Body size as a primary determinant of ecomorphological diversification and the evolution of mimicry in the lampropeltinine snakes (Serpentes: Colubridae)

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Abstract

Evolutionary correlations between functionally related character suites are expected as a consequence of coadaptation due to physiological relationships between traits. However, significant correlations may also exist between putatively unrelated characters due to shared relationships between those traits and underlying variables, such as body size. Although such patterns are often dismissed as simple body size scaling, this presumption may overlook important evolutionary patterns of diversification. If body size is the primary determinant of potential diversity in multiple unrelated characters, the observed differentiation of species may be governed by variability in body size, and any biotic or abiotic constraints on the diversification thereof. Here, we demonstrate that traits related to both predatory specialization (gape and diet preference) and predatory avoidance (the development of Batesian mimicry) are phylogenetically correlated in the North American snake tribe Lampropeltini. This is apparently due to shared relationships between those traits and adult body size, suggesting that size is the primary determinant of ecomorphological differentiation in the lampropeltinines. Diversification in body size is apparently not linked to climatic or environmental factors, and may have been driven by interspecific interactions such as competition. Additionally, we find the presence of a 'key zone' for the development of both rattle- and coral snake mimicry; only small snakes feeding primarily on ectothermic prey develop mimetic colour patterns, in or near the range of venomous model species.

Introduction

The underlying causes of phylogenetic correlations between traits are a central question in systematics and evolutionary biology (Losos, 1996). Although the evolution of coadaptive suites of functionally related traits is well known (e.g. Huey & Pianka, 1981; Brodie, 1992; Cooper, 2007), morphological integration of functionally unrelated traits may also occur through shared correla-

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tions with other characters of primary selective importance. For instance, studies have indicated that evolutionary correlations may exist between seemingly unrelated traits, such as vocalization and dietary preference in Galapagos finches, where both are tied to the underlying morphological variable of beak shape (Podos, 2001). Perhaps most fundamentally, body size is seen to be related to numerous aspects of ecomorphological diversification and morphological adaptation in organisms such as the squamates *Anolis* and three-spine sticklebacks (Losos, 1994; Nagel & Schluter, 1998). Although numerous studies have investigated the evolutionary roots of body size diversification (e.g. Moen & Wiens, 2009) and size-independent relationships among other traits (e.g.

Bickel & Losos, 2002; Bergmann *et al.* 2009), relatively few studies have directly investigated the effects of body size evolution on the diversification of traits in a quantitative context (see Losos, 1994, 1996).

As body size is one of the most fundamentally important aspects of organismal morphology (e.g. LaBarbera, 1989; Roy, 2008), the evolution of size may play a larger role in the development of phenotypic diversity that is not directly related to body mass (e.g. colour pattern, etc.) than is commonly discussed. For such traits, constraints on body size evolution may be ultimately responsible for both the potential diversity and observed differentiation of those traits. Thus, size may represent a single shared axis of diversification for traits which are correlated with gross morphology.

Such characters would thus share directional changes in and limitations on their diversification – not by being functionally related to each other, but rather by being correlated with and governed by body size. Therefore, potential adaptations and subsequent diversification in these traits may ultimately be controlled by body size evolution. Thus, evolutionary correlations between unrelated traits may be explained by shared relationships of those traits with body size (e.g. Bickel & Losos, 2002). These patterns could be dismissed as simple body size scaling (e.g. LaBarbera, 1989), suggesting that observed correlations between functionally unrelated characters do not represent meaningful evolutionary patterns. However, this may overlook the importance of body size in the evolutionary development of traits which are strongly related to size (e.g. Goodman *et al.*, 2009; Kozak *et al.*, 2009).

A thorough investigation of evolutionary relationships between multiple morphological and life-history traits may be most easily accomplished by studying organisms with reduced morphologies such as snakes, in which potentially confounding morphological variables such as limb elements, dermal armor and sexual ornamentations are absent (Vitt & Caldwell, 2009). The extreme reduction in body form in snakes leaves length (typically snout–vent length, SVL) as the primary determinant of adult body size, which varies by nearly two orders of magnitude ($\sim$10 cm to $\sim$10 m) in extant species, with a tendency towards moderate ($\sim$1.0 m) sizes (Boback & Guyer, 2003). One character most limited by body size is gape, the cross-sectional area of the open mouth (King, 2002). This affects predatory efficiency and thus may define dietary preference and limit prey size (Rodríguez-Robles *et al.*, 1999b; King, 2002). Other characters putatively related to body size include anti-predator traits, particularly Batesian mimetic colour patterns, as mimics should match the model in colour pattern as well as body size (review in Greene & McDiarmid, 1981). Thus, for snakes, body size may be a determining factor with respect to both obviously size-linked characters, such as predatory specialization and diet preference, and characters less clearly related to size, such as colour pattern.

A prime candidate group for such an analysis is the New World (NW) tribe Lampropeltini. The lampropeltinines comprise approximately 31 species, which shared a NW common ancestor in the mid-Miocene ($\sim$23 Ma; Burbrink & Lawson, 2007; Pyron & Burbrink, 2009a). They exhibit an extremely diverse array of ecomorphological forms and body size, and range from Canada to Central and South America (CA, SA; Williams, 1978; Conant & Collins, 1998; Stebbins, 2003; Pyron & Burbrink, 2009b). Diet compositions range from entirely ectothermic prey (*Lampropeltis extenuata*) to entirely endothermic ones [*Pituophis* and *Pantherophis* (part); Table 1]. Colour patterns in the group include classic cases of Batesian mimicry of venomous rattle and coral snakes, as well as drab cryptic patterns (Greene & McDiarmid, 1981; Garstka, 1982; Conant & Collins, 1998; Stebbins, 2003).

Excepting obviously coadaptive or functionally related suites of traits, there are three questions which can be asked about morphologically diverse groups such as the lampropeltinines with respect to the evolutionary history of trait diversification. First, do evolutionary relationships exist between seemingly unrelated traits, suggestive of an underlying axis of correlated diversification? Second, is this attributable to a key morphological variable such as body size (e.g. LaBarbera, 1989; Brown *et al.*, 1993; Martin & Palumbi, 1993)? Finally, was trait diversification related to abiotic (e.g. climatic or environmental; Smith *et al.*, 1995; Brakefield & French, 1999) or biotic (e.g. interspecific interactions; Losos, 1994; Robertson, 1998; Moen & Wiens, 2009) factors?

We tested for phylogenetic correlations in putatively unrelated suites of traits related to predatory specialization (e.g. gape size and diet preference) and adaptations for predator avoidance (e.g. Batesian mimetic colour patterns) in the Lampropeltini. With respect to the first question, a lack of a significant relationship would indicate the absence of correlated diversification, suggestive of unconstrained diversification across multiple axes of character space. Regarding the second question, we test if any significant relationships can be explained by shared correlations with body size, indicating an underlying axis of diversification in those traits, governed by body size. For the third question, we tested whether current climatic or environmental factors exhibit any relationship with ecomorphological diversity in the lampropeltinines.

