ON THE ROLE OF HISTORICAL CONSTRAINT IN EVOLUTION: AN EMPHASIS IN SALAMANDER EVOLUTION

By

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Abstract

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A fundamental challenge in evolutionary biology concerns estimating the extent to which ecological trade-offs impose constraints on evolution. In the first chapter, we test whether adaptive evolution of antipredator performance phenotypes in streamside salamanders was well predicted by stability in the selective regime experienced by salamander lineages. We found that the efficacy of antipredator phenotypes in salamanders is strongly related to historical duration, as well as consistency, of selection imposed by predatory fish on salamander larvae. Optimal circumstances for the evolution of effective antipredator responses in streamside salamanders seems to involve a long and consistent history of ecological contact with predatory fish.

Evolutionary biologists have long debated the relative influence of species selection on evolutionary patterns. As a test in the second chapter, we assess whether variation in species' ranges and in life-history contribute to patterns of diversification in salamanders. We find that paedotypy – wherein some organisms of a species mature in the gilled form without metamorphosing – is also associated with higher net diversification rates. Often dismissed as an insignificant process in evolution, this chapter, as well as the next, provides direct evidence for the role of species selection in lineage diversification of salamanders.

The third chapter explores whether interspecific hybridism promotes or limits lineage diversification in salamanders. Using character-mediated diversification analyses and treating hybridism as a binary evolutionary character, results suggest that hybridism acts to swallow diversity. While evidence is provided for species selection generally disfavoring hybridism in a clade of amphibians, this chapter concludes with a discussion of circumstances where hybridism may be macroevolutionarily advantageous.

The fourth chapter assesses whether coarse ecological differences among host populations are predictive of variation in forms of molecular selection in an emerging viral pathogen. We find elevated strengths of positive selection in pathogen strains associated with bait colonies of salamanders or in other captive-host environments. Given evidence of increased virulence associated with bait-associated strains of ATV, we argue that increased frequencies of tiger salamander epizootics in the western US are at least in part attributable to a relaxed virulence-transmission tradeoff in ATV-bait assemblages.

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INTRODUCTION

There are two senses of biological constraint, one of which has nearly been lost (Gould 2002): to constrain is either to compel or to confine. I do not regard these senses to be incongruous: to an unthinking evolutionary entity, it should not matter whether to be pushed from behind or led with a tether. Evolutionary constraint simply represents a state of affairs that permits only a narrowed set of outcomes while disallowing the remainder (see also Grantham 2004). If the recent analysis of a taxonomically broad dataset was insufficient evidence (see Estes and Arnold 2007), the very need for phylogenetic comparative methods is demonstrative of the pervasiveness of evolutionary constraint (Hansen and Houle 2004). Of particular interest to me is the nature and impact of constraint on the adaptive evolution of a lineage. Aside from fundamental inquiries into the strengths of particular constraints in lineages, I also take interest in the interrelation between constraint and novelty.

How are such evolutionary constraints as stabilizing selection upset, eroded, or otherwise relaxed so that evolutionary novelty can arise? Contrary to the controversial claim of novelty arising through speciation (as proposed by Eldredge and Gould 1972), Futuyma (1987) argues and Gould (2002) concedes that the origination of novelty may often precede speciation, only that speciation solidifies the independence of the newly founded lineage. Futuyma (1987), in discussing apparent bursts of morphological evolution associated with speciation events, describes how admixture erodes any sign of genetic divergence. Only through speciation are the distinctive forms (that are already present) preserved.

It may be likely that most populations maintain (or generate) sufficient variation so as to capably respond to ever changing (in both direction and strength) selective forces. Sufficient evidence of the claim is provided by the tremendous degree to which short term evolutionary rates appear to exceed those witnessed in geologic intervals (Lynch 1990; Williams 1992; Hansen and Houle 2004; Gingerich 2009). The apparent impediment to the generation of novelty would then seem to lie in something other than a lack of variation. But if sufficient variation exists and a “paradox of stasis” (Estes and Arnold 2007) permeates the fossil record (Eldredge and Gould 1972), we must ask: ‘Sufficient variation for what?’ It is interesting, and likely non-coincidental that adaptive radiation may well require the relaxation of

stabilizing selection (Roughgarden 1972; Lister 1976; Yoder et al., in press), an often sharp constraint. If there were no such constraints limiting the course of evolution of populations in exploring the adaptive landscape to happen upon empty peaks, we should expect few open niches. We also see tremendous rates of diversification in nearly every epoch, which seems to indicate a ubiquity of available but unused or underutilized niches at any given period. While in high dimensionality adaptive landscapes, populational shifting between adaptive 'peaks' may be much easier than we might intuit (Gavrilets 1999), certain constraints are generally hard to overcome, although are perhaps measurably different for different circumstances: it might be easier for a penguin lineage to re-evolve powered flight than for an annelid lineage to take to the air. It may be (only) through hybridism (Rieseberg et al. 1999; Seehausen 2004; Stelkens and Seehausen 2009), genetic drift, and transitory periods of maladaptive evolution (Mayr 1963; Rubinoff and Roux 2008) that the discontinuous novelty that arises might persist (Theissen 2009). It is through these processes that, at least initially, populations are able to escape the pull of the nearest adaptive peak, thereby becoming freed from (at least some) evolutionary constraints (Mallet 2007).

Herein, I explore multiple scales and manifestations of evolutionary constraint: temporal inconsistency of the selective regime; and selective processes operating at (and potentially conflicting among) different levels of biological organization, from strains of viruses to populations of organisms to lineages of salamanders. Analogously, I examine ecologic and genetic constraints in the evolution at infraspecific, specific, and supraspecific scales. The first, second, and fourth chapters investigate extrinsic (environmental) constraints on fitness or fitness components. The first chapter assesses whether histories of predator-induced selective regimes contribute to robust antipredator phenotypes in contemporary populations of salamander larvae. The second chapter considers fitness of higher-level clades, as potentially determined by aggregate or emergent species-level traits. The third chapter concerns the potential for a strong biotic interaction – hybridism – to act as a prime determinant of patterns in both macroecology and macroevolution. The concluding chapter explores dynamics of molecular evolution in a salamander pathogen as potentially driven by coarse ecological differences in host populations.

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CHAPTER FORMATS AND ATTRIBUTION

This dissertation comprises four chapters, each intended for separate publication. Formatting of chapters is therefore inconsistent, although each is formatted in compliance with restrictions and recommendations of the focal journal. I was the principal contributor to all chapters, with foremost involvement in study design, data collection, management, and analysis, as well as manuscript preparation. My dissertation advisor, Andrew Storfer, contributed in idea generation, study design, and manuscript preparation and revision; as such, he is a co-author on each chapter. The first chapter involved a collaboration with two additional researchers, John Niedzwiecki and B. Paul Nadler. Respectively, these colleagues contributed by supplying molecular data and by writing a Java script with which to parse data output. This first chapter is published (*Evolution* 63:2636-2647) and the second chapter has been accepted, pending satisfactorily addressed critiques, in *Systematic Biology*. The third and fourth chapters are in preparation for submission to the *American Naturalist* and to *Evolution* and are formatted accordingly.

CHAPTER ONE

DURATION AND CONSISTENCY OF HISTORICAL SELECTION ARE CORRELATED WITH ADAPTIVE TRAIT EVOLUTION IN THE STREAMSIDE SALAMANDER, *AMBYSTOMA BARBOURI*

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ABSTRACT

A fundamental challenge in evolutionary biology concerns estimating the extent to which ecological trade-offs may impose constraints on adaptive evolution. Novel ecological stressors may limit adaptive evolution of naïve lineages that have experienced historically different selective regimes. Regarded as recently derived from a pond-breeding ancestor, streamside salamanders face the novel and strong selection pressure of breeding in streams, some of which support predatory sunfish. A statistical phylogenetic approach was used to test whether adaptive evolution of antipredator performance phenotypes in streamside salamanders was positively associated with: a) estimated per-lineage duration of ecological contact with predatory fish; and, b) consistency of the antipredator selective-regime within salamander lineages. In treating salamander-fish contact as a stochastically evolving ecological trait, average durations of fish contact were computed for each salamander lineage on a set of chronograms. Selection consistency further considered the number of ecological transitions between states of the ecological trait. Historical selection in streamside salamanders can be generally characterized as unstable, apparently punctuated by the stochastic loss and reassociation with predatory fish in most lineages. We found that the efficacy of antipredator phenotypes in salamanders is strongly related to historical duration, as well as consistency, of selection imposed by predatory fish. Optimal circumstances for the evolution of effective antipredator responses involve a long and consistent history of ecological contact with predatory fish.

INTRODUCTION

A central challenge in evolutionary biology is to understand the extent to which historical constraints influence the capacity of populations to evolve in response to current selection pressures. Phylogenetic history can constrain contemporary evolution via trade-offs that result from adaptation to a selection regime that differs from current environmental conditions (Wilson 1975; Lande 1979; Sih et al. 2000; Orzack and Sober 2001). Adaptive evolution may otherwise be constrained functionally (Edwards and Naeem 1993), developmentally (Derrickson and Ricklefs 1988), environmentally (Newman 1992), or genetically (Wright 1951; Slatkin 1985, 1987; Schluter 2000). In contrast, genetic canalization for example (Kawecki 2000) may facilitate current adaptation by maintaining traits that are presently adaptive (i.e., exaptations *sensu* Gould and Lewontin 1979). Thus, understanding the course of adaptive trait-evolution among lineages necessitates examination under an explicitly historical context (Lande 1979; Estes and Arnold 2007).

If environments exert stable and consistent selection pressures (i.e., niche stability) on ecologically relevant traits, lineages should evolve toward local adaptive optima (Fisher 1930; Haldane 1930; Wright 1932; Kawecki and Ebert 2004). However, variable selection induced by niche instability can constrain population mean fitness if phenotype-environment tradeoffs exist and affected lineages fail to evolve phenotypic plasticity (Lynch 1987; Kawecki 2000; Huerta-Sanchez et al. 2008). Tradeoffs preventing fitness maximization across all temporally encountered environments for particular genotypes, should be observable through comparative phylogenetic analysis if one were able to: i) assess the historical variability of selective pressures on lineages; and, ii) estimate the trait optima associated with the different selective regimes. Despite the temporal lability of selection imposed by environmental variation expected in nearly every biotic system, few studies have tested whether fluctuating historical selective pressures constrain adaptive evolution in contemporary populations.

One of a pair of sister salamander taxa (streamside salamander, *Ambystoma barbouri*; smallmouth salamander, *A. texanum*) is well-suited for testing the effects of phylogenetic and ecological history on phenotypic trait evolution. The stream-breeding *A. barbouri* is hypothesized to have diverged during the

late Pleistocene from the pond-breeding *A. texanum* due to climatic warming, pond drying, and thus decreased availability of pond-breeding habitat (Petranka and Sih 1987; Kraus and Petranka 1989). Putatively monophyletic, streamside salamanders are endemic to a narrow geographic range centered in Kentucky with few peripherally-isolated populations in adjacent areas (Kraus and Petranka 1989; Fig. 1). In contrast, smallmouth salamanders have a widespread and largely contiguous geographic range throughout much of eastern United States (Kraus and Petranka 1989; Petranka 1998). It is currently presumed that *A. texanum* is the progenitor of *A. barbouri* (Kraus and Petranka 1989) because pond-breeding is the ancestral state for Ambystomatidae (Petranka 1998) and more widely distributed taxa are presumed to have greater speciation probabilities than narrowly distributed species (Darwin 1859; Rosenzweig 1995; Demastes et al. 2007; Eastman et al. 2007).

FIGURE 1. Sampling localities and geographic ranges of *A. barbouri* and *A. texanum*.

TABLE 1. Locality data and accession numbers for analysis of mtDNA sequence data.

species	accession	haplotype	north	west
A. barbouri	EU980549	AM	38.43	84.97
	EU980578	BC-a	37.89	84.38
	EU980583	BC-b	37.89	84.38
	EU980584	CC	37.89	84.39
	EU980589	CL	39.52	83.98
	EU980574	CR	37.79	84.55
	EU980587	DB	37.89	84.37
	EU980586	FC	37.77	84.57
	EU980545	GC	37.97	84.83
	EU980592	LV-a	37.37	88.46
	EU980590	LV-b	37.37	88.46
	EU980580	MC	37.88	84.45
	EU980591	MR	38.33	82.47
	EU980575	MU-a	37.88	84.45
	EU980582	MU-b	37.88	84.45
	EU980570	RL-a	35.82	86.48
	EU980571	RL-b	35.82	86.48
	EU980572	RL-c	35.82	86.48
	EU980585	RR-a	37.90	84.39
	EU980581	RR-b	37.90	84.39
	EU980588	RR-c	37.90	84.39
	EU980579	RU	37.90	84.39
	EU980577	SC-a	38.65	85.61
	EU980553	SC-b	38.65	85.61
	EU980548	SL	38.49	85.32
	EU980576	SW	38.20	84.75
	EU980573	TP	38.19	84.58
	EU980546	WC-a	38.02	84.84
	EU980547	WC-b	38.02	84.84
A. texanum	EU980561	BD-a	37.40	87.40
	EU980567	BD-b	37.40	87.40
	EU980568	BD-c	37.40	87.40
	EU980558	CM	38.76	92.20
	EU980550	DW-a	40.24	84.66
	EU980552	DW-b	40.24	84.66
	EU980589	GR-a	39.74	84.01
	EU980551	GR-b	39.74	84.01
	EU980569	LK	38.93	95.23
	EU980562	LV-a	37.37	88.46
	EU980563	LV-b	37.37	88.46
	EU980564	LV-c	37.37	88.46
	EU980565	LV-d	37.37	88.46
	EU980556	MF-a	39.43	81.47
	EU980557	MF-b	39.43	81.47
	EU980559	PR-a	37.21	88.12
	EU980560	PR-b	37.21	88.12
	EU980566	PR-c	37.21	88.12
	EU980554	RC	39.90	84.92
	EU980555	SC	38.69	85.67
A. bishopi	EU517608			
A. californiense	AY659995			
	NC006890			
A. cingulatum	EU517584			
A. mexicanum	AJ584639			
	AY659991			
A. tigrinum	AY659992			
	NC006887			
	EU980544*			

The invasion of ephemeral stream habitats (Maurer and Sih 1996) exposed *A. barbouri* to a new selective regime (Sih et al. 2000). In comparison to more permanent ponds, highly ephemeral stream habitats impose strong selection for rapid metamorphosis (Kats et al. 1988). As predicted, empirical laboratory and field studies support that apparently adaptive high larval activity rates are positively

correlated with high feeding and metamorphosis rates (Petranka and Sih 1987; Maurer and Sih 1996; Sih et al. 2000). However, some populations of *A. barbouri* breed in more permanent streams that support predatory fish. In these habitats, selection favors reduced larval activity and consequent predator avoidance (Kats et al. 1988; Sih et al. 2000, 2003). Thus, streamside salamander larvae face conflicting selection pressures in different streams (Storfer and Sih 1998; Storfer et al. 1999; Sih et al. 2000). *Ambystoma barbouri* thereby comprises two apparent ecotypes, whereby selection favors relatively high activity levels in ephemeral streams and low activity levels in fish-bearing streams.

Generally, streamside salamanders are observed to have poor antipredator avoidance behavior, and it is suggested this is attributable to contemporary gene flow between fish and fishless habitats (Storfer and Sih 1998; Storfer et al. 1999). It has also been hypothesized that *A. barbouri* may be phylogenetically constrained in their response to the novel selective force of fish predation due to its relatively recent divergence in a genus with a long evolutionary history of pond-breeding in fishless habitats (Petranka 1998; Sih et al. 2000).

Herein, we test two hypotheses to discern whether phylogenetic history, and specifically the history of selection in each deme explains observed variation in antipredator behavior among populations of *A. barbouri*. We test whether the efficacy of antipredator performance in *A. barbouri* is positively correlated with lineage-specific evolutionary *duration* of coexistence with fish, as well as *consistency* of historical selection induced by predatory fish. We distinguish between duration and consistency of selection because for example, a lineage that has coexisted with fish for half its existence and fish introduction occurring once should be better adapted than one coexisting for half its existence with fish, but with multiple fish introductions and losses. As such, estimating duration of coexistence alone may not accurately reflect the evolutionary history of a particular deme. We assume that a phenotype-by-environment negative correlation exists such that any single phenotype is not optimally fit in both selective regimes (i.e., fish-associated versus fishless), a result supported by multiple empirical studies (Kats et al. 1988; Storfer and Sih 1998; Sih et al. 2000). We attempt to measure adaptive 'lag load' (defined as the extent of deviation from optimal trait values; Bürger and Lynch 1995; Kawecki

2000) for fish-association in lineages of the streamside salamander. We predict that contemporary lineages having experienced both longer durations of coexistence with fish and consistent selection are expected to be closer to adaptive optima (Bjorkland 1996; Hansen et al. 2008) and therefore ought to exhibit more effective antipredator phenotypes than those with shorter durations and/or fluctuating selection regimes.

METHODS

Sampling Localities and Tissue Acquisition

We collected tissue samples from individual *A. barbouri* and *A. texanum* across their respective ranges (Fig. 1; Table 1) between 1999 and 2004. Both geographic forms (eastern and western; Kraus and Petranka 1989) of *A. texanum* were sampled, with sampling effort most concentrated in the eastern portion of its range, where this species is sympatric with *A. barbouri*. Collections for *A. barbouri* covered the entire species range, including peripherally isolated populations in western Kentucky, eastern West Virginia, and central Tennessee (Fig. 1). Tail clips (ca. 1 cm^2) or whole larval specimens were preserved in 95% ethanol and stored at -20ºC upon return to the laboratory. Taxonomic identification was determined in the field based on morphological characteristics following Kraus and Petranka (1989).

Total genomic DNA was isolated from each of 78 tissue samples using either a modified phenol-chloroform extraction protocol (Sambrook et al. 1989) or Puregene DNA extraction kits (Gentra Systems, Plymouth, MN). Maximal lengths of 913 nucleotides (bp), comprising both the mitochondrial control region and an adjacent intergenic spacer (ca. 350 bp), unique to the ambystomatids, were obtained by polymerase chain reaction (PCR) amplification using primers (DL3 and THR) developed by Shaffer and McKnight (1996). We performed PCR amplification as in Storfer et al. (2004). After successful amplification, products were purified using vacuum filtration with Montage SEQ_{96} kits (Millipore, Billerica, MA). Purified PCR products were cycle-sequenced using BigDye Terminator 3.1 (Applied Biosystems, Foster City, CA; ABI), sequenced with an ABI 377 automated sequencer. We obtained additional sequences electronically from GenBank for *A. cingulatum*, *A. bishopi* and for each of three species within the tiger salamander complex (*A. californiense*, *A. mexicanum*, and *A. t. tigrinum*; Table 1)

to be used as outgroups. An additional haplotype from a larval *A. t. tigrinum*, collected by JN, was also included as an outgroup. All sequence editing was performed first with visualization of electropherograms using sequence analysis (ABI) and alignment was performed in clustalx (vers. 1.83.1; Thompson et al. 1997) using default parameters; newly generated sequences were deposited in GenBank (for accession numbers, see Table 1).

Comparative Analysis

Appendix 1 presents a flow diagram of methods used, the details of which directly follow. Briefly, a statistical phylogenetic framework was used to evaluate the correlation between observed antipredator trait values for populations of *A. barbouri* with estimated measures of historical consistency and duration of selection toward optimal antipredator phenotypes.

At fourteen localities, four phenotypic traits related to antipredator performance (Storfer and Sih 1998; Storfer et al. 1999) were scored for *A. barbouri*, including: i) efficacy of antipredator-feeding trade-off using *Daphnia magna* as food and chemical cues from green sunfish (*Lepomis cyanellus*) as a predator (both *A. barbouri* and *A. texanum* exhibit antipredator responses to green sunfish cues as if the fish were present, see Kats and Dill 1998); ii) length of time surviving in the lethal presence of green sunfish; iii) responsiveness to a simulated predatory attack; and iv) extent of cryptic coloration, measured as a function of melanophore pigmentation of individuals (Storfer and Sih 1998; Storfer et al. 1999).

All experiments utilized streamside salamander larvae at roughly three weeks of age, reared from wild-collected eggs. Data from the feeding experiment were calculated as the difference in number of *D. magna* eaten in presence versus absence of the predator, *L. cyanellus*. Survival data were recorded as the average number of individuals from a given locality surviving the lethal presence of the predatory *L. cyanellus*, averaged across 20 time points. Response to physical perturbation was performed using a tap-test to simulate a predator attack; the number of taps sufficient to provoke an escape response of a larva was recorded. To estimate crypsis, the average number of melanophores was quantified from photographic images of the dorsocranial region of individual larvae; larvae with fewer melanophores better match the substrate in streams with sunfish (Storfer et al. 1999). Data from these performance

assays were log-transformed and constant of unity was added to raw data before transformation to avoid negative values. Further, values from the perturbation test and the coloration assay were represented as the inverse of the raw data, i.e. ln[1/(x+1)], to facilitate interpretability of results from subsequent analyses.

Topology generation

A set of credible chronograms was generated in BEAST (vers. 1.4.6; Drummond and Rambaut 2007) to test trait-evolution hypotheses for *A. barbouri*, later conducted in BAYESTRAITS (Pagel 1999; Pagel et al. 2004) using the generalized least-squares approach. A relaxed molecular-clock method, with log-normally distributed evolutionary rates, was used for chronogram estimates under a coalescent model of constant 'population' size (Drummond et al. 2006) using BEAST, aided by BEAUTI for input-file generation (both vers. 1.4.6; Drummond and Rambaut 2007). Nearly all priors were uninformative and were left as default; however, following Yang (1996) and Sullivan and Swofford (2001), the proportion of invariant sites was constrained to be zero. Sampling involved a total chain length of $5x10^6$ generations, with subsampling every 10^3 generations. The first 3001 samples were discarded as burnin, well after stationarity had been reached, as assessed qualitatively by change in likelihood scores using Tracer (vers. 1.4; Drummond and Rambaut 2007). For node constraints, a uniform prior distribution on the divergence of *A. californiense* from other members of the tiger salamander complex (*A. mexicanum* and *A. tigrinum* in this study) was used to calibrate chronogram estimates. This range (3.0 to 6.0 MYA) subsumes the estimates given by Wakabayashi and Sawyer (2001) for the secondary orogenic event of the Sierra Nevada range in California, the presumed evolutionary catalyst initiating the divergence of *A. californiense* from *A. tigrinum* (Shaffer and McKnight 1996). Given the lack of resolution of phylogenetic relationships within the Ambystomatidae, a wide range of values (0 to 33 MYA) was used as a uniform prior on the root node to account for this uncertainty (see Shaffer and McKnight 1996). The crown group is thought to have originated in the Oligocene (Larson et al. 2003), thus informing the lower bound. It should be noted that *absolute* divergence times are inconsequential to analyses conducted

herein, although we provide a conservative upper bound on the estimate for the evolutionary origin of *A. barbouri*.

Although presumed monophyletic based on a set of reproductive, behavioral, and morphological characters (Kraus and Petranka 1989), previous phylogenetic analyses by Shaffer et al. (1991) provided little resolution of the relationship between *A. barbouri* and *A. texanum*. A topological hypothesis of monophyletic *A. barbouri* was tested from the posterior distribution of trees from the BEAST run. This ensured that comparisons of trait evolution were made within a single group of closest common ancestry. The posterior probability for a clade containing all sampled *A. barbouri* haplotypes was evaluated using a constraint-filter in PAUP* (vers. 4.0b10; Swofford 1991).

Duration and consistency of selection

Lineage-specific durations, in absolute time of exposure to predatory fish (hereafter termed dwell times), and historical consistency of this selective pressure were estimated in SIMMAP (vers. 1.0; Bollback 2006). The state of a binary ecological trait was determined for each terminal node: either fish-associated versus fishless larval-habitat. Some localities of the streamside salamander support green sunfish (*L. cyanellus*), bluegill sunfish (*L. macrochirus*), or green-bluegill hybrids; populations of the smallmouth salamander are not known to coexist with these aquatic predators (Sih et al. 2000). Ecological trait-evolution was fitted with a continuous-time Markov model in SIMMAP (i.e., fish or fishless). A gamma prior distribution (α: 3.0, β: 2.0) was placed on the parameter describing transition-rate between character states (Huelsenbeck et al. 2003; Bollback 2006). The sum of durations in the character-state associated with exposure to fish was tabulated for each path from root to each terminal node, so to estimate historical duration of selection induced by predatory fish within each lineage. A random sample of 200 trees from the stationary phase of the BEAST analysis was used, upon which stochastic trait-mappings were generated. Ten realizations from the prior distribution of ecological-state frequencies (a symmetrical beta distribution, α: 1.0) were drawn per tree sample. For each tree in the sample, 10 realizations from prior distributions were used; dwell times were computed as the average durations across the 2000 simulated histories in SIMMAP. SIMMAP dwell times were interpreted by the Java program, TREDWE, written ad hoc

(available from www.wsu.edu/~storfer). TREDWE enables users to extract the evolutionary *duration* (i.e., dwell time) of each character state for all paths (root-to-tip) present in the mutational histories generated by SIMMAP. Values for dwell times were log-transformed for subsequent analysis.

Measures of selection consistency were determined in part by the total number of transformations between ecological states along each path. Per lineage lengths were interpreted by TREESTAT (vers. 1.1; Rambaut and Drummond 2007) and were averaged across the 2000 simulated character histories, generated in SIMMAP. To develop a quantitative measure of the consistency of selection toward optimal trait values, dwell time was divided by the log-transformed transitions between ecological states (fishless or fish). Thus, a lineage that had never been exposed to fish (both dwell time and transitions equal zero) would be expected to exhibit the most ineffective antipredator performance.

Association of selection with antipredator performance

Estimates of the duration (i.e., dwell times) and consistency of selection were regressed against measures of antipredator phenotypes in BAYESTRAITS. The random sample retained from the BEAST analysis (200 ultrametric trees) was pruned to leave only *A. barbouri* for which antipredator performance data were available (see Figure 2). The variance-covariance matrix of the continuous data is informed by a commensurate correction for 'phylogenetic heritability' (see Freckleton et al. 2002) via the parameter, λ (range: $\lambda \geq 0$; Pagel 1999; Pagel et al. 2004). Lineages with longer periods of shared evolutionary history are expected to exhibit similar trait-values (Felsenstein 1985), and this parameter describes the degree to which trait-values are associated solely due to phylogenetic relationship (Pagel 1999; Pagel et al. 2004).

Evolutionary regression of antipredator performance on both selection measures (i.e., consistency and duration) was performed in BAYESTRAITS under a Brownian motion model "A," adequate for modeling trait evolution due either to genetic drift or to variable selection (Housworth et al. 2004). MCMC sampling involved 5.5×10^6 generations, where the chain was sampled every 10^3 generations; the first 501 samples were discarded as burnin. A one-tailed t-test was performed to assess whether the regression slopes were significantly positive. The standard deviation of the slope was determined by the

raw data, whereas the slope (β), corrected for phylogenetic non-independence, was determined in BAYESTRAITS. The slope, simply, was the ratio of the trait-covariance (e.g., between dwell time and antipredator performance) to the variance of the variable along the abscissa (e.g., dwell time; Pagel 1999).

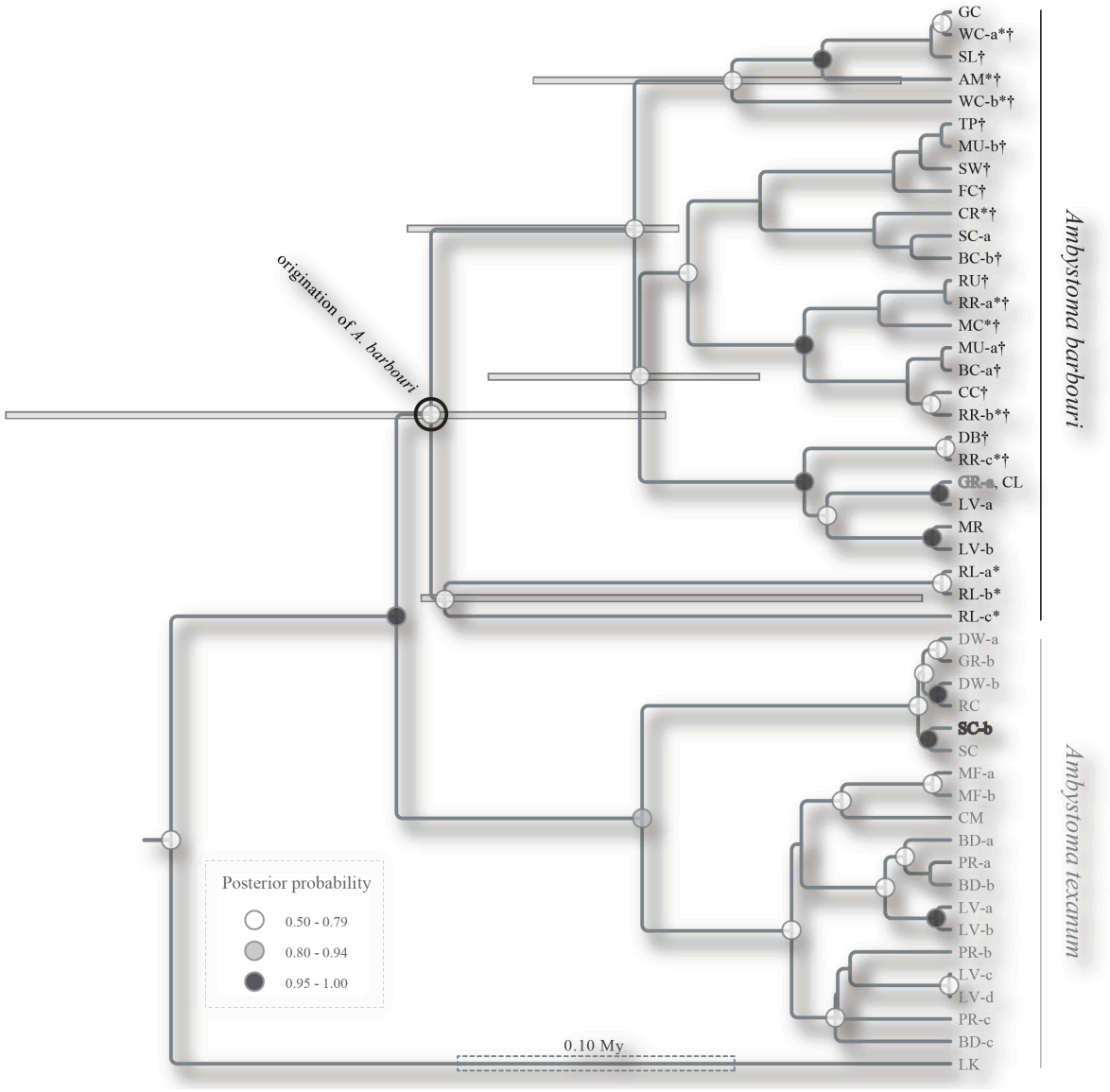


FIGURE 2. Tree estimate from Bayesian analyses conducted in BEAST. Posterior support for internal nodes denoted by arbitrary groupings of relative support (exceeding 0.49 posterior probability). Dark-shaded terminal taxa represent samples from *A. barbouri*; those light-shaded are *A. texanum*. Bolded taxon names (GR-a and SC-b) represent putative hybrids between the two species. Locality designations correspond to those in Figure 1. *: collection acquired from a fish-bearing locality; †: sample used in regression analyses using data for antipredator performance. Outgroups (see text) have been removed.

RESULTS

Topology Estimates and Phylogenetic Hypotheses

Reciprocal monophyly of *A. texanum* and *A. barbouri* is supported with the exception of two haplotypes (i.e., SC-b and GR-a; Fig. 2) that may be a result of interspecific hybridization. If these two haplotypes are permitted to be positionally unconstrained in the constraint filter-test implemented in PAUP, a large proportion of posterior trees sampled by BEAST (0.9665) are consistent with the monophyly of *A. barbouri* with respect to *A. texanum*.

Trait Evolution

Character-state reconstruction of the node shared by all sampled *A. barbouri* suggested streamside salamanders likely originated in habitats containing predatory fish (SIMMAP $p_{pos} = 0.9227$), not more recent than 0.11 MYA as determined by the posterior distribution of credible node heights from BEAST. However, fish-introductions appear to have occurred several times in the evolutionary history of *A. barbouri* in part because streamside salamander lineages currently in coexistence with fish lacked any posterior support as a monophyletic group, as determined in PAUP using a constraint filter-test (BEAST $p_{pos} < 0.0001$). Further, ecological trait histories simulated in SIMMAP suggested a strong bias toward loss of the fish-associated character-state in streamside salamanders. On average, 1.92 transitions with $CI_{\alpha=0.05}$ [1.57, 2.26] from fishless to fish-associated character states were estimated across each simulated tree-history; average tree-wide transitions between fish-associated to fishless states was 13.88 with $CI_{\alpha=0.05}$ [13.26, 14.49]. The stochastic loss of predatory fish is expected to be approximately 7-fold more frequent than is novel introduction to (or recolonization of) these predators in populations of *A. barbouri*.

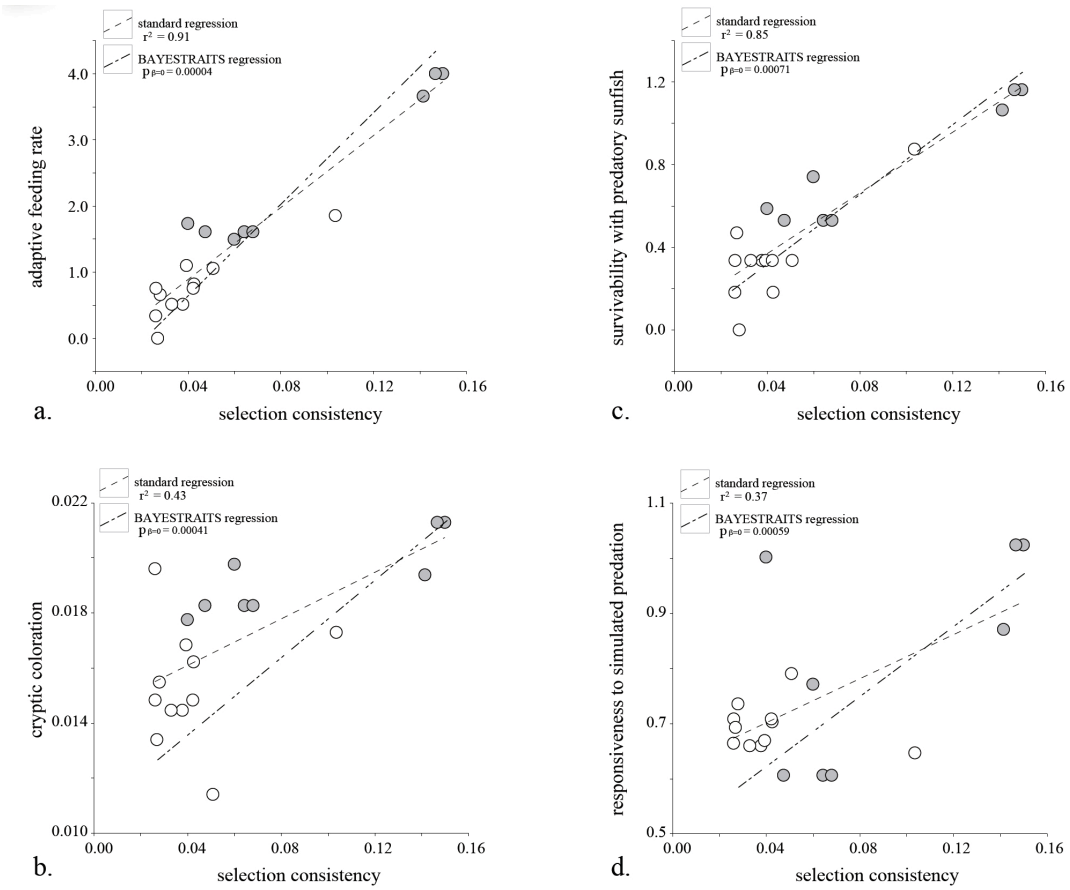


FIGURE 3. Regression analyses of four measures of antipredator performance against estimates of dwell time within lineages of the streamside salamander. Dwell times were estimated by stochastic mapping of character histories in SIMMAP, by treating fish-association of salamanders, or lack thereof, as an ecological trait evolving by Markov process. Estimates of absolute durations of evolutionary contact with predatory fish were recorded for each lineage and were used for regression analysis. Using BAYESTRAITS, all evolutionary regression coefficients were determined to be significantly positive ($p_{0.05(1),\,17} < 0.005$). Open symbols correspond to animals from fishless localities; closed symbols represent animals from habitat that presently supports predatory fish.

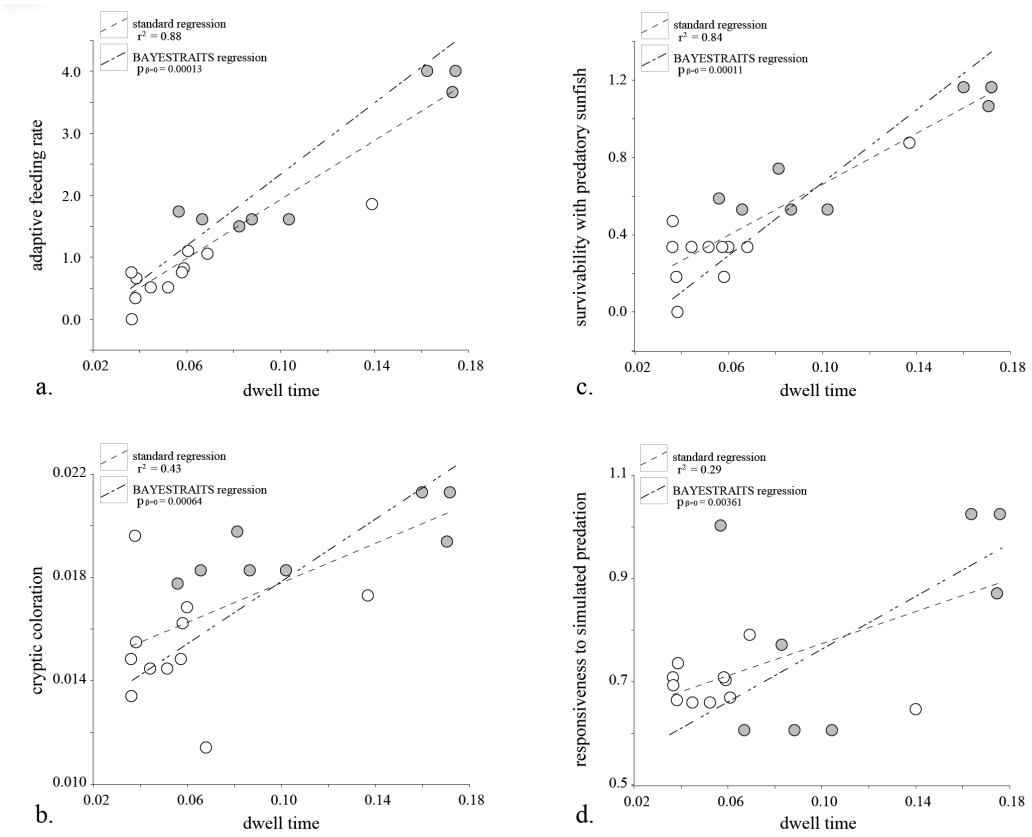


FIGURE 4. Regression analyses of four measures of antipredator performance against estimates of selection consistency. Total number of lineage-wide transitions between fishless and fish-exposed character states was used to scale per-lineage dwell times (as in Fig. 3), to quantify selection consistency. Both dwell times and ecological state-transitions were stochastically generated in SIMMAP on a set of credible topologies. All regression coefficients were significantly positive ($p_{0.05(1),\,17} < 0.001$). Symbols as in Figure 3.

TABLE 2. Estimated regression coefficients and phylogenetic heritabilities (λ) for four measures of antipredator performance and two predictor variables: duration of selection within lineages (i.e., dwell time) and selection consistency in lineages toward effective antipredator performance. One-tailed t-tests were performed to assess whether the regression slopes were significantly positive. The standard error of each slope was determined by the raw data, where slope, corrected for phylogenetic heritability, was estimated in BAYESTRAITS. For each analysis, ν = 17 and α = 0.05. *Marginal likelihood of each model was determined by the harmonic mean of likelihoods across all states in the post-burnin period of analysis in BAYESTRAITS.

	selection duration λ: 0.578 marg. like.*: 140.83		selection consistency λ: 0.631 marg. like.*: 143.89	
	$\hat{\beta}$	$p_{0.05(1),\,17}$	$\hat{\beta}$	$p_{0.05(1),\,17}$
survivability	7.947	0.00011	7.295	0.00071
responsiveness	2.163	0.00361	3.099	0.00059
feeding	27.960	0.00013	35.029	0.00004
crypsis	0.053	0.00064	0.063	0.00041

Strong support was estimated for positive relationships between the efficacy of antipredator phenotypes with both selection consistency and lineage-specific durations of predator coexistence (dwell times) from BAYESTRAITS evolutionary regression (Figs. 3 & 4; Table 2; all BAYESTRAITS regression analyses, $p_{\beta=0,\,(1)\,17\,df} < 0.004$). For measures along the axis of ordinates, larger values imply more effective antipredator performance (Storfer and Sih 1998; Storfer et al. 1999). Results indicate sizable differences in both antipredator performance, as well as ecological history within lineages of the streamside salamander (Figs. 3& 4). Coefficients of determination were larger for standard regression analyses of selection consistency (compare Fig. 3 with Fig. 4). Yet, the predictive power provided by the inclusion of the number of transitions between ecological states in this measure of historical selection appears to be small, relative to the sole consideration of dwell time. In all cases, estimates of phylogenetic heritability of trait values (λ) were non-zero, suggesting the influence of phylogenetic effect in shaping phenotypes expressed by individuals of contemporary populations (Table 2).

DISCUSSION

Results from this study point to the general conclusion that the extent to which lineages are adapted to contemporary selective pressures is strongly associated with both duration and consistency of a particular selective regime. Specifically, our results suggest that niche stability and consistent selection within lineages facilitates adaptation of *A. barbouri* to predatory fish, as a diverse theoretical literature would predict (Wilson 1975; Lande 1979; Harvey and Pagel 1991; Orzack and Sober 2001; Reznick and Ghalambor 2001), but few empirical works have tested (e.g., Michaud 2006). Much as Hansen et al. (2008) has provided evidence for lag loads owing to fluctuating multivariate selection, herein we present empirical evidence for the adaptive evolutionary costs associated with lineages experiencing fluctuating selection pressures due to a dichotomous ecological trait.

Depending on whether *A. barbouri* breed in the presence or absence of predatory fish (Kraus and Petranka 1989), divergent suites of phenotypic characteristics are selectively advantageous (Storfer and Sih 1998). Higher activity rates, correlated with both high feeding and developmental rates, enable larvae

to metamorphose quickly enough to escape a fishless, highly ephemeral stream (Kats et al. 1988). Fish-bearing streams (or stream reaches) are less ephemeral than fishless habitats (Sih et al. 1992; Storfer and Sih 1998), thereby relaxing the requirement for rapid metamorphosis. Further, lower activity rates benefit salamanders that are susceptible to predation from visual predators such as green sunfish (Kats et al. 1988; Storfer and Sih 1998; Sih et al. 2000). Thus, evolution of each ecotype appears to be influenced by the existence of separate adaptive optima for larval characteristics among fish and fishless lineages.

