

Ecological divergence and sexual selection drive sexual size dimorphism in new world pitvipers (Serpentes: Viperidae)

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Abstract

Hypotheses for the origin and maintenance of sexual size dimorphism (SSD) fall into three primary categories: (i) sexual selection on male size, (ii) fecundity selection on female size and (iii) ecological selection for gender-specific niche divergence. We investigate the impact of these forces on SSD evolution in New World pitvipers (Crotalinae). We constructed a phylogeny from up to eight genes (seven mitochondrial, one nuclear) for 104 species of NW crotalines. We gathered morphological and ecological data for 82 species for comparative analyses. There is a strong signal of sexual selection on male size driving SSD, but less evidence for fecundity selection on female size across lineages. No support was found for allometric scaling of SSD (Rensch's rule), nor for directional selection for increasing male size (the Fairbairn–Preziosi hypothesis) in NW crotalines. Interestingly, arboreal lineages experience higher rates of SSD evolution and a pronounced shift to female-biased dimorphism. This suggests that fecundity selection on arboreal females exaggerates ecologically mediated dimorphism, whereas sexual selection drives male size in terrestrial lineages. We find that increasing SSD in both directions (male- and female-biased) decreases speciation rates. In NW crotalines, it appears that increasing magnitudes of ecologically mediated SSD reduce rates of speciation, as divergence accumulates within species among sexes, reducing adaptive divergence between populations leading to speciation.

Introduction

Sexual size dimorphism (SSD) is a common pattern in nature and has been a major focus of research since the origins of evolutionary biology (Darwin, 1874). Numerous potential hypotheses for the ecological and evolutionary origins of SSD have been proposed (reviews in Hormiga *et al.*, 2000; Cox *et al.*, 2003; Stephens & Wiens, 2009), and a large body of research has clarified these mechanisms into three primary categories. These are (i) sexual selection on male size through mechanisms such as male–male combat (Cox *et al.*, 2003), (ii) selection related to fecundity on females, driven by a relationship between reproductive

capacity and size (Fairbairn & Shine, 1993), and (iii) ecological divergence in size mediated by intraspecific competition due to niche partitioning (Shine, 1989). Relatively few studies have considered all three explanations simultaneously, and those that have found mixed evidence for the various mechanisms (Hormiga *et al.*, 2000; Cox *et al.*, 2003; Stephens & Wiens, 2009).

To complicate matters further, SSD often scales allometrically and in different directions depending on which sex is larger (Rensch, 1960). Rensch's rule states that when males are larger (male-biased SSD, MBSSD hereafter), SSD is expected to increase with increasing body size, resulting in a hyperallometric relationship of male body size to female body size. Correspondingly, in taxa with larger females (female-biased SSD, FBSSD hereafter), SSD would be expected to decrease with increasing body size. This has been attributed to directional sexual selection for larger male body sizes, the 'Fairbairn–Preziosi hypothesis' (Fairbairn & Preziosi,

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1994; Abouheif & Fairbairn, 1997; Dale *et al.*, 2007; Stephens & Wiens, 2009). However, support for Rensch's rule and the Fairbairn–Preziosi hypothesis is not universal, and few studies have made links between the expected patterns of allometry and any mechanistic explanation (Kratochvíl & Frynta, 2002; Stephens & Wiens, 2009; Starostová *et al.*, 2010).

All of these processes can be addressed most powerfully in a comparative phylogenetic context. Previous studies have used such frameworks for testing the three classes of mechanisms for promoting the evolution of SSD (Hormiga *et al.*, 2000; Cox *et al.*, 2003; Stephens & Wiens, 2009). However, these previous studies were limited in some ways by (i) not including a majority of species in the groups examined, (ii) not using a time-calibrated phylogeny to place the evolutionary history of SSD in an explicit temporal context or (iii) lacking key information about the ecological correlates of intraspecific divergence.