As the geographic distribution of species richness in the group has been shown to be heavily influenced by climate (Pyron & Burbrink, 2009b), we investigated the possible influence of these ecological factors on phenotypic diversity in the group. If individual traits are not correlated with each other, variation might instead be related to geographic (e.g. latitude) or climatic factors. However, if correlations between traits exist due to shared relationships with body size, then variation in overall diversity may be explained through a relationship between ecological factors and body size, a common pattern in many

Table 1 Dietary composition, morphological measurements and mimetic colour pattern class for the 31 recognized species of Lampropeltini, and the OW outgroup *Coronella austriaca*.

Species	Diet (%)	SVL	Gape	PC1	Mimic	Diet reference	Mimicry reference
Arizona elegans	0.51	67.54	213.03	0.95	No	1, 2	–
Bogertophis rosaliae	0.00	100.92	482.03	−0.81	No	3, 1	–
Bogertophis subocularis	0.04	105.50	520.94	−1.20	No	1	–
Cemophora coccinea	0.77	36.92	72.18	2.66	Yes	1	9
Coronella austriaca	0.92	41.20	123.82	2.10	No	4	–
Lampropeltis alterna	0.83	53.01	256.93	1.06	Yes	5	19
Lampropeltis calligaster	0.14	76.31	273.32	0.45	No	6	–
Lampropeltis elapsoides	0.90	36.60	76.46	2.73	Yes	7, 8	9
Lampropeltis extenuata	1.00	33.30	35.32	3.34	No	9	–
Lampropeltis getula	0.59	91.09	388.35	−0.44	No	1, 10	–
Lampropeltis mexicana	0.75	52.15	248.92	0.98	Yes	11, 12	19
Lampropeltis pyromelana	0.59	72.44	252.95	0.69	Yes	1, 13	20
Lampropeltis ruthveni	0.75	63.30	210.16	0.98	Yes	From *L. mexicana*	19
Lampropeltis triangulum	0.13	67.85	202.77	1.09	No	14	–
Lampropeltis zonata	0.84	66.39	188.93	1.15	Yes	1, 15	20
Pantherophis alleghaniensis	0.07	113.64	605.91	−1.77	No	1	–
Pantherophis bairdi	0.00	84.00	423.51	−0.44	No	1	–
Pantherophis emoryi	0.14	80.82	390.63	−0.23	No	1	–
Pantherophis guttatus	0.14	96.26	387.30	−0.55	No	1	–
Pantherophis obsoletus	0.07	114.39	752.62	−2.44	No	1	–
Pantherophis slowinskii	0.14	82.96	359.32	0.07	No	1	–
Pantherophis spiloides	0.07	114.63	560.10	−1.52	No	1	–
Pantherophis vulpinus	0.00	112.41	585.22	−1.76	No	1	–
Pituophis catenifer	0.04	103.31	556.93	−1.36	No	1, 16	–
Pituophis deppei	0.00	98.13	496.49	−0.98	No	1	–
Pituophis lineaticollis	0.00	121.17	663.10	−2.17	No	1	–
Pituophis melanoleucus	0.26	134.47	834.37	−3.21	No	1	–
Pituophis ruthveni	0.18	139.83	863.27	−3.60	No	17	–
Pituophis vertebralis	0.00	107.21	516.18	−0.98	No	16	–
Pseudelaphe flavirufa	0.10	116.50	541.32	−1.52	No	17	–
Rhinocheilus lecontei	0.73	58.69	155.99	1.60	Yes	18	21
Senticolis triaspis	0.00	81.22	302.20	0.39	No	1, 17	–

[1]Rodríguez-Robles & de Jesús-Escobar (1999); [2]Rodríguez-Robles *et al.* (1999a); [3]Schulz (1996); [4]Luiselli *et al.* (1996); [5]Werler & Dixon (2000); [6]Fitch (1999); [7]Palmer & Braswell (1995); [8]H.W. Greene (unpublished data); [9]Conant & Collins (1998); [10]K.T. Wiseman and H.W. Greene (unpublished data); [11]Gehlbach & Baker (1962); [12]Smith (1944); [13]A.T. Holycross and C.W. Painter (unpublished data); [14]Brown (1979); [15]Greene & Rodríguez-Robles (2003); [16]Rodríguez-Robles (2002); [17]Rudolph *et al.* (2002); [17]Schulz (1996); [18]Rodríguez-Robles & Greene (1999); [19]Garstka (1982); [20]Stebbins (2003); [21]Savage & Slowinski (1992).

organisms including snakes (e.g. Smith *et al.*, 1995; Ashton & Feldman, 2003). A lack of ecological correlates of trait diversity may indicate that diversification was potentially driven by interspecific interactions, such as competition driving niche differentiation (e.g. Moen & Wiens, 2009). Pyron & Burbrink (2009a, b) presented a robust, multi-gene phylogenetic estimate of the Lampropeltini which sampled representatives of all currently described extant species, along with divergence time estimates and ancestral area reconstructions for the group which we use to test these hypotheses.

Methods and materials

Phylogeny and divergence time estimates

All subsequent analyses are based on the phylogenetic estimate and associated divergence times presented by

Pyron & Burbrink (2009a). The 31 commonly recognized extant species were included as terminal taxa. The analyses were based on a data set consisting of six mitochondrial genes (12-S, COI, cyt-*b*, ND1, ND2 and ND4) and four fragments of three nuclear genes (c-mos, Vimentin Introns 4 & 5 and SPTBN1), totalling 8294 bp of sequence data per species. Here, we use the topology and branch lengths from the maximum likelihood (ML) analysis performed in the program RAxML v7.0.3 (Stamatakis, 2006), and the Bayesian divergence time estimates from the program BEAST v1.4.8 (Drummond & Rambaut, 2007). In the phylogenetic analyses of Pyron & Burbrink (2009a), the nominal subspecies *Lampropeltis t. triangulum* was used as the terminal taxon to represent the complex. For these analyses, the morphological measurements, dietary compositions and colour pattern information are for *L. t. triangulum* only. A more detailed description of these methods is given in

Pyron & Burbrink (2009a) and Supporting Information Appendix S1.

Ancestral areas and ecological data

We used the occurrence data, climatic data and ML reconstructions of ancestral areas for internal nodes on the phylogeny presented by Pyron & Burbrink (2009b). Ancestral area reconstructions were performed in the program LaGrange 2.0.1 (Ree & Smith, 2008). The presence locality data set consists of 4564 occurrence records for all 31 species and the Old World (OW) outgroup taxon *Coronella austriaca*, which were used to calculate latitudinal midpoints, ranges and extents for all species. For the presence localities, climatic data was extracted from the 19 BIOCLIM variables in the WorldClim data set (Hijmans *et al.*, 2005), representing averages, ranges and extremes in yearly, quarterly and monthly temperature and precipitation projected at a 30-second spatial resolution. Ecological niche estimates were calculated using principal components analysis (PCA) to extract the primary axes of climatic variability from the 19 BIOCLIM variables. Here, we used the first PC axis, which accounts for the differences in niche between temperate and tropical lampropeltinine species (Pyron & Burbrink, 2009b).

