Mkv and BiSSE) to test for the irreversibility of viviparity and reconstruct ancestral character states.

BOID PHYLOGENY

Previous molecular and morphological studies have resolved many of the major lineages of snakes (Vidal and Hedges 2002; Wilcox et al. 2002; Lawson et al. 2004; Lee 2004; Vidal and Hedges 2004; Noonan and Chippindale 2006a; Wiens et al. 2008); however, although there are several well-supported lineages within the Boidae (boas), the systematics and biographic history of these snakes has only recently been studied in detail. For example, the Boidae has traditionally been subdivided into two subfamilies, the Boinae (*Corallus*, *Epicrates*, *Eunectes*, *Boa, Candoia, Acrantophis* and *Sanzinia*) and Erycinae (*Eryx*, *Lichanura* and *Charina*) based on morphological analyses, but molecular data suggest that *Eryx* is more closely related to the pacific island *Candoia* and the Neotropical boids than the other erycines *Lichanura* and *Charina*. Similarly, *Calabaria*, traditionally placed within the Erycinae by morphological data, has been found by some molecular analyses to be sister to the African *Acrantophis* and *Sanzinia* or the sister clade to all other boids (Vidal and Hedges 2002; Wilcox et al. 2002; Lawson et al. 2004; Lee 2004; Vidal and Hedges 2004; Noonan and Chippindale 2006a; Wiens et al. 2008).

Results from our phylogenetic analysis resolved most of the major lineages with strong support and confirmed a deeply nested position for *Eryx* within the larger Boidae family (Fig. 2) similar to other recent molecular phylogenetic analyses (Noonan and Chippindale 2006a; Wiens et al. 2008). Indeed, our tree based on the combined nuclear and mitochondrial data of Noonan and Chippindale (2006b) and Wiens et al. (2008) is nearly identical to these two previous studies. For example, the position of *Eryx* with respect to the other boids is well supported in our analyses and is the same as in Noonan and Chippindale (2006b) and Wiens et al. (2008). However, unlike Noonan and Chippindale (2006b), we find no support for the monophyly of *Calabaria* with the Malagasy *Acrantophis* and *Sanzinia*, but instead find *Calabaria* is strongly supported as the sister lineage to the remaining boids.

