

HOW DOES ECOLOGICAL OPPORTUNITY INFLUENCE RATES OF SPECIATION, EXTINCTION, AND MORPHOLOGICAL DIVERSIFICATION IN NEW WORLD RATSNAKES (TRIBE LAMPROPELTINI)?

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Ecological adaptive radiation theory predicts an increase in both morphological and specific diversification when organisms colonize new environments. Accordingly, bursts of morphological diversification, characterized by low within-subclade morphological disparity, may be associated with these increases in speciation rates. Conversely, increasing species density, reduction in available habitat, or increasing extinction rates are expected to cause rates of diversification to decline. We test these hypotheses by examining the tempo and mode of speciation in the lampropeltinine snakes, a morphologically variable group that colonized the New World ~24 million years ago and radiated throughout the Miocene. We show that specific diversification increased early in the history of the group, and that most morphological variation is partitioned among, rather than within subclades. These patterns provide further evidence for the hypothesis that morphological variation tends to be strongly partitioned among lineages when clades undergo early bursts of species diversification. A reduction in speciation rates may be indicative of density dependent effects due to a saturation of available ecological opportunity, rather than increases in extinction rates at the onset of the Pleistocene/Pliocene glacial cycles. This evidence runs counter to the general Pleistocene species pump model.

KEY WORDS: Density dependent models, diversification, ecological opportunity, morphological disparity.

Understanding the tempo and mode of diversification, in terms of both speciation and the evolution of morphological variation, are long-standing interests in biology (Simpson 1944, 1953; Raup and Jablonski 1985; Nee et al. 1994; Paradis 1997; Harmon et al. 2003; Nee 2006; Rabosky 2006a; Rabosky and Lovette 2008a,b). Studies of fossil taxa have commonly recovered a pat-

tern in which explosive bursts of speciation follow mass extinctions. The subsequent invasion of novel adaptive zones free from competitors is typically correlated with increased morphological variation (a prediction of the ecological opportunity hypothesis; Simpson 1953; Stanley 1973; Sepkoski 1998; Schlüter 2000, Rabosky et al. 2007). Molecular studies have corroborated paleontological data in this regard, showing that rapid cladogenesis often occurs early in the evolutionary history of a group, followed by a significant decline in the rate of

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diversification (Lovette and Bermingham 1999; Harmon et al. 2003; Kozak et al. 2005; Ruber and Zardoya 2005; Weir 2006; Harmon et al. 2008a; Phillipore and Price 2008; Rabosky and Lovette 2008; Rawlings et al. 2008).

The most common pattern, in which speciation is highest early in the history of the group, is often followed by a decline in diversification rate (Simpson 1953; Stanley 1973; Carlquist 1974; Sepkoski 1998; Lovette and Bermingham 1999; Harmon et al. 2003; Kozak et al. 2006; Weir 2006; Quental and Marshall 2009). Several hypotheses have been proposed to explain this trend. First, a decrease in speciation rates may be caused by saturation of the available ecological niche space, the inverse of the ecological opportunity hypothesis (Walker and Valentine 1984; Valentine 1985; Schlüter 2000; Rabosky and Lovette 2008b). Early rapid diversification is associated with greater ecological opportunity, and the decline in rates is caused by a shrinking of available ecological space as it becomes filled with new taxa. Therefore, an increase in species richness should be inversely associated with a decrease in speciation rate, described by density-dependent speciation models (e.g., Nee et al. 1992; Rabosky and Lovette 2008a). Alternatively, a decline in diversification rate could be due to an increase in extinction rate through time, which would yield a similar pattern to a decrease in speciation rate (Ricklefs and Cox 1972; Levinton 1979; Hubbell 2000; Paradis 2004; Rabosky and Lovette 2008a,b; Quental and Marshall 2009). Both of these explanations may produce the same net diversification (i.e., the difference in speciation and extinction; Rabosky and Lovette 2008a). However, several studies on birds and lizards indicated that speciation rates have decreased due to density-dependent effects without noticeable increases in extinction rates (Weir 2006; Phillipore and Price 2008; Rabosky and Lovette 2008a).

Combining measures of morphological variation with rates of species diversification may help clarify how trait diversity accumulates throughout the history of a clade. Although rules for the rates of specific and morphological diversification have been theorized for only a few organisms, several general trends have been noted. Early specific diversification may be accompanied by large ecomorphological changes (Foote 1997; Schlüter 2000; Harmon et al. 2003; Kozak et al. 2005). Alternatively, morphological convergence may occur consistently throughout the history of a clade, despite changes in rates of speciation (Jackman et al. 1997; Rüber and Adams 2001). Finally, morphological variance may increase after speciation rates have declined, indicating that a stable species assemblage has undergone subsequent ecomorphological specialization (Schlüter 2000).