Lineages that have *never* experienced predatory fish ought to be relatively close to an adaptive peak associated with highly ephemeral, fishless breeding-habitat (i.e., rapid developmental, feeding, and activity rates; Storfer and Sih 1998; Storfer et al. 1999). In contrast, lineages that have been in contact with predatory fish for most their evolutionary duration ought to have trait values corresponding to a separate adaptive peak (i.e., high larval survivability and reduced feeding rates in presence of predatory fish, generally reduced developmental and activity rates, effective background matching, and high responsiveness to predatory attack as measured in previous studies; Storfer and Sih 1998; Storfer et al. 1999). As predicted, all four measures of antipredator performance were positively correlated with dwell time of exposure to predatory fish (Fig. 3) and with the historical consistency of this selective pressure (Fig. 4). The four measures of antipredator performance are likely non-trivial components of fitness, as indicated by other studies (e.g., Storfer and Sih 1998; Sih et al. 2000). The antipredator behaviors and extent of crypsis measured are correlated with survivorship of larvae in lethal presence of predatory fish (Storfer and Sih 1998; Storfer et al. 1999), and both are strong predictors of metamorphosis for salamanders in streams with predatory fish (Sih et al. 2000). The absence of phenotypic plasticity in our four measures of antipredator performance is suggested by common garden experiments, indicating a genetic component to the measured traits and thereby suggesting the presence of distinct trait optima (Storfer and Sih 1998; Storfer et al. 1999).

Early *A. barbouri* seem to have evolved in contact with predatory fish (SIMMAP $p_{pos} = 0.9227$). In addition, it is likely that stochastic loss and recolonization of fish populations has typified the history of *A. barbouri*, given the strong support for non-monophyly of salamander lineages in contact with

predatory sunfish (Fig. 2). Although phylogenetic relatedness likely influences adaptive trait evolution in this system (Table 2), patterns of historical selection peculiar to each lineage are able to surmount this phylogenetic effect (Figs. 3 & 4). These results are in apparent conflict with those of Sih et al. (2003), which suggested *A. barbouri* had evolved for certain traits in the "wrong direction" upon evolutionary exposure to predatory fish. In contrast, the efficacy of antipredator behavior in *A. barbouri* is significantly positively associated with duration (i.e., dwell time), as well as consistency of apparent coexistence with fish. It is important to note that dwell time and selection consistency should be treated distinctly. That is, while dwell times may be equivalent among any two given lineages, one lineage may have a single fish introduction, while the other lineage may have multiple introductions and losses. The former lineage is expected to be closer to its adaptive optimum than the latter. Accordingly, including the number of ecological transitions appears to be more predictive of the efficacy of antipredator phenotypes than considering dwell time alone (Figs. 3 & 4).

These results dovetail previous work suggesting gene flow between fish and fishless habitats constrains antipredator performance in contemporary populations of streamside salamanders (e.g., Storfer and Sih 1998; Storfer 1999; Storfer et al. 1999; Sih et al. 2000; Garcia et al. 2003). That is, historical constraints also influence local adaptation of *A. barbouri* to predatory fish, but contemporary gene flow from fishless to fish-supporting localities also can swamp local adaptation by reducing the efficacy of antipredator behaviors and cryptic coloration (Storfer and Sih 1998; Storfer et al. 1999).

This study also suggests the possibility for hybridization between *A. texanum* and *A. barbouri* as a constraint on adaptive evolution. Preliminary molecular evidence suggests hybridization between *A. texanum* and *A. barbouri* because two mitochondrial haplotypes (SC-a, *A. barbouri* and GR-a, *A. texanum*; Fig. 1) are found in well-supported clades of the other species. *A. texanum* and *A. barbouri* are either sympatric or parapatric in these areas (Petranka 1982; Fig. 1), and *A. texanum* evolved in the absence of predatory fish (Sih et al. 1992). Much as gene flow between populations with conflicting selection pressures in *A. barbouri* has likely constrained local adaptation to predatory fish (Storfer and Sih 1998; Storfer et al. 1999), gene flow between ecologically diverged species may cause fitness reductions

in hybrids whereby intermediate phenotypes perform poorly (Mayr 1954; Templeton 1986; Coyne and Orr 2004). A few aspects of our study warrant caveats. First, ancestral state reconstruction should be treated with caution because incomplete sampling of all major lineages within *A. barbouri* is likely, despite nearly complete geographic sampling (Fig. 1). Much as in interspecific comparative analyses, differences in lineage-longevity resulting from the possession of a particular trait may bias reconstructed ancestral states (Schluter 2000). Second, whereas this study investigated historical patterns under which adaptive trait values of contemporary populations are constrained, the *current* selective regime should also be considered. The apparent non-random distribution of contemporary habitat-type (fishless versus fish-bearing) along axes of antipredator performance indicates the importance of contemporary selection pressures (Figs. 3 and 4). If current selective agents were inconsequential to the efficacy of antipredator phenotypes, one would expect that data would not be separable by current habitat-type, as seems to be the case. A notable exception appears with measures of behavioral responses to simulated predation (Figs 3d & 4d); some of the least responsive larvae were collected from fish-bearing localities. Third, the reliance on a single locus to estimate credible intraspecific phylogenies may result in inaccurate tree reconstruction. However, it seems quite unlikely that the use of additional genetic loci would be cause for major revision of our main result that duration and consistency of selection are positively associated with antipredator phenotypes. That is, our Bayesian analyses incorporated an extent of uncertainty in use of our data to reconstruct gene trees and historical selection regimes. Nonetheless, we must be more tentative with regard to putative evidence of hybridization. Lineage sorting notoriously muddles the detection of hybridization through phylogenetic methods, especially given only a single locus (Moore 1995; Arnold 1997).

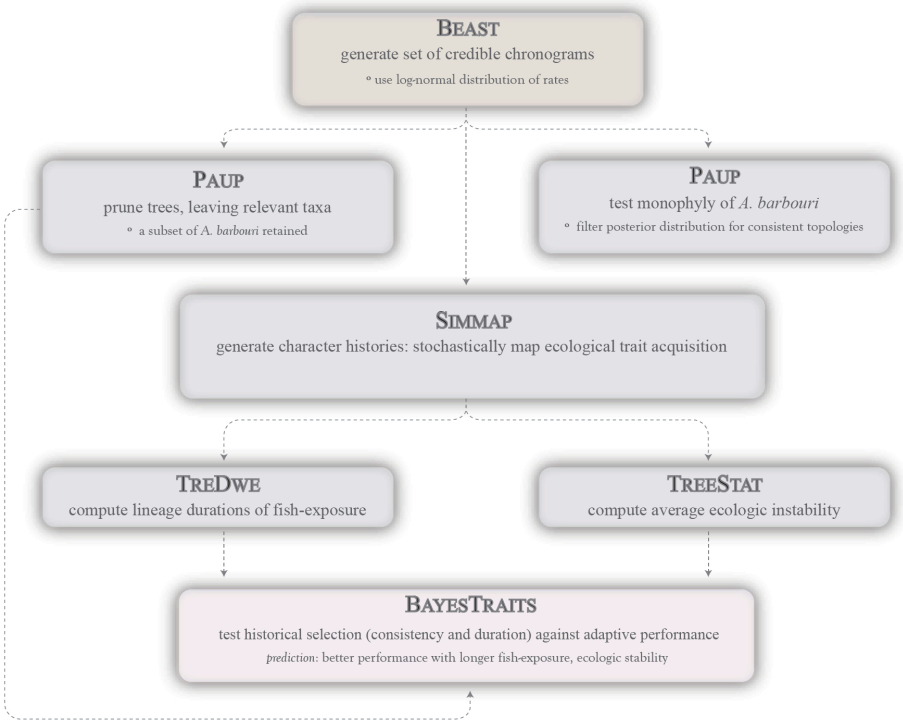
In addition to the above concerns, while we assume here that a set of phenotypic traits is statically optimal for larval streamside salamanders in the presence or absence of predatory fish, it is quite possible that these optima have shifted over the course of evolutionary contact between salamanders and fish (Roff 2000; Schluter 2000). However, the significant positive relationships between antipredator performance and measures of selection seem to indicate that trait optima have not markedly changed (Figs. 3& 4).

While some traits could be under perpetual directional selection (e.g., fecundity), unrecognized developmental constraints, antagonistic pleiotropic effects, or genetic correlations likely provide limitations on the evolution of such traits (Futuyma 1998).

CONCLUSIONS

Indicated by two ecotypes, populations of the streamside salamander appear to be under divergent selection, and the adaptive evolution of the species is strongly influenced both by contemporary processes (e.g., gene flow; Storfer and Sih 1998; Storfer 1999; Storfer et al. 1999; Sih et al. 2000; Garcia et al. 2003), as well as historical constraints. A historically unstable niche represents an important constraint on the adaptive evolution of antipredator phenotypes among lineages of *A. barbouri* experiencing periodic fish introductions and losses (Figs. 3 & 4). That is, our study suggests consistency, as well as duration of selection imposed by predatory fish is important in shaping adaptive trait evolution in the streamside salamander. In these analyses, we provide new software as a general means of interpreting and using estimates of lineage-specific durations within a particular character-state (i.e., dwell times) from SIMMAP (provided in a Java application called 'TREDWE' for interpreting the nexus-formatted output of stochastically-mapped character histories from SIMMAP; available at www.wsu/~storfer). This method may prove especially useful where dwell times are expected to bear heavily on the states or values of other traits.

Future work may test the relationship between historical patterns of selection and both behavioral and developmental traits associated with the fishless selective regime. Indeed, the rate of loss of fish is substantially larger than the estimate of transitions from fishless to fish-bearing larval habitat, suggesting that selection intensity on streamside salamander larvae may be somewhat weakened. If we are to assume disjoined adaptive peaks associated with each ecotype, historical selection-consistency and duration of the selective regime are expected to be equally important in the adaptive evolution of populations from fishless, but highly ephemeral, larval habitat.



APPENDIX. Overview of phylogenetic comparative methods used herein. See text for details.

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CHAPTER TWO

CORRELATIONS OF LIFE-HISTORY AND DISTRIBUTIONAL-RANGE VARIATION WITH SALAMANDER DIVERSIFICATION RATES: EVIDENCE FOR SPECIES SELECTION

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ABSTRACT

Evolutionary biologists have long debated the relative influence of species selection on evolutionary patterns. As a test, we apply a statistical phylogenetic approach to evaluate the influence of traits related to species distribution and life-history characteristics on patterns of diversification in salamanders. We use independent contrasts to test trait-mediated diversification while accommodating phylogenetic uncertainty in relationships among all salamander families. Using a neontological dataset, we find several species-level traits to be variable, heritable, and associated with differential success (i.e., higher diversification rates) at higher taxonomic categories. Specifically, the macroecological trait of small geographic-range size is strongly correlated with higher rates of net diversification. We further consider the role that plasticity in life-history traits appears to fulfill in macroevolutionary processes of lineage divergence and durability. We find that paedotypy – wherein some organisms of a species mature in the gilled form without metamorphosing – is also associated with higher net diversification rates as compared to lineages not exhibiting developmental plasticity. Often dismissed as an insignificant process in evolution, we provide direct evidence for the role of species selection in lineage diversification of salamanders.

INTRODUCTION

An enduring debate concerns the plausibility and strength of evolutionary processes that operate above the organismal level. Conflict among levels of selection generally provides some of the best evidence for the *existence* of multiple levels of selection (e.g., meiotic drive of the *t*-allele in house mouse, Lewontin 1970; Vrba 1989; Grantham 1995, 2007). But, despite being largely convinced of the possibility in principle, many have claimed higher-level selection exerts too weak an influence to represent a useful hypothesis to explain variation in evolutionary success among species (e.g., Fisher 1958; Mayr 1963; Dawkins 1982). Resolution of the debate, it seems, has primarily become an empirical issue.

As an explicit test of species selection, researchers have recently turned to testing the relationship of species-level traits with net diversification rates of lineages using molecular phylogenies (Barraclough and Savolainen 2001; Webster et al. 2003; Kruger 2008; Seddon et al. 2008). As Rabosky and McCune (2009) suggest, species selection could be defined as directionally consistent patterns of differential proliferation associated with species bearing a particular state of a character (see also 'broad-sense species selection' of Jablonski 2008a; and see 'emergent fitness' approach of Lloyd and Gould 1993; Gould and Lloyd 1999; Coyne and Orr 2004). Mechanistically, trait frequencies within lineages may evolve independently of trait frequencies in populations (Rabosky and McCune 2009). In the former case, phenotypic frequencies are determined by population genetic processes, whereas selection *among* lineages controls frequencies of higher-order traits in the latter example. Among-lineage selection is mediated by trait-based differential speciation and (or) extinction (see Rabosky and McCune 2009 for recent review).

A *repeatability* criterion thus distinguishes species selection from other macroevolutionary trends as can arise by species drift (see Gould 2002; Coyne and Orr 2004). Under this view, a repeatable pattern of sorting at the level of species is attributable to one of two causes: reductionistic macroevolution (i.e., the so-called 'effect hypothesis' of species selection: Vrba 1980; Gould 2002); or higher-level, 'strict-sense' species selection. Strict-sense species selection is defined by the requirement that the character mediating

differential success is a so-called emergent property, whose trait-state cannot be fully predicted from lower-level causes. The size and configuration of species' distributions is one such emergent property, both of which result from the *interaction* between organismal properties and the environment (Gould 2002; Jablonski 2008a).

Two plausible scenarios relating species diversification with geographic range size have been proposed, perhaps beginning with Darwin (1859), who argued that more widespread species often bear greater potential for speciation (Jablonski and Roy 2003). Conversely, others argue that dispersal-limited or stenotopic species may be more sensitive to environmental barriers, facilitating higher rates of vicariant or peripatric diversification than for broadly distributed taxa (Mayr 1963; Jablonski 1987, 2008a; Maurer and Nott 1998).

Both empirical evidence (e.g., Jablonski 1987; Jablonski and Roy 2003; Jablonski and Hunt 2006) and theoretical models (e.g., Gavrilets 1999, 2003; Mouillot and Gaston 2007) suggest a different pattern of lineage diversification associated with aspects of the distributional range, mediated either via effects on speciation or extinction probabilities. For example, Jablonski (1987) finds lineage duration in Cretaceous mollusks to be positively correlated with distributional range-sizes. In a similar work, Jablonski and Roy (2003) document a strong inverse relationship between speciation rates and range size in Cretaceous gastropods, further suggesting that the characteristics leading to larger distributional ranges may also limit speciation probabilities.

In a simulation study, Gavrilets (1999) finds consistent results, showing that speciation is more likely with increasing distributional fragmentation. Biological causes of this result might involve mutational accumulation of Bateson-Dobzhansky-Muller reproductive incompatibilities in allopatry, which can alone be sufficient for speciation (Gavrilets 2003, 2004). Whereas allopatry may not be sufficient for complete genetic isolation, it seems a fairly reliable indicator of reduced gene flow, especially for dispersal-limited taxa (Larson et al. 1984; Bolnick and Fitzpatrick 2007; Kozak et al. 2008). If not trumped by gene flow, local adaptation may also promote diversification via ecological speciation (Mayr 1963; Slatkin 1973; Schluter 2000, 2009; Nosil et al. 2009).

Either independently or through its association with distributional-range characteristics, life-history variation may be a potentially strong contributor to trait-mediated diversification (Hansen 1980; Jablonski 1986; Böhning-Gaese et al. 2006; Jablonski and Hunt 2006; Cooper et al. 2008). Phenotypic plasticity in timing and mode of amphibian metamorphosis is a particular life-history characteristic that has received a great deal of attention (e.g., Wilbur and Collins 1973; Werner and Anholt 1993). Paedotypic taxa (i.e., those that exhibit facultative metamorphosis; *sensu* Reilly et al. 1997) may be capable of exploiting a wider niche than obligately metamorphic or paedomorphic taxa (i.e., those that have truncated development and retain larval characteristics relative to their ancestors; see Reilly et al. 1997). Whereas paedotypic populations are expected to be under diversifying selection for particular traits (West-Eberhard 1989), the same need not be true for populations of terrestrial or fully aquatic (i.e., paedomorphic) species. When life-history variation is maintained within a population, intensity of competition among conspecifics may be lessened, for instance, if prey type preferences vary among morphotypes (Denoël 2004).

Developmentally-plastic lineages may experience mitigated risk of extinction and perhaps also elevated rates of speciation relative to metamorphic or paedomorphic taxa (Denoël et al. 2005). That is, maintained polymorphisms can lead to assortative mating along temporal, spatial, behavioral, or morphological axes (West-Eberhard 1989; Whiteman and Semlitsch 2005; Takahashi and Parris 2008). Divergence may otherwise occur allopatrically, following sweepstakes dispersal of metamorphosed organisms across unsuitable terrestrial habitat between ponds or springs.

Study System

The extant diversity of salamanders exhibits considerable life-history and distributional-range variation, and provides a tractable means of testing species selection due to the small size of the order (~560 spp.; Duellman and Trueb 1994; Petranka 1998). Whereas most salamanders are either metamorphic or direct developing (ca. 90%), several families comprise only paedomorphs (e.g., sirenids, cryptobranchids, amphiumids, proteids). All but one family, Rhyacotritonidae, has at least one paedomorphic or paedotypic species. For simplicity, we hereafter refer to the group of salamanders

exhibiting metamorphosis or direct development as terrestrial, to distinguish the life-history characteristic of these salamanders from both paedotypic and paedomorphic taxa.

We expect paedotypic lineages to experience higher rates of diversification than terrestrial lineages, which, in turn, are expected to be diversifying more rapidly than paedomorphic lineages. Using a phylogenetic comparative approach to test for evidence of species selection, we ask: Are patterns of lineage diversification associated with: i) differences in range size and continuity; and (or) ii) variation in life-history characteristics?

Similar to tests of natural selection at the organismal level (see Lewontin 1970), strong evidence for higher-level selection would be provided by trait variation among lineages, evidence of trait similarity owing to common ancestry, and consistent correlations of trait values with diversification rates among lineages (Coyne and Orr 2004; Jablonski 2008a; Rabosky 2009).

METHODS

Phylogeny estimation

Data from NCBI Genbank for four mitochondrial markers (two ribosomal subunits, 12S and 16S; cytochrome b; NADH dehydrogenase subunit 2, ND2) and one nuclear locus (recombination activation gene, RAG1) were concatenated for 45 salamander species to estimate both family-level rates of diversification and interfamilial evolutionary relationships (see Appendix 1). Rather than attempt to reiterate topological estimates of interfamilial relationships within salamanders, we relied on two conflicting but recent phylogenetic treatments of this amphibian order. In one analysis (see Fig. 1), interfamilial relationship was constrained to be congruent with the topological hypothesis of Frost et al. (2006; hereafter, FEA). In a complementary analysis, the topology of Wiens et al. (2005; hereafter, WEA) was used to constrain relationships amongst families. BEAST (vers. 1.4.8; Drummond and Rambaut 2007) was employed for both analyses. A birth-death tree prior, a general time reversible (GTR) model of sequence evolution with Γ-distributed among-site rate-heterogeneity, and log-normally distributed, phylogenetically-uncorrelated evolutionary rates were assumed for both analyses of trait

heritability. Under each topologically-constrained analysis, chain convergence among three independent runs and stationarity were assessed using AWTY (Wilgenbusch et al. 2004) and TRACER (vers. 1.4; Rambaut and Drummond 2007). Stabilization of tree likelihoods and sampled topologies occurred within several hundred thousand generations. Sampling began after $5x10^6$ generations, and samples were retained every 10^4 generations until $5x10^2$ trees were collected from the posterior distribution for each chain. Log and tree files were combined using LOGCOMBINER (vers. 1.4.8; Drummond and Rambaut 2007).

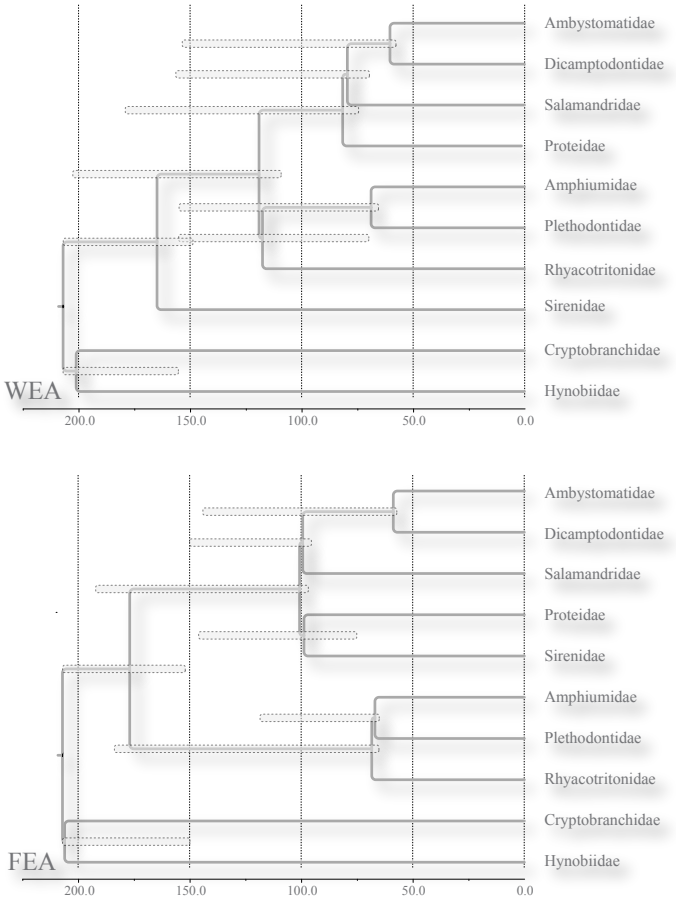

FIGURE 1. Topologies used for comparative analyses. Units along the abscissa are in millions of years before present. WEA: topology from Wiens et al. (2005); FEA: topology consistent with that proposed by Frost et al. (2006). Nodal constraints used for Bayesian inference of topology are as listed in Table 1. A hard constraint of 210 Ma. was placed on the root node, following Wiens (2007). Bars represent uncertainty (95% HPD) associated with divergence-date estimates.

Trait variation

We focus on two species-level traits in caudates and the potential relationship of each with differential success at the family level: distributional-range size and continuity and heterochronic (i.e., life-history) variation. Data for range size (total area in km^2) were obtained from resources provided by the global amphibian assessment (GAA; IUCN 2008). Range-size values were natural-log transformed. Data for a second measure of species' range variation -- range-continuity -- were recorded as range size scaled by number of distributional isolates. Thus, range-continuity data are an estimate of the average isolate size across the distribution of a species. We use data for all species included in the GAA database (530 of 563 recognized species). In light of recent concerns regarding skewness of such data (Webb and Gaston 2003; see also Hunt et al. 2005), we attempted to impose normality by transformation for these data associated with the distributional range. For range-size and range-continuity measures, assumptions of normality were assessed using Kolmogorov-Smirnov tests and normal quantile-quantile (QQ) plots using the R-package STATS. For family-level comparisons considering distributional-range characteristics, natural-log transformed data were averaged across species within each family.

For all but the following exceptions, life-history classification followed Duellman and Trueb (1994) for three discrete categories of heterochronic variation: *Ambystoma mavortium* is paedotypic (Collins 1981); and all *Dicamptodon* were scored as paedotypic (Nussbaum 1976; Petranka 1998) although one species is almost entirely metamorphic (i.e., *D. tenebrosus*) and *D. copei* is nearly exclusively neotenic.

We implemented three measures of heterochronic variation. The first assessed bias toward paedotypy or paedomorphy within families. The difference between paedotypic and paedomorphic species was scaled by total species richness in each family. Values ranged from -1 (exclusive paedomorphosis) to +1 (paedotypy). This measure of heterochronic variation (hereafter, *'heterochrony'*), however, cannot distinguish among families with only terrestrial species from those with even distributions of paedomorphic and paedotypic species. For this reason, two additional coding schemes were adopted to estimate family-level biases toward different developmental modes. *'Plasticity'* values considered the proportion of paedotypic species within families, whereas *'paedomorphy'*, values at the family level were

informed by the proportion of paedomorphic species within families. Each measure of heterochronic variation, while discrete at the species level, was thus continuous at the family level.

TABLE 1. Divergence constraints used for tree estimation. Most constraints employed uniform probability distributions (e.g., the most exclusive node subtending Hynobiidae and Cryptobranchidae was constrained to be present at some period between 210 and 161 Ma). Normal distributions (i.e., μ [σ]) were implemented where only point estimates of divergence times (μ) were available from the literature. In such cases, the standard deviation (σ) was taken as ten percent of μ.

taxon	*constraint*	*reference*
crown group salamanders (Caudata)	210.0 Ma.	following Wiens 2007
Cryptobranchidae + Hynobiidae	≥ 161.0 Ma.	Gao and Shubin 2003
Salamandroidea*	≥ 114.0 Ma.	Evans and Milner 1996
Amphiumidae + Plethodontidae	≥ 66.0 Ma.	Gardner 2003
Ambystomatidae + Dicamptodontidae	≥ 58.0 Ma.	Naylor and Fox 1993
Mertensiella + *Salamandra*	≥ 34.0 Ma.	Milner 2000
Notophthalmus + *Taricha*	≥ 24.0 Ma.	Milner 2000
Aneides + *Desmognathus*	≥ 19.0 Ma.	Tihen and Wake 1981
Bolitoglossinae**	≥ 5.0 Ma.	Clark 1985
Ambystomatidae	23.3-35.4 Ma.	Larson et al. 2003
Amphiumidae	13.0 [1.3] Ma.	Larson et al. 2003
Cryptobranchidae	15.8 [1.6] Ma.	Wiens 2007
Dicamptodontidae	2.0-5.0 Ma.	Steele et al. 2005
Hynobiidae	110.0 [11.0] Ma.	Zhang et al. 2006
Plethodontidae	60.7 [6.1] Ma.	Wiens 2007
Proteidae	102.6 [10.3] Ma.	Wiens 2007
Rhyacotritonidae	6.0-11.0 Ma.	Larson et al. 2003
Salamandridae	65.0-100.0 Ma.	Zhang et al. 2008
Sirenidae	8.9 [0.9] Ma.	Wiens 2007

* all caudate families excluding cryptobranchids, hynobiids, and sirenids; constraint relaxed for analyses using the Frost et al. (2006) topology
** here comprising *Batrachoseps*, *Bolitoglossa*, *Oedipina*, and *Pseudoeurycea*

Trait-state heritability

To test phylogenetic heritability of trait states, we use Pagel's (1999) λ_P, which is an off-diagonal multiplier of elements in the variance-covariance matrix of a phylogenetic tree. If trait evolution is uninfluenced by phylogenetic history, sister species should be no more similar than two species drawn from the topology at random. In the case where the topology perfectly predicts the covariance structure of traits among related taxa, $\lambda_P = 1$ (i.e., λ_P-fitted branches are unscaled). Nominal values of λ_P (approaching 0) for a particular trait would imply that observed trait values are drawn at random with

respect to the untransformed phylogeny estimate (i.e., λ_P-fitted internal branches are of zero length, corresponding to a star phylogeny; Pagel 1999). Where optimized λ_P-values for traits are statistically distinguishable from 0, we take this as evidence for non-trivial phylogenetic-signal (e.g., Waldron 2007, Eastman et al. 2009).

Insomuch as resolution at the specific level is presently incomplete for caudates, a collection of randomly-resolved, species-level trees was used for heritability analyses. Topology generation began with the unresolved tree of all salamander species (563 species as tips). Random resolutions of the salamander tree were generated in BEAST under a birth-death branch-length prior. All taxonomic classifications (i.e., genera and families) were assumed to represent monophyletic lineages, and family-level relationships were enforced as indicated below (Fig. 1, WEA). Temporal node-constraints followed Table 1; all other nodes were randomly generated under the birth-death prior.

Trait heritability was assessed in the R-package GEIGER (vers. 1.2-14; Harmon et al. 2008) on a random selection of 10^2 species-level trees retained from 10^6 generations of sampling from priors in BEAST. Using maximum likelihood, a λ_P value was fitted for each trait dataset (range size, range continuity, and the three character codings for heterochronic variation) using a tree from the random sample with which to optimize λ_P (Pagel 1999; Pagel et al. 2004). Hierarchical likelihood ratio tests were performed to test the explanatory contribution of an estimated λ_P to a model of trait evolution. Improved model fit by the incorporation of λ_P has been interpreted as evidence of 'phylogenetic heritability' -- that closely related taxa share more trait similarity than from an expectation of randomness (Waldron 2007; Eastman et al. 2009). Fit of different evolutionary models for the higher-level characters was tested using species-level trees.

While we expect estimates of phylogenetic heritabilities to be somewhat imprecise, this method allowed us to use the full trait-datasets and should provide a statistically conservative test of trait heritability. That is, if phylogenetic signal truly underlies these traits, similar character states would exhibit substantially more topological *underdispersion* in the true tree, relative to the degree of clustering among similar character-states as generated by random resolution (Symonds 2002). Yet, if on the true

tree, trait-states are substantially overdispersed with respect to phylogeny, our use of random resolutions could indeed yield inflated estimates of phylogenetic trait heritability (Abouheif 1998; Symonds 2002).

Trait-mediated diversification analyses

Topological data (see *Phylogeny estimation*) were used primarily to inform divergence dates of the most basal nodes of the salamander tree (i.e., subtending a family or higher clade). Divergence dates were then used to estimate rates of diversification within families as described below. Importantly, to develop accurate estimates of diversification within families, assurance that resolution of (true) basal nodes for each family is necessary from phylogenetic estimates. Within each family, topological estimates informing taxon selection to recover root nodes of crown groups followed references in Appendix 1 and Table 1. For five (of ten) families, exemplars from every contained genus or from every classified species were used for phylogenetic estimates. The Magallón and Sanderson (2001) procedure was used to estimate diversification rates of the ten caudate families. This method for estimating diversification rates assumes that rates of origination and extinction bear a positive relationship, a claim that has substantial support in the paleontological literature (e.g., Gould and Eldredge 1977; Stanley 1979, 1990; Van Valen 1985; Sepkoski 1998; Jablonski and Roy 2003). Two aspects of the Magallón and Sanderson (2001) procedure for estimating diversification rates are worthy of note. First, under an assumption of high relative-extinction *fraction* ($\varepsilon = \mu/\lambda$, where μ and λ are extinction and speciation probabilities, respectively), *absolute* rates of extinction need not be extremely high – if rate of origination itself is not particularly high. Second, under an assumption of a non-negligible extinction fraction ($\varepsilon > 0$), high diversification rate implies high absolute extinction risk but even higher probability of origination.

In implementing the Magallón-Sanderson method, an underlying assumption necessary to test is a positive relationship between clade age and species richness within families. If total diversification is limited by a carrying capacity of sorts (Walker and Valentine 1984, Ricklefs 2007), or is otherwise diversity-dependent, diversification rates would slow as groups become more saturated with lineages (Rabosky 2009a,b). As such, diversification rates would not be meaningfully estimable by the birth-death process, which assumes a temporal constancy in diversification rates (Rabosky 2009a, b). To test the

constancy assumption, we regressed crown-group age (averaged across the posterior distribution of trees) against Ln-transformed species richness for families (see Table 2). Results obtained from the base R-package STATS (vers. 2.10.0; R Development Core Team 2010) supported a strongly positive relationship between clade age and species richness ($t_{WEA} = 2.215$, $p_{WEA} = 0.0577$; $t_{FEA} = 2.196$, $p_{FEA} = 0.0593$). We interpret this result as consistent with temporally constant rates of diversification.

The posterior distribution of trees from BEAST (see *Phylogeny estimation*) was used to estimate net rates of diversification (sensu Magallón and Sanderson 2001). While the number of species in each family (Table 2) was treated as known, we included uncertainty in crown-group age for each family. For each topological estimate from the posterior distribution (a total of 1.5×10^3 trees), temporal placement of the basal crown-group node in each family was assessed in TREESTAT (vers. 1.1; Rambaut and Drummond 2007). From these estimates for each family, GEIGER was used to generate a distribution of credible rates of net diversification (r, where $r = \lambda - \mu$) under two scenarios of relative extinction. Following Wiens (2007) and Magallón and Sanderson (2001), estimates of clade diversification assumed one of two values for ε (i.e., relative-extinction fraction): 0.0 and 0.9.

TABLE 2. Family-level data used for phylogenetic independent contrasts. Leftmost continuous traits were used as predictors for statistical comparisons involving estimates of net diversification. Distributional range data are family-level averages of Ln-transformed *range sizes* (km^2) from species. *Range continuity* is range size scaled by the total number of distributional isolates. Values for the measure of '*heterochrony*' correspond to biases toward paedotypic (+1) or paedomorphic (-1) species. Larger values for '*plasticity*' correspond to higher frequencies of paedotypic species within families; similarly, larger values for *'paedomorphy*' denote higher proportions of paedomorphy. Species richness and crown-group age (not shown) were used to compute net diversification under two scenarios of relative extinction (ε, where ε was either 0.9 or 0.0; Magallón and Sanderson 2001). Rates of net diversification were determined from the posterior distribution of trees: times for crown-group diversification were averaged across all samples.

						net diversification pure-birth (ε = 0.0)		net diversification birth-death (ε = 0.9)		
family	range size	range continuity	heterochrony	plasticity	paedomorphy	FEA	WEA	FEA	WEA	species richness†
Ambystomatidae	13.26	11.75	0.0625	0.1875	0.1250	0.0961	0.0968	0.0471	0.0474	32
Amphiumidae	12.74	11.89	-1.0000	0.0000	1.0000	0.0319	0.0314	0.0093	0.0091	3
Cryptobranchidae	12.77	10.73	-1.0000	0.0000	1.0000	0.0241	0.0250	0.0070	0.0072	3
Dicamptodontidae	11.08	10.68	1.0000	1.0000	0.0000	0.2242	0.2420	0.0655	0.0707	4
Hynobiidae	12.74	12.17	0.0196	0.0196	0.0000	0.0288	0.0286	0.0155	0.0154	51
Plethodontidae	11.04	10.36	-0.0157	0.0079	0.0236	0.0871	0.0859	0.0599	0.0591	382
Proteidae	12.95	12.35	-1.0000	0.0000	1.0000	0.0116	0.0118	0.0037	0.0037	6
Rhyacotritonidae	10.15	9.74	0.0000	0.0000	0.0000	0.0846	0.0853	0.0247	0.0249	4
Salamandridae	12.87	11.76	0.0667	0.0667	0.0000	0.0392	0.0418	0.0225	0.0239	75
Sirenidae	12.89	12.19	-1.0000	0.0000	1.0000	0.0927	0.0927	0.0271	0.0271	4

† from AmphibiaWeb.org, accessed June 2008

We use phylogenetically-independent contrasts (hereafter PICs; Felsenstein 1985, 2008) to test for consistent relationships between trait values and differential success of lineages (Coyne and Orr 2004; Jablonski 2008a). PICs confer adequate rigor in performing statistical tests with hierarchically structured data, as phylogenetic comparative data necessarily are (Harvey and Pagel 1991; Martins et al. 2002; Housworth et al. 2004). PICs further appear robust to violation of certain model assumptions, such as branch-length error (Diaz-Uriarte and Garland 1998) and departures from Brownian motion trait-evolution (Oakley and Cunningham 2000).

Independent contrasts were calculated with the aid of APE (Paradis et al. 2004) to test correlations between emergent fitness (net diversification rates of families) and candidate traits for species selection (range size, range continuity, and heterochronic variation). PICs were performed on the full set of Magallón-Sanderson diversification-estimates (under both scenarios of ε), each of which was paired with a randomly selected tree from the posterior distribution, pruned to leave a single external branch per family. A total of $1.5x10^3$ PICs was generated for each hypothesis tested (twenty altogether): one of five higher-level characteristics; under one of two scenarios of ε; under an assumption of either WEA or FEA topology. Regression on independent contrasts was constrained to pass through the origin (Garland et al. 1992, 1999; Legendre and Desdevises 2009). Statistical significance of PICs was assessed by one-sample t-tests, performed in STATS on each correlation coefficient (ρ) with the null expectation of $\rho = 0$. To assess overall support for each test, accommodating topological uncertainty, probability values were averaged across the entire set of $1.5x10^3$ PICs.

Insofar as this method of generating continuous clade-data from discrete species-level characters is novel and unsubstantiated (see *Trait variation*), we conducted simulations over several draws from BiSSE parameter-space to test the ability of this method to recover underlying (i.e., simulated) evolutionary signal (Maddison et al. 2007, FitzJohn et al. 2009). The R-package DIVERSITREE (vers. 0.4-1; FitzJohn 2009) was in part used for these simulations. A detailed account of simulation conditions and results is provided in the Supplementary material. Briefly, simulations were conducted under trait-state dependent diversification. Trait-state variation and crown-group age was recorded for clades selected at random.

Using the continuous trait data for randomly chosen clades, we testing for trait-mediated diversification as previously described, correlating the PICs of clade-level trait-variation against the PICs of Magallón-Sanderson diversification rates.

To address the potential for heteroskedasticity in the regression models, we employed the Breusch-Pagan (1980) test for homogeneity of variance on model-fitted residuals using the R-package CAR (v1.2-13; Fox 2009). Several workers have suggested that a triangular constraint-envelope typifies relationships between body size and range size (see Brown and Maurer 1987; Brown 1995; Gaston 2003). In our case, it might be conceived that species with large and (or) continuous ranges may exhibit greater variance in net diversification. Further, perhaps developmental modes that are more habitat-generalized (i.e., of paedotypic species) may exhibit similarly large variance in rates of diversification. Tests for heteroskedasticity utilized linear models where values from each predictor variable (see Table 2) were paired with average family-level estimates of diversification under all four historical scenarios (i.e., WEA and FEA; pure-birth and high relative-extinction).

Association between range characteristics and heterochrony

Given the possibility for predictor collinearity, we tested the statistical independence and explanatory contribution of our predictor variables using analysis of deviance for two-predictor generalized linear models (GLM; McCullagh and Nelder 1989; Venables and Ripley 2002). GLMs were constructed from PICs, which were computed from raw summary data as given in Table 2: multivariate PICs were computed on the summary trees from the FEA and WEA posterior distributions (Fig. 1). Using minimal probability values, the two best predictors from univariate PIC regression were paired with other variables from the complementary higher-level trait (i.e., distributional range or heterochronic variation, depending upon the selected predictors). Analyses of deviance were conducted in STATS on GLMs assuming a Gaussian error distribution and identity link. Chi-square tests were implemented to test whether model fit was significantly reduced by elimination of predictor variables, comparing fit of single versus two-predictor GLMs. We interpret a non-significant result of these tests to indicate explanatory redundancy of the selected and secondary predictors (e.g., Jablonski and Hunt 2006; Venables and Ripley 2002).

To more specifically test for variable collinearity, phylogenetic analyses of variance (ANOVAs) were performed to ascertain whether variation in distributional range-size or continuity was statistically separable by life-history strategy. Phylogenetic ANOVA was performed with the package GEIGER under an assumption of Brownian-motion trait-evolution. A conservative phylogenetic ANOVA was conducted where correction for phylogenetic pseudoreplication was informed by untransformed tree structure; a separate phylogenetic ANOVA was performed on λ_P-transformed trees (see Pagel 1999), where λ_P was the fitted value for the dependent variable (either range size or range continuity) as described previously (see *Trait-state heritability*). Phylogenetic ANOVAs utilized 100 random-resolutions of the species-level tree with species-level data.

Batch analyses, simulations, and data-structure manipulation not achievable with use of publicly available software was accomplished with the aid of R and BASH shell scripts, all of which are available upon request.

RESULTS

Phylogeny estimation

To assess topological credibility of two hypotheses (i.e., WEA and FEA), analyses performed in BEAST were compared by log-Bayes-factor ($_{BF}$). While the WEA topology was preferred, support for this hypothesis was not overwhelming (FEA_{BF}: -1.215). Within the divergence constraints and topology imposed on MCMC tree searches, sequence data appeared relatively uninformative for divergence-date estimation; that is, highest posterior densities (HPDs) for divergence dates are quite broad (see Fig. 1). The skew in many of these HPDs may result from specification of a birth-death tree prior if different lineages have truly diversified at different rates. Further, the multiple sequence alignment for the data used herein was admittedly incomplete (see Appendix 1), likely contributing to the lack of phylogenetic signal deeper in the tree. Insofar as comparative analyses are based on a wide range of Bayesian credible samples, however, conclusions appear robust to topology.

TABLE 3. Estimates of phylogenetic heritability of higher-level traits, as assessed by Pagel's (1999) λ_P. Values of λ_P distinguishable from 0 are interpreted as a signature of trait heritability. Hierarchical likelihood-ratio tests compared models incorporating a constrained values of λ_P (i.e., $\lambda_P = 0$) to a model wherein λ_P was estimated. Probability values were drawn from a mixed Chi-square distribution with 0/1 degrees of freedom. The test statistic was twice the difference in likelihoods between compared models.

trait	*lambda-est* (λ_P)	lnL†			p-val$_{1/0\ mix\ df}$
		$\lambda_{P\ [Est]}$	$\lambda_{P\ [0]}$	$\lambda_{P\ [1]}$	
Heterochrony	0.800	-197.35	-303.90	-419.81	<0.00001
Plasticity	0.562	206.01	164.76	-148.35	<0.00001
Paedomorphy	0.825	249.30	50.95	-77.50	<0.00001
Range size	0.698	-1294.38	-1376.55	-1652.08	<0.00001
Range continuity	0.562	-1435.88	-1480.03	-1846.26	<0.00001

† averaged across 100 random resolutions in topology

Trait-state heritability

Each trait exhibited statistically supported signatures of phylogenetic heritability, as evaluated by a model selection approach. For each higher-level trait, model fit was improved by allowing an unconstrained λ_P (Table 3). Topological differences apparent in random resolutions in the species-level trees appears to have contributed little to estimates of phylogenetic trait-heritability. Owing to the phylogenetic heritability of traits investigated herein, we expect results to be robust to the absence of historical data on range continuities, sizes, and life-history deep within caudate lineages. That is, the underlying phylogenetic structure informed by tree inference and data observed at the tips (i.e., close phylogenetic relatives show similar distributions of trait values) suggests continuity in historical processes generating the observed data.

Natural-log transformation of distributional-range data improved normality substantially. For both range size and continuity, normal QQ plots appeared reasonable in the central masses of the distributions. Whereas we had evidence to reject normality for the range-continuity dataset even with data transformation (Kolmogorov-Smirnov test with Ln-transform; $p = 0.0015$), Ln-transformation was a considerable improvement (Kolmogorov-Smirnov test with raw data; $p < 2.2\text{X}10^{-16}$). We were unable to reject normality for the Ln-transformed range-size dataset ($p = 0.1585$). Severely right-skewed based on the raw data, data exhibited moderate left skew upon transformation.