We test here for the effects of sexual, fecundity and ecological selection on SSD, using size and ecological data and a well-sampled phylogenetic tree for New World (NW) pitvipers (Crotalinae [part]). Intraspecific combat has been shown to select for larger males and to be significantly associated with degree of SSD in snakes (Shine, 1978, 1994). Whether sexual selection acts in this manner is established by quantifying the relationship between male body size and SSD. We then examine the effects of fecundity selection by testing for a relationship between female body size and SSD, as larger females typically exhibit higher fecundity (Shine, 1994). Finally, differential resource availability and partitioning between macrohabitats or along gradients in latitude and elevation might be expected to result in ecological character displacement for body size (Bolnick & Doebeli, 2003). This results in SSD when different physiological optima are selected for according to different constraints acting upon each sex. We therefore examine geographical, elevational and macrohabitat data to quantify the influence of ecological selection on SSD to address the third established hypothesis for the evolution of SSD, the presence of ecological divergence.

New World crotalines are distributed throughout temperate North America, tropical Central America and tropical South America, with major elevational gradients in species richness, and major macrohabitat differentiation between arboreal and terrestrial species, in addition to frequent male combat and massive differences in female fecundity related to body size (Campbell & Lamar, 2002). Maximum adult total length (TL) in this group varies widely, from 50 cm in *Mixocoatlus barbouri* (Jadin *et al.*, 2011) to 360 cm in *Lachesis stenophrys* (Köhler, 2008). Given these attributes, NW crotalines present an excellent opportunity to study the correlates and drivers of SSD evolution. This study considers the effects of sexual, fecundity and

ecological selection on the origins of SSD in the group. For the purposes of this study, SSD is measured as the difference in maximum adult size between conspecific males and females.

In snakes, SSD can reach extremes: in multiple groups of colubroids, males can be up to 30% larger than conspecific females, whereas at the other end of the spectrum, some female boids can exceed conspecific males by up to 50% (Cox *et al.*, 2007). The NW crotalines exhibit the full spectrum of SSD, from FBSSD through monomorphism to MBSSD (Campbell & Lamar, 2002), which makes them a suitable candidate group with which to test the validity of Rensch's rule and the Fairbairn–Preziosi hypothesis. While the validity of Rensch's rule has been tested in snakes previously (Abouheif & Fairbairn, 1997), support was found to be mixed, no mechanistic hypotheses were tested, and crotalines were grouped with Old World viperines.

We assess allometric scaling of SSD in NW crotalines using phylogenetic regression to determine whether or not Rensch's rule is supported in the group. We use reconstruction of body size shifts along branches to assess support for the Fairbairn–Preziosi hypothesis. Directional selection for increasing male size may be expected to occur differentially between groups of lineages, for instance those that show opposing patterns of SSD or occupy different habitats, and therefore, examining the behaviour of body size in the context of the Fairbairn–Preziosi hypothesis may still provide key insights into the evolution of SSD in NW crotalines, even if Rensch's rule is not supported for the group overall.

Finally, we use recently developed algorithms linking continuous traits to diversification rates (FitzJohn, 2010) to determine whether SSD affects speciation. If sexual selection on male size or fecundity selection on female size drives SSD, we might expect a significant relationship between SSD and speciation, as high fecundity could be expected to influence speciation rate, or differential mate choice may lead to increased population segregation (West-Eberhard, 1983), and thus higher speciation. If ecological selection in different habitats is responsible for SSD, we would expect little relationship between SSD and speciation, as divergence between sexes would not be directly linked to divergence between populations. Finally, SSD may represent a trade-off between sex-specific and population-level selection pressures relating to resource partitioning, and thus, speciation rates may be reduced at extremes of SSD (Bolnick & Doebeli, 2003).