The phylogenetic relationship of *Casarea* has been particularly problematic for both morphological and molecular analyses. Traditionally the Bolyeriidae has been considered to be part of an unresolved polytomy with the Tropidophiidae and Caenophidia (Greene 1997), or with either the Pythonidae or Boidae (Zaher 1994); molecular data have supported a relationship with *Casarea* as sister to the pythoniforms (Lawson et al. 2004), sister to the Caenophidia (Vidal and Hedges 2004), sister to *Boa* + (*Acrochordus* + Caenophidia) (Lawson et al. 2005), or as sister to Boidae + pythoniforms (Vidal and Hedges 2002). Our data strongly support *Casarea* as sister to the Boidae + *Calabaria* (Fig. 2). Remarkably, a recent analysis of 20 nuclear loci failed to strongly support the phylogenetic position of *Casarea* (Wiens et al. 2008), but with the inclusion of additional data this relationship is well resolved.
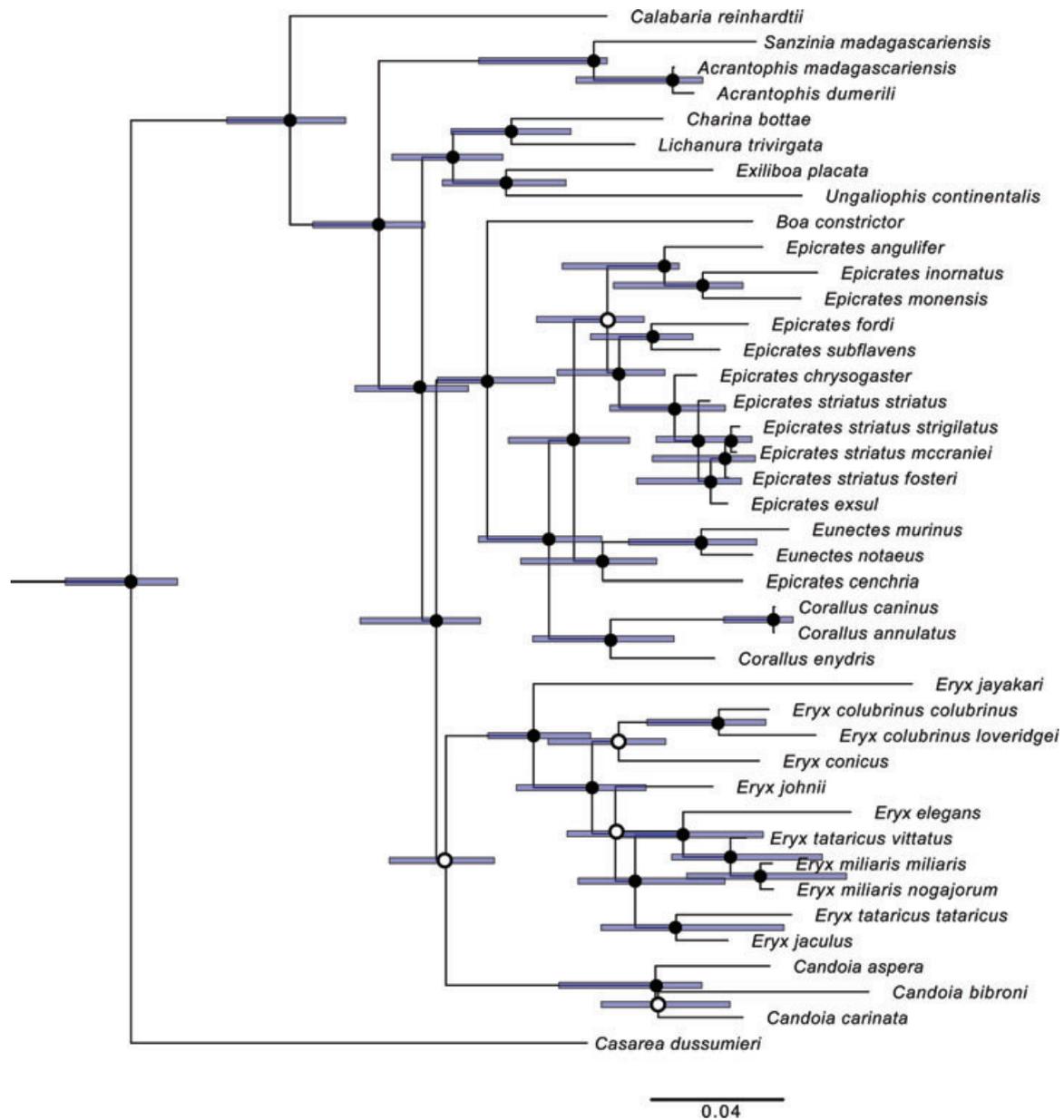
Figure 2. Bayesian phylogeny of boid snakes based combined nuclear and mitochondrial genes. Branch lengths are scaled to the number of substitutions per site, with the 95% confidence interval on branch length estimates shown as blue bars on nodes. Nodes with closed circles indicate nodes with Bayesian posterior probabilities (*PP*) ≥0.98, while open circles indicate nodes with *PP* < 0.98. Note that the out-groups (*Morelia, Aspidites* and *Loxocemus*) are not shown.

PHYLOGENETIC EVIDENCE FOR REVERSAL TO OVIPARITY

Snakes have evolved viviparity more than 30 times (Greene 1997), but although it has been suggested that reversions to oviparity may have occurred in some vipers (Greene 1997) and in some lizard species (deFraipont et al. 1996; Benabib et al. 1997; deFraipont et al. 1999; Schulte et al. 2000; Surget-Groba et al. 2006), no conclusive examples of reversal to oviparity have been identified. Intriguingly, there are at least two oviparous species within the otherwise viviparous Boidae, suggesting that reversions to

oviparity may have occurred in these lineages. Indeed, parsimony reconstruction of ancestral parity modes under an irreversible model, which allows only transitions from oviparity to viviparity, required five steps whereas an unordered and reversible model required only two steps (Fig. 3). Thus, the most parsimonious interpretation is that viviparity is reversible and oviparity has reevolved in *Eryx jayakari*. However, biasing the transition weights of the reversible model such that transitions to viviparity are twice as likely as reversals increases the cost of the reversible model from two to four steps, and biasing the reversible model such

Figure 3. Parsimony-based character reconstructions. (A) Character state reconstruction under a reversible model that allows both transitions from oviparity to viviparity and from viviparity to oviparity. Viviparous lineages are in black and oviparous lineages are in white. This model requires three steps. (B) Character state reconstruction under an irreversible model that does not allow transitions from viviparity to oviparity. This model requires five steps.

that transitions to viviparity are three times as likely as reversals increases the cost of the reversible model to five steps and equivalency with an irreversible model. Thus, the data could also be explained if gains are two to three times as likely as losses. In addition, a critical assumption of the parsimony method is that character states do not affect diversification rates, but violation of this assumption often leads to erroneous rejection of irreversibility (Goldberg and Igic 2008).