Trait-mediated diversification analyses

BiSSE simulations (see Supplementary material) revealed adequacy in summarizing the bias of discrete character-states as a continuous datum when analyzing trait-mediated diversification at the clade level. Statistically significant in many cases (Supplementary Figs. 1-2), the estimated association between trait-states and diversification was in the expected direction in nearly all cases. The estimator for these analyses, which in this case is the regression slope of independent contrasts, appeared statistically consistent. In these simulations, higher statistical power was afforded by i) greater magnitudes of bias in trait-mediated diversification, especially under high relative-extinction and ii) slight biases at the tree level toward the trait-state promoting rapid diversification (see Supplementary Figs. 1-2).

We were unable to reject the null hypothesis of homogenous variances across fitted values of models relating emergent fitness (i.e., net diversification) to our predictors (range size, range continuity, and heterochronic variation). Using the Breusch-Pagan (1980) method, we find support lacking for triangular (or otherwise heteroskedastic) relationships between these pairs of variables (χ^2 test; for all tests: $0.65 > p_{1\ df} > 0.12$). Neither topology nor extinction fraction seemed overly influential for regression slopes estimated between family-level diversification rates and traits potentially subject to species selection (Fig. 2; Table 4). This latter result is consistent with results of our simulations, indicating that relative-extinction fraction in the birth-death process (either assumed or as simulated) has little bearing on the ability to detect truly trait-mediated diversification (Supplementary Fig. 2).

Data do not appear to strongly support the hypothesized inverse correlation between paedomorphy and net diversification (Fig. 2; Table 4). Relationships between diversification and a set of three higher-level traits (heterochrony, plasticity, and range size), however, were significant in nearly all circumstances (Table 4). Support for the hypothesis of diversification mediated by range continuity was more marginal. Averaged probability-values, as well as the ranges of the correlation coefficients, lend support to the notion that evolutionary biases toward paedotypy and small distributional ranges (or isolates thereof) are associated with accelerated rates of diversification (Fig. 2; Tables 4, 6).

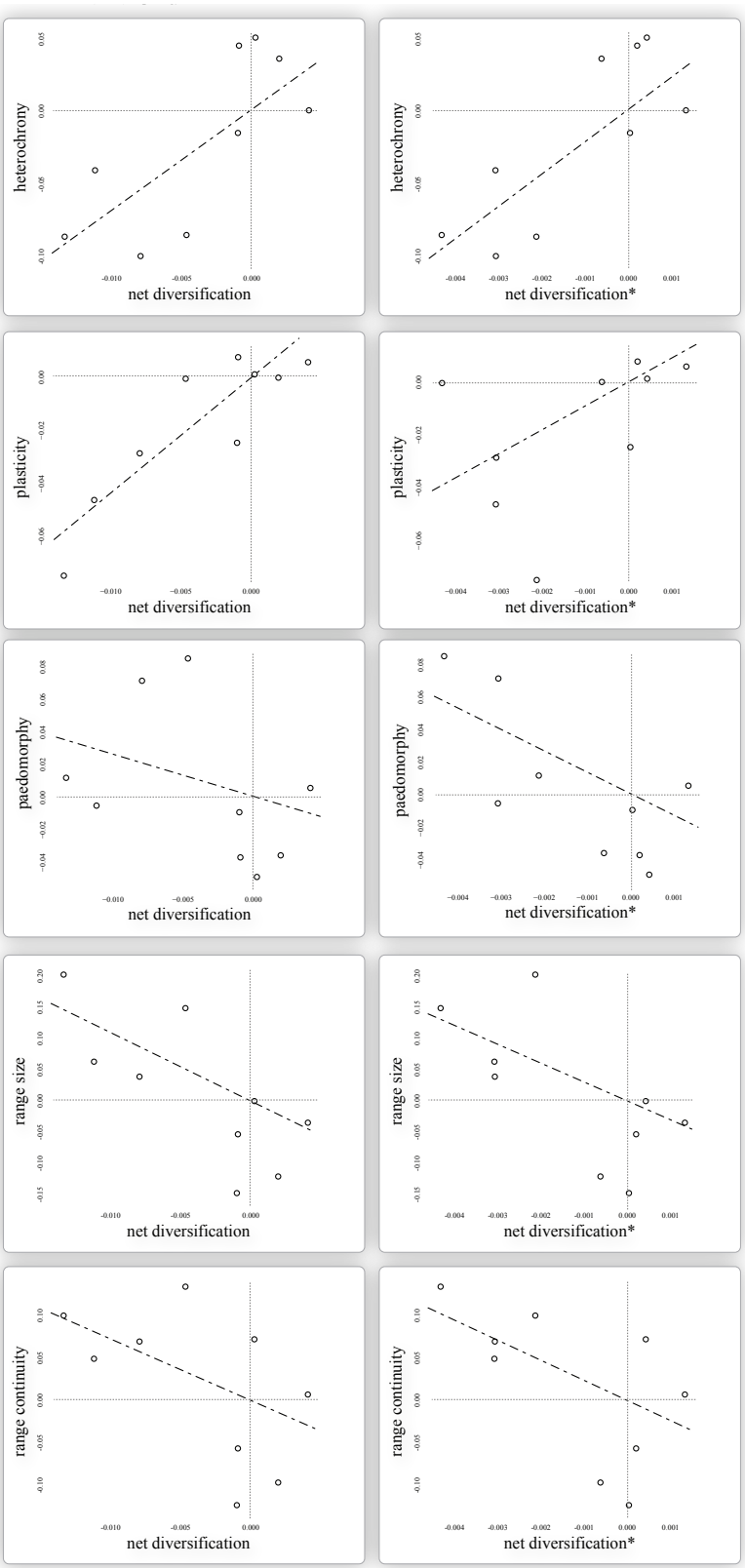

FIGURE 2. Caption on following page.

FIGURE 2 continued. Independent contrasts of diversification rate (sensu Magallón and Sanderson 2001) with three measures of life-history variation (heterochrony, plasticity, and paedomorphy), range size, and range continuity. Results were quite consistent between two topological hypotheses (WEA: Wiens et al. 2005; FEA: Frost et al. 2006). Depicted data are averaged diversification rates within families across entire posterior distribution of trees; PICs were computed using the WEA summary tree generated by TREEANNOTATOR (vers. 1.4.8; Drummond and Rambaut 2007). *: rightmost panels assume relative extinction fraction of 0.9; leftmost panels assume relative extinction proportion of 0.0 (i.e., pure birth). Dot-dashed lines represent slope of only summary PICs; ranges of slopes estimated across all PIC iterations for each test, can be found in Table 4. Larger values along the ordinates (from top to bottom) correspond to higher frequencies of paedotypic relative to paedomorphic species within a family; higher proportions of developmentally plastic (i.e., paedotypic) species; greater frequencies of paedomorphic species relative to clade size; larger distributional ranges; and more fragmented ranges. Statistical summaries across all PIC iterations for each test can be found in Table 4. Figure appears on following page.

TABLE 4. Independent contrasts (PICs) for primary tests conducted herein. PICs were calculated on trees under two topological hypotheses of the interfamilial relationships within Caudata (i.e., WEA from Wiens et al. 2005; FEA from Frost et al. 2006). Further, two scenarios of extinction fraction were utilized for net-diversification estimates (see text). Probability values and *t* test-statistics correspond to the average across all 1.5×10^3 PICs conducted for each test. The full ranges of estimated slopes (*m*) from the full set of PICs are given in square brackets. Summarized results are presented in Fig. 2 for the WEA topology.

Extinction fraction (0.0; pure-birth)						
	WEA			FEA		
	p-est.	*t-est.*	*m (interval)*	*p-est.*	*t-est.*	*m (interval)*
Heterochrony*	0.0112*	3.18	[4.22, 12.85]	0.0157*	2.97	[4.02, 13.71]
Plasticity*	0.0001*	5.94	[2.85, 6.27]	0.0004*	5.00	[2.77, 6.58]
Paedomorphy	0.1038	-1.36	[-7.44, -1.30]	0.1036	-1.36	[-7.64, -0.98]
Range size*	0.0289*	-2.17	[-21.22, -4.26]	0.0111*	-2.76	[-22.92, -5.38]
Range continuity	0.0604	-1.71	[-16.28, -2.74]	0.0296*	-2.16	[-16.07, -3.75]
Extinction fraction (0.9; birth-death)						
	WEA			FEA		
	p-est.	*t-est.*	*m (interval)*	*p-est.*	*t-est.*	*m (interval)*
Heterochrony*	0.0068*	3.49	[9.88, 31.72]	0.0117*	3.15	[12.60, 30.00]
Plasticity*	0.0190*	2.43	[1.48, 11.85]	0.0412*	1.95	[1.13, 12.42]
Paedomorphy*	0.0223*	-2.33	[-22.96, -7.11]	0.0217*	-2.35	[-22.30, -6.04]
Range size	0.0570	-1.75	[-42.42, -12.55]	0.0418*	-1.95	[-38.18, -10.67]
Range continuity	0.0610	-1.71	[-34.42, -10.38]	0.0418*	-1.95	[-31.10, -11.91]

The best predictors of differential rates of net diversification appeared to be two measures of heterochronic variation (*heterochrony* and *plasticity*; Table 4.) Results from our analyses of deviance were largely consistent with the univariate PICs (Table 6), supporting a primary role for heterochronic variation in trait-mediated differential success of salamander lineages. In few analyses of deviance was there substantial support for the independence of heterochronic and distributional range variation in predicting rates of net diversification (Table 6).

TABLE 5. Tests examining the relationship of heterochronic variation (the predictor variable) with observed variation in range size and range continuity. All species were scored as paedotypic, paedomorphic, or terrestrial (i.e., obligately metamorphosing or direct developing). Probability values for 'phylogenetic' analysis of variance (ANOVA), performed in GEIGER (vers. 1.2-14; Harmon et al. 2008). As recommended by Pagel (1999), degree of necessary phylogenetic-correction was determined by Pagel's λ_P (as in Table 3). All 100 random-resolutions of the species-level phylogeny were transformed with the point estimate of λ_P for either range size or range continuity. An additional ANOVA for each trait was performed without tree transformation.

trait	*p-est* λ_P*	*p-est*
Range size	0.126	0.238
Range continuity	0.248	0.432

* trees λ_P–transformed using estimate for each trait as in Table 3

TABLE 6. Results from analyses of deviance on generalized linear models (GLMs) constructed from PICs. Analyses of deviance were conducted on summary trees under two topological hypotheses for the interfamilial phylogeny of salamanders. Full, two-predictor models are shown on the left margin: *r* denotes Magallón-Sanderson (2001) estimates of net diversification under pure-birth; *r** signifies net-diversification estimates under birth-death, where relative-extinction fraction (ε) was assumed to be 0.9 (see Table 2). Predictor variables (*heterochrony*, *plasticity*, *range size*, and *range continuity*) are as described in text. Chi-square tests (1 df) compared the full GLM to a reduced model where one predictor was eliminated ('*dropped var.*'). Significance of Chi-square tests suggest explanatory importance of the dropped variable relative to the remaining predictor: in such cases, model fit was significantly diminished by model reduction. Significant results for both comparisons within a given model suggest statistical independence and predictive importance for both variables. Non-significant results for both predictors suggest explanatory redundancy of the predictors.

	Frost et al. 2006 topology (FEA)			Wiens et al. 2005 topology (WEA)		
full model	*dropped var.*	*scaled dev.*	*p-est*	*dropped var.*	*scaled dev.*	*p-est*
r ~ plasticity + range size	plasticity	8.38	0.0038 **	plasticity	11.59	0.0007 **
	range size	2.67	0.1021	range size	5.73	0.0167 **
*r** ~ plasticity + range size	plasticity	0.15	0.6979	plasticity	0.13	0.7156
	range size	1.65	0.1987	range size	3.91	0.0481 **
r ~ plasticity + range continuity	plasticity	12.26	0.0005 **	plasticity	15.01	0.0001 **
	range continuity	3.58	0.0584 *	range continuity	4.97	0.0259 **
*r** ~ plasticity + range continuity	plasticity	0.72	0.3960	plasticity	1.19	0.2746
	range continuity	2.01	0.1560	range continuity	3.52	0.0607 *
r ~ heterochrony + range size	heterochrony	1.24	0.2649	heterochrony	2.16	0.1413
	range size	2.32	0.1279	range size	2.39	0.1222
*r** ~ heterochrony + range size	heterochrony	2.99	0.0836 *	heterochrony	4.42	0.0355 **
	range size	0.08	0.7760	range size	0.77	0.3791
r ~ heterochrony + range continuity	heterochrony	2.59	0.1075	heterochrony	4.36	0.0368 **
	range continuity	0.70	0.4031	range continuity	0.39	0.5325
*r** ~ heterochrony + range continuity	heterochrony	3.29	0.0698 *	heterochrony	5.83	0.0158 **
	range continuity	0.17	0.6833	range continuity	0.73	0.3930

Association between range characteristics and heterochrony

Phylogenetic ANOVAs were used to determine whether particular developmental strategies were associated with variation in the distributional ranges of salamanders. As indicated in Table 5, life-history characteristics do not appear to be related to observed variation in range size and continuity (Table 5). As Pagel's λ_P-transformation is implemented to prevent over- (or under-) correction for phylogenetic relationship (Pagel 1999), we emphasize results from the λ_P-transformed trees (Table 5).

DISCUSSION

Our results have several implications for evolutionary biology. This study represents one of few neontological studies in which higher-level traits are found to be heritable, variable, and associated with differential success at a higher taxonomic category. Range size appears inversely correlated with diversification rates, and a peculiar life-history characteristic for some salamander taxa, paedotypy, is associated with accelerated rates of diversification. Regarded by some as the "most challenging and interesting of macroevolutionary phenomena" (Gould 2002), we suggest these patterns owe, at least in part, to species selection.

Distributional Range and Diversification

Whereas univariate PICs support a strong negative relationship between range size and net diversification rates, relationships were less clear with our measure of range continuity (Tables 4, 6). Apparently not solely determined by differences in life-history strategy (Table 5), other organismal traits may be influential in the extent and shape of species' distributions such as dispersal ability (Hansen 1980; Jablonski 1986, 2008a; Böhning-Gaese et al. 2006). Limited dispersal ability might increase the likelihood of origination and (or) extinction. Once allopatric isolates are established by vicariance, genetic isolation among patches might facilitate populational divergence through local adaptation (Slatkin 1973; Ibrahim et al. 1996; Storfer et al. 1999) or drift. Lower dispersal rates might allow most organisms to spend the majority of time in favorable habitat, thereby enhancing organismal viability (Gibbs 1998; Fahrig 2001). Conversely, reduced connectivity may allow inbreeding depression and the erosion of

genetic variability and consequently increasing extinction proneness (Titus and Gaines 1991; Hanski and Gilpin 1997; Holt and Gomulkiewicz 2004).

Bridged through gene flow and recruitment, populations of broadly distributed taxa may be less likely to speciate (Futuyma and Moreno 1988) but also less likely to suffer extinction (Jablonski 1986, 1987; Jablonski and Hunt 2006). Wide dispersion may serve to protect against stochastic perturbation resulting in dramatic habitat loss and consequent demographic vulnerability (Gomulkiewicz and Holt 1995; Hedrick et al. 1996; Hedrick 2001; Lande 1998). While the expectations regarding the relationship between distributional range variation and diversification rates seem relatively straightforward (but see Foote et al. 2008), there may be more complex interactions between lineage origination, extinction, and life-history variation.

Our data cannot inform how extinction proneness might be associated with variation in geographic range size. Yet, an inverse relationship between range size and extinction risk is both empirically supported (Stanley 1979, 1990; Van Valen 1985; Jablonski 1987; Sepkoski 1998; Jablonski 2008a; Liow et al. 2009) and predicted by theory (Gomulkiewicz and Holt 1995; Maurer and Nott 1998). If rates of origination and extinction are indeed generally positively correlated (e.g., Fig. 3; Stanley 1979, 1990; Gould and Eldredge 1977; Van Valen 1985; Sepkoski 1998; Jablonski and Roy 2003; Weir and Schluter 2007), a recent report by Cooper et al. (2008) would be quite consistent with results presented herein: in frogs, restricted geographic distributions appear to be a strong predictor of high extinction risk. Despite correlations of range size with the organismal traits of body size and clutch size, Cooper et al. (2008) find greater predictive capability of the emergent property of species (i.e., geographic range size) in explaining extinction risk of extant frogs.

While a causal relationship between geographical distribution and diversification is assumed, we cannot discount the possibility that rapidly diversifying lineages might *produce* species with small ranges, largely through non-ecological processes (e.g., see Schluter 2009). If the cause of rapid diversification is heritable, but is not range size itself, patterns observed herein would be misinterpreted. Alternatively, it may be that species have not yet reached equilibrium (or maximal) values of range size, especially for

rapidly diversifying lineages (Foote et al. 2008; Jablonski 2008a). Although we would need to assume that incipient species always begin with small geographical distribution, this would give the artifactual appearance of a macroevolutionary advantage for small range-size. Ploidal evolution for instance, or otherwise saltational speciation, must necessarily generate species with (at least initially) small ranges. Lineage diversification by polyploidization, although not altogether absent from caudate evolution (see Bogart et al. 2007), does not appear sufficiently ubiquitous to explain these results.

Heterochrony and Diversification

While large and continuous ranges are generally associated with lower rates of net diversification, lineages of paedotypic salamanders -- comprising some of the most broadly distributed taxa -- are also those diversifying most rapidly (Fig. 2; Table 4). Despite evidence for collinearity between the two categories of higher-level traits explored herein (i.e., distributional range and heterochronic variation; Table 6), phylogenetic ANOVAs did not support the partitioning of variation in range characteristics by life-history strategy (Table 5). An apparent relationship between distributional range and life-history in the raw data appears attributable solely to phylogenetic history (Table 5). Further work is thus necessary to reveal the underlying nature of collinearity between these higher-level traits. While expecting that trait-based differential success among salamander lineages involves an interaction between life-history and distributional range characteristics (among potentially other higher-level traits), we frame hypotheses for the potential role of paedotypy in the differential proliferation of lineages.

Many have argued for the selective advantage of developmental plasticity in highly heterogeneous and especially in unpredictable environments (e.g., Whiteman 1994; Denoël et al. 2005; West-Eberhard 2005; Takahashi and Parris 2008; Lande 2009). Especially in an underutilized niche with few competitors, evolution of broader reaction norms (e.g., paedotypy) may entail organismal selection that is reinforced by species selection. Evidence from univariate PICs and analyses of deviance support the role of developmental plasticity (e.g., salamander paedotypy), rather than the loss of obligate paedomorphosis, in explaining a great deal of variation in diversification rates (Tables 4, 6; Fig. 2). Increased

diversification of paedotypic lineages appears to be one such instance of species selection for variability (see Lloyd and Gould 1993).

Polyphenism in the eastern newt (*Notophthalmus viridescens* subspp.) is found to be adaptive in heterogeneous environments (Takahashi and Parris 2008), where potential competitors might have difficulty utilizing more specialized niches. If fitness tradeoffs are not too costly, these lineages might be expected to experience elevated rates of origination, perhaps especially if conditions are favorable for local adaptation. Paedotypic lineages may benefit from permanent, productive aquatic habitats but without being limited to such habitat, as are obligatory paedomorphs (Whiteman 1994). Especially for phenotypically plastic traits, adaptive populational response to environmental perturbation can occur on the order of a generation (e.g., Kingsolver 1995). Such rapid responses are witnessed empirically. For example, Ryan and Semlitsch (1998) find under higher maintained densities of *Ambystoma talpoideum*, experimental populations produce higher proportions of paedotypes. Paedotypes also often enjoy greater reproductive capacity and larger body size at maturity (Ryan and Semlitsch 1998). Importantly, body size is related to fecundity, wherein larger organisms are often more fecund (Tilley 1968). All else being equal, paedotypes reproduce earlier or are larger in size at maturity, both of which are associated with increased fitness (Ryan and Semlitsch 1998).

Developmentally-plastic lineages might also persist due to mitigated risk of extinction (Gould 1977, 2002). Gould (2002) analogizes the potential for conflict between organismal and species selection: he concocts the 'optimal fish' (perfectly adapted for a particular set of invariant environmental conditions) and the 'middling fish' (greater variability, but suboptimal in any particular environment). Where optimality of the optimal fish can only be driven by organismal selection, such success may not be upheld at the species level. Middling fish may represent a 'strategy' of risk-spreading (as does developmental plasticity), and such 'middling' lineages are likely to be more evolutionarily durable, owing to species selection for variability (Simpson 1953; Lloyd and Gould 1993; Gould 2002; Jablonski 2008b). We might expect relative-extinction fraction to meaningfully differ among these developmental modes (as in bottom panel of Fig. 3), where extinction risk may be buffered for paedotypic species.

Especially with larger datasets, such hypotheses could be tested using sophisticated models that relax assumptions of the constancy of diversification rates and relative extinction through time (e.g., Rabosky et al. 2007; Ricklefs 2007; Rabosky and Lovette 2008; FitzJohn et al. 2009; Rabosky 2009a, b).

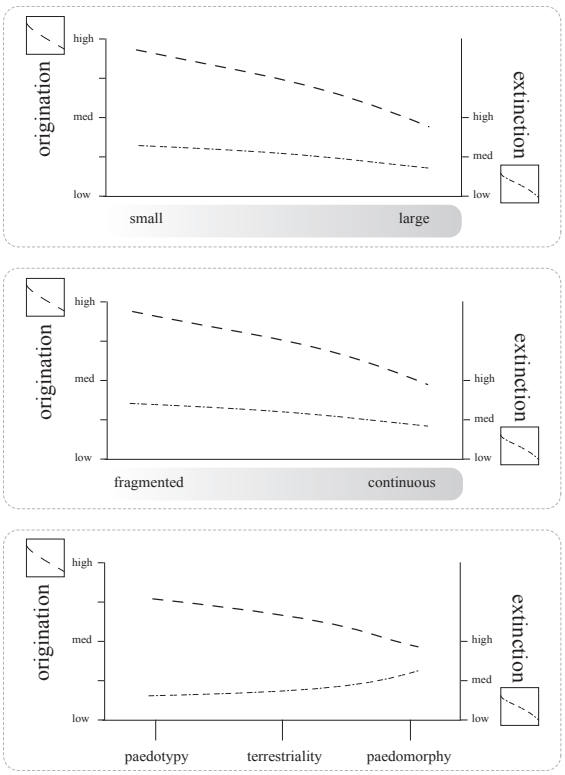

FIGURE 3. Shown are predictions of the differences in rates of origination and extinction for spatial arrangements of the distributional range (two uppermost panels) and life-history strategies (lower panel), characteristic of different salamander taxa. In each panel, upper dotted lines correspond to predicted rates of origination; lower, dot-dashed lines reflect predictions for extinction. Note that axes are devoid of units; relationships are only intended to represent qualitative hypotheses. Uppermost panels are fairly consistent with birth-death models assuming relative-extinction fraction (ε) is independent of trait states. The lowermost panel, however, would imply trait-state dependency of relative extinction (where $\varepsilon = \mu/\lambda$, and where μ is extinction probability and λ is speciation probability).

Paedomorphy appears to be an instance where organismal selection is in conflict with higher-level selection. Paedomorphic salamanders should be just as susceptible to poor-quality aquatic habitat as are metamorphosing salamanders. Where terrestrials have the physiological means to select more favorable habitat in which to develop and mature, paedomorphs should suffer more severely from local stochastic change. The organismal reproductive advantage afforded by paedomorphy (Ryan and Semlitsch 1998) may thus conflict with species selection for paedotypy (e.g., Fig. 3). However, the advantage of

paedotypy in heterogenous habitat with unpredictable ecological conditions appears to be a case where organismal and species selection are co-aligned (Whiteman 1994; Denoël et al. 2005; Pigliucci 2005; West-Eberhard 2005; but see DeWitt et al. 1998).

If plastic life-history facilitates proliferation at the species level and if evolutionary rates are truly skewed toward this characteristic, we might ask: Why do so few species exhibit paedotypy? Foremost, tradeoffs are likely to exist (Via et al. 1995; DeWitt et al. 1998; Pigliucci 2005). Developmentally plastic species are likely incapable of competing effectively in all commonly encountered habitat types. We regard either obligatory metamorphosis, direct development, or paedomorphy as reproductive stenotopy. As Takahashi and Parris (2008) have shown in the eastern newt (*Notophthalmus viridescens*), it appears that while polyphenic larvae are capable of adaptive developmental response to a particular favorable habitat (i.e., aquatic or terrestrial), populations of plastic organisms may lag behind the optimal life-history strategy presented experimentally. That is, when terrestrial habitats are favored, the developmentally plastic subspecies do produce more efts (terrestrial juveniles), but the response is not without exception (Takahashi and Parris 2008; see also Denoël et al. 2005).

In addition to tradeoffs, certain genetic constraints may bias the directionality of, or otherwise limit, life-history evolution (Via and Lande 1985; Via et al. 1995; DeWitt et al. 1998; Pigliucci 2005). Yet, at least in some ambystomatids, life-history variation (between metamorphosis and paedotypy) appears to be controlled by a locus of large effect (Voss and Shaffer 1996). Largely unstudied, negative pleiotropic effects and epistatic interactions may limit evolutionary potential of origin and maintenance of such plasticity. Even if plastic responses are in the 'proper' direction, environmental reversals or lagged response might render phenotypes suboptimal (DeWitt et al. 1998; Agrawal 2001; Eastman et al. 2009).

In a manner similar to the conflict between lower levels of selection (e.g., Lewontin 1970; Werren 1991), our results in part may be explained by potential conflict between selection at the organismal and species levels. If environmental conditions are adequately stable on ecological timescales, paedotypes may perform no better (perhaps worse) than paedomorphs in productive larval habitat owing to the costs of plasticity (DeWitt et al. 1998). Further, because of an apparent tradeoff exhibited by some paedotypes

where conditions suit either invariant mode of development (favorable terrestrial conditions; or permanent and productive aquatic habitat), members of paedotypic populations may well be outcompeted (Wilbur and Collins 1973).

CONCLUSIONS

Our data suggest that an emergent character – species range size – is inversely related to net diversification rate. Such emergent characters involve an interaction between lower-level properties and the environment and thus cannot be subject to selection at levels lower than that at which the characters emerge (e.g., the phenotype *cannot* be the unit of genic selection). Thus, for the emergent trait investigated herein -- distributional range characteristics -- we attribute differential diversification to 'strict-sense' species selection (Vrba and Gould 1986; Jablonski 2008b).

However, it is uncertain whether differential proliferation is a *primary* result of irreducible higher-level processes (species selection, *sensu stricto*) or simple correlations with organismal properties within taxa. Results suggesting an inverse relationship between range-size with diversification are consistent with previous empirical work, and range size may be correlated with organismal properties such as dispersal abilities (Jablonski 1987; Hunt et al. 2005; Jablonski and Hunt 2006). Even if dispersibility is demonstrably influential for generating much of the variance across distributional ranges, the interaction of this organismal trait with environment confounds the correspondence between the lower-level trait (dispersibility) and the higher order patterns (species' distributions and emergent fitness). We therefore anticipate primacy for the role of the species-level characteristic of range size in explaining variation in reproductive success of species.

The present work also represents one of few empirical explorations on the macroevolutionary consequence of developmental plasticity. It would seem that the costs of plasticity for organismal fitness (see DeWitt et al. 1998 for review) do not strongly exert influence at a macroevolutionary scale. Results appear consistent with extensive literature on the subject of phenotypic plasticity (e.g., Stearns and Koella 1986; West-Eberhard 1989, 2003, 2005; Agrawal 2001; Pigliucci 2001, 2005; Lande 2009; Svanbäck et

al. 2009). The macroevolutionary advantage of plasticity has been considered by a number of authors (West-Eberhard 1989; Agrawal 2001; Pigliucci 2005 and references therein), and several mechanisms concerning the contribution of plasticity to evolutionary divergence have been proposed. Plasticity may contribute to the evolution of novelty through genetic assimilation (Pigliucci 2001; Lande 2009) and correlated phenotypic-shifts in response especially to genetic perturbation (West-Eberhard 1989 and references therein). As suggested by the present work, highly plastic lineages may be more evolutionarily durable, as periods of maladaptation following environmental change should be shorter and weaker (West-Eberhard 1989; Lande 2009). More direct and quantitative measurement of plasticity maintained within populations and variance exhibited among populations is an intriguing prospect for future research in addressing how plasticity bears on macroevolutionary patterns (Pigliucci 2005; Losos 2009).

APPENDIX 1. Sequence data used herein. Taxon identifiers are NCBI GenBank identification numbers ('gi': GenInfo identifier); 'basal recovery': the most basal node within each family was assumed to be recovered if locus denoted with "*". Assumptions of recovery of basal nodes based on listed references (i.e., 'basal reference').

families	*markers*					*basal references*
AMPHIUMIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Amphiuma means		123286014	62736698		54610059	
Amphiuma pholeter			62736690		54610061	
Amphiuma tridactylum		1050570	62736694			
basal recovery			*			Karlin and Means 1994
AMBYSTOMATIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Ambystoma cingulatum/barbouri		90296353		118202000		
Ambystoma gracile/talpoideum				118202042	54610067	
Ambystoma jeffersonianum/opacum				83031788	54610065	
Ambystoma tigrinum/ordinarium		90296576	62736615		50957101	
Ambystoma unixexual				118202108		
basal recovery				*	*	Shaffer 1984; Shaffer et al. 1991; Shaffer and McKnight 1996
CRYPTOBRANCHIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Andrias		123286038			50957103	
Cryptobranchus		90296432			54610087	
basal recovery		*			*	Estes 1981; Duelman and Trueb 1986; Larson 1991; Larson and Dimmick 1993
DICAMPTODONTIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Dicamptodon aterrimus			62736619			
Dicamptodon copei					58202366	
Dicamptodon ensatus	1050606	90296287			123286212	
Dicamptodon tenebrosus		90296430	62736623		54610069	
basal recovery			*			Good 1989; Steele et al. 2005
HYNOBIIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Batrachuperus	51891006	123254214	51891101		123254612	
Hynobius	83319144	83319187	62736487	126470965	85725937	
Liua	51890997	17864746	51891083	84784280		
Onychodactylus	51891008	51891022	62736430		54610091	
Pachyhynobius	51890999	51891013	51891087			
Pseudohynobius	51891001	17864744	51891091	84784258		
Ranodon	62736606	34761149	62736514			
Salamandrella	51891002	51891016	62736674	56684402	54610095	
basal recovery	*	*	*			Zhang et al. 2006
PLETHODONTIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Aneides		70779707	47680456		54610041	
Batrachoseps		6457569	68144107	2351730	54610057	
Bolitoglossa		9622955		85838590	54610053	
Desmognathus		90296422	47680534	116242560	58202372	
Ensatina		123286043		46252591	58202380	
Eurycea		90296423	47680459	46391551	49887076	
Oedipina		6457557		6457413		
Plethodon		123286012	68144113	125380720	125380886	
Pseudoeurycea		19422050	62736651	62002493	54610055	
basal recovery		*	*	*	*	Chippindale 2004
PROTEIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Necturus		90296320			123286152	
Proteus	37900111	123286009			123286148	
basal recovery		*			*	Guttman et al. 1990
RHYACOTRITONIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Rhyacotriton cascadae		90296279			58202364	
Rhyacotriton kezeri					54610063	
Rhyacotriton variegatus		123286008			58202362	
basal recovery					*	Good et al. 1987
SALAMANDRIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Chioglossa			13561436		50957105	
Lyciasalamandra		94548892		94548900		
Mertensiella			13561496		38372037	
Notophthalmus	2804367	90296590		18265554	54610073	
Salamandra	60651039	73920388	13561444	28883301	50957115	
Salamandrina	62462013	62462017	62736663	62511785		
Taricha		90296613	62736659	75857975	123286222	
basal recovery	*	*	*	*	*	Steinfartz et al. 2006; Weisrock et al. 2006
SIRENIDAE	*12S*	*16S*	*ND2*	*cyt b*	*rag1*	
Pseudobranchus		90296352		57236075	54610083	
Siren		90296350	62736710	57236091	123286156	
basal recovery		*		*	*	Duellman and Trueb 1986; Larson 1991

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SUPPLEMENTARY MATERIAL

Intent of trait-mediated diversification simulations

With our empirical dataset, we were interested in part to determine whether diversification is mediating by different trait-states of discrete life-history strategies within salamanders (e.g., paedomorphosis or obligate metamorphosis). The lack of resolution and species-level sampling within the salamander phylogeny, however, prevented straightforward analysis of our hypotheses of trait-mediated diversification (e.g., with BiSSE; Maddison et al. 2007; FitzJohn et al. 2009). Rather, we tested whether clade-level biases toward a trait-state (e.g., facultative metamorphosis, or 'paedotypy') were associated with higher rates of net diversification, also analyzed at the clade-level. Significant correlations between phylogenetic independent contrasts (PICs) of trait-state biases and diversification rates within clades were interpreted as evidence for trait-mediated diversification. To address whether binary characters at a lower taxonomic level could be meaningfully summarized as a continuous datum when analyzed at a higher taxonomic level, we conducted BiSSE simulations to generate trees with true histories of trait-mediated diversification (Maddison et al. 2007; FitzJohn et al. 2009). We asked whether correlations between trait-state biases and diversification at the clade-level matched our expected dependence of diversification on states of a binary character, acting at the level of simulated species.

SUPPLEMENTARY TABLE 1. BiSSE parameters used for simulations. b[ ]: speciation rate of state; d[ ]: extinction rate of state; q01: rate of transition from state 0 to state 1; q10: reverse transition rate. Each row is a set of BiSSE parameters used to simulate 500 histories.

b[0]	b[1]	d[0]	d[1]	q01	q10
0.10	0.20	0.000	0.000	0.1	0.1
0.10	0.40	0.000	0.000	0.1	0.1
0.10	0.80	0.000	0.000	0.1	0.1
0.10	1.60	0.000	0.000	0.1	0.1
0.05	0.10	0.000	0.000	0.1	0.1
0.05	0.20	0.000	0.000	0.1	0.1
0.05	0.40	0.000	0.000	0.1	0.1
0.05	0.80	0.000	0.000	0.1	0.1
0.10	0.20	0.050	0.100	0.1	0.1
0.10	0.40	0.050	0.200	0.1	0.1
0.10	0.80	0.050	0.400	0.1	0.1
0.10	1.60	0.050	0.800	0.1	0.1
0.05	0.10	0.025	0.050	0.1	0.1
0.05	0.20	0.025	0.100	0.1	0.1
0.05	0.40	0.025	0.200	0.1	0.1
0.05	0.80	0.025	0.400	0.1	0.1
0.10	0.20	0.080	0.160	0.1	0.1
0.10	0.40	0.080	0.320	0.1	0.1
0.10	0.80	0.080	0.640	0.1	0.1
0.10	1.60	0.080	1.280	0.1	0.1
0.05	0.10	0.040	0.080	0.1	0.1
0.05	0.20	0.040	0.160	0.1	0.1
0.05	0.40	0.040	0.320	0.1	0.1
0.05	0.80	0.040	0.640	0.1	0.1

Simulation procedures

Base functions in the R-package DIVERSITREE (vers. 0.4-1; FitzJohn 2009) were used to generate simulated evolutionary histories under twenty BiSSE parameter sets (Table 1). Once trees were generated, we conducted trait-mediated diversification analyses, analogous to those described for our empirical dataset. All simulations and data processing were conducted in R (vers. 2.10.0, R Development Core Team 2010).

Parameters for simulation conditions are found in Supplementary Table 1, and simulation procedures are summarized as follows. Relative-extinction fractions for different simulations were drawn from the set (0.0, 0.5, and 0.8), where the relative-extinction fraction (ε) is d/b and where d and b are extinction and speciation probabilities respectively (Nee et al. 1994). Ratios of net diversification

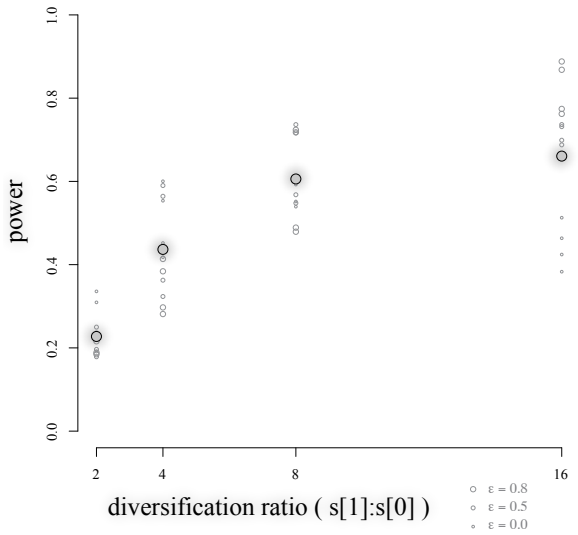
between states [i.e., $(b_1-d_1):(b_0-d_0)$] were 2, 4, 8, and 16. Speciation rate of state '0' varied between 0.05 and 0.10 (see Supplementary Table 1). Relative to our empirical dataset, state '0' in simulations could be analogous to the state of a paedomorphic species, state '1' to a non-paedomorphic species.

With a particular set of BiSSE parameters (see Supplementary Table 1), a randomly selected tree size, ranging between 200 and 600 tips, was evolved for each simulated history under diversification mediated by a binary character. Once generated, at least ten clades from each tree were sampled such that no tips were left unassigned to a clade and no tip belonged to more than one clade. Selected clades required membership of at least two tips: thus, trees where a single lineage was sister to the remainder of the lineages were discarded. For each selected clade, the proportion of species with state 1, extant species richness, and crown-group age were recorded.

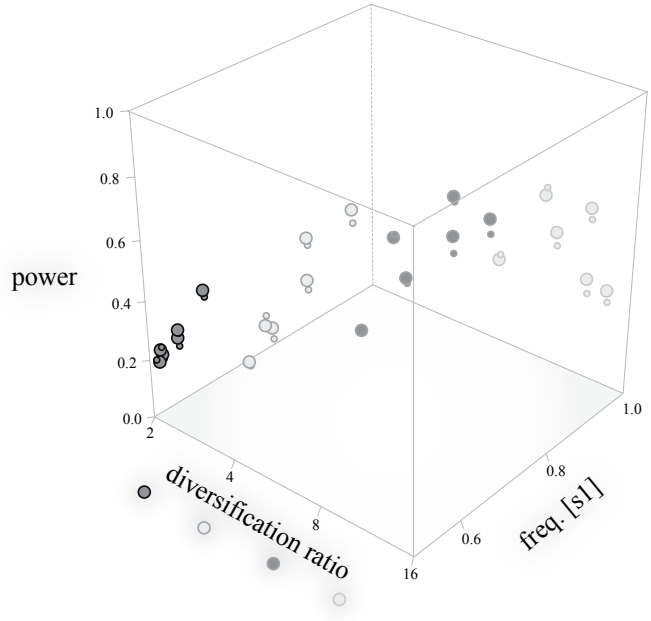
The Magallón and Sanderson (2001) method was used for estimating net diversification as implemented in the R-package GEIGER (vers. 1.3-1; Harmon et al. 2008).. This estimator requires extant clade size, crown-group age, and a relative-extinction fraction. We evaluated each diversification simulation under two relative-extinction fractions ($\varepsilon = 0.0$ or 0.9), assumed for each randomly selected clade. The R-package APE (vers. 2.4-1; Paradis et al. 2004) was used to compute independent contrasts (PICs) for the net diversification estimates and trait-state biases across entire simulated histories. A pruned, clade-level tree was used for these computations (i.e., if 10 clades were randomly selected from a particular simulated history, PICs were computed on a tree with 10 tips). Using STATS (vers. 2.10.0; R Development Core Team 2010). Linear regression with a forced intercept of zero was performed on PICs to assess the regression slope and significance of the relationship between diversification rate and character-state biases among clades. As state '1' was the rapidly diversifying trait-state in all simulations, larger contrasts between clade-level trait-state biases should be associated with larger contrasts in diversification rate estimates, thus yielding a predicted positive slope of these regressions.

Interpretation of simulation results

Power was assessed as the ability to reject the hypothesis of no association between character-state bias (assessed at clade level) and net diversification. Primary results suggest that the estimator (the slope of the PIC regression) is statistically consistent (Supplementary Figs. 1-2). Statistical power did not appear to be influenced either by relative-extinction fraction *used* in simulation ($t_{1,46} = 0.708$; $p = 0.483$) or relative-extinction fraction *assumed* for diversification analyses ($t_{1,46} = 0.354$; $p = 0.725$). Significant predictors of statistical power included the number of clades analyzed ($t_{1,46} = 7.083$; $p < 0.0001$), the ratio of net-diversification rates between binary character states (Supplementary Fig. 1-2; $t_{1,46} = 7.110$; $p < 0.0001$), and mean trait-state frequency across entire simulated trees (Supplementary Fig. 2; $t_{1,46} = 5.693$, $p < 0.0001$). Values for statistical comparisons were averaged across 500 simulated histories for the 48 distinct analyses (24 parameter combinations and two assumed relative-extinction fractions for trait-mediated diversification analyses). As might be expected (Maddison et al. 2007; Freckleton et al. 2008; FitzJohn et al. 2009), either very strong tree-level biases toward one character state (i.e., proportion of state '1' ~ 1) or a nearly balanced distribution of character states (i.e., proportion of state '1' ~ 0.5) limits our ability to recover the underlying signal of trait-mediated diversification using the method employed here (Supplementary Fig. 2).



SUPPLEMENTARY FIGURE 1. Results of character-associated diversification simulations, relating magnitudes of differences in trait-mediated diversification rates to statistical power. Simulations were conducted where state 1 diversified more rapidly (two- to sixteen-fold). Power was assessed as the frequency with which the null hypothesis of trait-independence in diversification was rejected. Each open point represents the mean for 500 simulations. Filled circles represent grand averages for each diversification ratio. Simulations were performed under three scenarios of relative extinction (ε = 0.0: smallest open points; ε = 0.5; and ε = 0.8: largest open points).



SUPPLEMENTARY FIGURE 2. Results of character-associated diversification simulations, showing an interaction between diversification ratio and tree-level mean frequency of the quickly diversifying trait-state (state 1). Size of points denotes the relative-extinction fraction assumed for the simulated data, regardless of the conditions in which the tree was simulated (ε = 0.0: smallest points; ε = 0.9: largest points). Shadings correspond to four different diversification ratios used to simulate trees; sets of points of the same shading are orthogonal to the 'diversification ratio' axis.

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CHAPTER THREE

On the role of hybridism in macroevolution: an empirical study in salamanders

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ABSTRACT

Hybridism has long received attention as having influence on lineage diversification, but its effects have rarely been tested using taxonomically broad datasets. In constructing a supertree for the order Caudata at ca. 60% of its extant diversity, we test the effects of hybridism on diversification and geographic aspects of species' ranges, using comparative methods. Our data suggest a positive but weak correlation between hybridizability and species' range-size, both of which appear phylogenetically heritable. We argue this association is a primary consequence of hybridism-mediated range expansion rather than the converse, inasmuch as hybridism does not appear predictive (or predicted by) proximity to the nearest species' distribution.