Materials and methods

Molecular data

We combined available molecular sequence data presented and analysed in previous studies (Castoe &

Parkinson, 2006, Douglas *et al.*, 2006; Castoe *et al.*, 2009; Fenwick *et al.*, 2009; Daza *et al.*, 2010; Jadin *et al.*, 2011), as well as unpublished sequences downloaded from the GenBank database. This is a minor update of the phylogeny presented in a previous study (Burbrink *et al.*, 2012a). Our taxonomy and species list is based on the August 2011 release of the Reptile Database (Uetz, 2011), from which we recognize 124 species of New World crotalines (Fenwick *et al.*, 2009; Jadin *et al.*, 2011; Pyron *et al.*, 2011).

We included one representative each from the Old World genera *Calloselasma*, *Deinagkistrodon*, *Garthius*, *Gloydus*, *Hypnale*, *Ovophis*, *Protobothrops*, *Trimeresurus*, *Trimesurus*, and *Tropidolaemus*, along with *Azemiops* as the sister to Crotalinae, as outgroups. We gathered data for 104 of the 124 species (84%) plus 11 outgroups (115 species total) from 8 genes, 7 mitochondrial: 12S (93 of 115 species, 81%), 16S (89, 77%), ATPase subunits 6 and 8 (ATP6/8; 23, 20%), cytochrome *b* (*cyt-b*; 111, 97%), NADH subunit 2 (ND2; 18, 16%), NADH subunit 4 (ND4; 97, 84%), NADH subunit 5 (ND5; 22, 19%) and one nuclear: oocyte maturation factor (*c-mos*; 6, 7%). Note that while some of these genes include sequence from relatively few species, these are either from important, difficult-to-resolve genera such as *Crotalus* (e.g. ND2) or distributed across numerous genera, from which little nuclear data have ever been analysed (e.g. *c-mos*). The total alignment length was 6700 bp, and the average sequence length was 2544 bp (38% complete), similar to previous studies (e.g. Castoe & Parkinson, 2006), with a range from 528 bp (8%) to 6342 bp (95%). Accession numbers are available in Appendix S1, and the final matrix and tree are available in the Dryad Digital Repository: doi: 10.5061/dryad.rf18s.

Phylogeny and divergence times

We followed previous studies for the phylogenetic and temporal analyses of these data (Castoe & Parkinson, 2006; Castoe *et al.*, 2009; Daza *et al.*, 2010; Burbrink *et al.*, 2012a). We determined the optimal model of sequence evolution using jMODELTEST (Posada, 2008). Although previous analyses have suggested that partitioning by codon position is generally an optimal strategy for some of these data (Castoe & Parkinson, 2006), preliminary analyses yielded poor mixing and convergence, so we partitioned by gene only. We performed simultaneous tree and date estimation in BEASTv1.61 (Drummond & Rambaut, 2007). We used four calibration points from previous studies (Castoe *et al.*, 2009; Daza *et al.*, 2010): (i) the origin of the genus *Agkistrodon* was given a minimum of 5.0 Ma (Holman, 2000) using an exponential distribution with an offset of 9.0 Ma and a mean of 1.0 [95% Highest Prior Density (HPrD) = 5.0–8.7 Ma], (ii) the *Crotalus atrox* species group (*C. atrox*, *C. ruber*, *C. catalinensis*) was constrained

to occur between 2.5 and 4.5 Ma (Castoe *et al.*, 2007, 2009) using a lognormal distribution (mean = 1.2101, SD = 0.14994), (iii) the origin of the genus *Sistrurus* was constrained to a minimum of 9.0 Ma (Parmley & Holman, 2007) using an exponential distribution with an offset of 9.0 Ma and a mean of 1.0 (95% HPrD = 9.0–12.7 Ma), and (iv) the MRCA of the Old and New World crotalines was given a mean age of 16.0 Ma (Holman, 2000), with a 95% Highest Prior Density from 9.8 to 26.1 Ma (mean = 2.7725, SD = 0.25). We ran two chains of 30 million generations each, discarding the first 5 million as burnin. The remaining 50 million were combined and summarized for the final topology.