Explicit evidence for a relationship between morphological diversification and speciation rates are lacking across a wide range of organisms. Nevertheless, it is predicted that bursts of nonoverlapping morphological divergence will typically occur early in the history of the group, if speciation rates are also highest early

(Harmon et al. 2003; Kozak et al. 2005). Subsequently, in the absence of increased extinction rates, declining speciation rates and declining morphological variation should be associated with a deficit of ecological opportunity.

Accordingly, we would expect that species invading new areas (e.g., islands, continents) will show a rapid burst of speciation in accordance with the ecological opportunity hypothesis, which would also be associated with large ecomorphological changes. Although a substantial number of organisms, including insects, birds, frogs, snakes, and lizards, dispersed from the Old World (OW) into North America (Sanmartín et al. 2001; Macey et al. 2006; Burbrink and Lawson 2007; Pramuk et al. 2008; Wüster et al. 2008), their patterns of specific and morphological diversification are lacking. We expect that colonization of the New World (NW) would be associated with a signature of early rapid diversification and low subclade morphological disparity. We evaluate this hypothesis using a well-studied group of snakes, the NW ratsnakes (tribe Lampropeltini).

The lampropeltinines represent many of the most common and widely recognized snakes in North America, including the ratsnakes (*Pantherophis*), pinesnakes (*Pituophis*), kingsnakes, and milksnakes (*Lampropeltis*). This group, composed of nine genera and 31 recognized species, has several useful attributes for examining both specific and morphological diversification through time. Previous research (Burbrink and Lawson 2007; Pyron and Burbrink 2009a,b) has demonstrated that the common ancestor of this group dispersed from the OW through Beringia to the NW approximately 24 million years ago (mya). The NW snake fauna was fairly depauperate at this time (Holman 2000), suggesting that the NW represented a relatively open niche space for advanced snakes (the Colubroidea). Currently, lampropeltinines inhabit most terrestrial habitats in the North America, including temperate and subtropical forests, grasslands, and deserts (Pyron and Burbrink 2009b). Morphological diversity in these snakes is high, with mean body sizes ranging from 33–139 cm, while possessing a large variety of color patterns and diets (Pyron and Burbrink 2009c). Lampropeltinines typically inhabit large species assemblages, with communities harboring up to 10 species of lampropeltinines, representing up to 37% of all local snake taxa in areas such as western Texas (Werler and Dixon 2000).

We test several aspects of the ecological opportunity hypothesis as it relates to rates of speciation, extinction, and morphological variation. To examine hypotheses regarding rates of diversification and morphological change through time, we first determine whether variation in the diversification rate of the lampropeltinines is consistent with elevated early rates of cladogenesis following their invasion into the NW, and a reduction in speciation rate toward the present as ecological opportunity decreased. Second, we examine models of speciation to determine if density dependence or increases in extinction rates account for a

Table 1. Log likelihood ($-\ln L$) and AIC scores for three sets of models examining diversification rates in lampropeltinine snakes. The first set of models examines the rate constant (Yule, birth-death) and rate variable models (DDX, DDL, Yule2, and Yule3). The second set is based on survival models with a single rate (A), monotonically decreasing or increasing rates following Weibull's Law (B), and two rates with a 5.2 mya breakpoint (C). The third set permits speciation variability (SPVAR), extinction variability (EXVAR), and both speciation and extinction variability (BOTHVAR).

	Rate constant and rate variable models						Survival models			Variable spec./ext. models		
	Yule	Birth-death	DDX	DDL	Yule2	Yule3	A	B	C	SPVAR	EXVAR	BOTHVAR
							(Weibull)	(5.2 mya)				
$-\ln L$	-24.32	-24.32	-21.68	-19.73	-18.44	-17.37	-102.47	-95.97	-95.38	-21.89	-24.36	-21.88
AIC	50.65	52.65	47.36	43.46	42.87	44.74	206.94	195.94	194.77	49.78	54.73	51.77
ΔAIC	7.77	9.78	4.49	0.59	0.00	1.87	12.17	1.17	0.00	0.00	4.95	1.99

reduction in speciation rates. Finally, we test the prediction that an increase in ecological opportunity following the initial colonization of the NW resulted in an increase in rates of morphological evolution.