Morphological and colour pattern data

To test hypotheses regarding morphological variation, we measured four primary variables related to adaptations for feeding morphology [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 *C. austriaca*. For the species *Lampropeltis ruthveni* and *Pituophis vertebralis*, we were unable to directly measure any specimens for the cranial morphology characters, but SVL measurements for both species were obtained from published works (Blanchard, 1921; Stull, 1940 respectively), for a total of 757 specimens (Table 1; Supporting Information Appendix S2). We corrected for allometry by regressing HH/SVL against SVL, and excluding individuals whose ratio occurred prior to the asymptote (Burbrink, 2001). We extracted the primary axes of gross morphological variation PCA, from the measurements of SVL, HW, JL and HH.

We calculated gape, the area of the open jaws with jaw length and head width as the major and minor axes, using the formula $(\pi \times JL \times HW)/4$ after King (2002). For *L. ruthveni* and *P. vertebralis*, we calculated the predicted mean value of gape for both species based on mean SVL, using the exponential function describing the curve which relates gape to SVL in the allometrically corrected data set. All morphological variables were log transformed (ln) prior to analyses. Batesian mimicry of both coral snakes (Greene & McDiarmid, 1981) and

rattlesnakes (Garstka, 1982) is observed in the lampropeltinines (Table 1). For the discrete phylogenetic correlation analyses of the evolution of Batesian mimicry, we coded the presence of coral and rattle snake mimicry as binary character (0 = nonmimic and 1 = mimic) based on both the presence of the tricoloured mimetic pattern class and published accounts or hypotheses of putative mimicry for the species (Table 1). As we are concerned with the potential expression of these patterns, polymorphic (i.e. species with potentially mimetic and nonmimetic individuals) were coded as mimics (1).

Dietary composition

For all 31 species and *C. austriaca*, we recorded diet preference as a continuous character, average proportion of ectothermic prey ranging from 0 to 1, based on published reports (Table 1). For species which represent recently elevated phylogeographic lineages (e.g. *Pa. guttatus, emoryi* and *slowinskii*; *Pa. obsoletus, alleghaniensis, spiloides* and *bairdi*), diet compositions for the original species were generalized across the lineages. Two poorly known Mexican species, *L. ruthveni* and *L. mexicana*, yielded only scattered reports of dietary records of ectothermic prey; analyses were run with both species given a null value of 0.5, and 0.75, representing the known proportion of ectothermic prey items. Results were similar for both analyses, and the latter are reported. Diet proportion was arcsine transformed prior to analyses.

Phylogenetic comparative analyses

We first reconstructed ancestral states for SVL, diet preference, mimicry and the PC axes explaining a significant portion of morphological variation to examine how the morphological and life-history traits varied over time. We then tested for significant associations between traits related to morphology (SVL, gape), predatory specialization (diet preference) and predator avoidance (mimicry) to assess evolutionary relationships between traits and potential relationships with underlying explanatory variables such as body size. All analyses were performed using both nonphylogenetic methods (linear and logistic regression of raw data; TIPS) and phylogenetic comparative methods (PCM) to assess potential differences between atemporal and evolutionary relationships between traits.

Analyses using PCM were conducted using Phylogenetic Generalized Least Squares (PGLS) (Martins & Hansen, 1997) and Felsenstein's independent contrasts (FIC; Felsenstein, 1985) implemented in the program compare v4.6b (PGLS relationships and PGLS ancestor models; Martins, 2004). In almost all cases, results from the three methods were highly similar (see Ord & Martins, 2006); thus, we report only the PGLS results, unless disagreement exists between the methods.

Ancestral states with respect to mimicry were inferred using the Discrete model (Pagel, 1994) implemented in MESQUITE v.2.5 (Maddison & Maddison, 2008). We used the ML topology and both the ML branch lengths and the chronogram branch lengths from Pyron & Burbrink (2009a), truncated to include only *C. austriaca*. Both yielded similar results; as we are interested in trait evolution in an explicitly temporal context, we report the results using the chronogram branch lengths.

For all regression analyses which involved multiple comparisons, we assessed significance using a Bonferroni correction of $\alpha_{0.05/n}$. We first estimated the size-dependent component of gape by regressing gape against SVL. We then tested for a size-independent relationship between gape and diet and mimicry by taking the standard residuals of gape regressed against SVL, and regressing those against dietary preference (% ectothermy) and mimicry (logistic regression of presence or absence). Significance for the latter two analyses was assessed using a Bonferroni-corrected $\alpha_{0.05/s} = 0.025$. We then tested whether significant phylogenetic relationships exist between the putatively unrelated traits of dietary preference and mimicry. Finally, we examined whether or not body size exhibited a significant relationship with dietary preference and the presence of mimicry, as predicted. Significance for the latter two analyses was again assessed using a Bonferroni-corrected level of $\alpha_{0.05/2} = 0.025$ to account for the multiple comparisons.

To test for a significant relationship between geography and the development and diversification of the morphological variables, we regressed the ecomorphological traits against the latitudinal midpoint of each species, calculated from the occurrence records from Pyron & Burbrink (2009b). For analysis of climatic factors, we used the first PC axis derived from the 19 BIOCLIM variables, which encompasses the primary climatic factors shown to have influenced current and historical distributions in the lampropeltinines (Pyron & Burbrink, 2009b). We then regressed the significant traits against each of the ecological and geographic variables. Analyses were performed using both nonphylogenetic (linear and logistic regression) and PCM analyses (PGLS and FIC), with Bonferroni corrections for multiple comparisons.

Results

Evolutionary history of diversity

As per Pyron & Burbrink (2009a,b), the NW MRCA of the lampropeltinines originated in the early Miocene (~23 Ma), and occupied a temperate niche in central NA. The PGLS reconstructions of gape, SVL and diet composition indicate that this ancestral lineage resembled the OW outgroup species *C. austriaca*, with an SVL of approximately 72 cm, and endotherms comprising a slight majority of the diet (Fig. 1). The Discrete reconstructions

of ancestral states with respect to the presence of mimicry using the Mk1 model selected using MESQUITE indicate that the early lampropeltinine lineages did not exhibit mimetic colour patterns (Fig. 1). However, a portion of known diversity in the *L. triangulum* group was excluded, including potential mimics in CA and SA, due to a lack of information of the phylogeography of the species. Preliminary results have indicated that the species is not monophyletic (see Bryson *et al.*, 2007; Pyron & Burbrink, 2009a); thus, we included only the nominate subspecies.