Morphological and ecological data

We assembled morphological data on the maximum total length (Max_TL), maximum male length and maximum female length (MTL and FTL, respectively) for all of the New World crotalines for which these data are available. Measures of maximum adult size were used in preference over average adult size, given that snakes exhibit indeterminate growth, and determining the size threshold of an 'adult' animal for the purpose of these analyses would be intractable. The potential inclusion of subadult individuals in an average measure would result in an unfair representation of SSD. Use of maximum size as opposed to average adult size has been shown to have no negative impact on results in other taxa with indeterminate growth (Stephens & Wiens, 2009) and has been used in other studies of biogeographical patterns in snakes (e.g. Terribile *et al.*, 2009). For specimens where the source material contained only snout-vent length (SVL), the total length was interpolated from tail length (TL)/SVL ratios of other specimens of the either the same species (for *Crotalus atrox*), or of closely related species. In the case of *Bothropoides jararaca*, *B. neuwiedi* and *B. pauloensis*, we used measurements from *B. alcatraz* (Marques *et al.*, 2002).

To address the role of ecological selection, we gathered data on the elevational range, latitudinal occurrence and macrohabitat preference of each species. We determined the upper and lower elevational thresholds of each species for which reliable records exist, and these data were then used to determine the elevational mid-point of the species range. For latitudinal analyses, species were broadly grouped into three geographical regions of occurrence: North, Central and South America. Finally, the species were categorized as either terrestrial or arboreal according to habitat preference. All data were obtained from an extensive literature search, with Campbell and Lamar (2002) and various works by L.M. Klauber (e.g. Klauber, 1937, 1972) contributing a large proportion of the data (among ~70 other published works). Although more

direct measures of sexual (e.g. intensity of combat) and fecundity (e.g. clutch size) selection might be desirable, both are strongly correlated with overall body size (Shine, 1978, 1994, 2000), data for which are far more prevalent for many poorly known crotaline species, particularly in the tropics. Data are available in Appendix S2. In total, of the 124 species of New World crotalines, relevant records and sequence data were available for 82 species, including representatives from all 15 currently recognized genera (Uetz, 2012).

Calculation of SSD

For comparative analyses, we used a ratio measure to quantify SSD as in many recent studies (Webb & Freckleton, 2007; Stephens & Wiens, 2009; Frýdlová & Frynta, 2010). We refer to Smith (1999) for a detailed and thorough justification of both the choice of measure and the method of calculation. The ratio measure of SSD was calculated using Smith's two-step extension of the Lovich-Gibbons ratio (Lovich & Gibbons, 1992; Smith, 1999). Using this method, SSD is calculated as follows:

If females are larger: (Larger Sex/Smaller Sex) – 1

If males are larger: – [(Larger Sex/Smaller Sex) – 1]

An estimation of ancestral dimorphism was made by reconstructing body size across the phylogeny using GLS ancestral state reconstruction in R 2.13.1 (R Development Core Team, 2011) in the 'Geiger' package (Harmon *et al.*, 2008). Male and female body sizes were independently reconstructed onto the phylogeny as log (MTL) and log(FTL), and then SSD was calculated for each node using the approach described above. Reconstructing changes in body size for each sex separately and then calculating SSD at each node from these values as opposed to recreating SSD directly onto the phylogeny allows for the assumption that different selective forces may have acted on each sex over time.

Phylogenetic comparative analyses

It has been shown that snakes exhibit conservatism in degree of SSD at both the generic and family level (Shine, 1978, 1994), and so phylogenetically independent contrasts (PICs) of SSD were used for all tests to negate phylogenetic bias (Felsenstein, 1985). To assess whether either the amount of SSD or the rate of evolution of SSD was influenced by categorical ecological and geographical variables, we used the phylogenetic area-rates tests (Burbrink *et al.*, 2012b). The variables examined were geographical area (North, Central or South America, as explained previously) and macro-habitat (either terrestrial or arboreal lifestyle). We performed PGLS regression using the package 'Caper'

(Orme *et al.*, 2012) in R 2.15.2 (R Development Core Team, 2012) to test for the signature of sexual selection (regressing SSD against male body size), fecundity selection (SSD regressed against female body size) and ecological variation (SSD regressed against elevational midpoint of range). PGLS analysis is a particularly suitable method as phylogenetic bias is taken into account. Maximum likelihood estimates of the branch length parameters delta, lambda and kappa were obtained to optimize the fit of the model in each comparison.