An alternate approach that can account for differences in gain/loss rates, branch lengths, and a character states effect on diversification rate is to reconstruct character states using maximum likelihood. We used two maximum likelihood models to test for the irreversibility of viviparity, maximum likelihood methods that compare an irreversible model to models that allow reversals, but do not account for the effect of the character on rates of speciation and extinction (Mk2 models), and likelihood models that jointly estimate transition rates between character states while accounting for that characters effect on speciation and extinction rates (BiSSE models). The irreversible Mk2 model (iMk2), which estimates the forward rate (oviparity-to-viviparity) directly from the data but constrained the reverse rate (viviparity-to-oviparity) to zero, was significantly worse than the reversible Mk2 model (rMk2) (Table 3). Like the parsimony method, however, Mk models are prone to falsely reject irreversibility when character states

influence diversification rates. Using the BiSSE model, which accounts for influence of character states on diversification rate, there is strong support for state-dependent diversification, with speciation rates in viviparous species ($\lambda_1 = 0.068$) estimated to be about ten times higher than in oviparous species ($\lambda_0 = 0.007$), but essentially no support for the irreversible model (Table 3).

The proportional likelihoods for the character state reconstruction at the most recent common ancestor of *Eryx* indicates there is a strong probability that the ancestor of this clade was viviparous (0.997), whereas the probability the *Eryx + Candoia* ancestor was viviparous is essentially one (Fig. 4). These data strongly support the conclusion that oviparity has reevolved in *E. jayakari*. Unlike the strong inference for parity reversal in *E. jayakari*, ancestral state reconstructions for reversal in *Calabaria* are indicate it did not regain oviparity; likelihood-based (rMK2) state reconstructions of the most recent common ancestor of *Calabaria* and the boids indicates there is only a 0.17 probability this ancestor was viviparous. Thus, we conclude there is strong statistical evidence for parity reversal in *E. jayakari* but only weak support for reversal in *Calabaria*.

LOST AND FORGOTTEN

The modern version of Dollo's law proposes that lost complex characters can never be regained or at least not regained in the

Table 3. Model comparisons testing for the irreversibility of viviparity. Speciation (λ), extinction (μ), and character transition (q) rate estimates are reported based on the time calibrated tree with a root age of 100. The likelihood of each model (ln L) are shown, followed by the ΔAICs, the best models are in bold. Note that the BiSSE model strongly rejects a single speciation rate for oviparous and viviparous species; therefore, our conclusions on reversibility are based on the BiSSE model, while the Mk and parsimony results are shown for comparison only. The No Common Mechanism (NCM) score is a scaling of the parsimony tree length into $-$log likelihoods and is shown for comparison to the other models. State 0, oviparity. State 1, viviparity.

Model	λ_0	λ_1	μ_0	μ_1	q_{01}	q_{10}	$-\ln L$	ΔAIC
BiSSE								
Free	**0.007**	**0.068**	4.1×10^{-6}	1.1×10^{-5}	**0.003**	**0.002**	**-167.61**	**0.00**
Single λ		0.044	2.5×10^{-6}	2.4×10^{-6}	0.139	0.022	-184.08	30.94
Irreversible	0.020	0.060	2.8×10^{-7}	2.0×10^{-5}	0.012	0.00	-182.85	28.48
Mk2								
Reversible	–	–	–	–	**0.003**	**0.002**	**-9.28**	**0.00**
Irreversible	–	–	–	–	0.014	0.000	-16.32	12.08
Parsimony	Cost gain		Cost reversal		Tree length		NCM	
Reversible	1		1		2		**-2.08**	**0.00**
Irreversible	1		∞		5		-4.16	2.16

same form. For example, the reevolved digits of *Bachia* and other lizards have different phalangeal formulas than species that have not undergone digit loss (Kohlsdorf and Wagner 2006; Brandley et al. 2008). Similarly, it has been suggested that if oviparous lineages descend from viviparous ones, then there might be differences in the new form of oviparity compared to primitively oviparous ancestors. During hatching, most oviparous amniotes use a specialized projection on the tip of the snout to break through the egg-shell. In squamates, this projection is a specialized tooth that projects outward from the premaxillary (Zug et al. 2001). Hatchling snakes use the egg-tooth to make several slits in the shell, but remain within the egg for up to 60 hours before emerging. Unlike all other oviparous snakes, but in common with all the other viviparous boids, *E. jayakari* and *E. muelleri* lack an egg-tooth (Staub and Emberton 2002). The loss of this character associated with oviparity suggests that the developmental pathway for egg-tooth formation has not yet been redeployed or may have been lost. Interestingly, while hatchling, *E. jayakari* and *E. muelleri* lack an egg-tooth and must forcibly push there way out of the egg, like other oviparous species they remain in the egg for several hours after rupturing it to absorb remaining yolk. Unlike the oviparous *Eryx* species, however, *Calabaria* hatchlings posses an egg-tooth, further suggesting it did not reevolve oviparity. Furthermore, both species of egg-laying sand boas have extremely thin shells (Staub and Emberton 2002; Staub and Treacher 2007), which may indicate differences in the structure of the reevolved egg-shell compared to the ancestral egg.