In the early Miocene (~18 Ma), the lampropeltinines diversified into three major clades (Fig. 1). Two clades (A and C; Fig. 1), the rat and pine snakes (*Bogertophis*, *Pituophis*, *Pantherophis* and *Pseudelaphe*), developed large adult body sizes (> 100 cm); one specimen of the Bull snake (*Pi. catenifer*) was the largest snake ever observed in NA at 267 cm (Devitt *et al.*, 2007). Both clades exhibit primarily endothermic diets. The third clade (B) comprised primarily of the king snakes (*Lampropeltis*) and relatives, evolved smaller adult body sizes and primarily ectothermic diets (Fig. 1). In the latter clade, the reconstructions of mimicry suggest that species may have exhibited putatively mimetic colour patterns as early as 16 Ma, with the first unambiguous reconstruction of a mimetic colour pattern occurring ~11.5 Ma (Fig. 1). The lineage leading to *Senticolis* appears to have undergone relatively little change (Fig. 1). The PCA scores revealed that > 95% of all variation in gross morphology occurs along the first axis (PC1), along which all four variables (SVL, HW, JL and HH) load strongly negatively (< −0.95). Plotted against time, the Miocene differentiation in gross morphology between clade B and clades A and C is clearly visible, whereas *Senticolis* exhibits little change from the centroid (Fig. 2).

Phylogenetic correlation of life-history traits

Analyses indicate that gape is strongly related to adult body size ($r = 0.96$, $P = 1.8 \times 10^{-19}$), suggesting that although snakes are gape-limited predators (Rodríguez-Robles *et al.*, 1999b; King, 2002), this limitation may be ultimately due to the underlying geometric relationship between gape and body size. There is no significant relationship between the standard residuals of gape against SVL and either diet composition ($r = 0.14$, $P = 0.18$) or the presence of mimicry ($r = 0.36$, $P = 0.08$), indicating that there is no body size-independent relationship between gape, and either dietary preference or mimetic colour pattern. Dietary composition (% ectothermy) shares a strong positive relationship with the presence of mimicry ($r = 0.56$, $P = 0.009$), suggesting that mimics are more likely to consume ectothermic prey. A significant relationship between these putatively unrelated traits suggests that both may be dependent on an underlying variable, such as body size.

Adult body size (SVL) exhibits a strong negative relationship with dietary preference (% ectothermy;
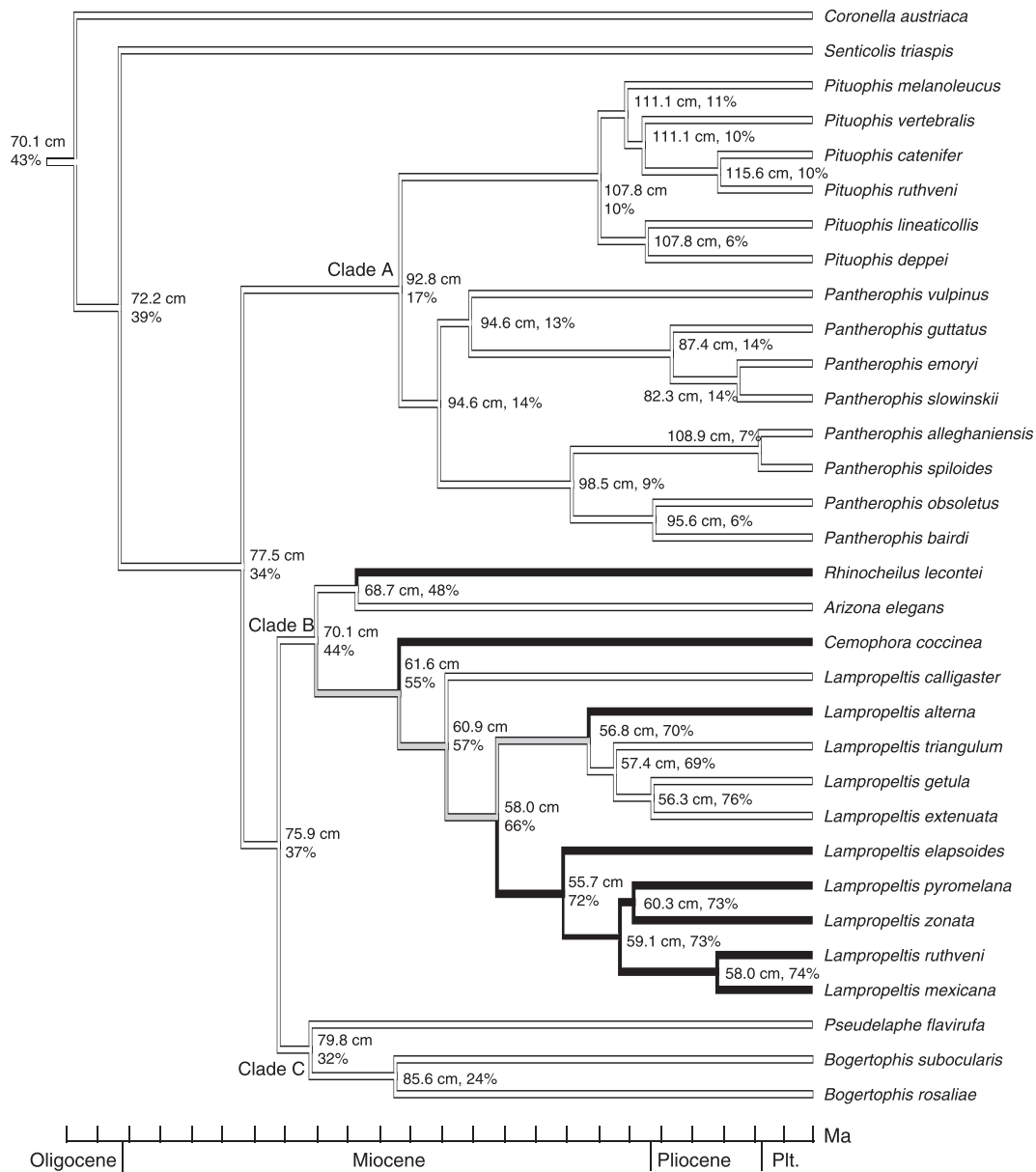
Fig. 1 Chronogram of the extant lampropeltinine species (see Pyron & Burbrink, 2009a; Supporting Information Appendix S1), showing Discrete ML reconstructions of the presence of putative mimetic colour patterns (black: tricoloured putative mimic, white: nonmimic, grey: ambiguous) and PGLS ancestor reconstructions of mean adult body size (SVL) and dietary composition (% ectothermy).