Material and Methods

PHYLOGENY AND DIVERGENCE TIME ESTIMATES

Analyses conducted here are based on the phylogenetic estimate and associated divergence times presented by Pyron and Burbrink (2009a). In that study, the 31 commonly recognized extant species of lampropeltinines were used as terminal taxa and sequenced for six mitochondrial genes (12-S, COI, cyt-b, ND1, ND2, ND4) and four fragments of three nuclear genes (c-mos, Vimentin introns 4 and 5, SPTBN1), yielding 8294 bp of sequence data per species. Branch lengths and tree topology were estimated using Maximum Likelihood (ML) analysis in the program RaxML version 7.0.3 (Stamatakis 2006), and the Bayesian divergence time estimates were performed in the program BEAST version 1.4.8 (Drummond and Rambaut 2007). The nominate subspecies *L. t. triangulum* was used as the terminal taxon to represent the very diverse *L. triangulum* complex in the phylogenetic analyses of Pyron and Burbrink (2009a). In the analyses here, the morphological measurements are for *L. t. triangulum* only.

MORPHOLOGICAL DATA

To examine hypotheses related to changes in morphological variation through time, we measured four primary variables related to dimension in head shape and body size in snakes (body size [SVL], head width [HW], jaw length [JL], and head height [HH]) for 728 specimens, comprising 29 of the 31 extant species of lampropeltinine species and *Coronella austriaca*. We were unable to directly measure cranial morphology for any specimens of *L. ruthveni* and *P. vertebralis*, however, SVL measurements for both taxa were obtained from published works (Blanchard [1921] and Stull [1940], respectively), yielding a total of 757 specimens (Table 1). Allometry was corrected by regressing HH/SVL against

SVL, and removing samples with ratios less than the asymptote (Burbrink 2001). All values were log-transformed prior to analysis, and principal components were obtained using the program Statistica version 6.1 (Statsoft, Inc. 2003). Mean principal components for the first two axes were obtained for all species and used in disparity through time analyses. These axes contained at least 95% of all variation and were extracted using the correlation matrix with varimax rotation.

LINEAGE DIVERSIFICATION THROUGH TIME

To understand the tempo of speciation in the Lampropeltini, we examined various speciation models that address variation in diversification rate through time. All tests of diversification described in this section were conducted using the packages Ape (Paradis et al. 2004) and Laser (Rabosky 2006b) in the statistical program R 2.9 (The R Foundation for Statistical Computing 2009). First, we compared lineage through time plots against a null Yule process model, and tested if diversification occurred early or later in time using Pybus and Harvey's γ statistic (Pybus and Harvey 2000) against a null distribution composed of 5000 samples under a constant rate, pure birth diversification model. Although our tree putatively represents all recognized species, it is likely that yet undiscovered taxa exist, particularly because recent phylogeographic studies have demonstrated that a few widespread species were actually composed of multiple taxa (Burbrink et al. 2000; Rodríguez-Robles and De Jesús-Escobar 2000; Burbrink 2002; Pyron and Burbrink 2009c). Because incomplete taxon sampling may significantly impact γ , we tested our value against a null distribution of 5000 samples, assuming that our tree represents 65%, 75%, 85%, 95%, and 100% of the total species of lampropeltinines.

To determine if rates of speciation change through time in these snakes or if density-dependent effects alter rates of speciation, we evaluated several models of diversification. We calculated Akaike's information criterion (AIC) values for the Yule and birth-death rate-constant models (RC) and compared these to rate-variable (RV) models that account for exponential

density-dependent effects (DDX) or linear density-dependent effects (DDL) and two different rate-shift Yule models (Yule2 and Yule3). Additionally, we calculated the difference in AIC values between the best-fit RC and RV models using our real tree and tested this against 1000 pure Yule trees (using a maximum likelihood estimate [MLE] of birth rate from our real tree of 0.089). If α is set at 0.05, then it is expected that the difference in AIC values between the RC and RV models using our real tree will be higher than the difference between RC and RV models in the simulations 95% of the time.

Because extinction is known to produce the spurious effect of increased rates of speciation later in the tree (Paradis 2004; Rabosky and Lovette 2008a), we also simulated 1000 birth–death trees to test the difference in RC and RV values. The combined birth (0.19) and death rates (0.04) were calculated using the MLE equations from Rosenzweig and Vetault (1992), modified by Etienne and Apol (2009), with the number of extant (31) and extinct (8) lampropeltinines, taken from the fossil record (Holman 2000). This simulation with missing species was estimated on both Yule and birth–death trees. We also conducted an analysis of diversification with survival models (Paradis 1997) to compare with these RC and RV results. Survival methods test three models using AIC scores: model A assumes no change, model B assumes diversification follows Weibull’s law, and model C assumes diversification changes with a specific breakpoint time. The parameter beta in model B determines if the monotonically changing diversification rate decreases with time (>1) or increases with time (<1).