WHAT SELECTIVE FORCES MAY FAVOR THE RE-EVOLUTION OF THE EGG?

The selective advantages of viviparity are well studied. For example, comparative studies have identified more than 100 in-

dependent origins of viviparity from oviparity in squamate reptiles (Blackburn 1982, 1985; Shine 1985; Lee and Shine 1998; Shine and Lee 1999), including more than 40 times in snakes (Greene 1997). Remarkably, most viviparous squamates occur in cold climates, and detailed phylogenetic analyses of oviparous and viviparous taxa indicate that viviparity is not just common in colder climates, but often evolves there (Shine 1985; Shine and Lee 1999; Shine 2004). These observations and direct experimental evidence suggest that viviparity is an adaptation to cold climates because pregnant females can maintain higher and more stable body temperatures than nest sites (Shine 2004).

Although viviparity may be advantageous in cold, unstable environments, there are costs associated with live birth (Tinkle and Gibbons 1977; Shine and Bull 1979; Seigel and Fitch 1984). Seigel and Fitch (1984) suggested that the longer reproductive season of viviparous species should lead to higher mortality than for oviparous species, to compensate viviparous snakes decrease relative clutch mass (RCM), effectively decreasing the reproductive burden on pregnant females. However, the consequences of reduced RCM include reduced offspring size, clutch size, food intake and locomotor performance, and an increase in predation risk (Tinkle and Gibbons 1977; Shine and Bull 1979; Seigel and Fitch 1984). These data suggest that the advantages of reducing RCM in viviparous species are strong.