$r = -0.84$, $P = 3.7 \times 10^{-10}$). Larger snakes show an increasing preference for endothermic prey, whereas smaller snakes exhibit primarily ectothermic diets. This is probably due to the increase in maximum possible prey volume for larger snakes, which are known to drop smaller prey items from their diets (Rodríguez-Robles et al., 1999b; King, 2002). As with dietary preference, the presence of mimicry exhibits a significant negative relationship with SVL ($r = -0.44$, $P = 0.015$). However, although the TIPS and PGLS results are significant at a Bonferroni-corrected level of $\alpha_{0.05/2} = 0.025$, the FIC

results are not ($r = -0.35$, $P = 0.054$). This appears to be due to the presence of the outlier *L. extenuata*, a very small nonmimic (Table 1). Removing the effect of *L. extenuata* by assigning it the same SVL value as its sister taxon *L. getula* (Fig. 1; Table 1) yields highly significant results for both PGLS ($r = -0.68$, $P = 3.3 \times 10^{-5}$) and FIC ($r = 0.59$, $P = 0.0006$). This suggests that body size is related to mimicry within a certain range of SVL values: that it is possible to be both too large as well as too small to be an effective mimic, probably based on model size (e.g. Greene & McDiarmid, 1981). These results suggest that
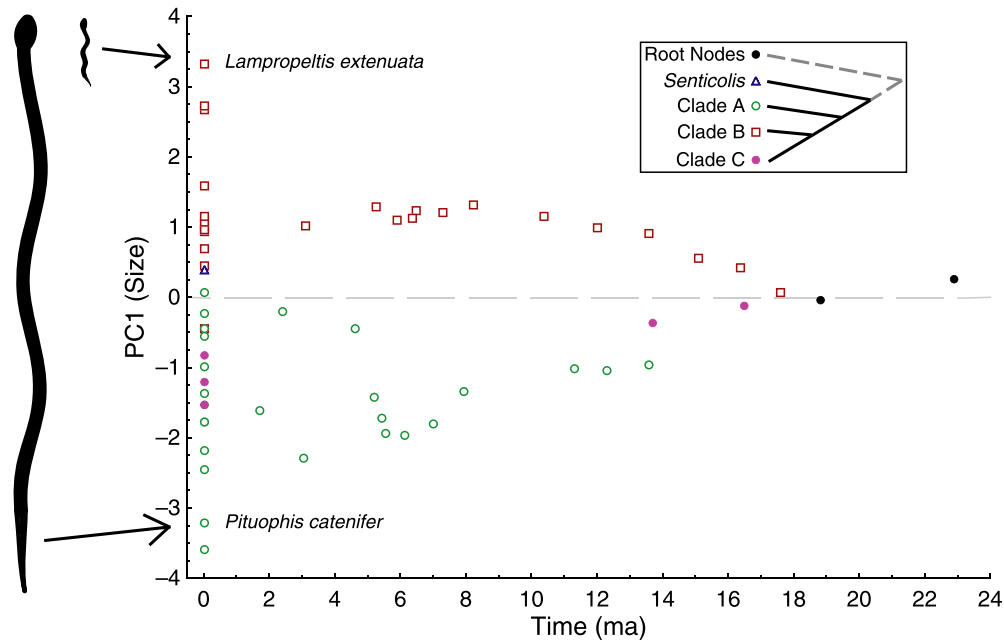
Fig. 2 Plot of the PC1 scores for the extant species and the PLGS ancestor reconstructions of ancestral states with respect to time (from the Bayesian divergence time analysis of Pyron & Burbrink, 2009a; Supporting Information Appendix S1), illustrating the mid- to late Miocene divergence in body size and cranial morphology. Cartoons on the left represent relative body size extremes in the Lampropeltini. The root nodes represent, from left to right, the MRCA of the Lampropeltini excluding *Senticolis* and the MRCA of all lampropeltinines respectively.

adult body size is the primary determinant of the presence, and probably the effectiveness of the display of mimetic colour patterns (see Greene & McDiarmid, 1981), as well as dietary composition (Rodríguez-Robles et al., 1999b; King, 2002).

Ecological correlates of diversity

Our analyses of the influence of climatic and geographic factors affecting evolution and diversity suggest that there is little impact of ecological variables on morphological diversity in the lampropeltinines. We found no significant relationship between the latitudinal midpoint of the species and SVL ($r = -0.07$, $P = 0.99$), diet ($r = 0.27$, $P = 0.33$) or mimicry ($r = 0.07$, $P = 0.99$). The same pattern holds for the measurements of ecological niche, where the PC1 axis exhibits no significant relationship with SVL ($r = 0.04$, $P = 0.99$), dietary preference ($r = -0.17$, $P = 0.33$) or mimicry ($r = 0.11$, $P = 0.62$). This indicates that the evolution of body size, diet preference and the presence of mimetic colour patterns are not strongly influenced by the primary ecological conditions experienced by the organisms, or by the geographic location of the species. Although we did not specifically test for the latter, the lack of climatic or geographic correlates of diversity suggests a role for interspecific interactions driving the evolution of these traits, a fundamental tenet of theories of competitive niche differentiation (see Losos, 2000).

Ecomorphological diversification and colour pattern evolution

The primary axis of diversification in the lampropeltinines thus appears to be defined by adult body size. Plotted against diet preference, this reveals the underlying axis of diversification in the Lampropeltini (Fig. 3). The hypothesized presence of extrinsic biotic constraints on the evolution of mimicry (e.g. the presence of a model species, and body size concordance between the mimic and the model; Greene & McDiarmid, 1981; Pfennig et al., 2001), along with the correlation between diet and body size, reveals the presence of a 'key zone' for mimicry (Fig. 3). Putative mimicry has developed or persisted only for lampropeltinine snakes within a certain size range (~40–70 cm) and exhibiting a primarily ectothermic diet. For comparison, adult sizes of the most common model species in NA are similar for both coral snakes; with *Micruroides euryxanthus* rarely larger than 50 cm, and *Micrurus fulvius* rarely larger than 80 cm; and rattlesnakes, with *Crotalus lepidus* rarely larger than 80 cm (Campbell & Lamar, 2004).

Thus, *L. extenuata* may be too small to be an effective mimic of the larger coral snakes, whereas *L. t. triangulum* exists well outside the range of any venomous coral snakes and has a primarily endothermic diet. Information on this optimal range for mimicry may be used to elucidate the phylogenetic origin of these colour patterns in the lampropeltinines. We determined whether or not
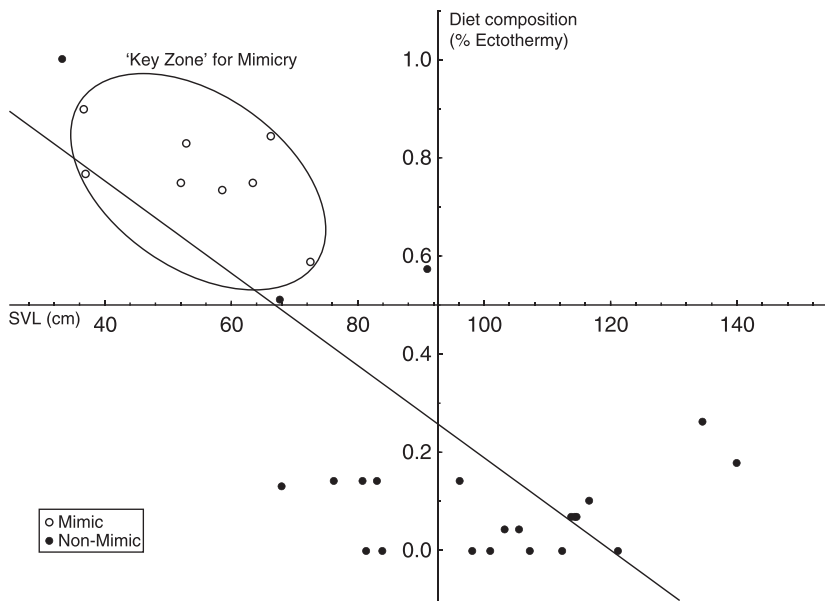
Fig. 3 Scatterplot of the raw values of SVL and dietary preference (% ectothermy), illustrating the primary axis of ecomorphological diversification in the lampropeltinines and the 'key zone' for mimicry. Open circles represent putative mimics, whereas closed circles nonmimics. The best fit regression line is for illustrative purposes.