Rate changes that involve a decrease in speciation through time might also be due to a recent increase in extinction rate. To test between these scenarios, we calculated log likelihood ($-\ln L$) and AIC values for the three models described in Rabosky and Lovette (2008b). The SPVAR model permits time varying speciation rates although extinction rates remain constant, whereas the EXVAR model holds speciation rates constant and allows for time varying extinction rates. The BOTHVAR model allows for both time varying speciation and extinction rates.

MODELS OF MORPHOLOGICAL EVOLUTION

To understand how morphological variation evolves in this group, we tested various models of evolution for continuous characters on the mean values of the first two principal components (PC1 and PC2) for all species. Using the package GEIGER (Harmon et al. 2008b) implemented in R 2.9 (The R Foundation for Statistical Computing 2009), we fit the most appropriate likelihood model of continuous character evolution for our data and calculated the differences in AIC scores. The models included Brownian motion (a constant variance process; Felsenstein 1985), Ornstein–Uhlenbeck (a random walk model with a central tendency and assessment of the strength of selection using α ; Butler and King 2004), Pagel’s lambda (multiplies all internal branches

by λ and examines the effect of phylogeny on character evolution where values closer to 1 evolve by Brownian motion, Pagel 1999; Freckleton et al. 2002), Pagel’s kappa (each branch is raised to the power of κ where values of 1 become a Brownian motion model and values of 0 become a speciational model with equal branch lengths), Pagel’s delta (assess character speed up, where values of δ greater than 1 indicate a speedup and those less than 1 indicate a slowdown; Pagel 1999), and early burst (fits a model in which the rate of character evolution speeds up or slows down exponentially through time; see GEIGER manual; Harmon et al. 2008b). The preferred model will be discussed in the context of how rates of speciation occur in this group of snakes.

DISPARITY THROUGH TIME

To examine the time course of morphological diversification, we assessed disparity through time using the package GEIGER (Harmon et al. 2008b) implemented in R 2.9 (The R Foundation for Statistical Computing 2009). This analysis will determine if the morphological variation in the lampropeltinines is concentrated in subclades and not equally represented throughout the tribe as a whole (disparity values closer to 0). The alternative, disparity values closer to 1, would suggest subclades contain a significant proportion of variation found throughout the entire group at that time. Disparity was calculated using average squared Euclidean distance among all pairs of points in our trees for PC1 and PC2. The disparity values from our real tree were compared to a null model composed of 1000 simulations using multivariate Brownian motion. The trend for lampropeltinines was compared against this null model and the morphological disparity index (MDI) was calculated. The MDI provides a measure against the median line of the null model, in which positive values indicate disparity is greater than expected and negative values indicate disparity is less than expected.

Results

PHYLOGENETIC HISTORY AND DIVERSITY THROUGH TIME

The most recent common ancestor (MRCA) of the lampropeltinines originated in the early Miocene (~23 mya) after dispersing from the OW to the NW (Burbrink and Lawson 2007). This group occupied a temperate niche in Central North American (Pyron and Burbrink (2009a,b) and diversified into three major clades (Appendix I) by the early Miocene (~18 mya). The first two clades, the rat and pine snakes (*Pituophis*, *Pantherophis*, and *Bogertophis*), developed large adult body sizes (>100 cm) and primarily endothermic diets, whereas the third clade, comprised primarily of the king snakes (*Lampropeltis*) and relatives, evolved smaller adult body sizes and primarily ectothermic diets (Pyron and Burbrink 2009d). The lineage through time plot

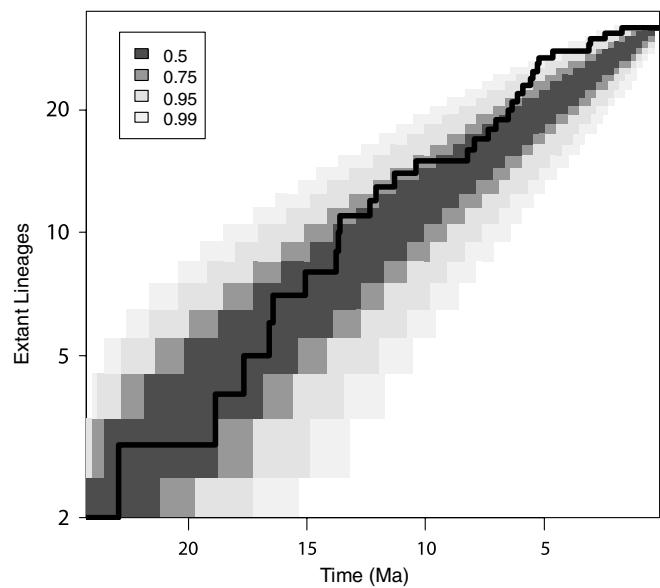


Figure 1. Lineages through time plot for lampropeltinine snakes (solid line) with a null Yule process model reflecting various confidence intervals (shaded). The x-axis represented in million years and is scaled from the chronogram of these snakes (Appendix I).