We use character-mediated diversification analyses (i.e., binary-state speciation and extinction, BiSSE) where we treat hybridizability of lineages as a binary evolutionary character. Results provide an inverse correlation between hybridization and diversification such that hybridism appears to erode diversity. Rate estimates for hybridism-mediated extinction greatly exceed those for hybrid speciation, resulting in a negative rate of net diversification for hybridizing lineages. As BiSSE analyses involve an assumption of phylogenetic heritability of the trait mediating diversification, we further discuss potential for discordance between two frequently used methods by which phylogenetic heritability is assessed. These include a randomization test introduced by Blomberg et al. (2003) and hierarchical likelihood-ratio tests involving a tree-transformation parameter, λ, from Pagel (1999). We conduct simulations to examine the adequacy of the Blomberg et al. (2003) independent-contrasts based randomization-test of phylogenetic heritability, applied to discrete data. Originally introduced for continuous data, we show that under many evolutionary circumstances this application for discrete data provides a powerful test of phylogenetic heritability and exhibits acceptable type-I error rates.

While we find evidence for the species-level disadvantage of hybridism in an amphibian lineage, we discuss circumstances where hybridism may offer macroevolutionary advantage. We conclude by discussing the possibility that a relationship between hybridization and diversification may be expected to be temporally inconstant, perhaps dependent upon ecological opportunity available to radiating lineages.

INTRODUCTION

As early as Linnaeus (1744), hybridization was recognized as an important force in speciation, but the relative importance of hybridization as a significant process in animal evolution has since been debated (Lotsy 1916; Fisher 1930; Mayr 1963; Dowling and DeMarais 1993; Seehausen 2004; Arnold 2006; Mallet 2007; Melo et al. 2009). Convincing arguments for the macroevolutionary influence of hybridism include facilitation of range expansion via *incorporation* of locally-adapted alleles (e.g., Lewontin and Birch 1966; Schmeller et al. 2005), or conversely that the *loss* of locally adapted alleles via introgression can lead to range contraction or loss of lineage independence (Rhymer and Simberloff 1996). As exemplified here, this theoretical discord underscores the need for a broad analysis of hybridism-effected macroevolution.

At the outset, hybridization ought not be regarded intrinsically as the "grossest blunder" organisms can make (Fisher 1930), but rather should be tested quantitatively (Arnold 1997). Although outbreeding depression and genetic assimilation are cited as primary constraints on maintenance and generation of biodiversity, elevated variation within hybrid populations may allow evolutionary hedging, perhaps ameliorating extinction proneness (Dobzhansky 1937; Mallet 2007; Seehausen et al. 2007). Support for this idea comes from a recent review showing evidence of transgressive segregation in at least one trait for better than nine-tenths of cases in a broad review of recent literature (Rieseberg et al. 1999). As a creative evolutionary process, introgressive hybridization is capable of "[g]enerating raw material for rapid adaptation, allowing niche divergence, and phenotypic novelty" (Rieseberg et al. 1999). Through the generation of novel adaptive variation, hybridism may thus provide accessibility to formerly inaccessible areas of an adaptive landscape (Mallet 2007), distorting 'genetic lines of least resistance' for an otherwise genetically cohesive and evolutionarily constrained group of organisms (*sensu* Schluter 2000; see also Gavrilets and Losos 2009).

Rates of speciation may also be hastened by hybridization if divergence has already commenced, perhaps in allopatry (Coyne and Orr 1989). Consequences of secondary contact in the diversification of a lineage might involve the generation of biodiversity through this proximal mechanism (*i.e.*, ecological

speciation or drift-mediated divergence may have been the ultimate cause, reinforcement acting only to complete the process; Coyne and Orr 2004). Further, under a 'mosaic model' (see Howard 1982, 1986; Harrison 1986), novel recombinant phenotypes may be viable in open or underutilized niches, or, alternatively, under the evolutionary novelty model, these may even outperform parental phenotypes in parental habitats (see Arnold 1997). Whether hybrid phenotypes are favored and directly selected (as in the latter examples) or are involved in reinforcement, secondary contact can thus lead to *formation* of biodiversity.

Upon secondary contact, recombination and introgression might otherwise erode the distinctiveness that arose in relative isolation (Futuyma 1987). If extensive, the two lineages may become fused into one, a process termed genetic assimilation or 'despeciation' (Sheppard et al. 2008). If assimilation contributes to broadened breadth in distribution of a now genetically-cohesive single lineage, hybridization might however foster lineage persistence by conferring a degree of geographic protection from extinction (Dobzhansky 1937; Seehausen et al. 2007). A well-known inverse correlate of extinction risk being range size (Stanley 1979; Van Valen 1985; Jablonski 1986; Sepkoski 1998; Cooper et al. 2008; Liow et al. 2009), assimilation should result in a fused and larger species' range, perhaps providing an additional buffer from stochastic causes of extinction.

Whether speciation, extinction, maintenance of a stable hybrid zone or mosaic, or range shift ultimately results from hybridization needs to be tested further with empirical data (Rieseberg 1991; Ellstrand and Elam 1993; Liou and Price 1994; Kirkpatrick and Barton 1997; Wolf et al. 2001). The relative balance of hybrid-mediated speciation and hybridization-induced loss of biodiversity is currently unknown for any taxonomically broad lineage. Recently developed comparative methods now allow tests of the potential macroevolutionary trends that arise from hybridism (e.g., Jockusch and Wake 2002; Seehausen et al. 2007; Gavrilets and Losos 2009).

Here we compile a phylogeny for the Caudata, sampling over 60% of extant salamander taxa (ca. 563 species in total; IUCN 2009; Alfaro et al. 2009). The size of this lineage should confer adequate power in tests of the macroecological and macroevolutionary roles of hybridism while still being

amenable to comprehensive survey of hybridization in the literature. We discern whether hybridism is associated with the spatial configuration of species' ranges in an effort to determine whether hybridization is to be understood simply as a 'mass-action' process. That is, we adopt as a null model of hybridism what was recently offered by Wiens (2006), that hybridization "...sometimes may be a consequence, rather than a cause, of rapid diversification."

Assuming a mass-action model of hybridization, we might expect greater rates of hybridism where many related species occur in close geographical proximity. On the contrary, if hybridism is a trait-based phenomenon wherein features peculiar to *some* lineages might modulate the probability of interspecific hybridism, we should expect to find i) either a weak or non-significant relationship between spatial segregation of species and hybridism and ii) a signature of phylogenetic heritability for this species-level trait, hybridism.

Expecting hybridization to elicit a marked spatial effect on species' ranges, we further attempt to discern whether variation in the latter is well predicted by the former. If hybridization induces no higher-level effect other than to expand distributional ranges, we should expect no evidence for hybridism-mediated diversification, but we should observe a positive correlation between species' range size and hybridism. Insofar as the relationship between hybridization and species' ranges may be confounded by other variables, we employ a model selection procedure in choosing the most predictive variable(s) for observed variation in species' ranges. Additional variables are those suspected or known to be strong correlates of distributional-range variation; these additional variables include body size, latitude, and two life-history characters (e.g., Lutz 1921; Rapoport 1982; Gaston and Blackburn 1996; Eastman and Storfer, forthcoming).

METHODS

1. Supertree Assembly

We used a modified glomogram approach (*sensu* Soltis et al. 2009) to construct a large-scale salamander supertree for our comparative analyses. Well-sampled phylogenies, available from the

literature for several of the ten salamander families, were used in supertree construction. For plethodontids, the most complete phylogeny to date was incorporated, which included approximately half the extant taxa within the family (see Adams et al. 2009). Other available subtrees included a nearly complete phylogeny from Weisrock et al. (2006) for salamandrids, a tree for hynobiids from Zhang et al. 2006 (ca. one third of the known diversity within the family), and a complete phylogeny for amphiumids from Bonnett et al. (2009).

Maximum-likelihood (ML) analyses were used where well-resolved and well-sampled phylogenies were unavailable from the literature. ML subtrees were estimated for several higher-level taxa (seven families and two genera; Appendix 1), all of which are well corroborated as individually monophyletic (Larson et al. 2003, Weisrock et al. 2005, Wiens et al. 2005, Frost et al. 2006, Zhang et al. 2006, Zhang and Wake 2009). To minimize the potential for topological biases introduced into our comparative analyses from heterotachy or among-lineage variation in molecular evolutionary rates, we estimated unknown portions of the supertree independently for each higher-level taxon. This method further permitted the use of much more complete sequence datasets for each estimated subtree (see Appendix 1), and topological searches under the ML criterion were performed with additional rigor (see below). We used hierarchical likelihood-ratio tests (hLRTs; Cunningham et al. 1998) to obtain a suitable process-model of sequence evolution for each ML analysis. In using hLRTs, we used dynamic model-selection to simplify parameterization of the model from GTR+Γ with unequal base frequencies. Through the selection procedure, model reduction occurred if fit of the simpler model was not significantly different from the more parameterized model. Parameter values were optimized in PAUP* (vers. 4.0b10; Swofford 1991) on the neighbor-joining tree inferred using paralinear distances (where distances are based on the determinant of the divergence matrix; Steel 1994; Lockhart et al. 1994; Lake 1994). The gamma-distributed rate-heterogeneity function was discretized into four rate-categories for all optimization iterations. Deletions, as informed by CLUSTALX (vers. 2.0.12; Thompson et al. 1997; Larkin et al. 2007), were treated as unknown character states.

For all but two exceptions, exhaustive searches for an optimal topology were conducted in PAUP* using a branch-and-bound search strategy, guaranteed to find the optimal ML tree (see Appendix 1). The exceptions involved heuristic ML-searches for the Ambystomatidae and *Hynobius*, using 500 random-addition sequence-replicates with tree bisection-reconnection branch swapping. A codon-aligned dataset for *Ambystoma* was culled of third positions due to apparent saturation. Relative divergence dates were estimated by rate smoothing under penalized likelihood, starting with midpoint-rooted ML trees (Sanderson 2002). Rate smoothing was conducted in the R-package APE (R: vers. 2.10.0, R Development Core Team 2009; APE: vers. 2.4-1, Paradis et al. 2004).

We used a 'backbone' phylogeny, provided by Zhang and Wake (2009), to assemble the subtrees into a salamander supertree. Assuming a crown-group age for Caudata of 214 MYA, we scaled subtrees by the highest posterior density of divergence-date estimates provided in Zhang and Wake (2009). BEAST was used for the original analysis to construct what is here used as the backbone chronogram and was based on 15 node calibrations (ibid.). Our assembled supertree is available in Newick format as Supplementary Data 1.

2. COMPARATIVE METHODS

2.1 ASSESSING PREDICTABILITY OF RANGE VARIATION

Measures of range variation

We used the IUCN (2009) global biodiversity database for all spatial data used herein. We considered three aspects of the species' ranges: range size, range fragmentation, and spatial proximity between species' distributions. Range size was simply the areal sum of all distributional isolates occupied by the species. In many cases, the species was assumed to be continuously distributed on the landscape, having a single distributional 'isolate.' The set of spatial polygons defining species' distributions were transformed using an equal-area Mercator projection to units of pixels; data projection was performed with the package SHAPEFILES (vers. 0.6; Stabler 2006). We computed the convex hull of the minimum continuous area of occupancy for each species, thereby subsuming all known localities into this minimum

area (see Fig. 1 for illustration). Given that range edges are well sampled, this convex hull represents the extent of a species' range if it were truly continuously distributed within the defined area (i.e., the dotted line in Fig. 1).

As a metric for range fragmentation, we scaled the sum of areas for each distributional isolate (i.e., our measure of 'range size') by the area defined by the convex hull. We used the formula 1-(a/h), where 'a' is the areal sum of known isolates and 'h' is the area of the convex hull to yield a statistic ranging between 0 and 1 for each species. This measure of range fragmentation thus represents a proportion of the total geographic extent for which a species is contiguously distributed. Spatial proximity was computed from UTM-transformed latitude and longitude data originally in decimal degrees.

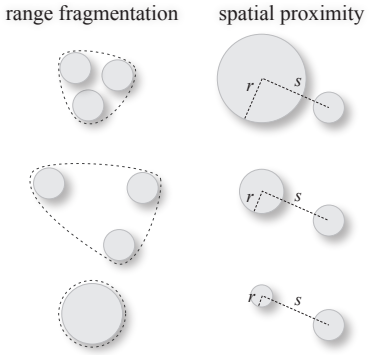

FIGURE 1. Illustrations of the manner in which two range characteristics were calculated. *Range fragmentation:* dotted lines are analogous to convex hulls for three species' distributions. To calculate range fragmentation for each species (as done herein), the sum of the isolates within each hull (a) is divided by the area of the convex hull (dotted line; h); this quantity is subtracted from a constant of one: 1-(a/h). Thus, the lowermost species' range is least fragmented (i.e., with a range fragmentation index of zero), the middle distribution considered most fragmented. The sum of known areas (colored circles) are equal for the three species. *Spatial proximity:* shown are three pairs of species' ranges. The distance between a species' range centroid and that of its nearest neighbor (s) is constant in these three illustrations. Assuming that the biotic interactions are likely to be strongest for the uppermost pair of species, we calculate a scaled measure of spatial proximity, proportional to the range radius (i.e., r) divided by the inter-centroid distance (s).

Inter-range distances for species were determined by the nearest range midpoint (i.e., centroid) of a heterospecific distributional range to the range centroid for each given species in units of *km*. To find an average distance from core to edge for each species, imposing an assumption that the distributions were roughly circular, we evaluated a species' range 'radius' as the square-root of a/π, where 'a' was species' range area (i.e., and where 'r' = a/π in reference to Fig. 1). To generate a metric that might be predictive of hybridism, we scaled range radii by inter-range distances. Larger values of our measure of spatial

proximity should thus be predictive of higher rates of hybridism under a mass-action hypothesis. To improve normality of this measure, spatial proximity data were Ln-transformed. Global patterns of all three range-characteristics are provided as Figure 2.

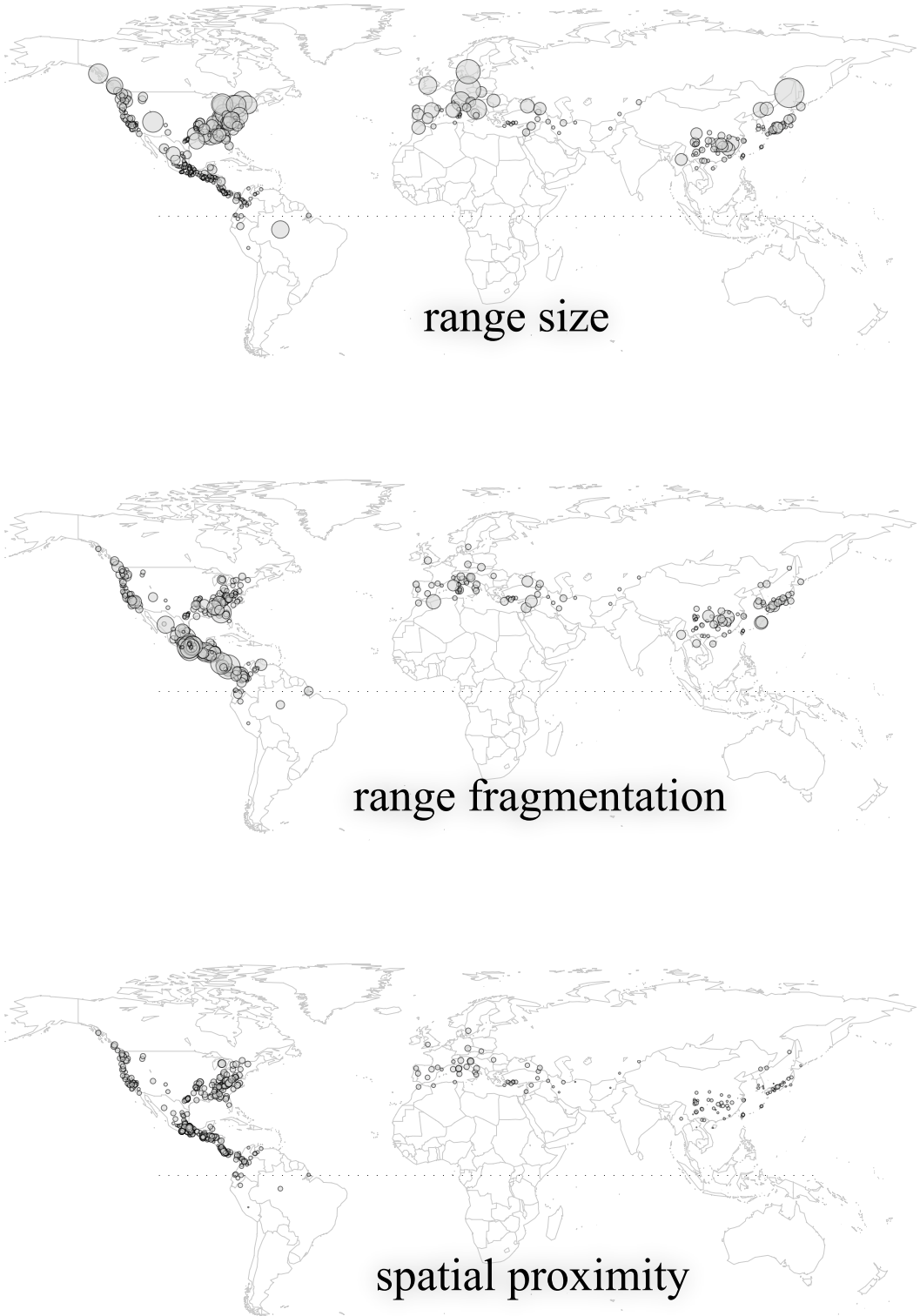

FIGURE 2. (Caption on following page.)

FIGURE 2. (From previous page.) Spatial patterns of variation for three range characteristics: range size (total geographic extent of a species' distribution); range fragmentation (minimum inclusive area of known areas of occupancy scaled by sum of occupied areas) and spatial proximity (range 'radius' scaled by distance from the midpoint of a species' distribution to the nearest centroid of another species; see Fig. 1). Size of points signifies the value for each range characteristic: larger points imply larger range sizes, greater distributional fragmentation, and greater distances between range midpoints respectively. For range size, depicted data are Ln-transformed equal-area Mercator-projected areas in units of pixels, where scaling of circles is proportional to map scale; for the spatial proximity histogram, data are Ln-transformed distances in *km*.

Potential predictors for variation in range characteristics

In assessing whether hybridism is meaningfully associated with species' range variation, we acknowledge the potential for other variables to either be confounding or perhaps better predictive of observed variation in species' distributions. We accordingly tested hybridism-mediated range-variation in the presence of several potential cofactors. The additional set of aggregate traits included two discrete life-history characters (*plasticity* and *paedomorphy*) and two continuous characters (mean *latitude* and *body size* within species). These additional variables are known or suspected to be strong correlates of distributional-range variation (e.g., Lutz 1921; Rapoport 1982; Stevens 1989; Gaston and Blackburn 1996; Reaka 1980; see also Eastman and Storfer, forthcoming).

To assess hybridism within salamanders, we consulted the primary literature using the search engines Google™ Scholar and ISI Web of Knowledge℠: we paired each generic epithet with the terms 'hybrid' and 'introgress' as well as dialectical and structural variants thereof (e.g., 'hybridisation', 'introgressed') to search for cases of non-artificial hybridization. Confirmed for roughly ten percent of extant caudates (55 of 563 species, IUCN 2009), documented hybridization appears to be restricted to five families (Ambystomatidae, Amphiumidae, Dicamptodontidae, Plethodontidae, and Salamandridae; see Appendix 2 for details and references). This measure of hybridism was binary, having scored each species as 'hybridizable' or not.

Our measure of plasticity considered whether species exhibit facultative metamorphosis (i.e., 'paedotypy'; see Reilly et al. 1997) in at least some populations. Similarly, we coded paedomorphy as a binary character, assessing whether organisms within species are invariably (or presumed to be) paedomorphic, where organisms retain larval features upon reaching sexual maturation, thereby forgoing metamorphosis. Coding of species for the binary measures of plasticity and paedomorphy followed

Eastman and Storfer (forthcoming). Body size data were Ln-transformed snout-to-vent lengths in *mm*. Sources for these data were Bonnett et al. (2009); Kozak et al. (2009); and Wiens and Hoverman (2008). Latitudinal data were converted to UTM (using the North American Datum of 1983, NAD83) from decimal degree centroids for species' ranges. Four bolittoglossine species (Plethodontidae) had southern mean species' latitudes, and upon UTM-projection, these points were represented as most distant from the center of origin (i.e., 0° latitude) despite the proximity of these species to other equatorial salamanders. Given the primarily northern distribution of salamanders, we converted these four southern centroids to negative UTMs in distance from the equator. To do this, we used the coefficients of regression ($r^2 = 1.00$) of the northern latitudes against projected UTM northings to compute the negative northings for these remaining four centroids. All species-level data are provided as Supplementary Data 2.

Univariate analyses

Treating each of the five aggregate traits as a predictor of variation in range characteristics, we regressed phylogenetic independent contrasts (PICs; Felsenstein 1985) for each predictor against PICs for each of the three measures of range characteristics. These analyses assessed the explanatory power of each predictor for observed variation in range characteristics. APE was used for all PIC calculations.

Despite the apparent robustness of PICs to model violation (e.g., Diaz-Uriarte and Garland 1996, 1998), tree transformations enforced better conformance of our contrasts to the assumption of Brownian-motion evolution. For continuous data, we considered several evolutionary models and one non-evolutionary model of trait variation (i.e., a 'white-noise' model, where species draw at random from a normal distribution of trait values; see Harmon et al. 2008). Evolutionary models included a random-walk with constant rate of change in trait values along the tree (i.e., constant-rate Brownian motion, 'CR'); a model where expected trait values and variance is informed by the correlation between trait covariances among species and their phylogenetic relationship ('Pagel's λ'; Pagel 1997, 1999); 'Pagel's δ,' modeling the temporal change in rate of trait evolution, whether concentrated more toward the root or tips; 'Pagel's κ,' a punctuational (or speciational) model of trait evolution; 'CC,' modeling the tendency for trait values to return to a medial value (i.e., also termed an Ornstein-Uhlenbeck process, or the Hansen

model; Hansen 1997; Butler and King 2004); and 'EB,' an 'early-burst' evolutionary model where trait evolution is concentrated toward the root of the tree (see Harmon et al., in press). Model fit for the three discrete characters was assessed for a constant rate ('CR') model of discrete trait evolution and the three models from Pagel (1999): λ, δ, and κ. While discrete characters are unable themselves to evolve by Brownian motion, the error terms for discrete characters may (Martins and Hansen 1996), enabling the use of independent contrasts for these data. Akaike's (1974) information criterion (AIC) was used to compare fit of the different models; model fit and model comparison was performed in GEIGER (vers. 1.3-1; Harmon et al. 2008, 2009).

PICs were computed using tree transformations of the supertree in accordance with the ML parameter-estimate for the model of character evolution best fit by the data (Garland et al. 1992; Diaz-Uriarte and Garland 1996, 1998; Pagel 1997, 1999; Blomberg et al. 2003). To illustrate, if α is the maximum likelihood estimate for the constraint parameter of a CC model, fitting a CR model to the data but transforming the tree by α returns the same likelihood as fitting the data under CC while estimating α. Branch lengths transformed in this manner are more consistent with Brownian-motion evolution (and with an assumption of PICs), where branch lengths are proportional to expected variances in trait values (Felsenstein 1985, 2008). In most cases, support for one model from the set of possible models was strong (Table 1), justifying the use of a single model for branch-length transformation for each dataset (see Supplementary Fig. 1 for examples of tree transformation under different models).

Univariate regression of PICs was performed to assess the 'best' predictor for each of the three range characteristics (range size, range fragmentation, and spatial proximity), where predictors were ranked by statistical significance in univariate regression analysis. Contrast regressions were variable in the size of datasets considered, given limitations for which data were available: datasets ranged from 190 (body size) to 339 (latitude) to 340 values (hybridism, paedomorphy, and plasticity), for which we had species-level data for both the predictor and dependent variables, as well as taxonomic representation in our supertree (see Supplementary Data 2).

TABLE 1. AIC weights for several models of character evolution. *WN*: a non-evolutionary 'white-noise' model where species draw from the same normal distribution of character values; *CR*: a 'constant-rate' evolutionary model (†: Brownian motion for continuous traits); *Pagel's λ*: an evolutionary model describing the correlation between trait covariances and tree structure (see Pagel 1997, 1999); *Pagel's δ*: an evolutionary model describing the temporal change in rate of trait evolution (larger values of δ suggest most change concentrated toward the tips of the tree); *Pagel's κ*: a punctuational model of trait evolution (smaller values of κ suggest evolution is concentrated at nodes); *CC*: an evolutionary model of 'constant constraint', where trait values have a tendency to return to a medial value (i.e., also termed an Ornstein-Uhlenbeck or 'OU' process, or the Hansen model; see Hansen 1997; Butler and King 2004); *EB*: an 'early-burst' evolutionary model where trait evolution is concentrated toward the root of the tree (see Harmon et al., in press).

	WN	CR†	Pagel's λ	Pagel's δ	Pagel's κ	CC	EB
range size	0.00	0.00	0.00	0.00	1.00 **	0.00	0.00
range fragmentation	0.73 *	0.00	0.00	0.00	0.00	0.27	0.00
spatial proximity	0.00	0.00	0.00	0.00	0.04	0.96 **	0.00
body size	0.00	0.13	0.00	0.00	0.00	0.87 *	0.00
latitude	0.00	0.00	0.00	0.00	0.00	1.00 **	0.00
hybridism	*n.a.*	0.10	0.04	0.15	0.71 *	*n.a.*	*n.a.*
plasticity	*n.a.*	0.06	0.66 *	0.03	0.25	*n.a.*	*n.a.*
paedomorphy	*n.a.*	0.01	0.30	0.00	0.68 *	*n.a.*	*n.a.*

Multivariate analyses

Given the possibility for predictor collinearity, we used analyses of deviance in two-predictor generalized linear models (GLM; McCullagh and Nelder 1989; Venables and Ripley 2002). Generalized linear models were constructed from PICs as previously described. Using minimal probability values, the best predictors from univariate PIC-regressions were paired with each remaining predictor in separate analyses of deviance, conducted in the base R-package STATS (vers. 2.10.0; R Development Core Team 2009). GLMs assumed a Gaussian error-distribution and identity link. Chi-square tests were implemented to determine whether model fit was significantly reduced by elimination of predictor variables, comparing fit of single versus two-predictor GLMs. We interpret a non-significant result of these tests to indicate explanatory redundancy of the primary and secondary predictors (e.g., Venables and Ripley 2002; Jablonski and Hunt 2006).

We further assessed statistical independence and explanatory contribution of our predictor variables using a stepwise model-selection procedure in multiple regression (see Kelly and Price 2004). Using Akaike's (1974) information criterion by which to evaluate model fit, we reduced the selected set of predictors from the fully parameterized model of five. After a nominal set of predictors was chosen, a 'forward-selection' procedure determined if statistical support was provided for variables returned to the

model. The datasets for multiple regression were informed by 190 species-level data entries, reduced to the size of the smallest dataset (i.e., body size). Model fitting was performed in the R-package MASS (vers. 7.3-3; Venables and Ripley 2002). Retained predictors were interpreted as those best predictive of variation in geographic range characteristics. As for our univariate comparisons, statistical significance of the predictors was assessed by one-sample *t*-test, comparing estimated regression slope against the null expectation that slope was zero. Directional hypotheses were clear for range size and spatial proximity, and one-tailed tests were implemented. We used two-tailed tests for correlations concerning range fragmentation.

2.2 Phylogenetic Heritability in Empirical Data

We assessed phylogenetic 'heritability' (i.e., phylogenetic signal) of traits by a randomization procedure recommended by Blomberg et al. (2003). We used a phylogenetic-heritability randomization-test, which essentially asks whether closely related species exhibit more trait similarity than expected by chance. Assuming that the trait of interest evolves along a given tree by Brownian motion, variances of phylogenetic independent contrasts are compared between the empirical dataset and randomly generated sets of data. If phylogenetic heritability underlies the trait investigated, the PIC variance from the empirical data will be exceeded by that estimated for the randomly generated datasets (Blomberg et al. 2003). Differences in the variances were assessed using a nonparametric test, comparing the observed PIC variance to a null distribution created by the PIC variances for each of 100 iterations of data randomization. Having three binary traits (i.e., *hybridism*, *plasticity*, and *paedomorphy*) that do not meet the assumption of Brownian motion evolution, we conducted simulations to assess the applicability of this Blomberg et al. (2003) randomization procedure for discrete data (see Supplementary Methods).

As described in section 2.1, a fitted Pagel's λ model describes the degree to which observed trait-states co-vary with tree structure, interpretable for both discrete and continuous data. For each dataset, we used a likelihood ratio test to compare an unconstrained λ-model (λ estimated) to one where λ was constrained to be 0, as a test for phylogenetic trait heritability. In the case where this trait-tree covariance

(i.e., λ) is distinguishable from 0, phylogenetic structure is predictive of observed trait-states or values, thereby providing evidence for phylogenetic heritability of the trait (e.g., Waldron 2007; Eastman et al. 2009).

2.3 Differential speciation and extinction, mediated by a binary character

To assess whether diversification is mediated by hybridism, we used binary-state speciation and extinction (BiSSE), implemented in DIVERSITREE (vers. 0.4-4; FitzJohn et al. 2009; FitzJohn 2009). The BiSSE method allows for the test of state-dependence in the processes governing diversification: speciation (λ_0 and λ_H) and extinction (μ_0 and μ_H). BiSSE also accounts for biases in transition rates between character states (q_{0H} and q_{H0}), which can have strong impacts on estimated state dependence of diversification if poorly estimated (Maddison et al. 2007; Goldberg and Igic 2008; FitzJohn et al. 2009). A Markov-chain Monte-Carlo method with slice sampling was used to explore credible estimates for the BiSSE parameters. Given the limited dimensionality of explored parameter space, stationarity was reached immediately; sampling was conducted for 10^3 generations to generate a posterior distribution of BiSSE parameter estimates.

We used a randomization procedure to test whether the posterior distributions of paired BiSSE parameters (i.e., λ_0 and λ_H; μ_0 and μ_H; q_{0H} and q_{H0}) were statistically separable. Here, hybridizable species were given state 'H' (see Appendix 1). For each set of paired BiSSE parameters, we conducted 10^3 randomization permutations comparing one draw from the posterior distribution of the BiSSE parameter under state '0' (e.g., λ_0) to a draw under state 'H' (e.g., λ_H). Expecting the sign of these comparisons to be random if posterior distributions were truly identical, we interpret the proportion of comparisons in a particular direction (e.g., $\lambda_1 > \lambda_0$) to be an approximate probability value. Thus, if $\lambda_0 \sim \lambda_H$ and $\mu_0 \sim \mu_H$, no support for character-state dependent diversification is provided.

An assumption of the BiSSE model is that the history of diversification is fitted by a birth-death process, whereby rates of speciation and extinction are temporally constant. We assessed the conformance of our supertree to this assumption by comparing fit of four diversification models: pure-birth, birth-death, and two implementations of diversity-dependent (temporally rate-variable)

diversification processes: 'DDX' and 'DDL' (see Rabosky and Lovette 2006). DDX assumes diversity-dependent diversification wherein diversification rate decays exponentially through time. A similar model, DDL, models logistic growth of the diversifying clade, where diversification rate decays linearly to zero as a 'carrying capacity' is approached and ultimately reached. Using AIC for model comparison, implemented in LASER (vers. 2.3; Rabosky 2009), the best fit model for our supertree was DDL, followed by pure-birth (ΔAIC = 8.83). Despite the support for a rate-variable model of diversification, we apply the BiSSE model to explore trait-mediated diversification expecting that qualitative differences in rates will still be informative but where parameter estimates may be somewhat inaccurate. This approach would seem reasonable if sampling across the tree were random with respect to the trait and to the phylogeny. The variation in levels of sampling within families appears not to deviate from chance using a contingency test ($\chi^2_{9\ df} = 5.35$, $p = 0.80$).

BiSSE analyses further require that trait states are heritable in a broad sense (i.e., 'phylogenetic heritability'), where topological structure of the phylogeny is in some meaningful way predictive of trait values; thus, phylogenetic 'inheritance' need not be underlain by genetics (see also Blomberg et al. 2003). This assumption was tested as previously described (see *Phylogenetic Heritability in Empirical Data*).

2.4 TOPOLOGICAL SENSITIVITY ANALYSES

The comparative methods used herein have the potential to be highly sensitive to branch-length error, inasmuch as our tests are based on a single supertree. Rather than accommodating topological uncertainty in a typical manner (e.g., by performing statistical tests across a *set* of credible trees), we test the sensitivity of our comparative methods to simulated error in branch-length estimates. We wrote a function in R to perturb branch lengths treewise, where the parameter governing branch-length transformation evolved by Brownian motion (hereafter referred to as 'jittered' trees; see Supplementary Fig. 2). We then assessed correlations between the absolute degree of stochastic branch-length transformation and the result of our comparative tests. We assessed the topological distance between the supertree and each of the jittered trees using the method of Billera et al. (2001), implemented in APE. If

results were largely insensitive to fairly extreme branch-length perturbations, these correlations should be weak or absent.

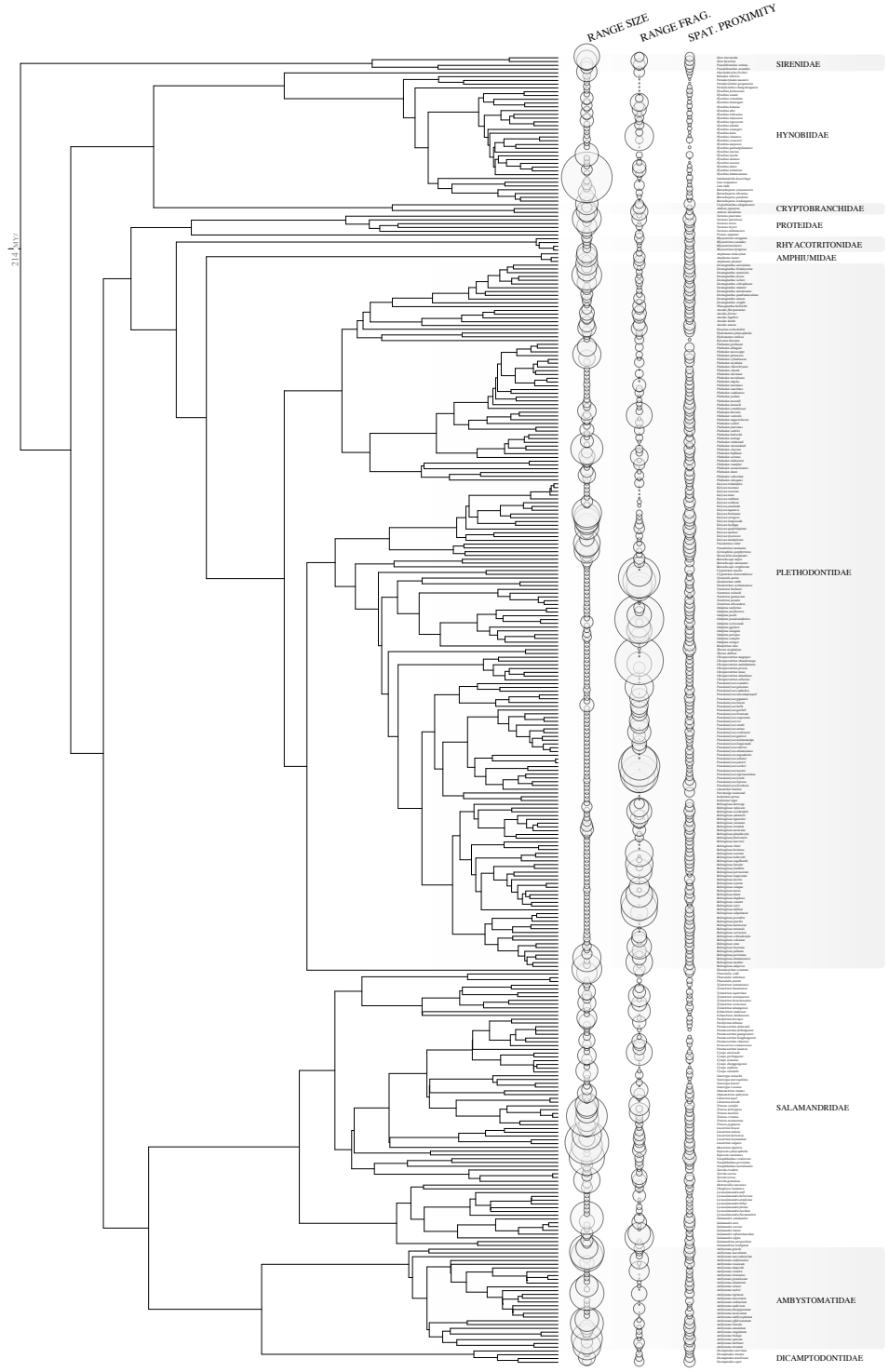

FIGURE 3. (Caption on following page.)

FIGURE 3. (From previous page.) The assembled supertree and phyletic patterns of variation in three range characteristics: *range size*, *range fragmentation*, and *spatial proximity* (see Fig. 1 caption or text for details). Rightmost labels are the ten recognized salamander families. Larger points signify larger range sizes, greater range fragmentation, and greater distances between range midpoints. Following Zhang and Wake (2009), a root age of 214 MYA was assumed. Greater than three-fifths of all recognized species within the Caudata (IUCN 2009) were sampled.

RESULTS

SUPERTREE ASSEMBLY

Our assembled tree (Fig. 3) included 347 species of 563 species within the extant salamander diversity (IUCN 2009). All portions of the tree were estimated either by maximum likelihood or by Bayesian methods. For regions of the tree that were previously published, divergence-date estimation was either performed simultaneously with topology search (e.g., using BEAST, Drummond et al. 2006) or from rate-smoothing under penalized likelihood (Sanderson 2003). Penalized likelihood was used to generate time-calibrated chronograms, starting from ML trees (for hynobiids excluding *Hynobius*, Zhang et al. 2006; and for plethodontids, Adams et al. 2009) or from Bayesian trees (for amphiumids, Bonnett et al. 2009; and salamandrids, Weisrock et al. 2006).

COMPARATIVE METHODS

Correlates of variation in range characteristics

To assess the degrees of independence of the three measures of range variation (*range size*, *range fragmentation*, and *spatial proximity*), we conducted correlation analyses for each pair of these variables. Our measure of range fragmentation did not appear correlated with spatial proximity ($t_{330} = -0.11$, $p = 0.91$). Well-supported associations, however, were apparent between range size and the other two range characteristics: spatial proximity ($t_{330} = 13.46$, $p < 0.0001$, $r = 0.59$) and range fragmentation ($t_{337} = 3.41$, $p = 0.0007$, $r = 0.18$).

Multivariate analyses

While univariate regressions of raw data exhibited highly significant correlations between several combinations of predictor and range characteristic, multiple regression models from PICs provided statistical support for many fewer variables associated with variation in range characteristics. Two of our aggregate species traits were retained as positive correlates of species' range sizes (latitude: $p = 0.0009$, r

= 0.23; hybridism: $p = 0.0498$, $r = 0.14$; Fig. 4; Table 2); correlation coefficients, r, are reported from the univariate analyses, p-values from multiple regression. Given some concern about leptokurtosis in our independent contrasts, we conducted Spearman's rank correlations from univariate comparisons with range size, which were fairly consistent with our parametric tests (latitude: $p < 0.0001$, $r_s = 0.31$; hybridism: $p = 0.0751$, $r_s = 0.08$).

TABLE 2. Results from analyses of deviance (ANODEV) on generalized linear models (GLMs) constructed from phylogenetic independent contrasts (PICs). Full, two-predictor models are shown on the left margin: the most predictive variable from univariate analyses (i.e., 'latitude', in all cases) is the first predictor in ANODEV. The first predictor was paired with the remaining four predictors for ANODEV. Chi-square tests (1 df) compared the full GLM to a reduced model where one predictor was eliminated ('*excluded var.*'). Significance of Chi-square tests and large deviances suggest predictive importance of the excluded variable relative to the full model: in such cases, model fit was significantly diminished by model reduction. Non-significant results for both predictors suggest either predictive redundancy or explanatory inadequacy of the independent variables.

full model	*excluded var.*	*df*	*scaled dev.*	*p-est*
RANGE SIZE				
range.size ~ latitude + body.size	full model	187		
	latitude	1	10.19	0.0014 ***
	body.size	1	1.13	0.2880
range.size ~ latitude + hybridism	full model	336		
	latitude	1	17.10	0.0000 ***
	hybridism	1	4.77	0.0290 **
range.size ~ latitude + paedomorphy	full model	336		
	latitude	1	19.10	0.0000 ***
	paedomorphy	1	0.05	0.8290
range.size ~ latitude + plasticity	full model	336		
	latitude	1	17.67	0.0000 ***
	plasticity	1	3.26	0.0709 *
RANGE FRAGMENTATION				
range.frag. ~ latitude + body.size	full model	187		
	latitude	1	0.96	0.3270
	body.size	1	0.02	0.8980
range.frag. ~ latitude + hybridism	full model	336		
	latitude	1	1.39	0.2390
	hybridism	1	0.06	0.8140
range.frag. ~ latitude + paedomorphy	full model	336		
	latitude	1	1.48	0.2240
	paedomorphy	1	0.00	0.9990
range.frag. ~ latitude + plasticity	full model	336		
	latitude	1	1.71	0.1910
	plasticity	1	0.85	0.3560
SPATIAL PROXIMITY				
spat.prox. ~ latitude + body.size	full model	185		
	latitude	1	11.68	0.0006 ***
	body.size	1	0.20	0.6562
spat.prox. ~ latitude + hybridism	full model	330		
	latitude	1	8.47	0.0036 ***
	hybridism	1	4.79	0.0286 **
spat.prox. ~ latitude + paedomorphy	full model	330		
	latitude	1	10.03	0.0015 ***
	paedomorphy	1	0.22	0.6391
spat.prox. ~ latitude + plasticity	full model	330		
	latitude	1	8.89	0.0029 ***
	plasticity	1	2.92	0.0873 *

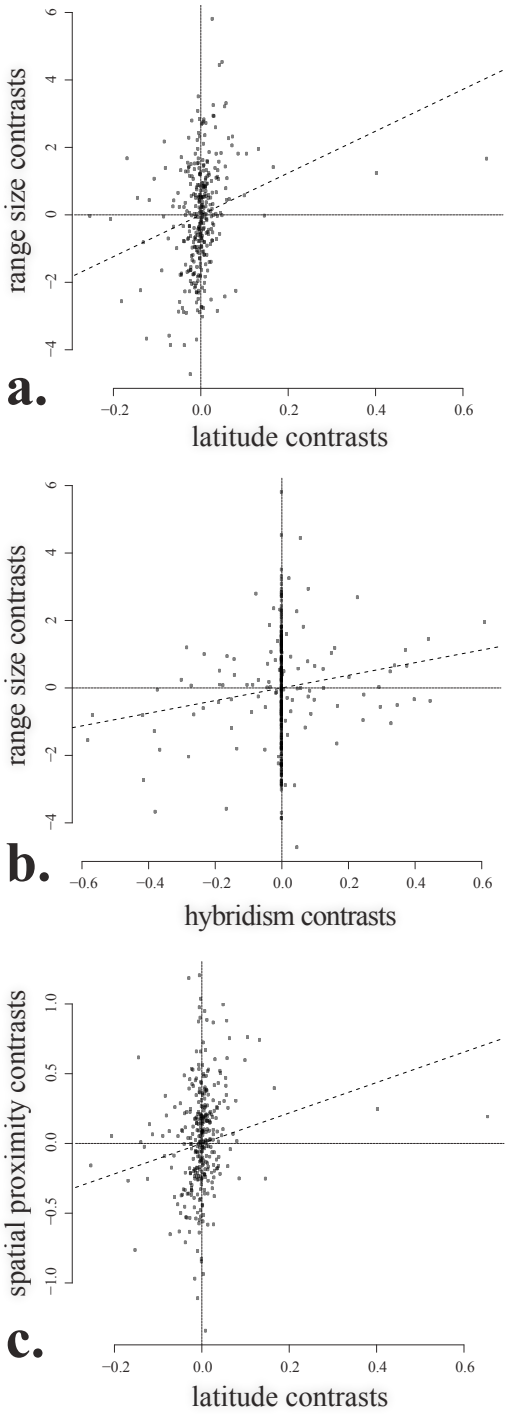

FIGURE 4. Results from univariate PIC analyses, showing predictors deemed explanatorily important from multiple regression analyses of PICs (see text for details). Latitude and hybridism are both weakly correlated with range size variation (latitude: $p = 0.0009$, $r = 0.23$; hybridism: $p = 0.0498$, $r = 0.14$; panels 'a' and 'b'). Non-parametric rank-correlation analyses yield similar results. Latitude is the sole predictor retained in a multiple regression model for spatial proximities between species' ranges and was significant in the univariate comparison ($p_{(2)} = 0.0006$, $r = 0.17$; panel 'c').