the reconstructed body sizes and diet proportions occur in the key zone for mimicry, and hypothesize that tricoloured patterns first evolved at the node subtending the genera *Cemophora* and *Lampropeltis*, despite the ambiguous reconstruction of mimicry at that node from the Discrete analysis. This would indicate that mimicry first evolved in the lampropeltinines during the mid-Miocene, approximately 13.7 Ma (10.6–17.0 Ma).

Discussion

Ecomorphological diversification

There are no large lampropeltinine snakes displaying the classic coral snake mimetic colour pattern or an ectothermic diet. By contrast, there are no small lampropeltinines with primarily endothermic diets. Obvious biological explanations for these patterns are not easily rendered without considering how these characters are related phylogenetically and correlated with body size. The relationships among the traits suggest a single underlying axis of radiation: that unconstrained diversification of these characters is not possible for these snakes. The major axis of diversification appears to be defined by adult body size (SVL), along which we observe the correlated diversification of gape, dietary composition and the development of mimetic colour patterns within a specific range of small body sizes and ectothermic diets (Fig. 3). We find a pattern of correlated trait evolution which is in turn explained by shared relationships of individual traits with body size. In most biological contexts, traits are size corrected in order to produce size-independent measurements for analysis. Here, size-dependent relationships between traits appear to be the primary

determinant of ecomorphological diversification in the lampropeltinines.

Other researchers have found similar patterns (e.g. Bickel & Losos, 2002; Pleguezuelos *et al.*, 2007) of correlations between functionally related traits but not between functionally unrelated traits when the effect of body size is removed. However, interpreting this as only revealing a null pattern attributable to body size scaling may overlook important aspects of evolutionary radiation related to the primary determinants of ecomorphological diversification (e.g. Schluter, 2000; Kozak *et al.*, 2009; Stephens & Wiens, 2009). If traits such as predatory specialization, predator avoidance strategies or morphological adaptations related to locomotor performance and/or habitat utilization are tied to single underlying variables such as body size (e.g. LaBarbera, 1989), diversification into available habitats and expansion into new niches will be dictated by any external constraints on body size (e.g. Glor *et al.*, 2003), whether abiotic (e.g. Smith *et al.*, 1995) or biotic (e.g. Moen & Wiens, 2009). Thus, size-dependent interactions between traits may reveal as many important evolutionary patterns regarding adaptive differentiation as size-independent relationships (e.g. Bickel & Losos, 2002; Ord & Martins, 2006; Goodman *et al.*, 2009).

Diversification in adult body size is inferred to have proceeded from an intermediate body size, bi-directionally towards both extremes in three different clades (Figs 1–3), a primary prediction for the effect of historical competition on body size evolution (e.g. Taper & Case, 1985; Losos, 1994; Moen & Wiens, 2009). Thus, ecomorphological diversity in the lampropeltinines may ultimately stem from interspecific interactions, due to the dependence of traits such as diet and colour pattern on body size. However, evolution in those traits may have been driven

by interspecific interactions affecting body size evolution, rather than direct selection on the individual traits stemming from competition. Although competitive differentiation along a body size continuum is a well known and potentially widespread phenomenon (see Kozak *et al.*, 2009; Moen & Wiens, 2009), the effect that this pattern may have on overall ecomorphological differentiation in multiple unrelated traits may have been overlooked.

In the lampropeltinines, the lack of any ecological correlates of diversity for adult body size, gape, diet or colour pattern suggests that adaptive differentiation was governed by interspecific interactions, such as competition. Although the present-day climate is not an absolute facsimile of past environments, the significant effect that climate has been inferred to have had on species diversification in the lampropeltinines (see Pyron & Burbrink, 2009b) suggests that estimating past effects using current variables is not unreasonable. Thus, the lack of any significant relationship, particularly with geography (which most strongly affects species richness in the group), indicates that ecomorphological diversification may have been influenced by biotic factors, rather than historical climatic or geographic effects. Potential factors include prey availability, the presence of model species for mimicry (e.g. Greene & McDiarmid, 1981) and potentially, interspecific interactions such as competition (e.g. Losos *et al.*, 1994; Moen & Wiens, 2009). However, more explicit tests for phylogenetic evidence of competitive differentiation (e.g. Moen & Wiens, 2009) will be required before any robust hypotheses of interspecific interactions driving the ecomorphological diversification of the lampropeltinines can be made.

Evolution of colour pattern and Batesian mimicry

The evolutionary constraints on the development of the tricoloured pattern are apparently related to both body size and diet preference, as the ringed pattern is exhibited only by species in a narrow range of body sizes and diet compositions (Fig. 2). This 'key zone' for mimicry thus appears to represent the optimal range for the development of the tricoloured ringed colour pattern. Within a range of adult body size from ~35 to 70 cm SVL, species consuming primarily ectothermic prey, such as the scarlet snake (*Cemophora coccinea*), scarlet kingsnake (*Lampropeltis elapsoides*), grey banded kingsnake (*L. alterna*) and mountain kingsnakes (*L. pyromelana* and *L. zonata*), all exhibit putatively mimetic colour patterns, even if closely related species do not (Fig. 1). By contrast, larger species of lampropeltinine (e.g. *Pantherophis* and *Pituophis*) tend to exhibit a drab, probably cryptic or break-up colour patterns (Schulz, 1996; Conant & Collins, 1998; Stebbins, 2003). Very small species, such as *L. extenuata*, also exhibit less colourful saddled (cryptic) patterns (Conant & Collins, 1998).

Body size evolution thus appears to affect phenotypic colour pattern traits as well as more obviously size-dependent traits such as gape and diet preference. Most strikingly, however, is the existence of a key zone for the tricoloured ring colour pattern, suggesting that both increases and decreases in size can affect the evolutionary development of putative Batesian mimetic antipredator strategies. Additionally, ecomorph development in these snakes appears to stem more from size-specific factors, rather than ecological or habitat factors, as in the squamate genus *Anolis* (Losos, 1994). Similar to the evolution of morphological diversity in *Anolis*, body size differentiation and by extension ecomorph adaptation appears to have resulted from interspecific interactions. Thus, rather than predicting a continuum of ecomorphological forms, the body size-dependent axis of diversification in the lampropeltinines appears to promote the development of distinct ecomorphs within certain ranges of body sizes.