(Fig. 1) suggests that increases in diversity occurred early and net rate of diversification slowed down just prior to the Pliocene. The observed negative value for γ (-2.67) is significantly different from the simulated distribution produced from a constant rate model ($P < 0.004$) that also indicates that diversification was highest early in the history of this group. The γ value remains significant at a maximum of $P = 0.002$, even when hypothetically failing to sample up to 35% of extant species. We note that if the missing species are not randomly distributed throughout the tree, but rather toward the tips, then we expect that γ might not be significantly negative and a prediction of early bursts of speciation might be questionable.

RV models are preferred to RC models (Table 1). The best RV model (Yule2) differs in AIC value from the best RC model (Yule) by 7.77. This ΔAIC value is greater than the distribution of differences between the best RC and RV models when data are simulated under both pure birth and birth–death models and when the number of missing taxa is equal to the number of extinct species ($P < 0.02$ in all simulations). Of the RV models, Yule2 is preferred with a change in speciation rate from 0.134 to 0.032 at 5.2 mya. We note that the DDL model differs in AIC from the Yule2 model by a value of only 0.59. Therefore, it is possible that density dependence may actually be the mode of evolution defining the decrease in speciation rate in these snakes.

Using the speciation (λ) and extinction rates (μ) estimated in the SPVAR model and same number of taxa as the lampropeltinine data, we simulated 1000 trees under the DDL model. The distribution of ΔAIC values for DDL and Yule2 models was

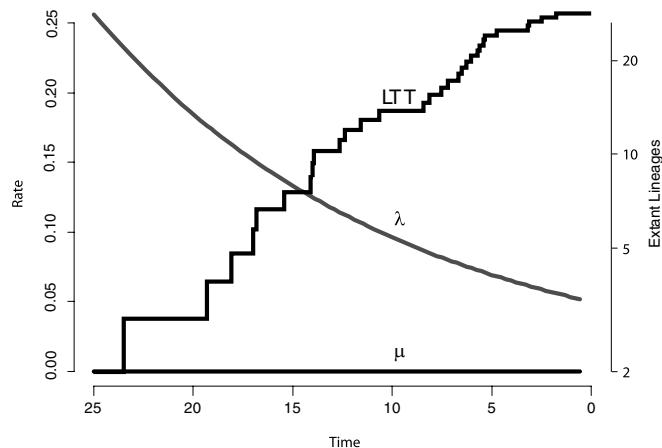


Figure 2. Rates of speciation (λ) through time with a constant extinction rate (μ), as calculated using the SPVAR model, and accumulations of lineages through time. The x-axis represented in million years and is scaled from the chronogram of these snakes (Appendix I).

estimated for our simulations and compared to our real ΔAIC for these models. Our value of 0.59 is not significantly greater ($P = 0.13$) than the distribution of these differences between the DDL and Yule2 models (mean = 1.78, SD = 2.72) when simulated under the DDL model. Therefore, we conclude that the DDL model may possibly account for the changes in speciation rates in the Lampropeltini. Additionally, these results confirming rate variability in the lampropeltinines were contrasted using survival models, where model C was preferred to the other models with a specific breakpoint at 5.2 mya (Table 1). In model B, Weibull's law indicates that speciation slowed down in this group ($\beta = 1.78$). As expected given the significant γ , models considering variable rates of extinction (EXVAR, BOTHVAR) did not provide a better fit to the tree over the variable speciation rate, constant extinction rate model (SPVAR). Therefore, as diversity increases in lampropeltinines, the rate of speciation decreases with a roughly constant pace of extinction (Fig. 2).

MORPHOLOGICAL EVOLUTION

Evolution of morphology occurred primarily along an axis of body size in this group (Pyron and Burbrink 2009d). PC1 explains $>95\%$ of the total variation in these snakes, and correlates highly with all four morphological variables ($r = -0.95$) whereas PC2 accounts for $<5\%$ variation. Brownian motion is the preferred model of evolution for PC1, whereas Pagel's λ is the best fit to PC2 (Table 2). The Brownian motion model does not address correlation between the tempo of morphological variation and the rate of species diversification in this group. Values for Pagel's δ were greater than 1 for PC1 (1.86) and PC2 (2.99), suggesting an increase in morphological evolution through time. However, neither of these values was significant.