While the benefits of viviparity are well studied, the advantages of oviparity have received little attention. Decreasing RCM may ameliorate some of the negative consequences of carrying offspring to term in viviparous species, but in resource limiting environments, the costs of viviparity may be too severe to overcome with additional decreases in reproductive output (as measure by RCM). For example, decreased food intake while pregnant may favor additional decreases in RCM in viviparous species, but at
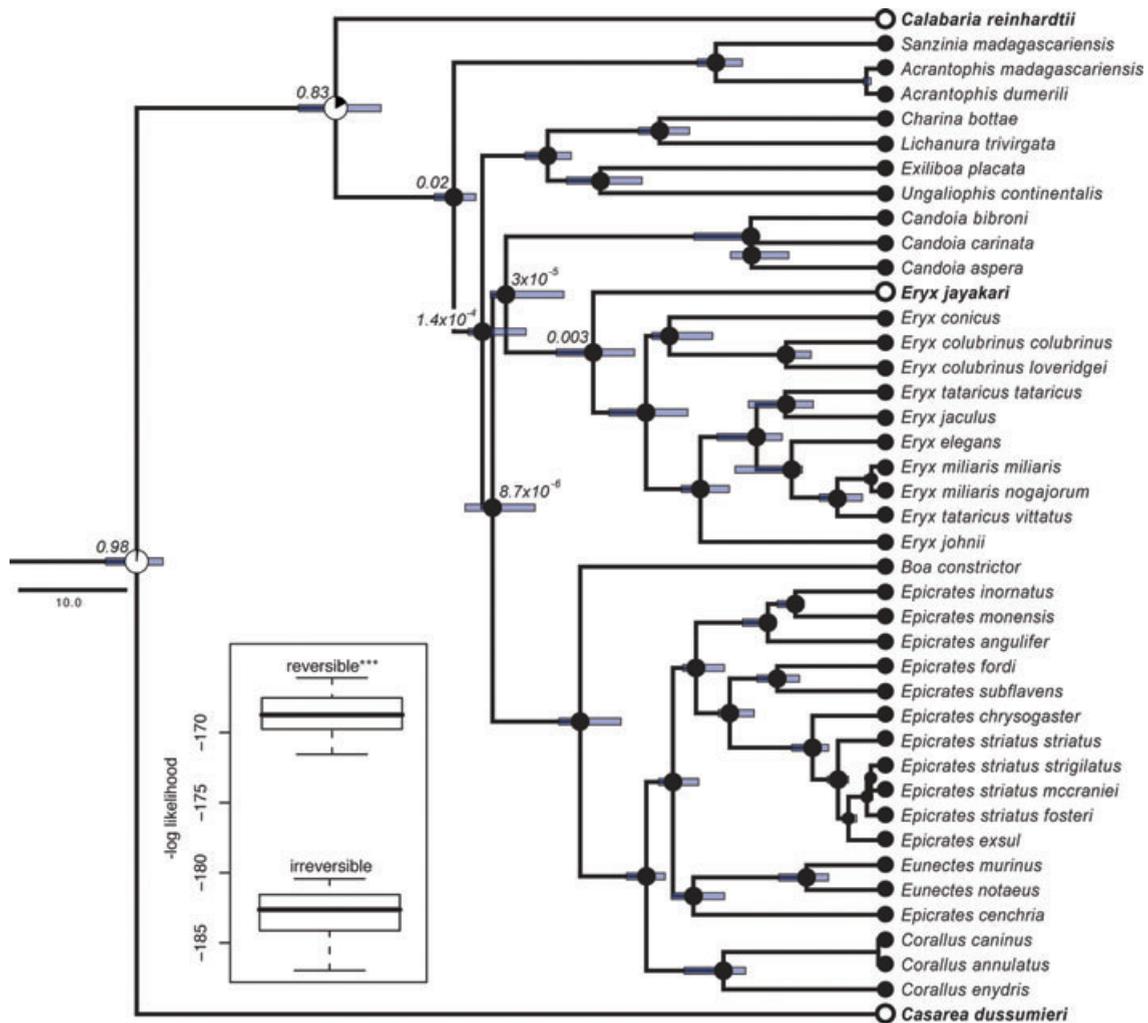
Figure 4. Likelihood-based character reconstructions. Character state reconstructions were estimated under a reversible Markov model (rMk2) using the Bayesian consensus phylogeny transformed to an ultrametric tree with a root age of 100 using penalized likelihood. Character states of terminal taxa and reconstructed ancestors are shown as pie charts drawn proportional to likelihood of the state at that node, numbers at nodes indicate the likelihood that node was oviparous and bars on nodes show the 95% confidence interval on the node age. White, oviparous; black, viviparous. Inset boxplot shows the distribution of likelihood scores generated with BiSSE for 100 trees under reversible and irreversible models. The likelihood difference between the BiSSE reversible and BiSSE irreversible models indicates that the BiSSE reversible model is significantly better than the irreversible model (ΔAIC = 28.48).

the cost of reduced clutch size or number. Similarly, the impact of carrying a litter to term may impose constraints on locomotor performance, particularly in fossorial species, which could be compensated for by reducing RCM but at the cost of decreased reproductive output. Therefore, selection can favor oviparity or decreased gestation times in viviparous species, which may lead to reversion to oviparity.

Interestingly, egg-laying sand boas span this continuum. The incubation period for *E. muelleri* eggs is only ∼14 days, but *E. jayakari* eggs take about ∼66 days to hatch (Staub and Emberton 2002; Staub and Treacher 2007). These differences suggest that the scenario often invoked to explain the origin of viviparity, prolonged egg retention leading to live birth, may have played

out in reverse during the reevolution of oviparity. As *Eryx* dispersed from their origin in Western Asia or India (Noonan and Chippindale 2006b) into the Arabian peninsula and Africa, *E. jayakari* and *E. muelleri*, respectively, invaded extremely arid deserts. In these resource-limiting environments, selection may have favored shorter gestation times and eventually reversion to oviparity over further reductions in reproductive output expected from decreasing RCM.

Conclusions

The reevolution of lost complex characters has been proposed for a growing number of characters, including insect wings, limbs,

eyes in snakes, and digits in lizards, among others. There have also been several studies suggesting that the transition from oviparity to viviparity may be reversible, particularly in squamate reptiles where the transition to viviparity has occurred more times than in any other lineage. However, convincing cases of reversion to oviparity have not previously been reported. Our reconstruction of ancestral parity modes strongly suggests that oviparity ree-volved in at least one species of Old World sand boa in the genus *Eryx*, nearly 60 million years after the initial boid transition to viviparity. Remarkably, like other viviparous boas hatchlings of oviparous *Eryx* lack an egg-tooth providing independent evidence that suggests oviparity is a derived state in these species. These re-sults suggest that the revolution of complex characters is possible and may be more common than generally recognized.

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