Latitude was retained as the sole predictor of spatial proximity, receiving strong statistical support in the multiple regression model ($p_{(2)} = 0.0006$, $r = 0.17$; Fig. 4c). Both paedomorphy and hybridism were selected as independent variables, positively and very weakly correlated with range fragmentation ($p_{(2)} = 0.1216$ and $p_{(2)} = 0.1566$, respectively; in both cases, $r < 0.02$).

Considering the variation in range fragmentation, the consistency of our multiple regression results and pervasive predictor redundancy in analyses of deviance (Table 2) would seem to indicate difficulty in explaining the variance of this range characteristic from our set of aggregate species-level traits. Range-size variation, however, appears to have two consistent positive correlates: latitude and hybridism, supported by both multiple regression and by analyses of deviance (Table 2). Similarly, latitude appears supported as the sole positive correlate of spatial proximity (Table 2; Fig. 4c).

Phylogenetic signal

Some interpretational inconsistency was apparent between the PIC variance for phylogenetic heritability and likelihood ratio tests of Pagel's λ (Table 3). While the PIC test for phylogenetic heritability appears to provide adequate power and acceptable Type I error rates for discrete data evolving under a continuous-time Markov process (i.e., constant-rate trait evolution; Supplementary Fig. 3), interpretation of results from this test and likelihood ratio tests of Pagel's λ are inconsistent for two traits from our empirical dataset (i.e., range fragmentation and plasticity; Table 3).

BiSSE analyses

Strong support for trait-mediated diversification was provided by BiSSE analyses of 'hybridizability,' treated here as a binary characteristic of species (Table 4). While not apparently caused by differential rates of speciation, elevated extinction proneness resulting from hybridism appears to drive the marked difference in estimated rates of net diversification (*r,* Table 4). Extinction rates (μ) associated with the state of being hybridizable are estimated to be many orders of magnitude larger than that for non-hybridizable lineages.

TABLE 3. Results from phylogenetic heritability analyses of our empirical data. *obs*: size of character dataset. *Blomberg's K* estimates substantially lower than unity suggest lability of character evolution. Values of *Blomberg's K* or *Pagel's λ* indistinguishable from zero suggest no phylogenetic signal in the distribution of character values. Values of *K* near unity are consistent with evolution of the trait by Brownian motion. An estimated *K*-statistic nearer to zero suggests evolutionary lability of the trait. *K*-estimates are not meaningful for discrete characters and were not computed for the three binary characters (hybridism, plasticity, and paedomorphy). *obs. var_{PIC}*: variance of independent contrasts for traits; *exp. var_{PIC}*: mean variance for independent contrasts, computed from many iterations of randomizing data across tips. p_{PIC}: results from one-tailed randomization tests, comparing the observed PIC variance to the distribution of expected PIC variances. p_{λ}: results from a likelihood ratio test for phylogenetic heritability, comparing a constrained model ($\lambda = 0$) to a model where λ is estimated. Significant results from the randomization test of PIC variances (p_{PIC}) or the λ-model comparisons (p_{λ}) indicate support for phylogenetic heritability of the trait.

	obs.	Blomberg's K	obs. var_{PIC}	exp. var_{PIC}	p_{PIC}	Pagel's λ	p_{λ}
range size	340	0.096	2.434	6.099	0.0000 **	0.851	0.0000 **
range fragmentation	340	0.105	0.034	0.030	0.8427	0.097	0.0028 **
spatial proximity	333	0.132	0.141	0.318	0.0000 **	0.805	0.0000 **
body size	196	0.139	0.003	0.011	0.0000 **	0.834	0.0000 **
latitude	339	0.173	0.003	0.032	0.0000 **	0.891	0.0000 **
hybridism	347	*n.a.*	0.014	0.020	0.0000 **	0.996	0.0000 **
plasticity	347	*n.a.*	0.003	0.002	0.8663	0.794	0.0000 **
paedomorphy	347	*n.a.*	0.008	0.017	0.0000 **	0.861	0.0000 **

TABLE 4. Maximum a posteriori (MAP) estimates of BiSSE diversification parameters in relation to hybridism. MAP estimates represent the mode of the posterior distributions for each parameter. λ: speciation rate; μ: extinction rate; ε: relative extinction fraction (μ/λ); *r*: net diversification rate (λ-μ); q_{0H}: transition rate toward hybridism; q_{H0}: rate of 'loss' of hybridism. 'H' denotes the character-state associated with hybridizability.

HYBRIDISM†		
parameter	*MAP estimate*	*p.value*
r_0	0.02938	0.038 **
r_H	-0.03956	
ε_0	0.00505	0.009 **
ε_H	2.34598	
λ_0	0.02953	0.412
λ_H	0.02939	
μ_0	0.00015	0.005 **
μ_H	0.06895	
q_{0H}	0.00521	0.071 *
q_{H0}	0.01319	

† state 'H' is regarded as the state of being 'hybridizable'

Topological sensitivity analyses

Many of the comparative methods used herein did not appear sensitive to our source of introduced branch-length error. Correlations between topological distances of the jittered trees from the

untransformed supertree and statistical significance in PIC regressions were non-significant for both predictors of range size: latitude and hybridism ($-1.46 < t_{48} < -0.28$; $0.15 < p < 0.79$).

Estimates for several BiSSE parameters (i.e., q_{0H}, q_{H0}, μ_0, λ_0, and λ_H, where 'H' is the state of being hybridizable) were systematically sensitive to the degree of branch-length error introduced (all $p < 0.05$). Yet the primary parameter of interest, this being the difference in trait-associated net-diversification rates (e.g., $r_0 - r_H$, Table 4), did not appear strongly sensitive in our jitter-tree analyses ($t_{48} = -0.14$; $p = 0.32$).

DISCUSSION

Our results suggest that hybridization exerts marked influence at the macroevolutionary scale: hybridism is phylogenetically heritable and, on average, disfavored at the species level. Alongside the prominent roles of such strong biotic interactions as interference competition, parasitism, or predation in shaping patterns of diversity (Jablonski 2008a and references therein), we provide empirical support for hybridism-mediated macroevolution. While we document an effect of hybridization in dampening diversification, we consider the possibility that the macroevolutionary outcome of hybridism may be temporally unstable throughout the diversification of a lineage.

Our data seem to further provide a detectable signature of the influence of hybridization on species' range evolution, as well as evidence consistent with the Rapoport effect (Lutz 1921; Rapoport 1982, Stevens 1989). In observing this positive correlation between species' range-size and latitude in salamanders, we discuss the likely ecological drivers of the pattern.

SPECIES SELECTION AGAINST HYBRIDISM

Hybridism and macroevolution

On average, hybridizable lineages appear to incur a severe cost at the scale of species: levels of diversity may be quickly eroded by higher rates of hybridization (Table 4). Given the negative estimate of net diversification for hybridizable lineages (i.e., μ exceeds λ), the extant diversity of salamanders

could only be reconciled with these results if hybridism infrequently arises and (or) isolating mechanisms ensure sympatric species remain in a non-hybridizable state. This would seem to be supported by the apparent inequality of transition rates between states of this character: the rate of 'loss' of hybridism (i.e., q_{H0} in Table 4) may be on the order of twice the reverse rate, a result that is statistically marginal (p = 0.071).

If hybridism were *simply* a result of a mass-action process, where species in closest proximity were most likely to hybridize, we should expect a measurable correlation between spatial proximity and hybridism. If we use multiple-regression model-selection (see Methods, section 2.1) to assess which traits are predictive of *hybridism*, we find range size and body size as the most informative variables for observed hybridization (range size: $t_{185} = 1.78$, $p = 0.0384$, $r = 0.14$; and body size: $t_{185} = 3.26$, $p_{(2)} = 0.0013$, $r = 0.23$). This result is consistent with the absence of hybridism in the multiple-regression model predicting spatial proximity. Certainly the proximity of species' distributions should have much (if not everything) to do with the probability that a particular pair of species will hybridize, but such a correlation in historical variation of these characters is not supported in our multivariate analyses. The AIC-selected CC model for spatial-proximity evolution may underrepresent meaningful variation in this character through time. Contrasts of historical range overlap may thus be poorly estimated. Additionally, a strong lineage-effect on the likelihood of interspecific mating may result in the lack of a statistical relationship between spatial proximity and hybridism. One such lineage-effect might be body size, which itself exhibits phylogenetic signal (Table 3) and appears to be a significant predictor of hybridism: if larger-bodied lineages tend to express greater vagility (Gaston 1994), the opportunities for heterospecific matings may be elevated.

Despite higher rates of hybridism potentially yielding a greater *absolute* number of favorable variants through the recombination of divergent genomes, we find a species-level disadvantage afforded hybridizable lineages (Table 4). Higher hybridism and mutability, for instance, both facilitate greater populational diversity, often at the fitness expense of individual organisms (Williams 1966; Sniegowski and Murphy 2006). While selection operating at different levels of organization have the potential to be

decoupled and some evidence exists for species selection on variability (Lloyd and Gould 1993; Jablonski 2008c; Eastman and Storfer, forthcoming), our results would suggest that selection against hybridism may be reinforced at organismal and higher levels.

We might expect species selection to disfavor hybridism in lineages that are no longer in a niche-filling phase of diversification (e.g. see McPeek 2008), as may be the case for salamanders (Table 4; see also Methods 2.3). Early in evolutionary radiations, however, hybridization may fulfill an altogether different role. Indeed, in many classic examples of adaptive radiation, we find diversification and hybridization both elevated: as in the Cichlidae (see Salzburger et al. 2002, Smith et al. 2003, Seehausen 2004); in Geospizinae (i.e., Darwin's finches; Grant and Grant 1992); in the Madiinae silverswords (Carr and Kyhos 1981, 1986); in *Aquilegia* (i.e., columbine; see Hodges and Arnold 1994); and *Heliconius* butterflies (see Gilbert 2003; Jiggins et al. 2008). Introgression and hybridization may be something of a regularity, perhaps especially for *radiating* lineages (Grant and Grant 1992; Masterson 1994; Seehausen 2004; Mallet 2007).

If stabilizing selection is generally weak upon entry of a lineage into a new adaptive zone (Simpson 1953; Yoder et al., in review), hybridism might indeed *facilitate* divergence and diversification in early radiations (Seehausen 2004; Mallet 2007). Costs of extreme or intermediate phenotypes should be weaker under these circumstances, given that selection surface(s) might be initially 'flat' with respect to variation present within populations. If the average organismal cost of *hybridization* is thereby relaxed, and if hybridization promotes isolation along behavioral, ecological, and (or) reproductive axes (e.g., Rieseberg 1991), novel genotypes that arise through this process may result in rapid early diversification. Certainly, this would require relative divergence of hybridizing lineages insofar as panmixis would forestall speciation (e.g., Futuyma 1987). The temporal stability in the macroevolutionary (dis)advantage of hybridism as well as lineage-effects in modulating rates of hybridization both seem deserving of further attention, in this and other systems.

EVOLUTION OF SPECIES' RANGES

Allopatric Speciation

Our data provide several lines of evidence in support of allopatric divergence as a major mode of salamander speciation. That the distributional range undergoes modification during salamander speciation is supported by the selected model of range-size evolution (i.e., a 'punctuational' or 'speciational' κ model; see Pagel 1997, 1999; Table 1; estimated $\kappa = 0.08$). Further, we find so-called 'antisignal' in PIC-variance tests of phylogenetic heritability of range fragmentation (Table 3). Antisignal in a trait is interpreted as an indication that related species have experienced character displacement (Blomberg et al. 2003). While this result conflicts somewhat with the *non*-evolutionary model that best fits our range-fragmentation data ('white-noise'; Table 1), antisignal here would seem to support the influence of allopatrically-isolated populations in speciation processes of salamanders. In particular, these results seem most consistent with a peripatric model of speciation, wherein ranges of descendants are asymmetrically split from an ancestral distribution (e.g., Mayr 1963; Eastman et al. 2007).

Whether hybrid-mediated speciation is a prominent signal underlying these results of range-evolution is a matter to resolved with additional studies. The fit of evolution of hybridism by Pagel's κ (Table 1) is consistent with this form of divergence, although it does not provide direct evidence for hybrid speciation. Formation of some evolutionary lineages via hybridization appears likely for some salamander lineages, including unisexuals within the *Ambystoma laterale* complex (Robertson et al. 2006), some *Plethodon* (Highton 1998), *A. tigrinum stebbinsi* (Jones et al. 1995), and possibly the progenitor of Sirenidae (Morescalchi and Olmo 1974). Because hybridization necessarily involves the interaction of lineages in space, it follows that this measure of biotic interaction might be most predictive of range-size variation, a hypothesis that our data support. Future efforts are required to fully tease apart the causal directionality that seems to link larger range sizes to higher frequencies of hybridization in salamanders, although the relationship is almost certainly bidirectional.

Statistical support that is spread between a non-evolutionary model ('WN') and an explicitly evolutionary model ('CC') seems to indicate relative inadequacy of *any* of our models in explaining

temporal change in range fragmentation (Table 1). Necessarily, there is a lower bound on our measure of range fragmentation as a species' range size cannot take a negative value (Fig. 1). And certainly, we expect some upper bound on the extent of range fragmentation that a species can withstand, beyond which either extinction or speciation ensues. The inability to adequately describe range-fragmentation evolution is consistent with the failure in our multiple regression analyses to recover reliable predictors for this character (e.g., Table 2). The small degree of evolutionary signal that we detect for range fragmentation (Table 3; Pagel's $\lambda = 0.097$) may well be solely driven by spatial autocorrelation, if neighboring species tend to be related and if potential for range fragmentation is also structured spatially. This would seem reasonable given that related salamanders do appear clustered in geographic space with respect to latitude (Table 3; Fig. 3).

Salamander Packing and Rapoport's Effect

All else being equal, organismal selection should favor resilience and broad climatic tolerances in extratropical regions relative to tropical regions (Stevens 1989). This is one of several explanations for the Rapoport effect, or the positive relationship between range size and latitude (see Gaston et al. 1998 for review). Despite weak correlations, the observed tendency toward larger ranges, broader latitudinal extents, and greater geographic overlap in extratropical regions provides support for the Rapoport effect in this system (Stevens 1989; Figs. 4-5). The direct relationship between latitude and spatial proximity of species' ranges suggests denser packing of salamander species in more temperate regions. While inter-centroid distances do tend to increase with latitude (e.g., the value '*s*' in Fig. 1; data not shown), larger range sizes in the more extreme latitudes seem to 'over-compensate' for the increasing distances between ranges in extratropical regions. With increasing latitude, growth in '*r*' seems to outpace that for '*s,*' referencing Fig. 1 (see also Figs. 4-5). Although space is only a single dimension of the niche, these data are consistent with smaller niches and less niche-overlap in the tropics (e.g., MacArthur 1965; Stevens 1989). While direct testing in this system is required to confirm hypothesized predictions for the roles of niche evolution and phenotypic plasticity in the Rapoport effect (Stevens 1989), we interpret these results as potentially driven by the breadth and instability of climatic conditions that lineages farther from the

equator may experience (e.g., Synder and Weathers 1975; Rapoport 1982; Stevens 1989, 1992; Gaston et al. 1998; but see Rohde 1996).

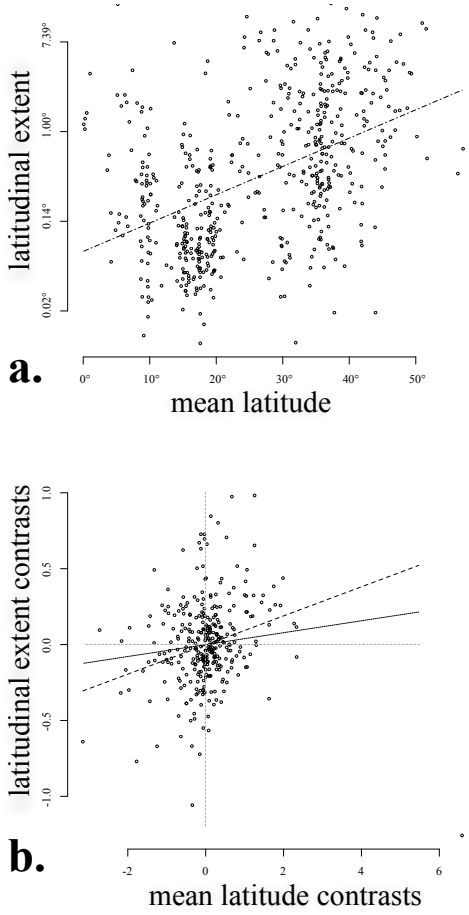

FIGURE 5. Originally surmised to involve the latitudinal breadth of species' ranges (Stevens 1989), we further assessed the Rapoport effect by testing whether latitudinal breadth was positively correlated with mean species' latitude (see also Fig. 4). Shown are comparisons of latitudinal extent and mean latitude in salamanders, in a test of Rapoport's rule for raw data (panel 'a') and phylogeny-corrected regression, using independent contrasts (panel 'b'). Results are consistent with Rapoport's rule, exhibiting a direct relationship between mean latitude and latitudinal extents of species' ranges. For raw data: $p < 0.0001$, $r = 0.46$. Regression lines in the lower panel show the trend with (solid line; $p = 0.0402$, $r = 0.11$) and without (dashed line; $p < 0.0001$, $r = 0.25$) inclusion of the datum in the lower right of the scatter plot. A Spearman's rank correlation provided similar support for the relationship in panel 'b' ($p = 0.0002$, $r_s = 0.20$).

Phylogenetic heritability

For two traits, the statistic chosen to evaluate phylogenetic heritability has an extreme effect on our interpretation of the result (Table 3). For range fragmentation and plasticity, strong support for phylogenetic heritability was provided by hLRTs of Pagel's λ tests, whereas support was lacking from a

test comparing expected and observed variances of PICs (Table 3). Yet in no case was a constant-rate model (e.g., Brownian motion) of character evolution supported by AIC weights (Table 1), which perhaps limits the utility of the randomization-test in assessing phylogenetic heritability of our data. While we confirm the applicability of the randomization test for phylogenetic heritability of binary characters evolving under a constant-rate process (Supplementary Fig. 3), we might expect that greater deviations from a continuous-time Markov process would commensurately yield poorer performance of this test.

Unfortunately, there seems to be no straightforward means of evaluating the degree of departure from Brownian motion for a particular dataset, because these deviations may be along one or more dimensions. Among other things, such discrepancies may be dependent upon tree balance, constraints on lability of trait evolution, mode of trait evolution (whether more gradualistic or punctuational), trends toward particular trait values, or non-constant evolutionary rates through time (e.g., Pagel 1997, 1999; Blomberg et al. 2003; Housworth et al. 2004; Estes and Arnold 2007; Revell et al. 2008). Where data are adequately fit by a constant-rate process of trait evolution, results from Pagel's λ and from Blomberg's K should be concordant (Blomberg et al. 2003; Revell et al. 2008). The same appears to be true for the PIC-based test of phylogenetic heritability. Where a constant-rate process generates data (even if data are discrete; Supplementary Fig. 3), this method should be powerful in detecting phylogenetic heritability, except for when the pace of binary-trait evolution exceeds the rate of the diversification process. We interpret this to result from an erosion in phylogenetic signal contained within the observed trait data (Supplementary Fig. 3). Given the lack of support for any of our traits evolving under Brownian motion (i.e., denoted 'CR' for continuous traits in Table 1), we feel Pagel's λ provides a more robust estimate for phylogenetic trait-heritability, this statistic being agnostic to any particular model of trait evolution assumed (Pagel 1997, 1999).

CONCLUSIONS

The macroecological consequences of variation in species' range sizes and shapes have long been an interest in organismal biology. Understanding variation in species' distributions has implications as far

reaching as processes governing community assembly (e.g., Chave and Leigh 2002), competitive dynamics (e.g., Webb 2002), host-pathogen interactions (e.g., MacColl and Chapman 2010), and extinction and speciation processes (e.g., Gaston 1998). While we often expect that species' ranges are in some predictable way influenced by the biotic interactions among related species (Jablonski 2008a, b), seldom is this tested quantitatively. Our work suggests that hybridization exerts marked influence in both macroecological and macroevolutionary patterns: hybridism appears to erode species diversity and appears important in range-size evolution. These data further provide initial evidence for a global pattern consistent with the Rapoport effect in salamanders (Rapoport 1982, Stevens 1989).

An acknowledged limitation of this study is our reliance on a single topology. In using a novel test to explore sensitivity of inferences to the assumed supertree, we find that the major conclusion of this study -- species selection against hybridism -- is largely insensitive to introduced branch-length error. We nevertheless recognize that the salamander phylogeny-estimate is unlikely to be static. Perhaps especially with the broader adoption of species-tree estimation procedures and their integration with methods for supertree construction, we anticipate some of the topological relationships within our assumed supertree to have been misled. Yet, given that BiSSE analysis involves (and requires) rich tree-shape information (i.e., waiting times and how these are distributed with respect to likely histories of trait evolution; Maddison et al. 2007; FitzJohn et al. 2009), we feel our estimate of the salamander phylogeny would need to be systematically and severely biased in topology in order to reverse the strong signature of hybridism-mediated macroevolution observed herein (Table 4).

With the advent of molecular-genetic methods and ever more sophistication in comparative and statistical phylogenetic methods, unanticipated discoveries of likely introgression (e.g., Eastman et al. 2009) and rigorous tests of hybridization generated by aberrant morphological patterns are now commonplace. Emerging methods allow for robust detection of hybridization (e.g., Joly et al. 2009) or direct estimation of reticulograms (e.g., Legendre and Makarenkov 2007). A necessary extension will be to accommodate use of explicitly reticulate trees in comparative tests.

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APPENDIX 1. NCBI accessions for taxa and respective markers used for supertree assembly.

		12S	ND2	COI	ND4	cytb	Dloop	RAG1
Ambystomatidae	*Ambystoma altamirani*						DQ241131.1	
	Ambystoma amblycephalum						DQ241132.1	
	Ambystoma andersoni						NC_006888.1	
	Ambystoma annulatum						EU517609.1	
	Ambystoma barbouri						EF184183.1	
	Ambystoma bishopi						EU517608.1	
	Ambystoma californiense						NC_006890.1	
	Ambystoma cingulatum						EU517584.1	
	Ambystoma dumerilii						NC_006889.1	
	Ambystoma flavipiperatum						DQ241138.1	
	Ambystoma gracile					AY691729.1*	AY186597.1	
	Ambystoma granulosum						DQ241140.1	
	Ambystoma jeffersonianum						EF184168.1	
	Ambystoma laterale						NC_006330.1	
	Ambystoma lermaense						DQ241142.1	
	Ambystoma mabeei						U36410.1	
	Ambystoma macrodactylum						U36414.1	
	Ambystoma maculatum					EF036637.1*	EU169900.1	
	Ambystoma mavortium						AF129976.1	
	Ambystoma mexicanum						AY659991.1	
	Ambystoma opacum					AY691730.1*	EF649892.1	
	Ambystoma ordinarium						DQ241169.1	
	Ambystoma rivulare						DQ241217.1	
	Ambystoma rosaceum						DQ241170.1	
	Ambystoma talpoideum						AF001420.1	
	Ambystoma taylori						DQ241173.1	
	Ambystoma texanum					EF036664.1*	EF184175.1	
	Ambystoma tigrinum					EF036665.1*	AY659992.1	
	Ambystoma velasci						DQ241201.1	
Cryptobranchidae	*Cryptobranchus alleganiensis*			AY916039.1				AY650141.1
	Andrias davidianus			AY916038.1				AY650142.1
	Andrias japonicus			AY915919.1				AY583346.1
Dicamptodontidae	*Dicamptodon atterimus*					AY728918.1*		
	Dicamptodon copei					DQ999064.1*	DQ999051	AY691695
	Dicamptodon ensatus					AY734600.1*	AY729015	EF107335
	Dicamptodon tenebrosus					DQ387951.1*	DQ388424	AY650132
Hynobiidae	*Hynobius abei*		62736478					
	Hynobius amjiensis		51891079			109716178		
	Hynobius boulengeri		62736499			126470977		
	Hynobius chinensis		51891103			109716186		
	Hynobius dunni		62736439					
	Hynobius formosanus		62736493			109716136		
	Hynobius guabangshanensis					118406753		
	Hynobius hidamontanus		62736466					
	Hynobius katoi					126470973		
	Hynobius kimurae		62736502					
	Hynobius leechii		51891085			35286629		
	Hynobius lichenatus		62736481			126470965		
	Hynobius naevius		62736472			169264751		
	Hynobius nebulosus		62736436			126470951		
	Hynobius nigrescens		62736490					
	Hynobius okiensis		62736454					
	Hynobius retardatus		62736505			193237664		
	Hynobius sonani		62736496			109716142		
	Hynobius stejnegeri		62736475					
	Hynobius takedai		62736487					
	Hynobius tokyoensis		62736484			126470933		
	Hynobius tsuensis		62736442					
	Hynobius yiwuensis					34419862		
Proteidae	*Necturus lewisi*	X86249.1						
	Necturus beyeri	DQ283151.1						
	Necturus maculosus	DQ283412.1						AY650137.1
	Necturus alabamensis							AY650136.1
	Necturus punctatus							EF107305.1
	Proteus anguinus							AY650138.1
Rhyacotritonidae	*Rhyacotriton cascadae*				AY691771.1	AY691727.1		AY691694.1
	Rhyacotriton kezeri				AY691772.1	AY691728.1		AY650129.1
	Rhyacotriton olympicus					AY764254.1		
	Rhyacotriton variegatus				AY691770.1	AY691726.1		EF107302.1
Sirenidae	*Pseudobranchus axanthus*					AY713284		
	Pseudobranchus striatus					AY713285		
	Siren intermedia					AY713291		
	Siren lacertina					AY691721		

APPENDIX 2. Cases of interspecific hybridization drawn from the literature and relevant references.

Ambystomatidae	
Ambystoma barbouri	Bogart et al. 2007; Eastman et al. 2009
Ambystoma californiense	Fitzpatrick and Shaffer 2007
Ambystoma jeffersonianum	Bogart et al. 2007
Ambystoma laterale	Bogart et al. 2007
Ambystoma mavortium	Fitzpatrick and Shaffer 2007
Ambystoma texanum	Eastman et al. 2009
Ambystoma tigrinum	Bogart et al. 2007
Amphiumidae	
Amphiuma means	Bonnett et al. 2009
Amphiuma tridactylum	Bonnett et al. 2009
Dicamptodontidae	
Dicamptodon copei	Baumsteiger et al., forthcoming
Dicamptodon tenebrosus	Baumsteiger et al., forthcoming
Plethodontidae	
Aneides ferreus	Jackman 1998
*Aneides vagrans**	Jackman 1998
*Batrachoseps gavilanensis**	Jockusch 2000; Jockusch and Wake 2002
*Batrachoseps luciae**	Jockusch 2000; Jockusch and Wake 2002
Bolitoglossa franklini	Wake and Lynch 1982
Bolitoglossa lincolni	Wake and Lynch 1982
*Desmognathus carolinensis**	Kozak 2003; Bonett 2002
*Desmognathus conanti**	Kozak 2003; Bonett 2002
Desmognathus fuscus	Kozak 2003; Bonett 2002
Desmognathus ochrophaeus	Kozak 2003; Bonett 2002
*Desmognathus orestes**	Kozak 2003; Bonett 2002
*Desmognathus santeetlah**	Kozak 2003; Bonett 2002
Eurycea cirrigera	Sweet 1984; Guttman and Karlin 1986; Kozak and Montanucci 2001
Eurycea neotenes	Sweet 1984; Guttman and Karlin 1986; Kozak and Montanucci 2001
Eurycea tridentifera	Sweet 1984; Guttman and Karlin 1986; Kozak and Montanucci 2001
Eurycea wilderae	Sweet 1984; Guttman and Karlin 1986; Kozak and Montanucci 2001
*Hydromantes ambrosii**	Nascetti et al. 1996; Tilley 1998
Hydromantes italicus	Nascetti et al. 1996; Tilley 1998
*Plethodon aureolus**	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
*Plethodon chattahoochee**	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon cheoah	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon chlorobryonis	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon cylindraceus	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon glutinosus	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon jordani	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon kentucki	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon metcalfi	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon montanus	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
*Plethodon petraeus**	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon shermani	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon teyahalee	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Plethodon yonahlossee	Highton 1995; Highton and Peabody 2000; Wiens et al. 2000
Salamandridae	
Lissotriton montandoni	Babik et al. 2005; Steinfartz et al. 2007
Lissotriton vulgaris	Babik et al. 2005; Steinfartz et al. 2007
Lyciasalamandra antyalyana	Johannesen et al. 2006
Lyciasalamandra billae	Johannesen et al. 2006
Taricha granulosa	Davis and Twitty 1964; Hedgecock and Ayala 1974; Kuchta and Tan 2005, 2006; Kuchta 2007
Taricha rivularis	Davis and Twitty 1964; Hedgecock and Ayala 1974; Kuchta and Tan 2005, 2006; Kuchta 2007
Taricha sierrae	Davis and Twitty 1964; Hedgecock and Ayala 1974; Kuchta and Tan 2005, 2006; Kuchta 2007
Taricha torosa	Davis and Twitty 1964; Hedgecock and Ayala 1974; Kuchta and Tan 2005, 2006; Kuchta 2007
Triturus carnifex	Schoorl et al. 1981; Brede et al. 2000; Babik et al. 2005
Triturus cristatus	Schoorl et al. 1981; Brede et al. 2000; Babik et al. 2005
Triturus dobrogicus	Schoorl et al. 1981; Brede et al. 2000; Babik et al. 2005
Triturus marmoratus	Schoorl et al. 1981; Brede et al. 2000; Babik et al. 2005

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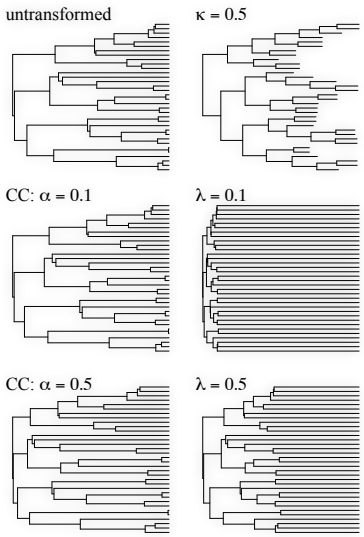
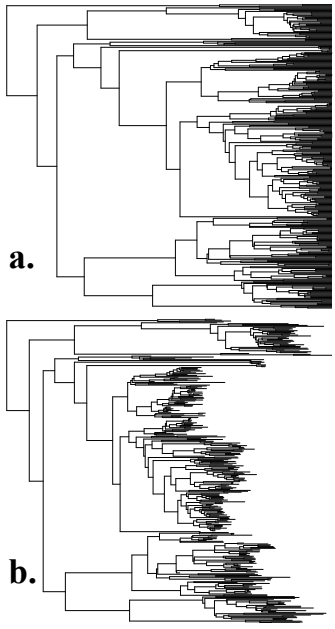
SUPPLEMENTARY MATERIALS



SUPPLEMENTARY FIGURE 1. Illustrations of the effects of three tree-transformation parameters. κ: the value by which branch lengths are raised; λ: a multiplier of off-diagonal elements in the phylogenetic variance-covariance matrix (see Pagel 1997, 1999). α: the 'constraint' parameter of an Ornstein-Uhlenbeck process (also termed a 'constant-constraints' model, '*CC*'; see Butler and King 2004 and Harmon et al. 2008). *untransformed*: the original, arbitrarily chosen tree from which transformations were generated.



SUPPLEMENTARY FIGURE 2. Topological structure of the assembled supertree (a.) and one instantiation of stochastic perturbation of the set of branch lengths from the supertree (b.). The degree of perturbation of each branch followed Brownian motion through the tree, where closely related lineages were likely to have branch lengths scaled to a similar extent. Tree perturbations were performed to mimic phylogenetic conservatism of molecular evolutionary rates. Perturbed trees were used to assess branch length sensitivity of our comparative tests, which assumed the phylogeny was without error.

Phylogenetic Signal Simulation

Simulations were conducted to assess the applicability of a randomization procedure, outlined by Blomberg et al. (2003), for testing phylogenetic signal (i.e., phylogenetic heritability) of discrete data. The application for discrete data represents a strong violation of one underlying assumption of the test, this being Brownian-motion (BM) trait-evolution. This phylogenetic-signal randomization-test essentially asks whether closely related species exhibit more trait similarity than that expected by chance. Assuming that the trait of interest evolves along a given tree by BM, variances of phylogenetic independent contrasts (PICs; Felsenstein 1985) are compared between the empirical dataset and randomly generated datasets; the expectation, if phylogenetic signal underlies the trait investigated, is that the PIC variance from the empirical data will be exceeded by that estimated for the randomly generated data (Blomberg et al. 2003).

Blomberg et al. (2003) found the randomization procedure to be robust to violations of the assumption of Brownian motion evolution (e.g., where a globally optimal trait-value is favored, as under an Ornstein-Uhlenbeck or 'OU' process of evolution). We attempted to determine whether the heritability test could be applied where trait evolution is known to strongly violate the BM assumption. We simulated character histories for a binary character, with equiprobable transition rates between states. Generating trees from several conditions under the birth-death process, we compared PIC variances between i) dataset where the character evolved along a tree to ii) datasets where species' traits were randomly drawn from either of the two character states. Trait histories that yielded no variation were excluded. We used a star phylogeny to generate expectations for trait 'evolution' where trait-states among species were wholly independent of phylogeny, thus exhibiting no phylogenetic signal. In this case, species drew trait-states at random and with equal probability. PIC variances were compared between the two scenarios of trait evolution (either with phylogenetic signal or not). Trait histories were simulated under several relative magnitudes of character evolutionary-rates, from where trait

evolution was comparatively slow in relation to the diversifying tree to where trait evolution was exceptionally fast (i.e., many orders of magnitude faster than the birth-death process).

We expected PIC variances in all cases of trait evolution truly bearing phylogenetic signal to be measurably smaller than for datasets where species were assigned character states at random. We anticipated statistical power to be minimal where i) binary character evolution outpaces the rate of the underlying birth-death process, thereby eroding trait conservatism within lineages; and where ii) number of taxa (i.e., tree size) was small. Simulations were conducted in R, with functions either written for this purpose or available in GEIGER (vers. 1.3-1; Harmon et al. 2008, 2009) or APE (vers. 2.4-1, Paradis et al. 2004).

SUPPLEMENTARY TABLE 1. Rates used for trait-heritability simulations. Units are expressed in events per million years for 'λ' (speciation rate) and 'μ' (extinction rate). Trait-state transition rates were taken from the set (0.005, 0.010, 0.100, 0.500). Forward and reverse transition rates between states of the binary character were equivalent. Relative influence of character state transition and diversification are shown as ratios in the rightmost four columns. Diversification rate is λ-μ. Simulations were conducted with trees bearing 20, 40, 80, or 160 extant species.

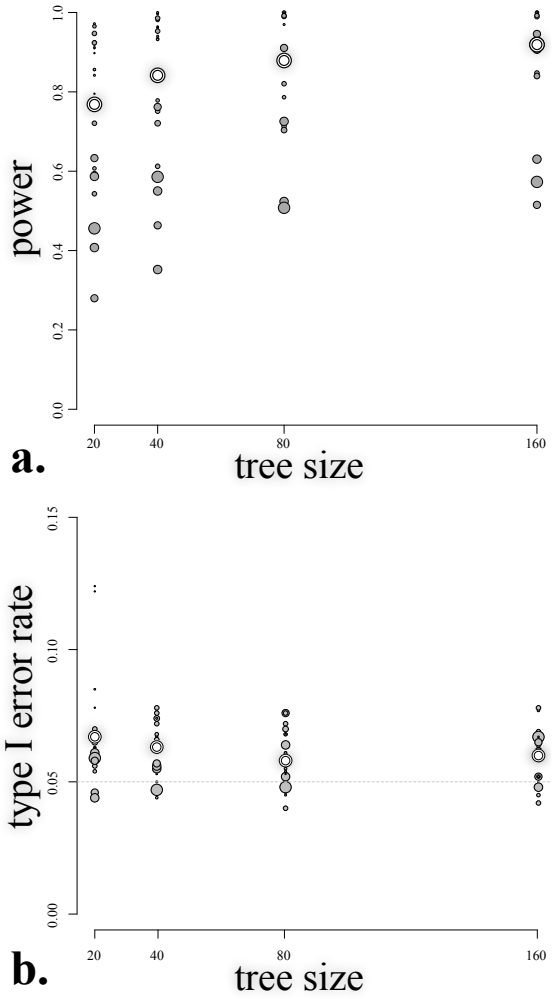
λ	μ	*relative transition rate*			
		q[0.005]	q[0.01]	q[0.1]	q[5.0]
0.100	0.000	0.05	0.1	1	5
0.100	0.050	0.10	0.2	2	10
0.100	0.090	0.50	1.0	10	50
0.010	0.000	0.50	1.0	10	50
0.010	0.005	1.00	2.0	20	100
0.010	0.009	5.00	10.0	100	500

Type-I error rates were assessed in a similar fashion. These analyses evolved character histories where phylogenetic signal was completely absent, being simulating on a star phylogeny. Similar to the assessment of statistical power, we compared PIC variances from the paired simulations by a randomization test, expecting these variances to be roughly equivalent. We recorded a type-I error for any simulation generating a two-tailed probability value of less than or equal to 0.05. For each combination of birth-death parameters and a transition-rate matrix (Supplementary Table 1), we initially simulated ten 'base' trees. For each base tree, 200 simulated character histories were generated by sampling states of a binary character without phylogenetic signal. Variances of PICs for simulated datasets were computed using the base tree.

The PIC variance for each of the first 100 simulated character histories was treated as an observed datum, compared by randomization test against all PIC variances from the second set of 100 simulated character histories. The proportion of randomization tests producing a *p*-value less than or equal to 0.05 was regarded as the type-I error rate for the given base tree.

Interpretation of Simulation Results

Results of these simulations indicate use of the Blomberg et al. (2003) randomization test of PIC variance for phylogenetic heritability is adequately powerful for use with binary data, exhibiting acceptable type-I error rates (Supp. Fig. 3). While type-I error rates on average exceed α=0.05, the degree of difference is often slight (mean type-I error rates across tree sizes were within the interval [0.058, 0.068]; Supp. Fig. 3). We used multiple regression to assess the contributions of tree size and diversification-scaled transition-rates (i.e., 'relative transition rate' in Supp. Table 1) on the power and type-I error rates of these tests. Smaller and smaller tree sizes appeared associated with higher Type-I error rates (relative transition rate: $t_{92} = -1.58$, $p = 0.0584$; tree size: $t_{92} = -1.78$, $p = 0.0391$). Statistical power was strongly related with both factors (relative transition rate: $t_{92} = -6.36$, $p < 0.0001$; tree size: $t_{92} = 3.28$, $p = 0.0007$) such that larger trees and lower relative transition rates afforded higher power (Supp. Fig. 3; see also Supp. Table 1).



SUPPLEMENTARY FIGURE 3. Statistical power (a.) and type-I error rates (b.) for the Blomberg et al. (2003) procedure for assessing phylogenetic signal, used here for a simulated binary character. Size of closed circles indicate the rate of transitions scaled by net diversification (i.e., 'relative transition rate'): larger circles represent simulations were transition rate was exceedingly rapid relative to the underlying diversification process. Smaller circles correspond to simulations where character evolution is outpaced by diversification, that is, relative transition rates are low (see Supplementary Table 1 for details). Correlation analyses suggest a strong influence of tree size ($t = 3.28$, $p = 0.0015$) and relative transition rate ($t = -6.36$, $p < 0.0001$) on statistical power. Neither factor was overwhelmingly predictive of type I error rates (for tree size: $t = -1.78$, $p = 0.0782$; for relative transition rate: $t = -1.58$, $p = 0.1168$). Grand means, across all simulations for a given tree size, are represented by bull's eyes. Average power ranged between 0.77 to 0.92 for smallest and largest tree sizes respectively. Type I error rates were on average slightly higher than an acceptable α of 0.05, ranging from 0.058 to 0.067 for tree sizes of 80 and 20 species, respectively.

SUPPLEMENTARY DATA 1. Salamander supertree, assembled from available literature sources and from ML-estimated subtrees. The tree is time-calibrated, where branch lengths are in millions of years before present (root: 214 MYA).