Table 2. ΔAIC scores for six models of morphological evolution in lampropeltinines snakes for principal components (PC) 1 and 2. Zero indicates the preferred model.

	Brownian	Lambda	Delta	Kappa	Ornstein–Uhlbeck	Early burst
PC1	0	0.065	0.97	1.8	0.91	2
PC2	3.5	0	1.89	2.8	0.62	5.5

Disparity through time (DTT) plots reveals that morphological disparity was lower than the simulated median line early in the evolution history of this group (Fig. 3). Prior to 5 mya, the true value becomes greater than the median line of the null. The MDI statistic for this group (-0.10) suggests that disparity for this group is less than expected. These results indicate that early in the history of these snakes, morphological variation was partitioned into separate subgroups, and that these subclades did not contain a significant proportion of the overall morphological variation found in the Lampropeltini during those times.

Discussion

The ecological opportunity hypothesis proposes that organisms freed from the burden of competition, such as through the invasion of novel and/or unoccupied habitats, will experience a “release” characterized by bursts of phenotypic or morphological evolution, and/or increased cladogenesis (Simpson 1953; Roughgarden 1972; Stanley 1973; Slatkin 1980; Schlüter 2000; Nosil and

Reimchen 2005). Prime examples of invasion of empty or underutilized ecological niches should be found in taxa that colonized the NW, and for which related groups were underrepresented (e.g., Sanmartín et al. 2001). However, hypotheses of increased specific diversification and morphological evolution have rarely been tested for organisms dispersing into the NW. Here, we show that dispersal North America by a group of snakes, where the existing snake fauna was relatively depauperate, was accompanied by early bursts of both specific diversification and morphological specialization (Fig. 1). Both rates have steadily declined, presumably as available ecological niches became saturated (Fig. 2). These results are a confirmation of several classic, but rarely tested hypotheses regarding adaptive ecological diversification (Schlüter 2000).

These elevated early rates of diversification are contrasted with a drastic reduction later. Beginning ~ 5 mya, rates of speciation were reduced to $\sim 26\%$ of their original value. This pattern of reduced speciation rates following an explosion in diversity has been observed in the fossil record and molecular phylogenetic studies of many groups of organisms (Lovette and Bermingham 1999; Harmon et al. 2003; Ruber and Zardoya 2005; Kozak et al. 2005; Weir 2006; Phillimore and Price 2008; Rawlings et al. 2008; Rabosky and Lovette 2008a; Wellborn and Broughton 2008), and attributed to a variety of factors, most important being an increase in extinction or a decrease in available ecological space and increased competition (Ricklefs and Cox 1972; Levinton 1979; Walker and Valentine 1984; Valentine 1985; Hubbell 2000; Schlüter 2000; Paradis 2004; Rabosky and Lovette 2008b). We addressed both of these factors in lampropeltinines, and found that models which included variable extinction rates are not well supported, whereas those with variable speciation rates provide a significantly better fit to the data (Table 1, Fig. 2). Therefore, we conclude that the decrease in diversification rate was not due to increasing extinction.

For species that diversify early in their evolutionary history, it should be expected that morphological disparity will be partitioned among rather than within clades (Harmon et al. 2003). It is also hypothesized that species that fill ecological niches early leave little room for morphological diversification within subclades. Our study lends support for these hypotheses, and indeed, the early burst of speciation in the Lampropeltini is associated low morphological disparity (Fig. 3). This indicates that morphological diversity is partitioned into subclades early, and occupies isolated islands of morphological space (Harmon et al. 2003; Kozak et al. 2005). Our study suggests that the early occupation of available ecological niches may lead to density-dependent effects on rates of diversification later due to a lack of ecological opportunity, which ultimately dampen rates of speciation.

Speciation rates in these snakes are inversely proportional to taxonomic diversity and appear to be unaffected by extinction