Conclusions

In the lampropeltinines, presumably functionally unrelated traits related to predatory specialization and predatory avoidance exhibit evolutionary correlations, which are due to the shared dependence of those traits on body size. Although the relationship between those traits disappears when body size is accounted for, dismissing the pattern as being due to simple scaling may overlook important aspects of ecomorphological diversification limited by variation in body size. Thus, the observed ecomorphological diversity of species assemblages with regard to numerous phenotypic, morphological and life-history traits may be determined by evolutionary constraints on body size. However, despite the wide range of ecological niches inhabited by the lampropeltinines, neither climatic nor geographic factors appear to influence body size or other traits, suggesting that both might be potentially influenced by interspecific interactions such as competition. Thus, although the geographic distribution of lampropeltinine species appears to have been driven by climatic factors, ecomorphological diversification in the group may have been driven by interspecific interactions such as competition. The evolution of mimetic colour patterns appears to be constrained by both body size and dietary composition, appearing in smaller snakes consuming primarily ectothermic prey, providing a prime example of the limitations on diversity imposed by an underlying trait such as body size.

Acknowledgments

References

Ashton, K.G. & Feldman, C.R. 2003. Bergmann's Rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* **57**: 1151–1163.

Bergmann, P.J., Meyers, J.J. & Irschick, D.J. 2009. Directional evolution of stockiness coevolves with ecology and locomotion in lizards. *Evolution* **63**: 215–227.

Bickel, R. & Losos, J.B. 2002. Patterns of morphological variation and correlates of habitat use in chameleons. *Biol. J. Linn. Soc.* **76**: 91–103.

Blanchard, F.N. 1921. A revision of the kingsnakes: genus Lampropeltis. *Bull. US Natl Mus.* **114**: 1–260.

Boback, S.M. & Guyer, C. 2003. Empirical evidence for an optimal body size in snakes. *Evolution* **57**: 345–351.

Brakefield, P.M. & French, V. 1999. Butterfly wings: the evolution of development of colour patterns. *BioEssays* **21**: 391–401.

Brodie, E.D., III 1992. Correlational selection for colour pattern and antipredator behavior in the garter snake *Thamnophis ordinoides*. *Evolution* **46**: 1284–1298.

Brown, E.E. 1979. Stray food records from New York and Michigan snakes. *Am. Midl. Nat.* **102**: 200–203.

Brown, J.H., Marquet, P.A. & Taper, M.L. 1993. Evolution of body size: consequences of an energetic definition of fitness. *Am. Nat.* **142**: 573–584.

Bryson, R.W., Pastorini, J., Burbrink, F.T. & Forstner, M.R.J. 2007. A phylogeny of the *Lampropeltis mexicana* complex (Serpentes: Colubridae) based on mitochondrial DNA sequences suggests evidence for species-level polyphyly within Lampropeltis. *Mol. Phylogenet. Evol.* **43**: 674–684.

Burbrink, F.T. 2001. Systematics of the North American rat snake complex (*Elaphe obsoleta*). *Herpetol. Monogr.* **15**: 1–53.

Burbrink, F.T. & Lawson, R. 2007. How and when did Old World rat snakes disperse into the New World? *Mol. Phylogenet. Evol.* **43**: 173–189.

Campbell, J.A. & Lamar, W.M. 2004. *The Venomous Reptiles of the Western Hemisphere*. Cornell University Press, Ithaca, NY.

Conant, R. & Collins, J.T. 1998. *Reptiles and Amphibians of Eastern and Central North America*, 3rd edn. Houghton Mifflin, Boston, MA.

Cooper, W.E., Jr 2007. Foraging modes as suites of coadapted suites of movement traits. *J. Zool.* **272**: 45–56.

Devitt, T., Crother, B.I., Meier, A., Burbrink, F.T. & Boundy, J.J. 2007. *Pituophis catenifer* (Bullsnake): Maximum Length. *Herpetol. Rev.* **38**: 209.

Drummond, A.J. & Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**: 214.

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.

Fitch, H.S. 1999. *A Kansas Snake Community: Composition and Changes over 50 years*. Krieger Publishing Company, Malabar, FL.

Garstka, W.R. 1982. Systematics of the Mexicana species group of the colubrid genus *Lampropeltis*, with an hypothesis [of] mimicry. *Breviora* **466**: 1–35.

Gehlbach, F. & Baker, J. 1962. Kingsnakes allied with *Lampropeltis mexicana*: taxonomy and natural history. *Copeia* **1962**: 291–300.

Glor, R.E., Kolbe, J.J., Powell, R., Larson, A. & Losos, J.B. 2003. Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). *Evolution* **57**: 2383–2397.

Goodman, B.A., Hudson, S.C., Isaac, J.L. & Schwarzkopf, L. 2009. The evolution of body shape in repsonse to habitat: is reproductive output reduced in flat lizards? *Evolution* **63**: 1279–1291.

Greene, H.W. & McDiarmid, R.W. 1981. Coral snake mimicry: does it occur? *Science* **213**: 1207–1212.

Greene, H.W. & Rodríguez-Robles, J.A. 2003. Feeding ecology of the California Mountain Kingsnake, *Lampropeltis zonata* (Colubridae). *Copeia* **2003**: 308–314.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Clim.* **25**: 1965–1978.

Huey, R.B. & Pianka, E.R. 1981. Ecological consequences of foraging mode. *Ecology* **62**: 991–999.

King, R.B. 2002. Predicted and observed maximum prey size – snake size allometry. *Funct. Ecol.* **16**: 766–772.

Kozak, K.H., Mendyk, R.W. & Wiens, J.J. 2009. Can parallel diversification occur in sympatry? Repeated patterns of body-size evolution in co-existing clades of North American salamanders. *Evolution* **63**: 1769–1784.

LaBarbera, M. 1989. Analyzing body size as a factor in ecology and evolution. *Annu. Rev. Ecol. Syst.* **20**: 97–117.

Losos, J.B. 1994. Integrative approaches to evolutionary ecology: *Anolis* lizards as model systems. *Annu. Rev. Ecol. Syst.* **25**: 467–493.

Losos, J.B. 1996. Phylogenies and comparative biology, Stage II: Testing causal hypotheses derived from phylogenies with data from extant taxa. *Syst. Biol.* **45**: 259–260.

Losos, J.B. 2000. Ecological character displacement and the study of adaptation. *Proc. Natl Acad. Sci. USA* **97**: 5693–5695.

Losos, J.B., Irschick, D.J. & Schoener, T.W. 1994. Adaptation and constraint in the evolution of specialization of Bahamian Anolis lizards. *Evolution* **48**: 1786–1798.