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SUPPLEMENTARY DATA 2. Data used herein as space-delimited text. *Supertree* denotes whether a species was sampled ('1') or not ('0') in the assembled supertree. *SVL* is our measure of body size (snout-to-vent lengths in original units of mm). *Lon* and *lat* and longitude and latitude, respectively, in decimal degrees; *utm.y* is the UTM-projected latitudes in km. *Area*, *frag*, and *prox* are the three spatial measures of species' range distribution: range size, range fragmentation, and spatial proximity. See text for details of each metric and for transformations used for comparative analyses.

species family supertree hybridism plasticity paedomorphy SVL area frag prox lon lat utm.y
Ambystoma_altamirani AMBYSTOMATIDAE 1 0 1 0 NA 0.03991 0.78365 1.32749079184435 -99.35 19.11 2113.03566
Ambystoma_amblycephalum AMBYSTOMATIDAE 1 0 0 0 NA 0.00051 1 -1.23954834923814 -101.35 19.64 2173.3445
Ambystoma_andersoni AMBYSTOMATIDAE 1 0 0 0 NA 0.00053 1 -1.98643905623538 -102.21 19.76 2187.33229
Ambystoma_annulatum AMBYSTOMATIDAE 1 0 0 0 84.89 5.40744 0.7296 4.48051011137594 -92.9 36.73 4064.92581
Ambystoma_barbouri AMBYSTOMATIDAE 1 1 0 0 75.27 2.76936 0.44867 1.75528709330358 -85.56 37.47 4147.9871
Ambystoma_bishopi AMBYSTOMATIDAE 1 0 0 0 NA 0.94914 0.64652 2.02125980284774 -85.73 30.78 3405.91047
Ambystoma_bombypellum AMBYSTOMATIDAE 0 0 0 0 NA 0.00239 1 -0.403531686433 -99.51 19.87 2197.17646
Ambystoma_californiense AMBYSTOMATIDAE 1 1 0 0 91.8 0.67925 0.23778 1.05939976357962 -121.45 37.16 4113.7471
Ambystoma_cingulatum AMBYSTOMATIDAE 1 0 0 0 53.9 3.11906 0.6656 2.67347112506966 -82.52 31.22 3454.97991
Ambystoma_dumerilii AMBYSTOMATIDAE 1 0 0 1 NA 0.00015 1 -1.8514360650492 -101.63 19.57 2166.02183
Ambystoma_flavipiperatum AMBYSTOMATIDAE 1 0 0 0 NA 0.00636 0.42046 -1.64964217064012 -103.44 20.56 2274.23083
Ambystoma_gracile AMBYSTOMATIDAE 1 0 1 0 77.87 19.3997 0.45509 3.24985558397945 -126.89 50.96 5647.49731
Ambystoma_granulosum AMBYSTOMATIDAE 1 0 0 0 NA 0.00347 1 -0.164391256005798 -99.66 19.65 2172.88518
Ambystoma_jeffersonianum AMBYSTOMATIDAE 1 1 0 0 82.15 25.65208 0.73238 2.58666172160292 -79.99 41.85 4633.61545
Ambystoma_laterale AMBYSTOMATIDAE 1 1 0 0 54.9 101.85168 0.45852 3.13124087177399 -76.31 45.38 5026.00004
Ambystoma_leorae AMBYSTOMATIDAE 0 0 0 0 NA 0.00098 1 -0.57181423181437 -98.71 19.28 2131.83483
Ambystoma_lermaense AMBYSTOMATIDAE 1 0 0 1 NA 0.00223 1 -0.385467760249168 -99.7 19.4 2145.23678
Ambystoma_mabeei AMBYSTOMATIDAE 0 0 0 0 66.1 4.08085 0.72011 2.45726517343176 -77.28 36.4 4030.7317
Ambystoma_macrodactylum AMBYSTOMATIDAE 1 0 0 0 60 86.21607 0.64894 1.78758215813906 -133.06 56.32 6243.38698
Ambystoma_maculatum AMBYSTOMATIDAE 1 0 0 0 81.38 129.10531 0.59389 4.12131747619602 -79.84 43.64 4832.54372
Ambystoma_mavortium AMBYSTOMATIDAE 1 1 0 0 65.56 NA NA NA NA NA NA
Ambystoma_mexicanum AMBYSTOMATIDAE 1 0 0 1 NA 0.00284 1 1.19662914239438 -99.02 19.15 2117.42493
Ambystoma_opacum AMBYSTOMATIDAE 1 0 0 0 110.85 75.10755 0.77735 4.38595777257207 -84.46 38.87 4305.41818
Ambystoma_ordinarium AMBYSTOMATIDAE 1 0 1 0 63.5 0.12187 0.9998 0.952214649784628 -100.77 19.49 2156.00429
Ambystoma_rivulare AMBYSTOMATIDAE 1 0 0 0 NA 0.00628 0.20796 -0.159645033661136 -99.56 18.85 2084.32365
Ambystoma_rosaceum AMBYSTOMATIDAE 1 0 0 0 NA 5.04878 0.78987 2.53917230368785 -107.11 26.69 2953.83826
Ambystoma_silvensis AMBYSTOMATIDAE 0 0 0 0 NA 0.03154 0.05481 NA -106.52 26.44 2925.30823
Ambystoma_talpoideum AMBYSTOMATIDAE 0 0 1 0 71.85 23.83279 0.51581 3.99614261859505 -86.6 34.69 3838.73901
Ambystoma_taylori AMBYSTOMATIDAE 1 0 0 0 NA 0.00044 1 0.262017083534599 -97.48 19.37 2142.47071
Ambystoma_texanum AMBYSTOMATIDAE 1 1 0 0 96.61 46.10841 0.59476 3.65208297913001 -86.86 35.72 3952.9022
Ambystoma_tigrinum AMBYSTOMATIDAE 1 1 1 0 NA 120.58791 0.30113 2.2220513728866 -111.49 37.26 4123.82826
Ambystoma_velasci AMBYSTOMATIDAE 1 0 0 1 84.52 20.3461 0.80481 1.86561452861134 -103.49 24.6 2721.49661
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Amphiuma_pholeter AMPHIUMIDAE 1 0 0 1 214 1.72767 0.45866 2.3207461265806 -85.33 30.33 3356.53519
Amphiuma_tridactylum AMPHIUMIDAE 1 1 0 1 554 14.75011 0.64933 2.85642876035232 -90.86 31 3431.56858
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Andrias_japonicus CRYPTOBRANCHIDAE 1 0 0 1 NA 2.08634 0.38838 -1.3205824590412 133.42 33.83 3744.42999
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Aneides_ferreus PLETHODONTIDAE 1 1 0 0 NA 5.83179 0.33352 2.41261028483501 -124.45 46.25 5122.84705
Aneides_flavipunctatus PLETHODONTIDAE 1 0 0 0 65.3 2.01363 0.50814 2.02269602510017 -122.64 38.82 4296.86375
Aneides_hardii PLETHODONTIDAE 1 0 0 0 54 1.03988 0.85661 0.245646171683635 -105.62 33.09 3661.43548
Aneides_lugubris PLETHODONTIDAE 1 0 0 0 79.3 4.12508 0.39884 2.43140427747121 -119.16 35.27 3905.12567
Aneides_vagrans PLETHODONTIDAE 0 1 0 0 68.3 2.747 0.18787 1.30934315802212 -125.47 49.34 5468.18894
Batrachoseps_aridus PLETHODONTIDAE 0 0 0 0 NA NA NA NA NA NA NA
Batrachoseps_attenuatus PLETHODONTIDAE 1 0 0 0 44.6 2.24374 0.45548 2.07679855160172 -121.95 38.89 4305.09406
Batrachoseps_campi PLETHODONTIDAE 0 0 0 0 NA 0.01276 1 -0.634770177177536 -117.95 36.75 4067.56056
Batrachoseps_diabolicus PLETHODONTIDAE 0 0 0 0 NA 0.08018 0.65642 1.1107318783301 -120.62 38.24 4235.12371
Batrachoseps_gabrieli PLETHODONTIDAE 0 0 0 0 NA 0.02037 0.39245 -1.24413126282534 -117.13 34.07 3769.92518
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Batrachoseps_gregarius PLETHODONTIDAE 0 0 0 0 NA 0.29949 0.73586 1.79175498330032 -119.25 36.61 4053.96785
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Batrachoseps_luciae PLETHODONTIDAE 0 1 0 0 38.8 0.06043 0.83529 0.361371742622965 -121.66 36.28 4015.83851
Batrachoseps_major PLETHODONTIDAE 1 0 0 0 53.3 0.96733 0.4254 0.686106971490653 -116.93 32.54 3600.29541
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Batrachoseps_robustus PLETHODONTIDAE 0 0 0 0 NA 0.05451 1 0.722784628653802 -118.1 35.95 3978.96216

Batrachoseps_simatus PLETHODONTIDAE 0 0 0 0 NA 0.00669 0.27122 0.622361772443717 -118.54 35.81 3963.96971
Batrachoseps_stebbinsi PLETHODONTIDAE 0 0 0 0 NA 0.01304 0.13648 -0.0347523141484947 -118.57 35.44 3922.97194
Batrachoseps_wrightorum PLETHODONTIDAE 1 0 0 0 44.6 0.56559 0.89947 NA -122.08 44.73 4953.36941
Batrachuperus_karlschmidti HYNOBIIDAE 0 0 0 0 NA 0.09772 1 -2.64268168492514 102.02 29.96 3318.09172
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Batrachuperus_pinchonii HYNOBIIDAE 1 0 0 0 105.15 4.5225 0.73745 -0.372417608049269 102.27 29.21 3234.34077
Batrachuperus_tibetanus HYNOBIIDAE 1 0 0 0 NA 8.73006 0.6351 -1.42810134576469 102.88 32.65 3614.47305
Batrachuperus_yenyuanensis HYNOBIIDAE 1 0 0 0 83.23 0.50564 1 -0.727848987321078 102.15 28.27 3130.40872
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Bolitoglossa_alvaradoi PLETHODONTIDAE 0 0 0 0 NA 0.09176 0.38588 2.50832166446909 -83.76 10.09 1116.641
Bolitoglossa_anthracina PLETHODONTIDAE 0 0 0 0 NA 0.02828 0.97653 2.17934892180438 -82.54 8.94 988.57259
Bolitoglossa_biseriata PLETHODONTIDAE 1 0 0 0 NA 1.2855 0.14336 1.99010339142863 -78.4 7.41 819.91399
Bolitoglossa_borburata PLETHODONTIDAE 0 0 0 0 NA 0.07175 0.17979 -0.408310051507657 -68.84 10.62 1173.96771
Bolitoglossa_bramei PLETHODONTIDAE 0 0 0 0 NA 0.00068 0.1922 1.02470917516122 -82.59 8.88 981.96009
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Bolitoglossa_celaque PLETHODONTIDAE 1 0 0 0 NA 0.02556 0.20307 0.322704806891858 -88.16 14.32 1583.43061
Bolitoglossa_cerroensis PLETHODONTIDAE 1 0 0 0 NA 0.01904 1 2.07722906010123 -83.78 9.59 1061.3166
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Bolitoglossa_compacta PLETHODONTIDAE 0 0 0 0 NA 0.0132 0.99883 2.78581608317382 -82.66 8.93 987.52388
Bolitoglossa_conanti PLETHODONTIDAE 1 0 0 0 NA 0.00915 0.04944 0.0023296086752504 -88.88 14.89 1647.0128
Bolitoglossa_copia PLETHODONTIDAE 0 0 0 0 NA 0.00034 1 -2.18017903263873 -80.62 8.66 957.28397
Bolitoglossa_cuchumatana PLETHODONTIDAE 0 0 0 0 NA 0.07582 0.59389 1.29870125399204 -91.02 15.37 1700.22435
Bolitoglossa_cuna PLETHODONTIDAE 0 0 0 0 NA 0.00984 0.70151 -0.70599431256827 -77.61 8.76 969.31564
Bolitoglossa_decora PLETHODONTIDAE 1 0 0 0 NA 0.00046 1 2.97798362070933 -86.65 15.11 1670.52254
Bolitoglossa_diaphora PLETHODONTIDAE 1 0 0 0 NA 0.00117 1 2.94050323719571 -88.24 15.51 1715.12061
Bolitoglossa_digitigrada PLETHODONTIDAE 0 0 0 0 NA 0.00063 1 -4.37590887672182 -73.9 -12.52 -1389.81451650263
Bolitoglossa_diminuta PLETHODONTIDAE 0 0 0 0 NA 0.00022 1 0.432199453155466 -83.7 9.68 1071.20752
Bolitoglossa_dofleini PLETHODONTIDAE 1 0 0 0 103.35 0.05946 0.05327 1.36844045051668 -88.95 15.65 1731.18119
Bolitoglossa_dunni PLETHODONTIDAE 1 0 0 0 NA 0.00493 0.11878 1.42254880460756 -88.65 15.32 1694.39427
Bolitoglossa_engelhardti PLETHODONTIDAE 1 0 0 0 45.75 0.03246 0.70743 2.49932146565167 -91.73 14.9 1647.65514
Bolitoglossa_epimela PLETHODONTIDAE 0 0 0 0 NA 0.00785 0.54929 1.11781469185166 -83.65 9.81 1085.55202
Bolitoglossa_equatoriana PLETHODONTIDAE 0 0 0 0 NA 0.53333 0.70518 0.655313033450348 -76.72 0.09 9.95222
Bolitoglossa_flavimembris PLETHODONTIDAE 0 0 0 0 NA 0.07078 0.85577 3.52013539017849 -91.73 14.84 1641.01752
Bolitoglossa_flaviventris PLETHODONTIDAE 1 0 0 0 NA 0.01954 0.53806 1.29479259492356 -92.11 15.02 1660.73079
Bolitoglossa_franklini PLETHODONTIDAE 1 1 0 0 NA 0.08601 0.62356 1.54041270915882 -92.22 15.24 1685.02132
Bolitoglossa_gomezi PLETHODONTIDAE 0 0 0 0 NA 0.00278 0.34147 1.22909207281972 -82.85 8.8 973.24379
Bolitoglossa_gracilis PLETHODONTIDAE 1 0 0 0 NA 0.00213 0.99994 1.56732430933102 -83.73 9.71 1074.55423
Bolitoglossa_guaramacalensis PLETHODONTIDAE 0 0 0 0 NA 0.00423 0.96597 -1.23765817172888 -70.2 9.23 1020.50205
Bolitoglossa_hartwegi PLETHODONTIDAE 1 0 0 0 NA 0.09253 0.46242 1.18813497094782 -91.76 16 1769.33136
Bolitoglossa_heiroreias PLETHODONTIDAE 0 0 0 0 NA 0.00146 0.30827 -0.683144249497868 -89.36 14.53 1607.65818
Bolitoglossa_helmrichi PLETHODONTIDAE 1 0 0 0 NA 0.14666 0.88293 2.23400029313033 -90.03 15.39 1703.65787
Bolitoglossa_hermosa PLETHODONTIDAE 1 0 0 0 NA 0.0093 0.99345 0.566285735560839 -100.49 17.44 1928.84814
Bolitoglossa_hiemalis PLETHODONTIDAE 0 0 0 0 NA 0.00258 0.76186 -0.875864765031733 -76.42 4.16 459.95487
Bolitoglossa_hypacra PLETHODONTIDAE 0 0 0 0 NA 0.00216 0.6815 -0.151167190906498 -76.09 6.46 714.18348
Bolitoglossa_jacksoni PLETHODONTIDAE 0 0 0 0 NA 3e-04 1 4.04192213341295 -91.28 15.87 1755.31202
Bolitoglossa_lignicolor PLETHODONTIDAE 1 0 0 0 NA 0.26183 0.25316 0.803144344056477 -81.9 8.01 885.51198
Bolitoglossa_lincolni PLETHODONTIDAE 1 1 0 0 NA 0.03918 0.11095 1.77537462309197 -91.62 15.54 1718.5316
Bolitoglossa_longissima PLETHODONTIDAE 1 0 0 0 NA 0.00035 1 -2.20641511890671 -85.93 14.96 1654.17934
Bolitoglossa_lozanoi PLETHODONTIDAE 0 0 0 0 NA 0.00413 1 0.0740326247688842 -75.74 5.32 588.08592
Bolitoglossa_macrinii PLETHODONTIDAE 1 0 0 0 NA 0.11629 0.91667 1.07946152871687 -96.83 16.03 1773.46891
Bolitoglossa_magnifica PLETHODONTIDAE 0 0 0 0 NA 0.00199 1 0.562073070903237 -82.51 8.8 973.07587
Bolitoglossa_marmorea PLETHODONTIDAE 1 0 0 0 NA 0.01665 0.9993 2.62374552378044 -82.62 8.84 977.55042
Bolitoglossa_medemi PLETHODONTIDAE 1 0 0 0 NA 0.05462 0.11871 0.548549037130661 -77.56 7.24 801.07683
Bolitoglossa_meliana PLETHODONTIDAE 0 0 0 0 NA 0.05789 0.44572 3.26028744319603 -90.39 15.1 1671.05263
Bolitoglossa_mexicana PLETHODONTIDAE 1 0 0 0 61.9 4.19194 0.37408 1.97943644232789 -88.12 18.37 2031.48638
Bolitoglossa_minutula PLETHODONTIDAE 1 0 0 0 NA 0.03816 0.87605 3.3166015916677 -82.68 8.94 988.63986
Bolitoglossa_mombachoensis PLETHODONTIDAE 0 0 0 0 NA 0.00025 1 -2.65286359203077 -85.91 11.77 1301.35147
Bolitoglossa_morio PLETHODONTIDAE 1 0 0 0 51.88 0.39863 0.75904 2.12853703313221 -91.02 15.07 1667.02517
Bolitoglossa_mulleri PLETHODONTIDAE 0 0 0 0 70.23 0.03358 0.1616 1.01341503515958 -90.72 15.88 1756.99196
Bolitoglossa_nicefori PLETHODONTIDAE 0 0 0 0 NA 0.08015 0.73173 -0.380721435965794 -73.01 6.93 766.47167
Bolitoglossa_nigrescens PLETHODONTIDAE 0 0 0 0 NA 0.02655 0.90457 1.96280295189727 -83.88 9.65 1068.04875
Bolitoglossa_oaxacensis PLETHODONTIDAE 0 0 0 0 NA 0.04395 0.99955 1.96436907366046 -96.97 16.44 1818.69422
Bolitoglossa_obscura PLETHODONTIDAE 0 0 0 0 NA 0.00024 1 0.295864975707711 -83.78 9.72 1075.70566
Bolitoglossa_occidentalis PLETHODONTIDAE 1 0 0 0 34.85 0.2437 0.13591 3.97898409446615 -90.38 15.14 1675.49368
Bolitoglossa_odonnelli PLETHODONTIDAE 1 0 0 0 NA 0.06959 0.48312 1.44709898116008 -89.17 15.51 1715.91519
Bolitoglossa_oresbia PLETHODONTIDAE 0 0 0 0 NA 0.00025 1 -1.47237307384582 -87.9 14.73 1628.65674

Bolitoglossa_orestes PLETHODONTIDAE 0 0 0 0 NA 0.00571 0.54858 0.286113808711358 -71.11 8.66 957.90729
Bolitoglossa_palmata PLETHODONTIDAE 1 0 0 0 46.9 0.1418 0.34088 -0.00704834427075437 -78.02 -1.03 -116.829534948146
Bolitoglossa_pandi PLETHODONTIDAE 0 0 0 0 NA 0.00072 1 -1.67682385471444 -74.45 4.19 463.1506
Bolitoglossa_paraensis PLETHODONTIDAE 0 0 0 0 NA 0.53647 0.3228 -1.22196151837899 -50.08 0.28 30.95243
Bolitoglossa_peruviana PLETHODONTIDAE 1 0 0 0 NA 2.05509 0.63719 0.768844140324627 -77.04 -3.87 -431.475083043422
Bolitoglossa_pesrubra PLETHODONTIDAE 1 0 0 0 NA 0.01977 0.79306 2.39134334050235 -83.58 9.52 1053.3985
Bolitoglossa_phalarosoma PLETHODONTIDAE 0 0 0 0 NA 0.00998 0.0848 -0.11977975873311 -76.82 6.69 739.85217
Bolitoglossa_platydactyla PLETHODONTIDAE 1 0 0 0 79.73 1.5663 0.74699 2.97896599754065 -96.14 18.7 2070.04986
Bolitoglossa_porrasorum PLETHODONTIDAE 1 0 0 0 NA 0.00787 0.13276 0.778036844783054 -87.25 15.4 1702.58414
Bolitoglossa_ramosi PLETHODONTIDAE 0 0 0 0 NA 0.1828 0.29828 1.60037415040105 -75.23 5.73 633.36345
Bolitoglossa_riletti PLETHODONTIDAE 1 0 0 0 NA 0.0082 0.992 1.38399282177007 -97.91 17.02 1882.09076
Bolitoglossa_robusta PLETHODONTIDAE 0 0 0 0 100.1 0.1749 0.37056 2.76825429250143 -84 9.89 1094.73173
Bolitoglossa_rostrata PLETHODONTIDAE 1 0 0 0 44.73 0.05816 0.10881 1.52391740651036 -91.53 15.34 1696.46792
Bolitoglossa_rufescens PLETHODONTIDAE 1 0 0 0 36.64 2.05632 0.22425 1.79473690918094 -91.18 16.81 1859.42794
Bolitoglossa_salvinii PLETHODONTIDAE 0 0 0 0 51.1 0.04761 0.3752 0.592976466289134 -90.1 14.07 1557.3969
Bolitoglossa_savagei PLETHODONTIDAE 0 0 0 0 NA 0.01139 0.99706 -1.90366982697011 -73.83 10.96 1211.80494
Bolitoglossa_schizodactyla PLETHODONTIDAE 1 0 0 0 NA 0.31028 0.32346 1.51736530797682 -81.04 9.24 1021.38635
Bolitoglossa_silverstonei PLETHODONTIDAE 0 0 0 0 NA 0.11789 0.24135 1.84865469139748 -76.33 6.53 721.98673
Bolitoglossa_sima PLETHODONTIDAE 1 0 0 0 NA 0.09787 0.76247 1.23077031554957 -78.89 0.53 58.62097
Bolitoglossa_sombra PLETHODONTIDAE 0 0 0 0 NA 0.00498 0.90863 1.43502134594216 -82.84 8.98 993.14839
Bolitoglossa_sooyorum PLETHODONTIDAE 0 0 0 0 NA 0.0183 1 2.35271105183478 -83.64 9.51 1052.34149
Bolitoglossa_spongai PLETHODONTIDAE 0 0 0 0 NA 0.00299 0.95887 -0.0373590094213036 -71.38 8.63 954.76342
Bolitoglossa_striatula PLETHODONTIDAE 1 0 0 0 NA 2.91927 0.74835 3.74887010062211 -84.46 11.01 1218.26116
Bolitoglossa_stuarti PLETHODONTIDAE 0 0 0 0 NA 0.06851 0.86693 1.03785837282475 -92.13 16.08 1777.98036
Bolitoglossa_subpalmata PLETHODONTIDAE 1 0 0 0 55.28 0.04212 0.26248 1.43862848448178 -84.83 10.51 1162.62177
Bolitoglossa_synoria PLETHODONTIDAE 1 0 0 0 NA 0.00408 0.9972 -0.201661179645388 -89.08 14.36 1588.55168
Bolitoglossa_tatamae PLETHODONTIDAE 0 0 0 0 NA 0.00459 0.23041 -0.546936316429965 -76.04 5.93 655.57463
Bolitoglossa_taylori PLETHODONTIDAE 0 0 0 0 NA 0.02197 0.99995 0.0931879223061414 -77.63 7.85 868.62518
Bolitoglossa_vallecula PLETHODONTIDAE 0 0 0 0 NA 0.2346 0.44412 2.0938348893921 -75.49 5.43 600.21815
Bolitoglossa_veracrucis PLETHODONTIDAE 0 0 0 0 NA 0.11925 0.70468 1.6033696147847 -94 17.28 1910.80615
Bolitoglossa_walkeri PLETHODONTIDAE 0 0 0 0 NA 0.04075 0.27936 0.50396845534494 -76.56 3.62 400.27314
Bolitoglossa_yucatana PLETHODONTIDAE 1 0 0 0 56.9 1.33998 0.39323 1.40918196959744 -88.89 19.28 2132.8917
Bolitoglossa_zapoteca PLETHODONTIDAE 0 0 0 0 NA 0.00207 1 1.2728182718588 -95.87 16.27 1800.95633
Bradytriton_silus PLETHODONTIDAE 1 0 0 0 42.6 0.00035 1 4.11899747332658 -91.28 15.87 1755.31202
Calotriton_arnoldi SALAMANDRIDAE 1 0 0 0 NA 0.00519 0.99488 -1.65449959717749 2.4 41.79 4626.6347
Calotriton_asper SALAMANDRIDAE 1 0 0 0 57.87 1.24617 0.66108 1.08604861755255 0.77 42.65 4724.36089
Chioglossa_lusitanica SALAMANDRIDAE 1 0 0 0 45.85 2.44482 0.54114 1.14854490158417 -7.71 42.29 4682.78147
Chiropterotriton_arboreus PLETHODONTIDAE 1 0 0 0 NA 0.00039 1 0.240125895042565 -98.69 20.71 2290.08403
Chiropterotriton_chiropterus PLETHODONTIDAE 0 0 0 0 33.3 0.00248 0.99834 2.6842270875601 -96.99 19.13 2116.42796
Chiropterotriton_chondrostega PLETHODONTIDAE 1 0 0 0 24.05 0.01925 0.0194 0.523588725673241 -98.72 21.01 2323.27992
Chiropterotriton_cracens PLETHODONTIDAE 0 0 0 0 NA 0.00122 1 -1.38699698299202 -99.2 23.12 2556.81793
Chiropterotriton_dimidiatus PLETHODONTIDAE 1 0 0 0 25.1 0.00632 0.99707 0.215304901090863 -98.74 20.16 2229.20837
Chiropterotriton_lavae PLETHODONTIDAE 1 0 0 0 30 0.00199 1 0.788564937972332 -97 19.59 2167.34024
Chiropterotriton_magnipes PLETHODONTIDAE 1 0 0 0 NA 0.00995 0.99465 -0.321637859814793 -99.17 21.29 2354.25284
Chiropterotriton_mosaueri PLETHODONTIDAE 0 0 0 0 NA 0.01174 0.99985 -0.345063775098693 -99.33 20.76 2295.62168
Chiropterotriton_multidentatus PLETHODONTIDAE 1 0 0 0 29.6 0.12692 0.13945 0.951354347957454 -99.32 21.77 2407.40387
Chiropterotriton_orculus PLETHODONTIDAE 0 0 0 0 NA 0.24857 0.45578 2.19614936984954 -98.53 19.38 2142.94173
Chiropterotriton_priscus PLETHODONTIDAE 1 0 0 0 47.97 0.07826 0.41449 0.0680209732014294 -100.06 24.83 2746.54044
Chiropterotriton_terrestris PLETHODONTIDAE 0 0 0 0 NA 0.00086 0.99646 0.635518720104496 -98.65 20.67 2285.66571
Cryptobranchus_alleganiensis CRYPTOBRANCHIDAE 1 0 0 1 308.5 20.5296 0.52504 3.73743110298128 -85.05 38.7 4285.29151
Cryptotriton_adelos PLETHODONTIDAE 0 0 0 0 NA 0.00167 0.04977 0.605839993499071 -96.73 17.83 1972.83804
Cryptotriton_alvarezdeltoroi PLETHODONTIDAE 1 0 0 0 NA 0.00123 0.13278 -1.15714449600298 -92.88 17.18 1899.4717
Cryptotriton_monzoni PLETHODONTIDAE 0 0 0 0 NA 9e-05 1 -2.3085201352944 -89.26 14.98 1657.35391
Cryptotriton_nasalis PLETHODONTIDAE 1 0 0 0 29.6 0.00838 0.99579 2.43302294913688 -88.2 15.52 1716.20247
Cryptotriton_veraepacis PLETHODONTIDAE 0 0 0 0 NA 0.00116 0.25891 -0.185848195643356 -90.15 15.27 1690.19672
Cryptotriton_wakei PLETHODONTIDAE 0 0 0 0 NA 0.00021 1 3.20029044403332 -88.7 15.37 1699.96827
Cynops_chenggongensis SALAMANDRIDAE 1 0 0 0 NA 0.00088 1 -4.81079086334867 101.5 24.12 2669.77959
Cynops_cyanurus SALAMANDRIDAE 1 0 0 0 NA 2.05557 0.88508 0.210134061454214 102.78 25.11 2778.96974
Cynops_ensicauda SALAMANDRIDAE 1 0 0 0 60.82 0.06365 0.12561 -1.78112492347655 128.36 27.22 3010.96468
Cynops_orientalis SALAMANDRIDAE 1 0 0 0 NA 19.11939 0.78798 -0.641954537041578 116.87 28.75 3180.29527
Cynops_orphicus SALAMANDRIDAE 1 0 0 0 NA 0.10215 1 -3.66881966311917 115.89 23.61 2611.4888
Cynops_pyrrhogaster SALAMANDRIDAE 1 0 0 0 63.78 10.81218 0.49492 0.712694010640677 134.61 34.88 3859.80512
Cynops_wolterstorffi SALAMANDRIDAE 0 0 0 0 NA 0.0218 0.97535 -2.06306518401453 102.68 24.84 2749.22698
Dendrotriton_bromeliacius PLETHODONTIDAE 0 0 0 0 NA 0.00372 0.99904 1.18508649146135 -91.82 14.92 1649.81436
Dendrotriton_cuchumatanus PLETHODONTIDAE 1 0 0 0 NA 0.00034 0.12575 0.625982239793258 -91.49 15.59 1724.15803
Dendrotriton_megarhinus PLETHODONTIDAE 0 0 0 0 NA 0.0011 1 -1.63290074985858 -93.61 16.2 1791.15538
Dendrotriton_rabbi PLETHODONTIDAE 1 0 0 0 NA 0.00605 0.07031 2.0654162065006 -91.45 15.59 1724.18884
Dendrotriton_sanctibarbarus PLETHODONTIDAE 0 0 0 0 NA 0.00261 0.99395 3.34167647345818 -88.12 14.92 1649.78099
Dendrotriton_xolocalcae PLETHODONTIDAE 0 0 0 0 32.95 2e-04 1 -2.05099109858505 -92.63 15.41 1703.70875
Desmognathus_abditus PLETHODONTIDAE 0 0 0 0 NA 0.04599 0.7279 0.518846688130016 -84.97 35.85 3969.2147

Desmognathus_aeneus PLETHODONTIDAE 1 0 0 0 25.9 1.73117 0.43208 2.48363290222797 -85.29 33.85 3746.8403
Desmognathus_apalachicolae PLETHODONTIDAE 0 0 0 0 42.7 0.68305 0.50692 2.91396277584287 -84.83 31.03 3434.95001
Desmognathus_auriculatus PLETHODONTIDAE 1 0 0 0 53.9 19.46696 0.41621 2.807115226019 -88.7 30.92 3421.97504
Desmognathus_brimleyorum PLETHODONTIDAE 1 0 0 0 76.5 2.11914 0.77035 3.99195185980119 -94.17 34.62 3831.52638
Desmognathus_carolinensis PLETHODONTIDAE 0 1 0 0 45.6 0.42749 0.76328 3.16849958423872 -82.52 35.74 3956.17685
Desmognathus_conanti PLETHODONTIDAE 0 1 0 0 56.8 NA NA NA NA NA NA
Desmognathus_folkertsi PLETHODONTIDAE 0 0 0 0 71.4 0.00122 1 -1.09456796468377 -83.93 34.75 3849.23235
Desmognathus_fuscus PLETHODONTIDAE 1 1 0 0 57.23 62.7081 0.69985 4.54386378005266 -83.84 35.95 3982.13317
Desmognathus_imitator PLETHODONTIDAE 1 0 0 0 44.5 0.35726 0.73771 3.81105586269431 -83.6 35.53 3934.93153
Desmognathus_marmoratus PLETHODONTIDAE 1 0 0 0 79.95 1.31089 0.68049 3.01151244594498 -82.82 35.66 3947.7649
Desmognathus_monticola PLETHODONTIDAE 1 0 0 0 61.8 12.43253 0.65295 3.4693923742953 -84.91 33.48 3706.45705
Desmognathus_ochrophaeus PLETHODONTIDAE 1 1 0 0 40.4 12.61056 0.62413 2.01102989625792 -76.99 41.92 4642.80978
Desmognathus_ocoee PLETHODONTIDAE 0 0 0 0 43.61 1.53712 0.56999 2.10699603174229 -84.68 34.28 3795.63968
Desmognathus_orestes PLETHODONTIDAE 0 1 0 0 38.9 0.84388 0.75254 3.65469770179207 -81.42 36.5 4039.49019
Desmognathus_planiceps PLETHODONTIDAE 0 0 0 0 NA NA NA NA NA NA NA
Desmognathus_quadramaculatus PLETHODONTIDAE 1 0 0 0 69.59 3.17552 0.67696 4.22528698959748 -82.28 35.96 3980.26944
Desmognathus_santeetlah PLETHODONTIDAE 0 1 0 0 45.2 0.40037 0.73451 3.86801851846349 -83.55 35.57 3939.25067
Desmognathus_welteri PLETHODONTIDAE 1 0 0 0 66.6 0.837 0.55573 1.75759695978985 -83.14 36.68 4061.50929
Desmognathus_wrighti PLETHODONTIDAE 1 0 0 0 29.12 1.29451 0.6992 4.02920816559207 -82.36 35.87 3970.38404
Dicamptodon_aterrimus DICAMPTODONTIDAE 1 0 1 0 NA 2.71531 0.79791 1.70860681814959 -115.74 46 5094.81963
Dicamptodon_copei DICAMPTODONTIDAE 1 1 1 0 83.25 1.362 0.48391 4.04144372571218 -123.29 46.91 5195.20373
Dicamptodon_ensatus DICAMPTODONTIDAE 1 0 1 0 108 0.4529 0.63648 0.840957227243818 -122.51 37.93 4198.16173
Dicamptodon_tenebrosus DICAMPTODONTIDAE 1 1 1 0 91.6 8.09335 0.59801 2.20839497293232 -122.13 47.68 5281.10135
Echinotriton_andersoni SALAMANDRIDAE 1 0 0 0 82.5 0.07139 0.1732 -1.72374568422107 128.58 27.4 3030.81031
Echinotriton_chinhaiensis SALAMANDRIDAE 1 0 0 0 NA 0.00168 1 -3.47993947289745 121.78 29.79 3296.14012
Ensatina_eschscholtzii PLETHODONTIDAE 1 0 0 0 64.5 10.25944 0.29195 3.91225993703535 -121.89 45.4 5027.98643
Euproctus_montanus SALAMANDRIDAE 1 0 0 0 NA 0.3561 0.81161 3.53537187031356 9.07 42.15 4666.43322
Euproctus_platycephalus SALAMANDRIDAE 1 0 0 0 NA 0.08571 0.35206 1.83933990807796 9.35 40.09 4437.80521
Eurycea_aquatica PLETHODONTIDAE 1 0 0 0 36 NA NA NA NA NA
Eurycea_bislineata PLETHODONTIDAE 1 0 0 0 40.4 59.5679 0.66098 2.86304047227055 -74.08 43.98 4870.06311
Eurycea_chamberlaini PLETHODONTIDAE 0 0 0 0 NA NA NA NA NA NA NA
Eurycea_chisholmensis PLETHODONTIDAE 0 0 0 0 32.9 0.002 1 -0.792618812371934 -97.47 31 3430.60704
Eurycea_cirrigera PLETHODONTIDAE 1 1 0 0 36.09 34.96383 0.6755 5.47542343222684 -84.28 35.23 3901.94207
Eurycea_guttolineata PLETHODONTIDAE 0 0 0 0 51 26.41589 0.70001 3.45118375306847 -83.99 33.83 3747.3306
Eurycea_junaluska PLETHODONTIDAE 1 0 0 0 43.5 0.25655 0.8261 2.54327147372578 -83.82 35.57 3939.91859
Eurycea_latitans PLETHODONTIDAE 0 0 0 1 36 0.25439 0.67827 1.74630567020485 -98.71 29.99 3317.71274
Eurycea_longicauda PLETHODONTIDAE 1 0 0 0 59.1 32.1905 0.63256 3.60906343477375 -83.51 39.86 4415.23521
Eurycea_lucifuga PLETHODONTIDAE 1 0 0 0 62.9 17.35346 0.53959 2.62590823982032 -87.6 36.94 4088.38468
Eurycea_multiplicata PLETHODONTIDAE 1 0 1 0 41 5.10535 0.7657 2.77520362573153 -93.61 35.74 3955.28293
Eurycea_nana PLETHODONTIDAE 1 0 0 1 26 0.00165 1 1.27231096024826 -97.95 29.85 3302.62718
Eurycea_naufragia PLETHODONTIDAE 0 0 0 0 29 0.00172 1 -0.0880857355003057 -97.72 30.67 3393.72966
Eurycea_neotenes PLETHODONTIDAE 1 1 1 0 35 0.00123 0.95484 -0.188327080214031 -98.69 29.63 3277.82773
Eurycea_pterophila PLETHODONTIDAE 0 0 0 0 NA 0.0105 0.97404 0.370579444917191 -98.23 30.05 3324.57572
Eurycea_quadridigitata PLETHODONTIDAE 1 0 0 0 30.2 25.33303 0.44958 5.62441016657848 -86.75 31.6 3496.1273
Eurycea_rathbuni PLETHODONTIDAE 1 0 0 1 54.3 0.00186 0.94887 1.33221156015457 -97.97 29.89 3307.04232
Eurycea_robusta PLETHODONTIDAE 0 0 0 1 NA 0.00059 1 0.387845472335645 -97.91 29.91 3309.31209
Eurycea_sosorum PLETHODONTIDAE 1 0 0 0 29.2 0.00086 1 1.68468273335862 -97.77 30.27 3349.34469
Eurycea_spelaea PLETHODONTIDAE 1 0 1 0 50.7 4.34437 0.81162 4.37106259681359 -92.93 36.84 4077.12565
Eurycea_tonkawae PLETHODONTIDAE 0 0 0 0 30.5 0.02724 0.96272 1.29309535972767 -97.74 30.5 3374.86671
Eurycea_tridentifera PLETHODONTIDAE 1 1 0 1 37.6 0.008 0.50715 0.747886605933724 -98.52 29.69 3284.5321
Eurycea_troglodytes PLETHODONTIDAE 0 0 0 1 NA 0.11243 0.92346 1.02440272448737 -99.11 29.35 3246.76872
Eurycea_tynerensis PLETHODONTIDAE 1 0 0 1 33.3 0.86216 0.61273 1.52092711889218 -94.34 36.35 4023.60358
Eurycea_waterlooensis PLETHODONTIDAE 0 0 0 0 NA 0.00018 1 0.902694964179953 -97.75 30.27 3349.36569
Eurycea_wilderae PLETHODONTIDAE 1 1 0 0 33.94 2.85701 0.77221 4.42502985471585 -82.37 35.78 3960.41372
Gyrinophilus_gulolineatus PLETHODONTIDAE 0 0 0 0 NA 0.13197 0.88306 1.40642956728316 -84.27 35.75 3959.65929
Gyrinophilus_palleucus PLETHODONTIDAE 0 0 0 1 NA 0.62337 0.4031 2.17430391880933 -86.96 34.95 3867.49908
Gyrinophilus_porphyriticus PLETHODONTIDAE 1 0 0 0 80.2 32.27348 0.70422 4.99536454265505 -82.65 37.32 4131.64901
Gyrinophilus_subterraneus PLETHODONTIDAE 0 0 0 0 NA 0.00012 1 -1.46645624260565 -80.55 37.76 4179.2825
Haideotriton_wallacei PLETHODONTIDAE 0 0 0 1 25.45 0.00309 0.02598 NA -84.61 31.09 3442.03356
Hemidactylium_scutatum PLETHODONTIDAE 1 0 0 0 40.2 63.92892 0.41151 3.95211366637098 -83.47 40.56 4492.84794
Hydromantes_ambrosii PLETHODONTIDAE 0 1 0 0 NA 0.04969 0.33649 NA 10.63 43.88 4859.83672
Hydromantes_brunus PLETHODONTIDAE 0 0 0 0 57.3 0.0047 0.82934 -1.25262910361142 -120.08 37.6 4165.44726
Hydromantes_flavus PLETHODONTIDAE 0 0 0 0 NA 0.02616 0.99707 NA 9.58 40.44 4476.75642
Hydromantes_genei PLETHODONTIDAE 0 0 0 0 70.6 0.08842 0.89906 NA 8.68 39.28 4347.89813
Hydromantes_imperialis PLETHODONTIDAE 0 0 0 0 NA 0.09976 0.98962 NA 9.26 39.86 4412.25121
Hydromantes_italicus PLETHODONTIDAE 1 1 0 0 51.9 0.82794 0.64545 NA 12.13 43.57 4828.11962
Hydromantes_platycephalus PLETHODONTIDAE 1 0 0 0 52.8 1.89816 0.63018 2.16791281196198 -120.01 38.51 4266.64267
Hydromantes_sarrabusensis PLETHODONTIDAE 0 0 0 0 NA 0.00118 0.98185 NA 9.45 39.3 4350.16539
Hydromantes_shastae PLETHODONTIDAE 0 0 0 0 54 0.01841 0.8483 -0.960978833712023 -122.18 40.81 4517.98928
Hydromantes_strinatii PLETHODONTIDAE 0 0 0 0 NA 0.4238 0.54801 NA 8.42 44.32 4907.57924