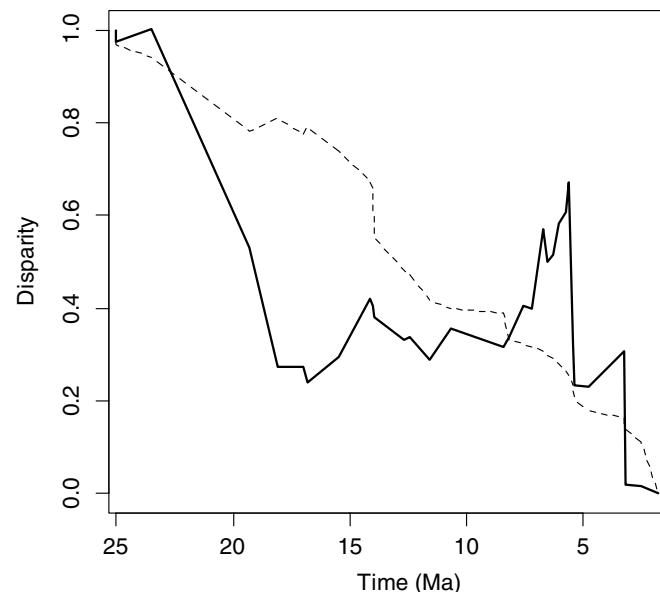


Figure 3. Disparity through time plots for the lampropeltinines snakes (solid line) against the median of simulations (dashed). Time is represented in millions of years and scaled from the chronogram of these snakes (Appendix I).

rates. Therefore, it is likely that diversification decreased with time due to an increase in competition for novel ecological niches. Models with variable diversification rates are supported over constant rate models in lampropeltinines, but between the best RV models with low AIC values, the DDL model of speciation cannot be rejected in favor of the Yule model with two rates of diversification (Yule2; Table). Even when trees are simulated under the DDL model, the Yule2 model provides an equally good explanation for the data. Density-dependent effects may have played a role in reducing diversification rates in these snakes. Several other studies on vertebrates have demonstrated this pattern (Weir 2006; Phillipmore and Price 2008; Rabosky and Lovette 2008a). Such a pattern is not unexpected in the Lampropeltini, because they exist in large communities in North America, often representing a majority of snakes in those assemblages (Conant and Collins 1998).

Alternatively, speciation rates may have slowed not due to density effects, but other extrinsic factors. In the less likely scenario that the Yule2 model best accounts for diversification in this group, an explanation beyond density-dependent effects should be offered. The Yule2 model highlights a noticeable change in speciation rate at ~5 mya in the Lampropeltini that coincides with a decrease in temperatures in North America and the beginning of a series of glacial cycles that characterized the Pliocene and Pleistocene (Zachos et al. 2001). Thus, it is possible the altered and reduced habitat during the glacial cycles of the Pliocene and Pleistocene in North America (Hopkins et al. 1982; Watts 1980; Bennett 1990; Jackson et al. 2000) did not increase extinction rates or competition in lampropeltine snakes, but rather limited space needed to maintain elevated speciation rates (see Rosenzweig 2000 for a discussion of area and speciation rate; Fig. 2). Precedence for this hypothesis was presented by Zink and Slowinski (1995), who suggested that displaced or reduced habitats due to the effects of glacial cycles caused diversification in North American birds to decrease prior to the Pleistocene (see Avise and Walker 1998 for an alternate view). Regardless of cause, our study provides evidence that speciation rate decreased prior to the Pliocene/Pleistocene glacial cycles, which are thought to have increased diversification in North American vertebrate faunas during the Quaternary (Rand 1948; Avise 2000; Johnson and Cicero 2004).

The lampropeltinine snakes entered the NW ~24 mya, when the fossil record indicates advanced snakes were not yet the major component of the terrestrial snake fauna (Holman 2000). Therefore, it seems likely that an empty niche for these terrestrial advanced snakes had yet to be filled. Our results indicate that diversification occurred rapidly after entering the NW, and that morphological variation was quickly partitioned among subclades to fill these niches. The greatest diversification took place in the central part of the United States in the mid-Miocene

(Pyron and Burbrink 2009b), thus the low disparity in morphology may have been associated with adaptations to varying proportions of ectothermic and endothermic diets (e.g., Pyron and Burbrink 2009d), and modification of forest habitats and the development of grasslands (Zubakov and Borzenkova 1990). Morphological disparity increases rapidly prior to the Pliocene, near the same time that rates of speciation decrease. It is unclear if these events are related, as disparity is known to decrease towards time zero (Harmon et al. 2003).

Our research has demonstrated that colonization and diversification of the underutilized ecological niche space of the NW promoted an early and rapid burst of specific and morphological diversification in lampropeltine snakes. Associated with a rapid burst of speciation are low within-clade morphological disparity, and an inverse relationship between the rate of speciation and taxonomic diversity. Furthermore, density-dependent effects, without an increase in extinction rates, may account for a decrease in speciation rates prior to the Plio/Pleistocene glacial period. This research confirms several classic, but rarely tested aspects of the ecological opportunity hypothesis (e.g., Schlüter 2000), indicating that competitive release in novel environments may foster rapid diversification (e.g., Nosil and Reimchen 2005; Harmon et al. 2008a), and that subsequent competition may have a strong influence on the ecomorphological evolution of species assemblages (e.g., Kozak et al. 2005; Pyron and Burbrink 2009d).