Luiselli, L., Capula, M. & Shine, R. 1996. Reproductive output, costs of reproduction and ecology of the smooth snake, *Coronella austriaca*, in the eastern Italian Alps. *Oecologia* **106**: 100–110.

Maddison, W.P. & Maddison, D.R. 2008. Mesquite: A Modular System for Evolutionary Analysis, version 2.5. Available at: http://mesquiteproject.org.

Martin, A.P. & Palumbi, S.R. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proc. Natl Acad. Sci. USA* **90**: 4087–4091.

Martins, E.P. 2004. compare, version 4.6b. Computer Programs for the Statistical Analysis of Comparative Data. Indiana University, Bloomington, IN. Distributed by the author at: http://compare.bio.indiana.edu/.

Martins, E.P. & Hansen, T.F. 1997. Phylogenies and the comparative method: a general approach to incorporating

phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**: 646–667.

Moen, D.S. & Wiens, J.J. 2009. Phylogenetic evidence for competitively-driven divergence: body-size evolution in Caribbean treefrogs (Hylidae: *Osteopilus*). *Evolution* **63**: 195–214.

Nagel, L. & Schluter, D. 1998. Body size, natural selection, and speciation in sticklebacks. *Evolution* **52**: 209–218.

Ord, T.J. & Martins, E.P. 2006. Tracing the origins of signal diversity in anole lizards: phylogenetic approaches to inferring the evolution of complex behaviour. *Anim. Behav.* **71**: 1411–1429.

Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters.. *Proc. Roy. Soc. B* **255**: 37–35.

Palmer, W.M. & Braswell, A.P. 1995. *Reptiles of North Carolina*. University of North Carolina Press, Chapel Hill, NC.

Pfennig, D.W., Harcombe, W.R. & Pfennig, K.S. 2001. Frequency-dependent Batesian mimicry. *Nature* **410**: 323.

Pleguezuelos, J.M., Fernández-Cardenete, J.R., Honrubia, S., Feriche, M. & Villafranca, C. 2007. Correlates between morphology, diet and foraging mode in the Ladder Snake *Rhinechis scalaris* (Schinz, 1822). *Contrib. Zool.* **76**: 179–186.

Podos, J. 2001. Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**: 185–188.

Pyron, R.A. & Burbrink, F.T. 2009a. Neogene diversification and taxonomic stability in the snake tribe Lampropeltini (Serpentes: Colubridae). *Mol. Phylogenet. Evol.* **52**: 524–529.

Pyron, R.A. & Burbrink, F.T. 2009b. Can the Tropical Conservatism Hypothesis explain temperate species richness patterns? An inverse latitudinal biodiversity gradient in the New World snake tribe Lampropeltini. *Glob. Ecol. Biogeogr.* **18**: 406–415.

Ree, R.H. & Smith, S.A. 2008. Maximum-likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* **57**: 4–14.

Robertson, D.R. 1998. Implications of body size for interspecific interactions and assemblage organization among coral-reef fishes. *Aust. Ecol.* **23**: 252–257.

Rodríguez-Robles, J.A. 2002. Feeding ecology of North American Gopher Snakes (*Pituophis catenifer*, Colubridae). *Biol. J. Linn. Soc.* **77**: 165–183.

Rodríguez-Robles, J.A. & de Jesús-Escobar, J.M. 1999. Molecular systematics of New World lampropeltinine snakes (Colubridae): implications for biogeography and evolution of food habits. *Biol. J. Linn. Soc.* **68**: 355–385.

Rodríguez-Robles, J.A. & Greene, H.W. 1999. Food habits of the Long-nosed Snake (*Rhinocheilus lecontei*), a ''specialist'' predator? *J. Zool.* **248**: 489–499.

Rodríguez-Robles, J.A., Bell, C.J. & Greene, H.W. 1999a. Food habits of the Glossy Snake, *Arizona elegans*, with comparisons to the diet of sympatric Long-nosed Snakes, *Rhinocheilus lecontei*. *J. Herpetol.* **33**: 87–92.

Rodríguez-Robles, J.A., Bell, C.J. & Greene, H.W. 1999b. Gape size and evolution of diet in snakes: feeding ecology of erycine boas. *J. Zool.* **248**: 49–58.

Roy, K. 2008. Dynamics of body size evolution. *Science* **321**: 1451–1452.

Rudolph, D.C., Burgdorf, S.J., Conner, R.N., Collins, C.S., Saenz, D., Schaefer, R.R., Trees, T., Duran, C.M., Ealy, M. & Himes, J.G. 2002. Prey handling and diet of Louisiana Pine snakes (*Pituophis ruthveni*) and Black Pine snakes (*P. melanoleucus lodingi*), with comparisons to other selected colubrid snakes. *Herpetol. Nat. Hist.* **9**: 57–62.

Savage, J.M. & Slowinski, J.B. 1992. The colouration of the venomous coral snakes (family Elapidae) and their mimics (families Colubridae and Aniliidae). *Biol. J. Linn. Soc.* **45**: 235–254.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.

Schulz, K.D. 1996. *A Monograph of the Colubrid Snakes of the Genus Elaphe Fitzinger*. Koeltz Scientifc Books, Havlickuv Brod, Czech Republic.

Smith, H.M. 1944. Snakes of the Hoogstraal Expeditions to northern Mexico. *Field Mus. Nat. Hist. Zool. Ser.* **29**: 135–152.

Smith, F.A., Betancourt, J.L. & Brown, J.H. 1995. Evolution of body size in the Woodrat over the past 25,000 years of climate change. *Science* **270**: 2012–2014.

Stamatakis, A. 2006. RaxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.

Stebbins, R.C. 2003. *A Field Guide to Western Reptiles and Amphibians*, 3rd edn. Houghton Mifflin, Boston, MA.

Stephens, P.R. & Wiens, J.J. 2009. Evolution of sexual size dimorphisms in emydid turtles: ecological dimorphism, Rensch's rule, and sympatric divergence. *Evolution* **63**: 910–925.

Stull, O.G. 1940. Variations and relationships in the snakes of the genus *Pituophis*. *Bull. US Natl Mus.* **175**: 1–225.

Taper, M.L. & Case, T.J. 1985. Quantitative genetic models for the coevolution of character displacement. *Ecology* **66**: 355–371.

Vitt, L.J. & Caldwell, J.P. 2009. *Herpetology: An Introductory Biology of Amphibians and Reptiles*, 3rd edn. Elsevier, San Diego, CA.

Werler, J.E. & Dixon, J.R. 2000. *Texas Snakes*. University of Texas Press, Austin, TX.

Williams, K.L. 1978. *Systematics and Natural History of the American Milk Snake, Lampropeltis triangulum*. Milwaukee Public Museum, Milwaukee, WI.

Supporting information

Additional supporting information may be found in the online version of this article:

Appendix S1 Detailed description of methods for phylogenetic inference and divergence time estimation, from Pyron & Burbrink (2009a).

Appendix S2 Museum numbers and morphological measurements for the 757 lampropeltinine specimens examined in this study.

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