Hydromantes_supramontis PLETHODONTIDAE 0 0 0 0 NA 0.02592 0.80996 NA 9.55 40.15 4444.55108
Hynobius_abei HYNOBIIDAE 1 0 0 0 57.77 0.08427 0.34339 -3.16527549511959 135.87 35.87 3969.87934
Hynobius_amjiensis HYNOBIIDAE 1 0 0 0 NA 0.00085 0.99466 -5.88347736052936 119.14 30.48 3373.92249
Hynobius_arisanensis HYNOBIIDAE 0 0 0 0 NA 0.17594 0.9966 -2.47821349043111 120.88 23.24 2571.67271
Hynobius_boulengeri HYNOBIIDAE 1 0 0 0 NA 0.39057 0.24432 -2.15836216057755 133.63 33.54 3711.99473
Hynobius_chinensis HYNOBIIDAE 1 0 0 0 NA 0.15372 0.10027 -3.13462292291126 114.95 29.25 3237.42657
Hynobius_dunni HYNOBIIDAE 1 0 0 0 65 0.23741 0.35932 -2.17283006733252 131.97 32.85 3638.56983
Hynobius_formosanus HYNOBIIDAE 1 0 0 0 NA 0.10121 1 -0.798765575316707 121.2 23.97 2652.07464
Hynobius_guabangshanensis HYNOBIIDAE 1 0 0 0 NA 0.00928 1 -3.70236672991568 112.33 27.2 3009.28668
Hynobius_hidamontanus HYNOBIIDAE 1 0 0 0 NA 0.09539 0.60657 -3.12170866461899 137.57 36.6 4053.57702
Hynobius_hirosei HYNOBIIDAE 0 0 0 0 NA NA NA NA NA NA
Hynobius_katoi HYNOBIIDAE 1 0 0 0 NA 0.02797 0.60203 -3.79414053070797 138.09 35.12 3890.2286
Hynobius_kimurae HYNOBIIDAE 1 0 0 0 NA 2.17225 0.49207 -1.37959841628035 136.5 35.66 3947.27529
Hynobius_leechii HYNOBIIDAE 1 0 0 0 51.65 23.92644 0.65016 -0.0508482320071649 128.15 42.01 4651.23616
Hynobius_lichenatus HYNOBIIDAE 1 0 1 0 70.65 3.39207 0.73208 -1.1811894712182 140.36 38.71 4284.79029
Hynobius_maoershanensis HYNOBIIDAE 0 0 0 0 NA 0.00839 0.91278 -4.2757937247395 110.43 25.87 2861.41135
Hynobius_naevius HYNOBIIDAE 1 0 0 0 74.1 NA NA NA NA NA
Hynobius_nebulosus HYNOBIIDAE 1 0 0 0 63.1 2.38193 0.35346 -1.4169244440822 132.94 34.08 3772.94229
Hynobius_nigrescens HYNOBIIDAE 1 0 0 0 67.25 4.21954 0.60916 -1.07204658978922 139.14 38.17 4226.31258
Hynobius_okiensis HYNOBIIDAE 1 0 0 0 NA 0.00945 0.85392 -4.93855740459557 133.28 36.25 4013.05013
Hynobius_quelpaertensis HYNOBIIDAE 0 0 0 0 NA 0.41583 0.27953 -2.23306155136577 127.28 34.48 3816.7225
Hynobius_retardatus HYNOBIIDAE 1 0 0 0 NA 3.64345 0.57142 -2.95263467103355 144 43.33 4801.83504
Hynobius_sonani HYNOBIIDAE 1 0 0 0 NA 0.03685 1 -1.30393654909944 121.28 23.94 2648.65056
Hynobius_stejnegeri HYNOBIIDAE 1 0 0 0 61.2 0.20538 0.93457 -2.24529340859123 130.92 32.38 3584.17884
Hynobius_takedai HYNOBIIDAE 1 0 0 0 NA 0.11899 0.50301 -3.08518964374566 137.03 36.88 4083.48524
Hynobius_tokyoensis HYNOBIIDAE 1 0 0 0 NA 0.37515 0.51888 -2.49604381131903 140.05 35.75 3956.63667
Hynobius_tsuensis HYNOBIIDAE 1 0 0 0 64.5 0.02544 0.69207 -4.29164811686577 129.31 34.36 3802.11685
Hynobius_turkestanicus HYNOBIIDAE 0 0 0 0 NA 0.1268 0.9368 -2.96044167405035 72.52 40.34 4468.44833
Hynobius_yangi HYNOBIIDAE 0 0 0 0 NA 0.01259 0.71622 -3.98174838878433 129.13 35.19 3894.12149
Hynobius_yatsui HYNOBIIDAE 0 0 0 0 NA NA NA NA NA NA
Hynobius_yiwuensis HYNOBIIDAE 1 0 0 0 NA 0.64684 0.58099 -0.503286885250166 121.49 29.73 3289.82269
Ixalotriton_niger PLETHODONTIDAE 1 0 0 0 NA 0.00018 1 NA NA NA NA
Ixalotriton_parvus PLETHODONTIDAE 1 0 0 0 NA 0.00034 1 NA -94.17 16.57 1832.35098
Karsenia_koreana PLETHODONTIDAE 1 0 0 0 NA 0.13979 0.51563 -3.69018010967299 127.35 35.72 3954.14846
Lineatriton_lineolus PLETHODONTIDAE 1 0 0 0 NA 0.01396 0.62802 NA -96.95 19.08 2110.94161
Lineatriton_orchileucos PLETHODONTIDAE 0 0 0 0 NA 0.00252 0.99993 NA -96.38 17.69 1957.82153
Lineatriton_orchimelas PLETHODONTIDAE 0 0 0 0 32.15 0.03447 0.72312 NA -95.04 18.46 2042.29464
Lissotriton_boscai SALAMANDRIDAE 1 0 0 0 30.5 9.61172 0.68394 1.83305074488977 -6.97 40.24 4456.37284
Lissotriton_helveticus SALAMANDRIDAE 1 0 1 0 40.4 57.87306 0.43184 1.23443011892944 -3.08 51.7 5727.67532
Lissotriton_italicus SALAMANDRIDAE 1 0 0 0 NA 3.23498 0.52153 1.69280925776239 15.52 40.82 4518.90588
Lissotriton_montandoni SALAMANDRIDAE 1 1 0 0 44.3 6.84954 0.40343 1.00097271702249 18.01 49.08 5440.65738
Lissotriton_vulgaris SALAMANDRIDAE 1 1 0 0 40.64 477.10821 0.49586 3.12275362890843 12.68 50.17 5560.10994
Liua_shihi HYNOBIIDAE 1 0 0 0 74.5 1.90184 0.46805 -2.16672903516176 112.72 31.76 3515.12181
Liua_tsinpaensis HYNOBIIDAE 1 0 0 0 NA 0.4626 0.98566 -2.15710409247501 107.98 32.97 3651.90668
Lyciasalamandra_antalyana SALAMANDRIDAE 1 1 0 0 NA 0.0082 0.87956 0.0702606734829796 30.53 36.95 4092.17764
Lyciasalamandra_atifi SALAMANDRIDAE 1 0 0 0 NA 0.06764 0.92521 -0.404801198995063 31.65 36.91 4085.73984
Lyciasalamandra_billae SALAMANDRIDAE 1 1 0 0 NA 0.00235 0.89118 -0.55459873957409 30.55 36.75 4069.93979
Lyciasalamandra_fazilae SALAMANDRIDAE 1 0 0 0 NA 0.02654 0.68759 -0.297077689865278 28.89 36.7 4063.25848
Lyciasalamandra_flavimembris SALAMANDRIDAE 1 0 0 0 NA 0.01397 0.94316 -0.617948117682874 28.38 36.97 4092.43457
Lyciasalamandra_helverseni SALAMANDRIDAE 1 0 0 0 NA 0.01486 0.37749 -1.63684492119727 27.1 35.62 3941.80691
Lyciasalamandra_luschani SALAMANDRIDAE 1 0 0 0 NA 0.0689 0.64799 -0.236660114089408 29.73 36.21 4010.69794
Mertensiella_caucasica SALAMANDRIDAE 1 0 0 0 73.9 1.07153 0.59092 0.484151068375916 40.48 41.1 4550.91394
Mesotriton_alpestris SALAMANDRIDAE 1 0 1 0 47.06 74.51454 0.40639 3.10609435988295 9.9 45.4 5027.78105
Necturus_alabamensis PROTEIDAE 1 0 0 1 131.93 0.28044 0.96214 1.05763811117645 -86.99 33.88 3748.85086
Necturus_beyeri PROTEIDAE 1 0 0 1 137.92 13.73169 0.63375 2.82065664032642 -91.12 31.9 3530.89387
Necturus_lewisi PROTEIDAE 1 0 0 1 NA 1.07071 0.8597 2.62853042139746 -77.93 35.77 3962.40108
Necturus_maculosus PROTEIDAE 1 0 0 1 186.17 55.0534 0.32283 5.48798297117117 -84.43 44.12 4888.41418
Necturus_punctatus PROTEIDAE 1 0 0 1 126.1 6.39615 0.6963 1.80130941518731 -79.82 34.1 3773.87263
Neurergus_crocatus SALAMANDRIDAE 1 0 0 0 75.23 0.52295 0.99529 -1.09874500056745 44.34 37.34 4132.79516
Neurergus_kaiseri SALAMANDRIDAE 1 0 0 0 NA 0.00562 0.64499 -3.49780345420873 48.66 32.83 3634.86777
Neurergus_microspilotus SALAMANDRIDAE 1 0 0 0 NA 0.02118 0.90568 -2.69992006794119 46.37 35 3873.90088
Neurergus_strauchii SALAMANDRIDAE 1 0 0 0 74.3 0.70319 0.5759 -0.478924057944089 40.18 38.37 4247.52786
Notophthalmus_meridionalis SALAMANDRIDAE 1 0 0 0 44.4 2.75757 0.55532 1.37456834426237 -98.01 24.25 2682.26231
Notophthalmus_perstriatus SALAMANDRIDAE 1 0 1 0 43.25 2.00432 0.55501 2.5202395552224 -82.56 30.67 3394.06901
Notophthalmus_viridescens SALAMANDRIDAE 1 0 1 0 54.18 140.42648 0.51639 5.95617319842157 -84.24 44.16 4893.35
Nototriton_abscondens PLETHODONTIDAE 1 0 0 0 NA 0.02304 0.91734 2.22463032528952 -84.08 10.09 1116.7936
Nototriton_barbouri PLETHODONTIDAE 1 0 0 0 NA 0.01542 0.1426 1.11434049763599 -87.39 15.3 1691.54542
Nototriton_brodiei PLETHODONTIDAE 0 0 0 0 NA 0.00087 1 3.9109832844989 -88.7 15.37 1699.96827
Nototriton_gamezi PLETHODONTIDAE 0 0 0 0 NA 0.00013 1 -1.45174327341439 -84.77 10.31 1140.53879
Nototriton_guanacaste PLETHODONTIDAE 1 0 0 0 NA 9e-04 0.99995 -1.79743198598663 -85.46 10.95 1210.88079

Nototriton_lignicola PLETHODONTIDAE 0 0 0 0 NA 0.00228 0.99515 3.778335736942 -86.65 15.12 1671.62861
Nototriton_limnospectator PLETHODONTIDAE 0 0 0 0 NA 0.0025 0.99101 3.32014672872796 -88.13 14.91 1648.68021
Nototriton_major PLETHODONTIDAE 0 0 0 0 NA 0.00015 1 -1.27487316976297 -83.46 9.81 1085.3934
Nototriton_picadoi PLETHODONTIDAE 1 0 0 0 26.4 0.00641 0.82768 2.17951484581096 -83.92 9.74 1078.0489
Nototriton_richardi PLETHODONTIDAE 1 0 0 0 NA 0.00616 0.72474 2.85277077967733 -84.04 10.14 1122.36829
Nototriton_saslaya PLETHODONTIDAE 0 0 0 0 NA 0.00543 1 -0.195500765724885 -85.05 13.72 1517.61175
Nototriton_stuarti PLETHODONTIDAE 0 0 0 0 NA 0.00046 1 -1.09453988511579 -88.67 15.63 1728.71213
Nototriton_tapanti PLETHODONTIDAE 0 0 0 0 NA 0.00012 1 -0.0507086145722616 -83.78 9.77 1081.23994
Nyctanolis_pernix PLETHODONTIDAE 1 0 0 0 39.9 0.00399 0.03404 -0.0516546752142287 -90.82 15.63 1729.20759
Oedipina_alfaroi PLETHODONTIDAE 0 0 0 0 NA 0.14434 0.86496 1.18426581161269 -82.98 9.66 1068.45167
Oedipina_alleni PLETHODONTIDAE 0 0 0 0 NA 0.1354 0.49704 2.39274356045776 -83.43 8.77 970.28928
Oedipina_altura PLETHODONTIDAE 0 0 0 0 NA 0.00044 0.99916 0.754896794444308 -83.96 9.7 1073.65901
Oedipina_carablanca PLETHODONTIDAE 0 0 0 0 NA 0.01691 0.9104 1.66268600951378 -83.66 10.14 1122.08386
Oedipina_collaris PLETHODONTIDAE 0 0 0 0 NA 0.01972 0.00669 -0.138484704337027 -81.84 9.79 1082.3092
Oedipina_complex PLETHODONTIDAE 1 0 0 0 31.4 1.39629 0.22329 1.48852396641961 -78.74 6.26 692.48386
Oedipina_cyclocauda PLETHODONTIDAE 1 0 0 0 49.3 3.50774 0.58706 3.03989328452206 -84.67 13.66 1511.33582
Oedipina_elongata PLETHODONTIDAE 1 0 0 0 NA 0.25607 0.13084 1.81213031201887 -89.22 15.86 1754.70914
Oedipina_fortunensis PLETHODONTIDAE 0 0 0 0 NA NA NA NA NA NA NA
Oedipina_gephyra PLETHODONTIDAE 1 0 0 0 NA 0.00295 0.17263 0.0109904526145946 -87.08 15.55 1719.16156
Oedipina_gracilis PLETHODONTIDAE 0 0 0 0 NA 0.21807 0.44746 4.63614044698694 -84.03 10.13 1121.27125
Oedipina_grandis PLETHODONTIDAE 0 0 0 0 NA 0.00728 1 1.62487183152817 -82.77 8.97 992.00443
Oedipina_ignea PLETHODONTIDAE 0 0 0 0 NA 0.05675 0.23184 1.14696536082277 -89.65 14.43 1596.93116
Oedipina_maritima PLETHODONTIDAE 0 0 0 0 NA 5e-05 0.84109 -2.73449058760177 -81.56 9.1 1005.95554
Oedipina_pacificensis PLETHODONTIDAE 1 0 0 0 NA 0.18937 0.50498 2.56047826685608 -83.51 8.89 983.6262
Oedipina_parvipes PLETHODONTIDAE 1 0 0 0 39.75 0.94695 0.32871 1.83727502481653 -78.37 8.1 896.29126
Oedipina_paucidentata PLETHODONTIDAE 0 0 0 0 NA 6e-05 1 -0.156120148035346 -83.96 9.74 1078.08709
Oedipina_poelzi PLETHODONTIDAE 1 0 0 0 NA 0.01218 0.14301 1.75160558331706 -84.11 10.01 1107.90857
Oedipina_pseudouniformis PLETHODONTIDAE 1 0 0 0 NA 0.02557 0.01827 1.3800355632701 -84.55 11.16 1234.77905
Oedipina_savagei PLETHODONTIDAE 1 0 0 0 NA 0.00765 0.82036 1.73521943288767 -82.88 8.86 979.89704
Oedipina_stenopodia PLETHODONTIDAE 0 0 0 0 NA 0.01531 0.89774 2.75460025755463 -91.74 14.81 1637.69265
Oedipina_stuarti PLETHODONTIDAE 0 0 0 0 NA 0.00059 0.95734 -2.16644002824596 -87.64 13.27 1467.08327
Oedipina_taylori PLETHODONTIDAE 0 0 0 0 NA 0.21112 0.21668 1.37840383823473 -88.58 14.09 1558.25284
Oedipina_tomasi PLETHODONTIDAE 0 0 0 0 NA 0.00026 1 2.18846453880757 -88.23 15.51 1715.11442
Oedipina_uniformis PLETHODONTIDAE 1 0 0 0 49.8 0.17098 0.43426 3.17473134071866 -83.71 9.56 1057.9348
Ommatotriton_ophryticus SALAMANDRIDAE 1 0 0 0 NA 11.58421 0.44735 1.67442878350545 41.09 42.49 4706.30009
Ommatotriton_vittatus SALAMANDRIDAE 1 0 0 0 49 2.11442 0.25635 0.0487898601139652 35.68 33.11 3666.67929
Onychodactylus_fischeri HYNOBIIDAE 1 0 0 0 NA 15.04001 0.44572 -0.282983302107401 130.55 42.44 4699.79511
Onychodactylus_japonicus HYNOBIIDAE 0 0 0 0 64.5 6.29199 0.4286 0.441996070356014 134.97 35.05 3878.58838
Pachyhynobius_shangchengensis HYNOBIIDAE 1 0 0 0 107.65 0.43336 1 -2.00170933334232 115.71 31.26 3459.13496
Pachytriton_brevipes SALAMANDRIDAE 1 0 0 0 99.75 13.80449 0.75676 -0.95561311074485 115.47 26.46 2927.53555
Pachytriton_labiatus SALAMANDRIDAE 1 0 0 0 NA 7.03375 0.3566 -1.14194604283322 114.94 27.54 3047.93957
Paradactylodon_gorganensis HYNOBIIDAE 1 0 0 0 NA 0.00159 1 -4.2851521174316 55.03 36.95 4091.13962
Paradactylodon_mustersi HYNOBIIDAE 1 0 0 0 73.27 0.01116 1 -4.29660862724101 68.91 34.59 3827.58091
Paradactylodon_persicus HYNOBIIDAE 0 0 0 0 NA 1.57002 0.46057 -0.54702691947046 50.36 36.81 4073.98658
Paramesotriton_caudopunctatus SALAMANDRIDAE 1 0 0 0 68.6 1.151 0.41956 -1.81512078061812 109.66 26.5 2931.75479
Paramesotriton_chinensis SALAMANDRIDAE 1 0 0 0 64.25 7.10338 0.32098 -0.382134500270455 113.24 27.47 3040.4912
Paramesotriton_deloustali SALAMANDRIDAE 1 0 0 0 NA 0.67488 0.97478 -1.65913336748102 105.04 21.94 2426.18642
Paramesotriton_fuzhongensis SALAMANDRIDAE 1 0 0 0 NA 0.17469 0.77208 -3.14904811291763 111.31 24.74 2736.19438
Paramesotriton_guangxiensis SALAMANDRIDAE 1 0 0 0 NA 0.01281 1 NA 107.45 21.9 2423.7787
Paramesotriton_hongkongensis SALAMANDRIDAE 1 0 0 0 78.45 0.59735 0.75922 -2.78578922061937 113.83 22.36 2475.41574
Paramesotriton_laoensis SALAMANDRIDAE 1 0 0 0 NA 0.01532 0.38548 -4.92895188778667 102.89 18.99 2101.05292
Parvimolge_townsendi PLETHODONTIDAE 1 0 0 0 19.34 0.01906 0.99366 2.75447370095065 -97.01 19.15 2118.61791
Phaeognathus_hubrichti PLETHODONTIDAE 1 0 0 0 108 0.03277 0.37107 2.29923471951161 -86.84 31.6 3496.11129
Plethodon_ainsworthi PLETHODONTIDAE 0 0 0 0 NA 2e-05 1 -4.08713325095899 -89.28 31.95 3537.1643
Plethodon_albagula PLETHODONTIDAE 1 0 0 0 67.6 11.79631 0.56381 2.03658321024564 -95.46 32.53 3601.85396
Plethodon_amplus PLETHODONTIDAE 1 0 0 0 61 0.0153 0.99572 0.711873296919539 -82.37 35.49 3928.24746
Plethodon_angusticlavius PLETHODONTIDAE 1 0 0 0 43.4 2.47229 0.80116 2.48575293292773 -93.28 36.27 4013.93252
Plethodon_asupak PLETHODONTIDAE 0 0 0 0 NA 0.00299 0.67021 0.0183910056831882 -123.06 41.78 4625.35217
Plethodon_aureolus PLETHODONTIDAE 0 1 0 0 55.5 0.03194 1 1.97631831484796 -84.14 35.24 3903.40996
Plethodon_caddoensis PLETHODONTIDAE 1 0 0 0 43.7 0.02731 0.53204 0.766007279150384 -93.93 34.36 3802.46521
Plethodon_chattahoochee PLETHODONTIDAE 0 1 0 0 61.3 NA NA NA NA NA NA
Plethodon_cheoah PLETHODONTIDAE 1 1 0 0 52.5 0.00579 1 0.635614036165008 -83.56 35.35 3914.86715
Plethodon_chlorobryonis PLETHODONTIDAE 1 1 0 0 52.7 NA NA NA NA NA NA
Plethodon_cinereus PLETHODONTIDAE 1 0 0 0 45.3 89.73038 0.57891 3.93940796035351 -78.7 43.93 4866.67079
Plethodon_cylindraceus PLETHODONTIDAE 1 1 0 0 71 7.54525 0.79271 2.40332644686589 -79.71 36.36 4024.65181
Plethodon_dorsalis PLETHODONTIDAE 1 0 0 0 40.9 10.87515 0.6269 2.58344516784291 -88.2 35.71 3952.44788
Plethodon_dunni PLETHODONTIDAE 1 0 0 0 58.2 3.54307 0.75823 1.90361015128169 -123.89 43.95 4866.70467
Plethodon_electromorphus PLETHODONTIDAE 0 0 0 0 NA 4.72542 0.75434 2.46022701606903 -82.41 39.77 4403.1811
Plethodon_elongatus PLETHODONTIDAE 1 0 0 0 53.8 1.03243 0.80828 2.02643445520152 -123.64 41.78 4625.54842
Plethodon_fourchensis PLETHODONTIDAE 0 0 0 0 NA 0.00718 0.62673 1.14821873883812 -94.06 34.68 3838.06862

Plethodon_glutinosus PLETHODONTIDAE 1 1 0 0 68.5 50.15835 0.63016 3.32822877981206 -77.5 39.57 4383.01996
Plethodon_grobmani PLETHODONTIDAE 1 0 0 0 58.2 NA NA NA NA NA
Plethodon_hoffmani PLETHODONTIDAE 1 0 0 0 48.6 2.47032 0.63451 2.78790864073895 -79.06 39.34 4356.3033
Plethodon_hubrichti PLETHODONTIDAE 1 0 0 0 53.8 0.00193 0.94838 -1.02597605410737 -79.54 37.51 4152.45242
Plethodon_idahoensis PLETHODONTIDAE 1 0 0 0 44.1 4.39467 0.74704 1.94934997597321 -115.44 47.1 5217.45806
Plethodon_jordani PLETHODONTIDAE 1 1 0 0 53.9 0.0725 0.84714 1.73886695959088 -83.31 35.65 3947.58838
Plethodon_kentucki PLETHODONTIDAE 1 1 0 0 59.8 1.14236 0.67776 3.43642607858526 -82.55 37.52 4153.68927
Plethodon_kiamichi PLETHODONTIDAE 0 0 0 0 66.7 0.15443 0.86158 2.0514369109961 -94.7 34.9 3863.27275
Plethodon_kisatchie PLETHODONTIDAE 0 0 0 0 NA 0.96877 0.5502 1.0649432369621 -92.57 32.6 3607.02601
Plethodon_larselli PLETHODONTIDAE 0 0 0 0 NA 0.7973 0.41686 1.24240674186773 -121.37 46.2 5117.56114
Plethodon_meridianus PLETHODONTIDAE 1 0 0 0 61.5 0.033 0.96476 0.379454427061359 -81.69 35.61 3940.91243
Plethodon_metcalfi PLETHODONTIDAE 1 1 0 0 54.8 0.29229 0.70676 1.77118675123561 -82.66 35.15 3890.93956
Plethodon_mississippi PLETHODONTIDAE 1 0 0 0 64 NA NA NA NA NA
Plethodon_montanus PLETHODONTIDAE 1 1 0 0 55 0.26789 0.36607 1.8873778160647 -81.98 36.6 4050.94778
Plethodon_neomexicanus PLETHODONTIDAE 1 0 0 0 62.1 0.14116 1 -0.752837129344189 -106.52 35.93 3977.25241
Plethodon_nettingi PLETHODONTIDAE 1 0 0 0 45.1 0.05216 0.607 0.689289508272233 -79.63 38.78 4293.25502
Plethodon_ocmulgee PLETHODONTIDAE 0 0 0 0 59.3 NA NA NA NA NA
Plethodon_ouachitae PLETHODONTIDAE 1 0 0 0 55.2 0.04206 0.64427 1.40111503651593 -94.7 34.69 3839.98173
Plethodon_petraeus PLETHODONTIDAE 0 1 0 0 70.7 0.0121 1 0.669519708869079 -85.43 34.76 3847.5516
Plethodon_punctatus PLETHODONTIDAE 1 0 0 0 64.1 0.4503 0.79979 2.72905224965368 -78.93 38.69 4284.41079
Plethodon_richmondi PLETHODONTIDAE 1 0 0 0 52.5 3.93226 0.80743 3.85983951112455 -82.95 37.38 4138.81313
Plethodon_savannah PLETHODONTIDAE 0 0 0 0 NA NA NA NA NA NA
Plethodon_sequoyah PLETHODONTIDAE 0 0 0 0 64.7 0.00228 0.28606 -0.94600086254878 -94.49 34.17 3782.00902
Plethodon_serratus PLETHODONTIDAE 1 0 0 0 38.5 7.17189 0.25101 1.67680314082921 -91.25 34.39 3806.78834
Plethodon_shenandoah PLETHODONTIDAE 1 0 0 0 46.9 0.00985 1 -0.0407821885571293 -78.33 38.57 4272.44272
Plethodon_sherando PLETHODONTIDAE 0 0 0 0 NA 0.00144 0.8195 -1.17241449877181 -79.06 37.93 4199.82389
Plethodon_shermani PLETHODONTIDAE 1 1 0 0 55.1 0.03685 0.55658 1.31813400882875 -83.78 35.21 3899.87458
Plethodon_stormi PLETHODONTIDAE 0 0 0 0 NA 0.09403 0.89195 1.74256125212638 -123.16 41.95 4644.23727
Plethodon_teyahalee PLETHODONTIDAE 1 1 0 0 69.7 0.32189 0.49029 2.24691435416712 -83.23 35.29 3907.48525
Plethodon_vandykei PLETHODONTIDAE 1 0 0 0 53 1.00134 0.42717 3.88763617334914 -123.18 46.91 5195.17863
Plethodon_variolatus PLETHODONTIDAE 0 0 0 0 58.3 NA NA NA NA NA
Plethodon_vehiculum PLETHODONTIDAE 1 0 0 0 47.2 8.89318 0.51683 2.92826351104239 -123.73 48.15 5333.23016
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Plethodon_virginia PLETHODONTIDAE 0 0 0 0 NA 0.10046 0.886 1.97897505516087 -78.96 38.89 4306.54871
Plethodon_websteri PLETHODONTIDAE 0 0 0 0 NA 0.81665 0.09827 1.59206351525664 -87.37 33.14 3666.86854
Plethodon_wehrlei PLETHODONTIDAE 1 0 0 0 57.5 4.76293 0.48423 3.61096614063932 -82.3 37.69 4172.21624
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Plethodon_yonahlossee PLETHODONTIDAE 1 1 0 0 84.5 0.46641 0.74363 4.58607507382857 -81.7 36.26 4013.01424
Pleurodeles_nebulosus SALAMANDRIDAE 1 0 0 0 NA NA NA NA NA NA
Pleurodeles_poireti SALAMANDRIDAE 1 0 0 0 NA 0.07128 0.74775 -0.96516006328691 7.55 36.88 4082.54241
Pleurodeles_waltl SALAMANDRIDAE 1 0 0 0 90.43 15.24974 0.54494 1.23289627351204 -6.76 34.99 3874.22749
Proteus_anguinus PROTEIDAE 1 0 0 1 146.8 1.7206 0.55821 3.37829004186947 13.71 45.19 5004.86713
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Pseudoeurycea_gadovii PLETHODONTIDAE 1 0 0 0 27.6 0.02889 0.3954 0.777038083385649 -97.64 19.12 2114.6617
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Salamandra_lanzai SALAMANDRIDAE 1 0 0 0 NA 0.03731 0.85989 -0.484859357973876 6.97 44.91 4974.95808
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Thorius_munificus PLETHODONTIDAE 0 0 0 0 NA 0.00107 0.96974 -0.0522493884890259 -97.12 19.64 2172.73228
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Thorius_troglodytes PLETHODONTIDAE 1 0 0 0 NA 0.00426 0.87415 5.429167737533 -97.22 18.68 2066.35534

Triturus_carnifex SALAMANDRIDAE 1 1 0 0 NA 23.62558 0.19471 2.40145957749463 6.9 41.94 4645.24819
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Triturus_marmoratus SALAMANDRIDAE 1 1 0 0 NA 24.96202 0.61982 2.35711960584076 -1.62 41.9 4639.5946
Triturus_pygmaeus SALAMANDRIDAE 1 0 0 0 54.9 7.00939 0.57813 1.19794808518355 -3.21 38.69 4282.39747
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Tylototriton_hainanensis SALAMANDRIDAE 1 0 0 0 NA 0.05971 0.51822 -4.39540187523227 109.34 18.82 2081.72902
Tylototriton_kweichowensis SALAMANDRIDAE 1 0 0 0 87.12 1.06946 0.73906 -0.545484924139177 105.05 26.16 2893.40405
Tylototriton_taliangensis SALAMANDRIDAE 1 0 0 0 NA 0.85239 1 0.707498429909785 102.75 28.61 3166.84823
Tylototriton_verrucosus SALAMANDRIDAE 1 0 0 0 69.35 10.04101 0.30748 -1.51426539503156 96.83 22.41 2479.82321
Tylototriton_vietnamensis SALAMANDRIDAE 1 0 0 0 NA 0.83285 0.99011 -1.55397403916466 105.92 21.82 2413.18763
Tylototriton_wenxianensis SALAMANDRIDAE 1 0 0 0 NA 1.56004 0.1808 -1.58304560148096 107.57 29.83 3302.721

CHAPTER FOUR

GEOGRAPHIC VARIATION IN MOLECULAR SELECTION AMONG NATURALLY AND ARTIFICIALLY- SELECTED STRAINS OF AN EMERGING AMPHIBIAN PATHOGEN

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ABSTRACT

Emerging infectious diseases (EIDs) are often recognized as they increase in virulence, geographic range, or host-species range. When EIDs move into new host populations or are associated with wildlife trade, they are often faced with novel selection pressures. To test for variation in molecular selection among strains of an emerging ranavirus of tiger salamanders in western North America, we assembled a large sequence dataset. We explore whether coarse ecological differences among host populations are predictive of variation in forms of molecular selection. Model-selection analyses provide support for strong positive selection acting globally among strains on four *Ambystoma tigrinum* virus (ATV) genes, which are generally involved in evasion of host defense and recognition or in cooption of host resources. Further, we show that artificial selection imposed in captive-host populations alters the form of molecular selection among these genes in different ATV strains. In particular, we find elevated strengths of positive selection in a set of ATV strains associated with bait colonies of salamanders or in other captive-host environments. Functional-genomic study is needed to determine the relationship of genotype at these loci to phenotypic variation in performance among ATV strains, particularly with regard to virulence.

To dovetail prior work in the ATV-salamander system, we introduce a method for assessing host-pathogen codivergence. Results from these analyses support previous inferences of strong tree concordance in the system. One potential correlate of pathogen emergence is recent elevation in rates of lineage diversification, which we assess with phylogenetic methods. Pathogen emergence in this system appears decoupled from lineage diversification but may be driven by altered selective regimes imposed on ATV in captive-host environments. Given evidence of increased virulence associated with bait-associated strains of ATV, we argue that increased frequencies of tiger salamander epizootics in the western US are at least in part attributable to a relaxed virulence-transmission tradeoff in ATV-bait assemblages.

INTRODUCTION

Emerging infectious diseases pose a major threat to human, wildlife, and livestock health (Daszak et al. 2000; Smith et al. 2008). Although some pathogens can play important roles in maintaining community diversity and ecosystem function (McCallum & Dobson 1995; Hudson et al. 2002; Lafferty et al. 2006), other pathogens may threaten biodiversity by causing host extinction, especially in perturbed systems (Hudson & Greenman 1998; de Castro & Bolker 2005; Smith et al. 2006). Outcomes of host-pathogen coevolutionary dynamics ranging from stable coexistence to local extinction can result from variation in ecological and genetic characteristics among host populations (Benkman 1999; Nuismer et al. 2000; Gomulkiewicz et al. 2000; Brodie et al. 2002; Lively et al. 2004; Thompson 1994, 2005).

As EIDs move to new host populations, geographic variation in the host genotypes can differentially drive pathogen evolution. Pathogens can evolve quickly, due to high mutation rates, large effective population sizes and short generation times, as in the case of widespread antibiotic resistance among bacterial species or the evolution of HIV in a single, infected patient (Holmes 2009). Increasingly, EIDs are associated with wildlife trade (Smith et al. 2008), which may impose artificial selection on pathogen phenotypic traits. For example, a ranavirus of tiger salamanders collected from bait shops was shown to be more virulent than those collected from natural populations (Storfer et al. 2007). In this case, it was argued that the unnaturally high densities of bait salamander populations imposed artificial selection on virulence evolution, since viral transmission was essentially guaranteed in the bait populations. This is in contrast to the expected virulence-transmission tradeoff, whereby high virulence typically causes host damage and reduces opportunities for transmission (Lenski and May 1994; Bull 1994; Bull and Ebert 2008). Thus, maintenance of EIDs in captive populations may alter inherent fitness tradeoffs that exist in natural populations. Further, large-scale geographic movements of EIDs into new host populations can also affect evolutionary trajectories of pathogens and and (or) their epidemiology. As an example, movement of cattle throughout Britain is associated with high incidence of tuberculosis (Woolhouse 2005). Taken together, these modern influences on emerging pathogens can affect their molecular evolution, resulting in spatial variability in adaptive genetic variation.

Recent analyses have shown that viral pathogens are more likely to emerge than fungi, bacteria, or helminths (Cleaveland et al. 2001). In an analysis of 335 recent disease emergence events, 85 were attributable to viruses, and most emergences occurred in developed countries (Jones et al. 2008; Woolhouse 2008). Aside from emergence of drug resistant bacterial strains, which is expected to be higher in developed relative to under-developed countries, disease emergence has also been attributed directly to development, which may alter host-pathogen ecological relationships (Cleaveland et al. 2001; Woolhouse et al. 2005). Nipah virus provides an excellent example, whereby deforestation causes carrier fruit bats to forage farther and with increasing frequency into human-inhabited areas, where they drop partially eaten fruit into pig pens (Chua et al. 2000). As the virus is typically vectored in bat saliva, pigs infected by eating partially eaten pieces of fruit may harbor the virus, which can become infectious to humans (Chua et al. 2000). It is this unique combination of events resulting from ecological alteration of natural host-pathogen dynamics that has led to disease emergence in this system (Epstein et al. 2006).

Study System

Recent changes in development, and particularly increases in aquaculture and wildlife trade has led to emergence of ranaviruses (Iridoviridae: *Ranavirus*), globally distributed pathogens of poikilothermic vertebrates and insects (Chinchar 2002; Storfer et al. 2007; Greer and Collins 2008). Ranaviruses have recently been classified as emerging and notifiable disease agents due to increases in incidence, virulence and geographic range (Chinchar 2002; OIE 2008). A group of ranaviruses, collectively referred to as ATV, are the proximal cause of *Ambystoma tigrinum* epizootics primarily along the western cordillera of North America (see Fig. 1; Jancovich et al. 1997, 2005; Green et al. 2002; Storfer et al. 2007).

Although recent work has shown that there are viral host-switching events resulting from human movement of infected bait salamanders (Storfer et al. 2007; Picco and Collins 2008), several independent lines of evidence suggest that tiger salamanders and ATV are coevolving in most locations. First, when three host switches attributable to bait movement are excluded from analyses, there is complete phylogenetic concordance in shared splits between salamander and virus trees and a strong correlation of

nodal depths (Storfer et al. 2007), providing at the very least support for codivergence (see Huelsenbeck and Rannala 1997; Page 2003). Second, there is an inverse correlation between ATV-induced disease prevalence and cannibalism rates among salamander populations throughout Arizona (Pfennig et al. 1991). A fitness cost to cannibalistic morphs is enhanced risk of acquiring ATV from conspecifics (Pfennig et al. 1991; Bolker et al. 2008). Common garden experiments suggest these patterns are genetically-based and not phenotypically-plastic responses to varying exposure to ATV. Rather, frequencies of cannibalism appear to result from past selective pressures (Parris et al. 2005), consistent with the interpretation by Storfer et al. (2007) that ATV is largely an endemic pathogen of tiger salamanders. Third, tiger salamanders appear to be the principal host and reservoir of ATV (Jancovich et al. 2001) and are often the only suitable hosts inhabiting breeding ponds where epizootics have been documented. It follows, then, that tiger salamander populations exert the primary extrinsic selective force on ATV, and accordingly, molecular variation in genes under selection should be driven by localized variation in host-ATV ecological dynamics. Indeed, previous work has shown spatial molecular variation among putative ATV virulence genes whereby different ATV lineages exhibit different signatures of positive and (or) purifying selection (Ridenhour and Storfer 2008).

The present study expands on previous molecular work, as well as evidence of increased virulence among host switch strains relative to coevolved ATV strains (Storfer et al. 2007). By expanding the number of ATV strains and putative viral-performance genes sequenced, we use a comparative phylogenetic framework to: 1) more thoroughly evaluate host-pathogen concordance and identify host-switch versus endemic ATV strains; 2) test for molecular selection among nine putative virulence genes identified in the genomic sequence of ATV (Jancovich et al. 2003; Chinchar 2006); 3) test whether patterns of molecular selection on these nine genes differ between human-associated ATV strains and strains isolated from natural *A. tigrinum* populations; and, 4) test whether phylogenetic analyses can provide evidence of pathogen emergence.

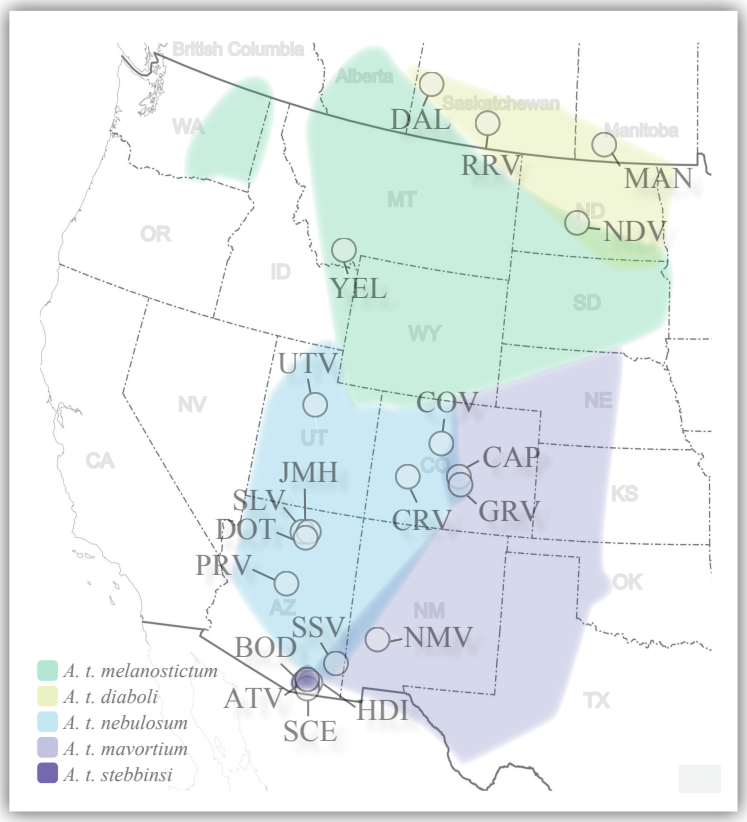

FIGURE 1: Distribution of 22 strains of ATV, overlain on distributional ranges of western subspecies of *Ambystoma tigrinum* (colored patches). Overlapping colors denote contact zones, of which there are several: between *A. t. stebbinsi* and both *A. t. nebulosum* and *A. t. mavortium*; between *A. t. mavortium* and *A. t. nebulosum*; and between *A. t. melanostictum* and *A .t. diaboli*.

METHODS

Virus culture and DNA amplification

FHM (fat-head minnow) or EPC (*Epithelioma papilloma cyprini*) cells were used for virus propagation in Eagle's minimum essential medium (MEM; Cellgro, USA) supplemented with 2% fetal bovine serum (FBS; HyClone, USA). Procedures followed Jancovich et al. (2001, 2005). EPC cells were infected with ATV at 18°C for 1 hour and culture flasks (75 cm2) were rocked every 10 min. Upon viral infection, media was supplemented with penicillin-streptomycin (1%) and fungizone (0.1%). After one hour, MEM with 10% FBS was added to the flasks, and cells were incubated at 18°C–21°C. When the cell monolayer was completely lysed, virus and infected cells were harvested and processed for PCR.

After three freeze-thaw cycles to release virions from cells, centrifugation (15 min at 2500*g*) was performed to pellet culture cells and debris. A modified PCI (pheno-chloroform-isoamyl alcohol) extraction was initially used to recover viral DNA. Samples of 2.0 mL supernatant were treated with 6 µL proteinase K (20 mg/mL) and incubated overnight at 37°C; RNase A (2 µL) treatment and incubation at 37°C for 2 h followed. In each step after chemical addition, samples were centrifuged for 12 min at 2500*g*; the aqueous layer was retained for each subsequent step in the PCI extraction. Samples were initially extracted with 2.0 mL phenol (pH 8.0); the aqueous layer was further extracted with 2.0 mL phenol/chloroform/isoamyl alcohol (25:24:1); lastly, the retained sample was extracted with 2.0 mL chloroform/isoamyl alcohol (24:1). DNA was precipitated by addition of 200 µL sodium acetate (3 M, pH 5.2) and two volumes pure ethanol (2-4 mL). After centrifugation (30 min at 2500*g*), samples were set on ice for 10 min. Alcohol and acetate were removed, and pellets were washed with 70% ethanol. Pellets were air dried, and DNA was resuspended in Tris-EDTA buffer (pH 8.0).

RCA (rolling circle amplification; Fire and Xu 1995) was performed with a Qiagen (Valencia, CA) REPLI-g mini kit to attempt to uniformly amplify genomic DNA. Using supplementary protocol (RG14) from Qiagen, QIAamp® DNA mini and QIAtip®-20 kits were used for DNA purification, resulted in obtainment of 3-5 µg of genomic DNA per strain. Quantitation of DNA concentration was performed using NanoDrop™ spectrophotometry (Thermo Fisher Scientific, Waltham, MA).

DNA Sequencing

Capillary sequencing on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA) was performed for nine genes identified by Chinchar (2002) as genetic factors strongly contributing to viral performance: 1) a viral ortholog of eukaryotic translation-initiation factor, eif2-α (*EIF*[1]), putatively involved in host interferon downregulation (Essbauer et al. 2001; Majji et al. 2006); 2) a tumor necrosis factor (*TNF*); 3) RNase III (*RNA*), involved in binding and cleaving dsRNA and possibly in RNA interference; 4) a β-hydroxy steroid oxidoreductase (*BOH*[1]), involved in host immune suppression via corticosteroid upregulation (Reading et al. 2003); 5) a caspase recruitment domain (*CRD*[1]) reputedly involved in downregulation of apoptosis, thereby increasing host cell longevity for virus replication

(Bouchier-Hayes and Martin 2002); 6) a ribonucleotide reductase (*RDR*) and 7) a deoxynucleoside kinase (*DNK*), both of which are implicated in utilization of host dTTP for viral DNA synthesis; 8) a dUTPase (*UTP*); and 9) a DNA methyltransferase gene (*DMT*), which is involved in methylation of the viral genome to protection from exogenous digestion and is potentially involved in viral recombination. *DMT* also likely prevents host Toll-like receptor-9 activation, which plays a fundamental role in pathogen recognition and activation of innate immunity. Involvement of these genes in pathogenesis has been verified with knockout experiments in ATV (B. Jacobs, pers. comm.), other ranaviruses (*EIF*; Majji et al. 2006), closely related poxviruses (*BOH*; Reading et al. 2003), or herpes viruses (*UTP*; Cattone et al. 2002). Knockout strains were less virulent than were intact, wildtype strains. The functional roles of *RDR* and *DNK* are widely conserved among many viruses and are targets for antinucleoside drug-therapies, such as those used to combat infection from human immunodeficiency virus.

Superscripted genes above were previously sequenced by Ridenhour and Storfer (2008). We performed PCR amplification of these gene regions under the thermal conditions and with the primers as found in Appendix 1. Dataset alignment was conducted in CLUSTALX (vers. 2.0.12; Thompson et al. 1997; Larkin et al. 2007).

Phylogeny Estimation

An aligned length of ~12.6 kb was used for initial phylogeny estimation. A set of credible chronograms for ATV was generated in BEAST (vers. 1.5.0; Drummond and Rambaut 2007). In BEAST, we implemented a relaxed molecular-clock method with log-normally distributed evolutionary rates, under a coalescent model of constant 'population' size, to conduct tree searches (Drummond et al. 2006). Nearly all priors were uninformative and were left as default; however, following Yang (1996) and Sullivan and Swofford (2001), the proportion of invariant sites was constrained to be zero. Because our molecular-selection analyses were insensitive to absolute divergence dates, the root node of ATV was arbitrarily constrained to be 1.0 time units in the past. In total, MCMC sampling involved $5x10^7$ generations, with subsampling every 10^4 generations. The first 201 subsamples were discarded as burnin from each chain, well after stationarity had been reached, as assessed qualitatively by change in

likelihood scores using TRACER (vers. 1.4; Drummond and Rambaut 2007). To assess sampling convergence, five independent MCMC runs were used.