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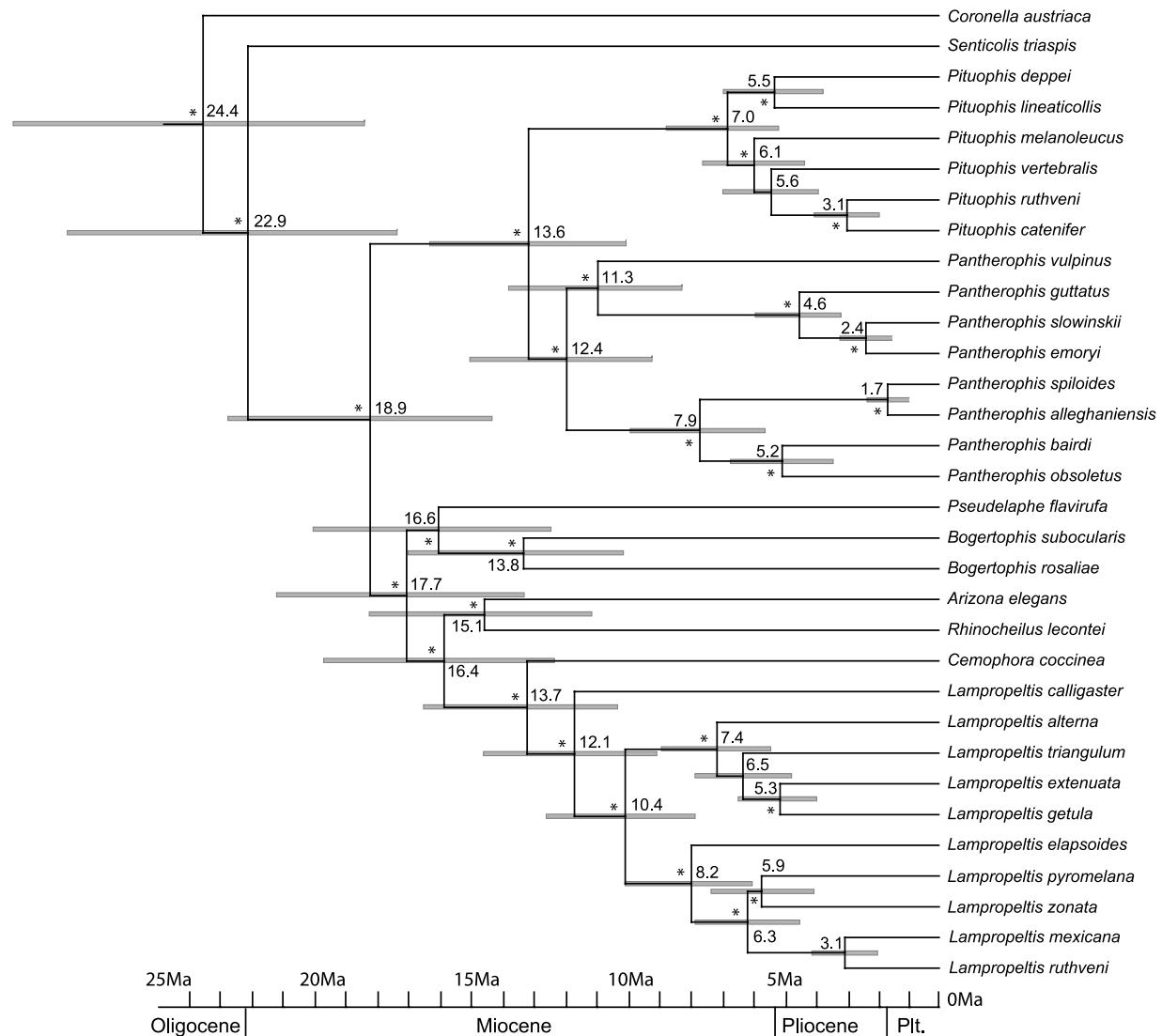
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Appendix 1

CHRONOGRAM OF LAMPROPELTININE SNAKES



Appendix I. Bayesian inference chronogram of the Lampropeltini inferred using the program BEAST version 1.4.8 (Drummond and Rambaut 2007). Asterisks at nodes represent >95% Posterior Probability support. Bars indicate the 95% Highest Posterior Density for the inferred divergence time estimates. Topology has been truncated to show only the immediate outgroup *Coronella austriaca*. Support and methodology used to create this tree can be found in Pyron and Burbrink (2009a).