Owing to our a priori expectations for signatures of positive selection in our dataset as observed for *BOH* and *CRD* in Ridenhour and Storfer (2008), and in light of the possibility for positively selected sites to confound phylogeny estimation (Drummond and Suchard 2008), we pruned the nucleotide dataset after conducting positive-selection analyses (described below; hereafter termed the 'nearly-neutral dataset'). These initial molecular-selection analyses were based on coding regions of the nine putative performance genes; trees for these analyses were based on the entire sequence dataset, ~12.6 kb. Taking a conservative approach, we culled from the sequence alignment nucleotide sites with strong signatures of positive selection and those for which patterns of molecular selection could not be evaluated. The reduced dataset included ~5.9 kb aligned sites. Phylogeny estimation was repeated as described above, and our final analyses of molecular selection were based on trees estimated from this nearly-neutral dataset.

Tests of tree concordance between host and virus

Based on strong support for codivergence between ATV and associated tiger salamander populations (Storfer et al. 2007), we developed a preliminary estimate of the evolutionary timeframe for ATV by using the evolutionary history of the host species. Following Shaffer et al. (2004), we used a calibration point of 5 MYA to represent the divergence time between California tiger salamanders (*Ambystoma californiense*) and their closest extant relatives, including tiger salamanders (*A. tigrinum*). This calibration corresponds to the timing for the secondary orogenic event of the Sierra Nevada range in California (Wakabayashi and Sawyer 2001) and presumed evolutionary catalyst initiating the divergence of *A. californiense* from *A. tigrinum* (Shaffer and McKnight 1996). We used BEAST to generate a distribution of credible crown group ages for tiger salamanders, from the nucleotide dataset provided by Storfer et al. (2007) in addition to several other sequences (*A. californiense*: AY682357 and DQ241128; *A. mexicanum*: NC_005797; *A. dumerilii*: NC_006889; and *A. laterale*: NC_006330). Two independent runs, each of $2x10^7$ generations with retained subsamples every 10^3 generations, were conducted. The

first 5001 trees from both runs were discarded as burn-in. Data from 11 populations of tiger salamanders, for which we had a corresponding viral strain, were available from Storfer et al. (2007). We used the distribution of root node-heights for *A. tigrinum* as proxy for the crown-group age-distribution for ATV.

To test tree concordance of the expanded dataset herein, we used the Paradis et al. (2004) package APE (v. 2.4) to compute tree distances between host and virus phylogenies. Virus and host trees were pruned to leave only corresponding pairs for which we had data for both virus and salamander. Both sets of trees had 11 tips each (CRV, BOD, JMH, DOT, SLV, COV, UTV, RRV, NDV, MAN, and SSV; each virus sequence was matched with a corresponding exemplar from syntopic tiger salamander populations). We further pruned taxa that were inferred to result from host-switching events (BOD, COV, and SSV: see Storfer et al. 2007). In APE, we implemented the Billera et al. (2001) method for tree comparison, accounting for unshared bifurcations as well differences in node heights. To develop a null distribution of tree distances under no evolutionary association of the two taxa, we initially generated $2 \cdot 10^3$ random coalescent trees in APE, each with eight tips. Using this set of trees, 10^4 tree distances between randomly selected coalescent trees were used to construct the null distribution. We then sampled a total of 10^3 draws at random from the posterior distributions of ATV and tiger salamander trees; we again used 10^4 pairings to construct the empirical distribution of tree distance between host and virus. An additional test of topological congruence, presumably more stringent, used the following rationale: under strict codivergence, topological variation *between* the posterior distributions of trees for two putatively codiverged taxa should not exceed the topological variation *within* either posterior distribution. To construct a distribution of tree distance under this rationale, tree distances were computed from draws *within* the posterior density of trees for either ATV or tiger salamanders. From each of the two posterior distributions, $5 \cdot 10^3$ comparisons were used to construct a null distribution of tree distances under an assumption of strict codivergence.

To standardize trees for tree-distance comparisons, simulated and empirical trees were all scaled such that the height of the root node was 1.0. Congruence of the distributions was assessed by non-parametric randomization ('NPR') tests. For NPR tests, values drawn at random from each tree-distance

distribution were compared. If distributions are congruent, the collection of differences should not depart from a mean of 0. We interpreted the quantile at which differences were centered at 0 to be an approximate p-value. That is, if nine-tenths of comparisons with the null distribution were larger than expected, $p_{rand} = 0.1$. These analyses resulted in discovery of an additional apparent host switch: UTV.

Assessing molecular selection

Given the observed phenotypic variation among ATV strains (Storfer et al. 2007), and previously documented molecular variation (Ridenhour and Storfer 2008), we expect signatures of molecular selection to vary among ATV strains as a result of ecological differences among host populations. For tests of molecular selection, we used the highest clade-credibility tree from the posterior distribution for ATV (see previous section). To test whether whole genes and amino acid sites within genes exhibit strong signatures of molecular selection, we used PAML (vers. 4.3; Yang 2007). We derived posterior probabilities of amino-acid sites having experienced substantially strong positive selection using both Bayes-empirical (BEB; Yang et al. 2005) and naïve-empirical Bayesian approaches (NEB; Yang 2007). The strength of molecular selection (ω) ranges from 0 to $+\infty$. Values of ω close to zero are characteristic of sites under strong purifying selection, values indistinguishable from unity suggest neutral evolution, and values greater than unity suggest sites are under positive selection.

As a gene-wide test of positive selection (Nielsen and Yang 1998), we compared statistical fit of a 'nearly-neutral' model ('m_1') of codon evolution to a parametrically richer 'positive-selection' model ('m_2'). The nearly-neutral model involves two codon-classes ($0 < \omega_R < 1$; $\omega_N = 1$), whereas the positive selection model additionally allows some codons with $\omega_S > 1$. These models are so-called *site* models insofar as different branches in a phylogeny are expected to draw from the same distributions of ω. We used hierarchical likelihood-ratio tests and Bayes-empirical Bayesian approaches (BEB) to test for positive selection across the nine genes in the dataset. We interpreted substantial support for positive selection if either hLRTs were significant (α=0.05) or if BEB posterior probabilities (p_{pos}) for at least two codons were $\geq$0.80. The use of these criteria in combination, hLRT and p_{pos}, seemed to partition genes by a natural break in statistical support for positive selection (Table 1). To test for the signature of purifying

selection in the dataset, we also used hLRTs to assess whether the 'nearly-neutral' model was significantly different from a null model ('m_0') with only one site class ($\omega_N = 1$).

Tests of selection in host-switch versus coevolved strains

We conducted a pair of complementary tests to determine whether human-associated strains of ATV evolve differently from naturally coevolved (i.e., endemic) strains. For these tests of molecular selection, we partitioned the dataset into two subsets: i) genes with a global signature of positive selection (from *site* models as described above); and ii) genes that either exhibited no departure from neutrality or had only a signature of purifying selection. In each case, the branch-site model was compared to m_1, a nearly-neutral site model described above.

Branch-site models were implemented in PAML to test whether human-associated strains face different selective pressures relative to other strains. We classified strains, *a priori*, into two groups: bait and non-bait strains. Previous work suggests host-switches from bait-associated strains for BOD, COV, and SSV (Jancovich et al. 2005; Storfer et al. 2007). We also included two known human-associated strains not included in previous analyses – AXO (isolated from the Indiana University axolotl colony, a source of salamanders for scientific research) and ORV (isolated from a Phoenix bait shop). We hypothesize that these six (BOD, COV, SSV, UTV, AXO and ORV) human-associated strains (referred to as 'bait strains,' for simplicity) are under different molecular selection pressures due to selection for increased virulence in unnaturally high-density host populations; these expectations are supported by previous experiments (Storfer et al. 2007).

Testing recent emergence of ATV

We develop a method to test evidence of ATV emergence by evaluating topological structure of phylogenetic trees. We first estimated relative extinction rates (ε) for a sample of 1000 trees from the posterior distribution of ATV trees. This analysis was conducting in the R-package LASER (v. 2.3; Rabosky 2009; see also Nee et al. 1994). In GEIGER (v. 1.3-1; Harmon et al. 2008, 2009), we then simulated trees under the diversification conditions, sampling from the empirical distribution of ε-estimates and constraining tree size to be consistent with our empirical dataset. To explore the extent of

departure from uniformity in diversification rate of ATV, we estimated the γ-statistic of Pybus and Harvey (2000) for the set of simulated trees and for the sample of trees from the posterior distribution of ATV. The observed and null γ-distributions were compared for congruence using NPR procedures. A potentially confounding phenomenon for this method is referred to as 'pull of the recent' (Nee et al. 1994), where extinction is expected to leave a predictable topological signature. Temporally recent branches (i.e., 'tips') of a molecular phylogeny are necessarily unsampled by extinction, thus giving the appearance of rapid lineage-accumulation toward the present if extinction is nontrivial in the true evolutionary history. This issue was avoided on account of the null distribution of γ-values having been generated from trees with rates of relative extinction drawn from the empirical distribution of ε for observed ATV trees. We thus distinguish phylogenetic from epidemiological emergence, with the former *potentially* underlying the latter. Differences in observed and expected γ-values were compared by a NPR test, as previously described.

RESULTS

Of the 5863 base pairs from Ridenhour and Storfer (2008) and an additional 6736 nucleotides sequenced here, 269 sites are parsimony-informative and 202 others are unique among 22 strains of ATV. Upon pruning the dataset of positively-selected sites, the phylogeny estimate changed little with respect to topology or branch lengths (summary tree from the nearly-neutral dataset is shown as Fig. 2). While positively selected codons were necessarily polymorphic, the remainder of the dataset appeared to contain sufficient phylogenetic signal so as to not be overwhelmed by potentially misleading sites under positive selection. Most internal relationships are well-supported ($p_{pos} > 0.95$), and in general the summary topology is quite consistent with previous phylogenetic estimates (i.e., Storfer et al. 2007; Ridenhour and Storfer 2008). Most of the more tipward relationships are congruent with those reported by Ridenhour and Storfer (2008), although some deeper relationships are somewhat inconsistent. Many of these inconsistencies involve nodes that receive little support here ($p_{pos} < 0.70$) or in the summary tree of Ridenhour and Storfer (2008; ML bootstrap support < 80%).

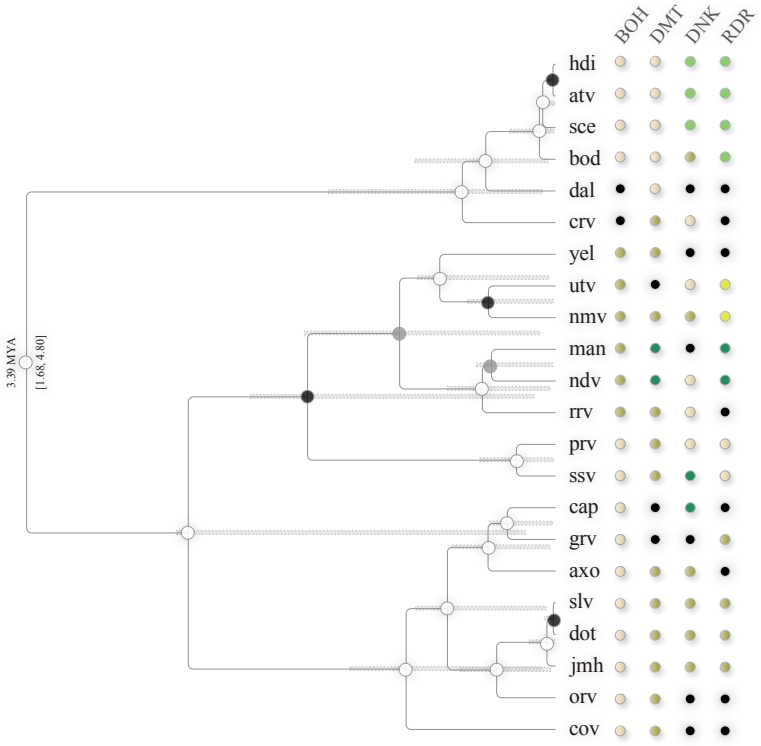

FIGURE 2: Ultrametric summary of Bayesian phylogeny estimate for ATV viral strains based on sequences from 9 open reading frames associated with viral host immune evasion. The dataset used for phylogeny estimation in BEAST was culled of positively-selected codons as described in text. Open circles denote posterior probabilities (p_{pos}) ranging from 0.95 to 1.00; shaded circles, from 0.70 to 0.94 p_{pos}; and for closed circles, 0.50 to 0.69 p_{pos}. For all but the root node, bars indicate highest posterior densities of node heights (95% HPD). If calibrated by presumed divergence of tiger salamanders, the root node of the tree is estimated to occur in the range indicated with brackets, with a mean crown-group age for ATV at 3.39 MYA. Indicated to the right of the tree are amino acid sequences for four immune evasion genes determined to be subject to substantial positive selection as estimated in PAML. A black dot indicates that a viral strain has a unique amino acid sequence for that gene. Dots of the same color within columns indicate identical amino acid sequences shared by those viral isolates. As shown in the pattern of colored circles, two sets of *multigene* amino acid sequences are identical among multiple viral strains: (JMH, DOT, and SLV) and (HDI, ATV, and SCE).

Tests of tree concordance between host and virus

When host-switching events are pruned from the ATV and salamander trees, distributions of empirical tree-distances (i.e., between the collections of virus and host trees) compared with those generated by comparing random coalescent trees were incongruent (p_{rand} = 0.0055; Fig. 4). This result provides support for a non-random degree of concordance between the phylogenies of ATV and *A. tigrinum*. Similarly, the observed distribution of tree distances did not significantly depart from expectations under a scenario of strict codivergence (p_{rand} = 0.1085; Fig. 4). In accord with results of Storfer et al. (2007), we suggest that these two results in combination provide substantial support for tree concordance between host and virus trees.

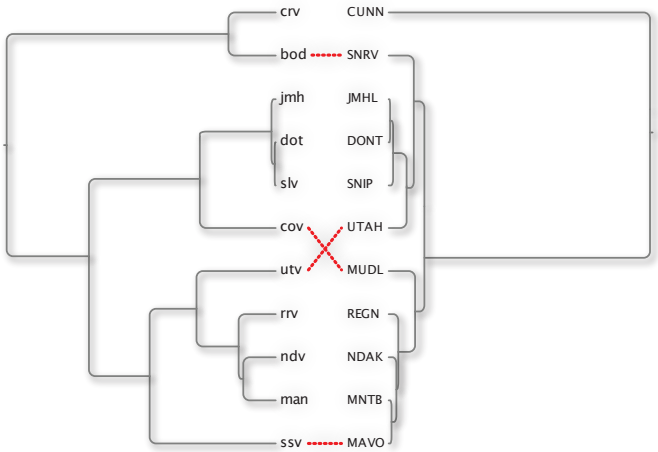

FIGURE 3: Comparison of virus (leftward) and host (rightward) summary trees. Inferred host-switches are indicated with red, dotted connections between a syntopic pair of ATV and tiger salamander. Evidence for *BOD*, *COV*, and *SSV* as host-switch strains is provided by Storfer et al. (2007) and supported by these trees; support for a host-switch involving *UTV* is provided herein (see Fig. 3). Depicted trees are summaries of the two posterior distributions, pruned to leave tips that correspond between the two datasets. Branch lengths are proportional to time.

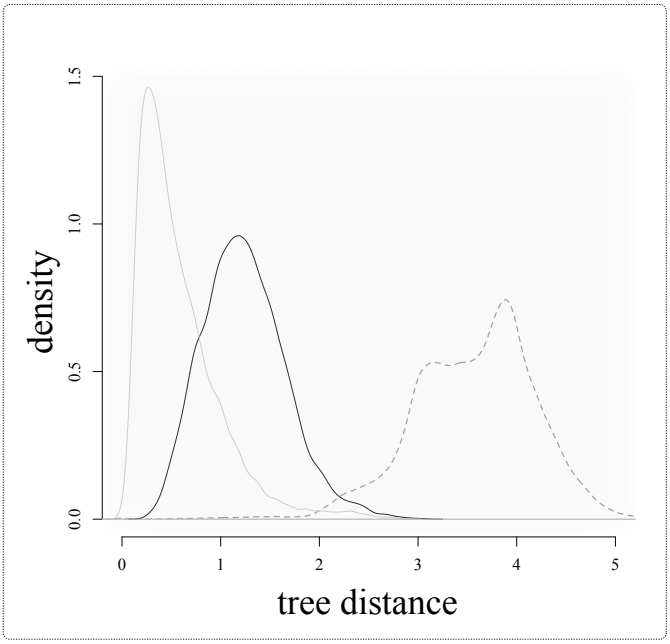

FIGURE 4: A test of topological concordance between ATV and tiger salamander trees. Two methods were used to generate a null distribution of expected tree distances: represented by the dashed line, a collection of random coalescent trees were simulated and compared for resulting tree distances; in the leftmost gray distribution, tree distances from *within* each posterior distribution of trees were collected. The resulting distributions were compared to tree distances computed for random draws from *between* the posterior distribution of trees for ATV and tiger salamanders (black distribution), after lineages resulting from likely human-mediated host-switches had been removed. Results provide evidence for codivergence between these taxa. Comparisons between the empirical (black) distribution with the dashed (rightmost) distribution suggest topological similarity between ATV and salamander trees is significantly greater than expected under an assumption of independent evolution. Comparisons between the empirical and gray distributions suggest that tree distances between the phylogenies for ATV and *A. tigrinum* do not significantly depart from strict codivergence. See text for further detail.

Assessing molecular selection

Using hierarchical likelihood-ratio tests and Bayes-empirical Bayesian approaches, we provide evidence for a positive selection within *BOH*, *DMT*, *DNK,* and *RDR* (Table 1; Fig. 2). We interpreted support for positive selection to be substantial if either hLRTs were significant (α=0.05) or if posterior probabilities (p_{pos}) for particular amino-acid sites from the BEB approach were equal to or in excess of 0.80. For analyses of purifying selection, hLRTs were implemented and compared the 'nearly-neutral' model against a null model with only one site class ($\omega_N = 1$). We observed signatures of purifying selection for *DMT, DNK, EIF, RDR, and RNA*. Results were fairly consistent with Ridenhour and Storfer (2008) for the three markers investigated therein: *BOH*, *CRD*, and *EIF*. Two of these three genes (*BOH* and *CRD*) were found to be under strong positive selection, whereas *EIF* appeared under strong purifying selection (Ridenhour and Storfer 2008); results here are congruent with the exception that neither hLRT nor posterior probabilities provide strong support for positive selection within *CRD* (Table 1).

TABLE 1. Molecular selection results from PAML. Site-model parameters included $0 < \omega_R < 1$; $\omega_N = 1$; and $\omega_S > 1$, where ω signifies the strength of molecular selection. m_0: a neutral model (involving ω_N only); m_1: a nearly-neutral model (involving ω_R and ω_N); m_2: a positive selection model (involving ω_R, ω_N, and ω_S). '*BEB'*: codons with posterior probabilities (in parentheses) of positive selection greater than or equal to 0.50, assessed under the Bayes-empirical Bayesian approach (Yang 2005). '*p.purSel'*: result of hLRT comparing m_0 and m_1; small *p*-values provide evidence for purifying selection. '*p.posSel'*: result of hierarchical likelihood ratio test (hLRT) between m_1 and m_2. Small *p*-values provide evidence for positive selection. '*-lnL'*: log-likelihood values under each model. Rightmost column provides interpretations for each gene in regard to positive and purifying selection. If an amino acid site had a BEB posterior probability in excess of 0.80 but had a non-significant hLRT (*p.posSel*), we interpret this result to be weak evidence of positive selection within the locus.

locus	BEB	*p*.purSel	*p*.posSel	*-lnL*(m0)	*-lnL*(m1)	*-lnL*(m2)	interpretation
beta-hydroxy oxidoreductase (BOH)	24 (0.50); 34 (0.85); 39 (0.85); 43 (0.85)	0.2923	0.0147	265.3	264.1	259.8	positive selection
caspase recruitment domain (CRD)	35 (0.60)	0.6662	0.8262	481.4	481.0	480.8	no departure from neutralality
cytosine DNA-methyltransferase (DMT)	81 (0.84); 153 (0.84)	0.0000	0.1981	1034.2	1023.2	1021.6	purifying & weak positive selection
deoxynucleotide kinase (DNK)	44 (0.96); 45 (0.98)	0.0000	0.0182	1062.6	1045.8	1041.8	purifying & positive selection
eukaryotic initiation factor (EIF)		0.0000	0.9990	1165.6	1153.1	1153.1	purifying selection
ribonucleoside-diphosphate reductase (RDR)	204 (0.81); 243 (0.80); 381 (0.79); 443 (0.79); 552 (0.94)	0.0000	0.0990	2826.1	2814.8	2812.5	purifying & weak positive selection
ribonuclease III (RNA)		0.0000	0.9998	1714.6	1704.5	1704.5	purifying selection
tumor-necrosis factor receptor (TNF)		0.1562	1.0000	372.9	371.0	371.0	no departure from neutralality
deoxyuridine triphosphatase (UTP)	34 (0.69)	0.2390	0.4728	676.7	675.3	674.5	no departure from neutralality

Tests of selection in host-switch versus coevolved strains

Using genes with global signatures of positive selection from preceding analyses (*BOH*, *DMT*, *DNK*, and *RDR;* Table 1), support for statistical separability of ω_S parameters by strain type (i.e., bait

versus non-bait) was lent by likelihood ratio test in a branch-site analysis of molecular selection ($hLRT_{3df}$; $p = 0.0068$). This test compared the branch-site model (where ω_S was independently estimated for bait and non-bait strains) to a nearly-neutral substitution model (m_1) where *all* branches in the tree were assumed to have the same proportion of sites under ω_R and ω_N and the same strength of ω_R. Under the branch-site model, estimated strengths of positively-selected sites (ω_S) were markedly different between the two groups of ATV strains (for bait strains, $\omega_S = 63.7$; for non-bait strains, $\omega_S = 5.3$), although less than one percent of codons in the dataset were estimated to be under positive selection. Proportions (f_ω) and selective strengths (ω) for the remaining two site-classes were as follows: $f_{\omega R} = 0.799$ and $\omega_R = 0.0$; $f_{\omega N} = 0.198$ and $\omega_N = 1.0$.

Support for the branch-site model was non-significant for the dataset comprising the remaining five genes, all of which appeared nearly neutral in the global site-model analysis ($hLRT_{3df}$; $p = 0.3548$; see Table 1). Despite this result, branch-site estimates revealed large selective strengths for human-associated strains ($\omega_S = 171.0$), compared to the freely estimated ω_S for strains not associated with humans ($\omega_S = 0.0$).

Testing recent emergence of ATV

Accounting for the effect that extinction has in producing large and positive γ-estimates, we find that while expected values of γ were slightly lower than those observed for ATV, this result was non-significant ($p_{rand} = 0.339$; see Fig. 5). This result suggests that lineage diversification of ATV cannot be distinguished from a temporally constant process. The tendency for γ-estimates for ATV to be positive seems to result solely from a high degree of lineage turnover in ATV (Figs. 5 & 6). Estimated rates of relative extinction (ε) tend to be close to unity, suggesting that rates of lineage splitting (λ) are nearly matched by rates of lineage extinction (μ), where $\varepsilon = \mu / \lambda$ (Nee 2001). Mean and median estimates for relative extinction were $\varepsilon = 0.79$ and $\varepsilon = 0.85$, respectively (Fig. 6).

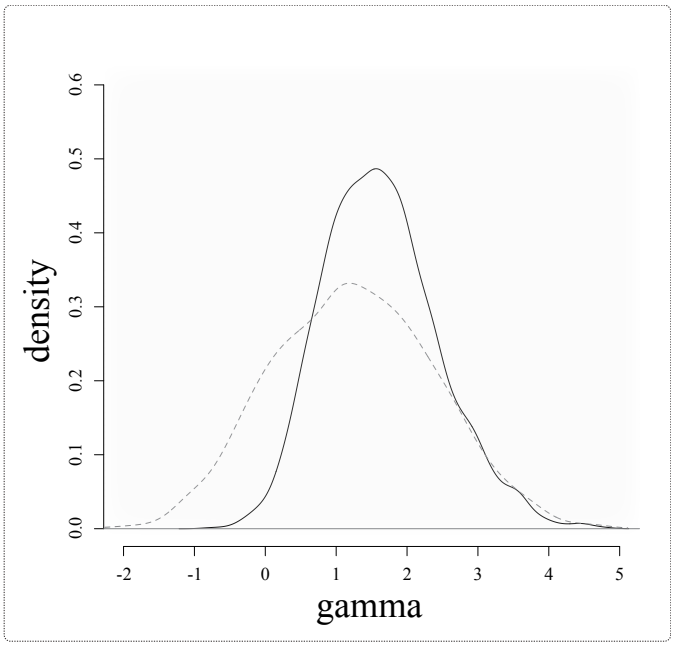

FIGURE 5: A test of rate constancy of diversification through time. Dashed function is a density plot generated by simulation under uniform rate of diversification (and where diversification parameters were estimated from our empirical dataset). The mean γ of the null distribution is greater than 0 as a result of simulations having been conducted where magnitude of relative extinction was estimated from the empirical set of trees, whose γ-density plot is shown in the dark, unbroken function. The means of the depicted distributions do not differ significantly, suggesting that any apparent departure from uniform diversification in ATV is likely attributable to pull-of-the-recent.

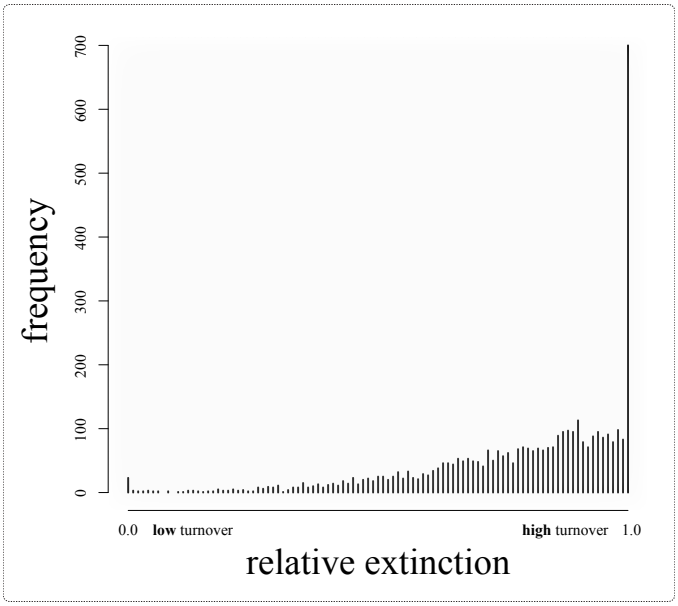

FIGURE 6: Frequency distribution of rates of lineage turnover across the set of posterior trees for ATV. Relative extinction (ε) is the ratio of rates of lineage extinction relative to lineage splitting; high values of turnover approach $\varepsilon \sim 1$. By contrast, a pattern consistent with a pure-birth process of diversification (i.e., where the signature of extinction is negligible) would be supported where $\varepsilon \sim 0$.

DISCUSSION

Emerging infectious diseases are often recognized when they increase in geographic range or switch to new host species (Daszak et al. 2000; Smith et al. 2008). Geographic or host range expansions pose evolutionary challenges for pathogens to adapt in new host populations. Novel host-pathogen phylogenetic concordance analyses on our expanded dataset show a high degree of concordance, with ATV undergoing four host-switches, consistent with results in Storfer et al. (2007). We also show spatial variation in molecular selection in putative virulence genes for strains of *Ambystoma tigrinum* virus, a primary source of epizootics amongst western tiger salamander populations (Chinchar 2002; Jancovich et al. 2005). As predicted, response to selection experienced by human-associated strains differs from endemic strains, potentially resulting in emergence of highly virulent strains via maintenance within high-density tiger salamander bait colonies. In introducing a novel method for testing for deviations from a temporally-uniform rate of diversification, we fail to show lineage accumulation increasing toward the present for ATV as might be expected of an emerging pathogen. As such, we can conclude that ecological variation among host populations seems to be driving recent ATV evolution. The emergence of this iridovirus thus appears not to be underlain by recent lineage diversification but possibly through repeated introductions of ATV strains that experienced artificial selective regimes in human-maintained populations of hosts.

Tree Concordance

Tree concordance analyses and inferred substitution rates support an apparent long duration of association between ATV and tiger salamanders, on the order of millions of years. Maximal sequence divergence (uncorrected *p*-distance) was ~0.745% per lineage for ATV. If time is bounded by the time-calibrated salamander phylogeny (1.68 to 4.80 MYA), the range of molecular evolutionary rates for ATV would be 4.7×10^{-9} to 1.3×10^{-8} substitutions per codon per year for each lineage, which is within but at the lower end of the range reported by Duffy et al. (2008) for dsDNA viruses. Despite the consistency of inferred rates of molecular evolution in ATV with those of other dsDNA viruses, abundant evidence is provided for rate variation through time and among branches of a phylogeny (Lopez et al. 2002; Kumar

2005). The degree to which rates may vary through time and among lineages is currently understudied in this system and perhaps more generally (e.g., see Ho et al. 2005, 2007). While historically, rates of substitution may be quite low for ATV, these values are not likely to characterize lineages for their entire evolutionary history. Indeed, we expect changes in selective pressures faced by human-associated strains to have occurred quite recently. If tree height for ATV is truly on the order of millions of years, rates of directional evolution would need to be extreme in the very recent period of salamander-human contact in order to be detectable in our branch-site analyses of molecular selection (see Results).

Analyses based on our nearly-neutral dataset suggest a high degree of host-pathogen phylogenetic concordance with four host switches. One additional host switch was discovered (in strain UTV; Fig. 3) relative to the three (SSV, COV, BOD) found in previous analyses of a smaller (1.5 kb) dataset (Storfer et al. 2007). Whereas movement of viruses in the formerly discovered three host-switch localities can be attributed directly or indirectly to human movement of bait salamanders (Storfer et al. 2004, 2007), direct evidence of bait movement into Utah is lacking. Nonetheless, Picco and Collins (2008) have documented extensive commercial movement of salamanders by those involved with the bait trade, and anglers frequently release unused bait into ponds, which are not often where the salamanders originated.

When host-switching events are pruned from the tree, observed tree-distances between the putatively codiverged taxa are substantially smaller than expected under random coalescence (p_{rand} = 0.0055; Fig. 4). Paired with inferences made from topological distances from trees generated under necessarily perfect concordance, these tests in combination are likely to provide a rigorous means by which hypotheses of codivergence can be assessed. For the case of ATV, observed tree distances did not significantly depart from expectations under perfect tree concordance (p_{rand} = 0.1085; Fig. 4). These results appear to support strong inference for codivergence between ATV and *A. tigrinum*.

Our implemented method of tree concordance does not explicitly account for topological variation that is expected to result from the coalescent process (e.g., Kingman 1982). We may thus expect many circumstances where two taxa exhibit incongruous gene-trees but would be consistent with a true history of codivergence. If due solely to the coalescent process, we expect most of the branch-length

inconsistencies to be associated with short internal branch-lengths in the true 'codivergent' tree (i.e., analogous to the distinction between the set of gene trees contained within a single species tree; e.g., Maddison and Knowles 2006). Even if the *topologies* are markedly different as a result of coalescent processes operating in each taxon, this method is expected to perform powerfully (Billera et al. 2001; e.g., Fig. 3). For instance, consider the Newick formats of three trees, where roman letters signify species and branch lengths follow colons: τ_1 ((a:1.9, b:1.9):0.1, c:2); τ_2 ((a:1, b:1):1, c:2); τ_3 ((a:1.9, c:1.9):0.1, b:2). In terms of tree distances computed under the Billera et al. (2001) method, τ_1 and τ_3 are most concordant, despite differing in topology. We feel this is a great advantage of this implementation in the context of the coalescent, although we caution that it is most meaningful when comparing ultrametric trees, where branch-lengths are related to time since divergence.

Molecular Evolution

In addition to investigating evolutionary history between salamanders and ATV, an additional goal of these analyses was to distinguish endemic ATV strains (as indicated by nodes of concordance with the host phylogeny) with host-switch strains for comparisons of molecular evolution. A total of six ATV strains (four host-switch strains and two human associated strains – AXO from the Indiana University axolotl colony and ORV isolated from a Phoenix bait shop) were considered human-associated strains in comparisons of molecular evolution with 'endemic' strains (the remainder of ATV strains analyzed herein).

Selection varied geographically among ATV strains. Five of the nine genes showed evidence of either (or both) purifying selection or positive selection (Table 1). Specifically, we found *BOH* under positive selection; this gene is associated with corticosteroid upregulation and thereby suppression of lymphocyte proliferation in other viruses (Reading et al. 2003). Four haplotypes were observed for *BOH* overall, and notably two unique haplotypes were found in locations DAL (Saskatchewan, Canada) and CRV (Colorado, USA). These two locations share several amino acid substitutions that are distinct from the remaining strains and may suggest different selection processes on this gene in those particular host populations (Fig. 2). Interestingly, the *DMT* gene, associated with viral-genome methylation and Toll-like

receptor 9 downregulation in the host, had six haplotypes overall, with unique haplotypes in three host populations (UTV, CAP, GRV) that differed from those where amino-acid sequences in *BOH* are unique. Ribonucleotide reductase (*RDR*) and *DNK*, both of which are implicated in utilization of host dTTP for viral DNA synthesis, showed the most variation, with six and eight unique haplotypes, unshared by any other strain, respectively.

When observing data across all four genes that showed evidence of positive selection, the virus isolated from host population DAL showed the most unique haplotypes (three of four), which may suggest coevolution is tightest in this host population (Fig. 2). Several isolates showed two unique haplotypes (CRV, YEL, CAP, GRV, ORV, COV), four isolates showed one (UTV, MAN, RRV, AXO), and several showed none (HDI, SCE, ATV, BOD, NMV, PRV, SSV, SLV, DOT, JMH). These unique haplotypes do not appear phylogenetically structured, which may suggest local variation in host selection on virus strains. Future work may test the relationship between molecular signatures within these genes and their correlation with viral performance and fitness.

Although four of the nine genes did not show evidence of departure from neutrality, significant likelihood ratio tests, however, appear related to gene length. Notably, signatures for three of the four shortest genes were indistinguishable from neutrality. Rather than an indication of functional or adaptive insignificance of these shorter genes, results may be attributable to insufficient statistical power to detect signatures of selection for these genetic loci. This could potentially be remedied by increasing sample size of ATV strains analyzed.

Selection among ATV strains varied with respect to coarse ecological differences among host populations. Strengths of positive selection differed significantly between human-associated virus strains and naturally coevolved strains, as identified by phylogenetic concordance analyses. Estimates of strong positive selection for human-associated ATV strains, by virtue of large estimated ω_S-values (see Results), may be attributable to several potentially complimentary mechanisms. First, tiger salamanders are maintained under high density and are often replenished in cases of mortality in bait shops (Storfer et al. 2007; see also Picco and Collins 2008). Second, there may be more viral genetic variation in animal

facilities maintained by humans, providing the necessary material upon which selection can act. If strains from distinct areas are found in the same bait-shop, multiple infections may result, which could enable recombination among distantly related strains and the origination of novel variation. Further, although prevalence of ATV in bait-shops varies seasonally (Picco and Collins 2008), viral reproduction may be more continuous in thermally controlled aquaculture of bait colonies, supporting larger and relatively stable population sizes of viruses. If constraints on adaptive evolution of ATV exist in natural populations of hosts, such constraints may be somewhat relaxed in colonies of bait salamanders. That is, with abnormally high rates of transmission to susceptible hosts, the selective regime of ATV may be altered due to interstrain competition or relaxation of the virulence-transmission tradeoff (Lenski and May 1994; Ebert and Bull 2008). Especially virulent strains may be favored in these circumstances because viral transmission in captive environments is virtually guaranteed (Lively 2009).

Interstrain competition is unlikely, however, because Picco and Collins (2008) find no molecular evidence for multiple strains present in any particular bait-shop using fairly extensive sampling (~30 salamanders per bait-shop per month). In fact in many cases, shared alleles from both major capsid protein (495 bp) and a region including a 16-bp polymorphism (207 bp) were observed across several different bait-shops within three western states. While more substantial genomic sampling would be required to wholly characterize the genetic structure of ATV and their evolution in bait-shops, current evidence would not appear to support the competition hypotheses as underlying elevated ω_S in bait-associated strains. Extensive work needs to be carried out to explore the possibility of multiple infection, strain diversity, and short-term evolution of this pathogen within both bait-shops and natural populations of tiger salamanders. Since none of the human-associated strains used herein were *isolated* from bait colonies of tiger salamanders, we cannot determine whether the modification in the form of selection has occurred either (or both) in the transition into an artificial assemblage of hosts (i.e., as within a bait-shop) or upon reentry into non-captive tiger salamander populations.

We suspect that the anthropogenic upset of the epidemiology of this system, accredited to the commercial trade of salamanders, may be a principal source of epizootics. Many emerging diseases have

been attributed to human-induced ecological change, such as agricultural development (Cleaveland et al. 2001) and wildlife trade (Jones et al. 2008). The tiger salamander-ATV system, in a sense, combines both of these factors. Tiger salamander breeding ponds in western North America are often in landscapes characterized as cattle grazing areas, with salamander breeding occurring in human-dug earthen cattle drinking ponds (Collins et al. 1988). These landscapes are easily accessible by roads, which may influence movement of bait salamanders. Accordingly, a previous study showed that hybridization of the principal bait salamander subspecies (*A. t. mavortium*) with an endemic and endangered subspecies (*A. t. stebbinsi*) was more likely to occur proximal to roads in south-central Arizona (Storfer et al. 2004). Although variable in prevalence, ATV has been detected in more than four out of every five bait-shops in Arizona in recent years (Picco and Collins 2008). Accelerated molecular evolutionary rates of human-associated ATV strains, coupled with typical maintenance of high densities in captive environments and consequent selection for increased virulence are cause for concern regarding long distance movement of bait salamanders that may be infected with ATV. Indeed, movement of bait salamanders appears to have compromised the genetic integrity of native and federally threatened California tiger salamanders (*A. californiense*; Fitzpatrick and Shaffer 2006) as well as endangered Sonoran tiger salamanders (*A. t. stebbinsi*; Storfer et al. 2004).

In general, EIDs are increasingly recognized as agents that can lead to extinction (DeCastro and Bolker 2005), as in the amphibian chytrid fungus (Stuart et al. 2004; Fisher et al. 2009). In fact, one study suggests EIDs are now the sixth leading cause of extinction (Smith et al. 2006). Although density-dependent pathogens such as ATV are not predicted to drive host populations to extinction, artificially selected high virulence in captive host populations may lead to "short-sighted virulence" (Levin and Bull 1994) and potentially localized tiger salamander extirpations. While single base-pair substitutions can underlie drastic changes in virulence (Bull 1994), functional genomic study of ATV is needed to illuminate the connection between molecular and phenotypic evolution in this system.

Emergence

While for this empirical dataset we cannot distinguish pathogen emergence for ATV from an effect of high relative extinction (Figs. 5-6), we nonetheless provide a tree-based test of accelerating lineage accumulation while accounting for a known artifact afflicting phylogenetic analysis of extant taxa. That is, the anticipated problem of the 'pull of the recent' (see Nee et al. 1994) is accounted for in our analyses by simulating a null distribution of the test statistic under a constant diversification process that is informed by the empirical data (i.e., γ, Pybus and Harvey 2000). While a bias toward large negative γ-values is known to afflict analyses testing for negative departures of γ from zero, we do not expect the same problem to arise in this method. Because incomplete sampling is likely to drive more *negative* γ-values (Harmon et al. 2008), we expect the method presented herein to be robust to incomplete sampling in that we focus on the opposite tail of the γ-distribution. Simulation work is needed to clarify the power of this test. It may well be that under biologically plausible scenarios, the ability to detect accelerating lineage accumulation is limited (e.g., see Nee 2001; Rabosky and Lovette 2008).

For this system, however, we interpret a lack of a phylogenetic signal of pathogen emergence as consistent with recent ecological changes that have occurred. As discussed previously, recent agricultural development, captive breeding of salamanders and consequent artificial selection and introductions has likely altered the evolutionary dynamics of ATV. As such, recent epidemiological emergence of ATV seems driven by a combination of environmental or genetic cofactors and appears dissociated from the process of diversification in ATV.

CONCLUSIONS

Our results document evidence of: 1) an apparent long coevolutionary history of western tiger salamander subspecies and ATV overall, with recent host-switching events attributable to movement of infected salamanders as fishing bait; 2) variation in selection signatures within putative host immune evasion genes among ATV strains isolated from different host populations; 3) greater strengths of positive selection in human-associated ATV strains relative to endemic (i.e., coevolved) strains; and, 4) a lack of

phylogenetic signal of recent ATV diversification, consistent with recent changes in ecological selection pressures. Similar to other EIDs, ATV has experienced artificial selection through the bait trade and long distance movements by humans, possibly resulting in higher frequencies and potentially greater severities of epizootics.

Overall, spatial and localized variation in genes under selection may help explain observed variation in phenotypic qualities (e.g, transmissibility, virulence, infectivity) among strains of ATV. An apparent shift in selective regime experienced by human-associated strains may provide some direction for understanding the magnitudes of virulence exhibited by strains having diverged in bait colonies. Functional genomic work of this pathogen would benefit our understanding of the link between amino acid substitutions and phenotypic traits, particularly in the evolution of virulence.

locus	forward.primer	reverse.primer	ATVref.from	ATVref.to	gene.length	amp.length	anneal. (ºC)
deoxynucleotide kinase (DNK)	CAAAGATGCTGGTAACCCGT	GTATTGAAGACGTGGGCGAT	22479	23066	587	1388	58.5
cytosine DNA-methyltransferase (DMT)	TCCCACAGCATTGTGTTTGT	GAACGGTCTAACGGGACAGA	24268	24912	644	1403	58.5
ribonuclease III (RNA)	GTGTTGAAAATTATCGGCGG	CGATAGCAATAAAGACATCCACA	27224	28345	1121	1415	55.0
tumor-necrosis factor receptor (TNF)	GGTGTCTGACCCCGTTAGAG	CCATCCCCTGTCTTTTCTCA	31637	31888	251	463	55.0
deoxyuridine triphosphatase (UTP)	CTCCAAAGTTTTGCATGGCT	TTTTACTTTGATGCCGTCCC	40631	41068	437	655	55.0
ribonucleoside-diphosphate reductase (RDR)	CAGGAGGAGGTGACTGCTCT	CGCCTTTAGGGTGTCGTAGT	74878	76575	1697	1985	58.5

APPENDIX 1. Primer sequences for genes amplified and sequenced herein. '*ATVref.from*' and '*ATVref.to*': refer to primed region corresponding to genomic positions of the Genbank ATV reference sequence (NCBI: NC_005832). '*gene.length*' refers to the length (bp) of the open reading frame; '*amp.length*' denotes length (bp) of fragment amplified through PCR between primers. '*anneal.*' corresponds to annealing temperature in degrees Celsius. Gene lengths for the remaining three loci (BOH, CRD, and EIF; from Storfer and Ridenhour 2008) were 162, 288, and 780 bp, respectively.

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