Model fitting for evolutionary traits was performed on logMTL and logFTL in R 2.15.2 using the 'Geiger' package (Harmon *et al.*, 2008). The small sample version of the Akaike information criterion (AICc) was used to select the most appropriate model of trait evolution; we then transformed the branch lengths to fit a Brownian model to avoid violating the assumptions of subsequent analyses (Burnham & Anderson, 2004). The evolutionary models compared were a random walk or Brownian motion process (BM), the single selective peak of the Ornstein-Uhlenbeck (OU) model, a monotonic rate decrease represented by a linear model, a rapid burst of trait evolution followed by exponential decrease as described by the Early Burst (EB) model and two diversity-dependent models based on linear and exponential decreases with increasing diversity (DDL and DDX, respectively). To test for a relationship between speciation rate and magnitude of SSD, we used the Quantitative State Speciation and Extinction (QuaSSE) function of the 'diversitree' package (FitzJohn, 2010) in R 2.15.2. We tested constant, linear, sigmoidal and hump-shaped models of speciation rate in response to SSD. Ratio SSD forms a continuous variable, with 0.0 representing the mid-point or monomorphism.

Rensch's rule and allometric dimorphism

The allometry of SSD was established using PGLS regression as previously described. Once again, raw body size measurements were log-transformed and log male body size was regressed against log female body size. Under Rensch's rule, male body size scales hyperallometrically to female body size, and so a significant hyperallometric relationship would be expected if Rensch's rule is supported in this group.

To evaluate the applicability of the Fairbairn-Preziosi hypothesis, patterns of shifts in body size along branches were quantified using ancestral state reconstructions of body size, performed using R 2.15.2. Evolutionary model fitting for logMTL and logFTL resulted in the selection of the OU model (Brownian motion with a selective constraint) in both cases (logMTL – AICc = 81.07276, Δ AICc = 1.11; and logFTL – AICc = 74.79221, Δ AICc = 1.59). Branches were transformed to fit a Brownian model, and PIC reconstruction of ancestral body size for each node was

performed for logMTL and logFTL separately to allow for independent changes in body size to occur. Our approach differs from that of Stephens and Wiens (2009) in that we use the methodology described above to calculate ancestral SSD at each node (based on the reconstructed logFTL and logMTL) as opposed to reconstructing SSD value onto the phylogeny. Using the nodal SSD for each branch, we determined (i) the direction of SSD, (ii) the changes in male and female body size and (iii) in which sex the magnitude of the change was greatest. Analysis of these patterns allows us to accept or reject the Fairbairn–Preziosi hypothesis for the evolution of Rensch’s rule (Stephens & Wiens, 2009). The behaviour of SSD in MB and FB lineages was considered separately to negate effects of bias caused by differential patterns of allometry between the groups.

In this way, there are eight possible combinations for each branch, only two of which would support the FP hypothesis as valid in crotalines (Combination 1 = lineage exhibits MBSSD, SSD increases, and change in male body size is greater; Combination 2 = lineage exhibits FBSSD, SSD decreases, and change in male body size is greater). A chi-square goodness-of-fit test, implemented in R 2.15.2, was used to test whether or not there are more branches with this pattern than would be expected by chance, and at the same time allowing bias into any of the other categories to be revealed.

To elucidate whether the pattern of SSD evolution differs between arboreal and terrestrial lineages, we used the macrohabitat preference of the species to reconstruct ancestral habitat use across the phylogeny. We employed the ‘ace’ function of the ‘Ape’ package in R 2.15.2 which is suitable for discrete variables (Paradis *et al.*, 2004), likelihood tests selected the symmetric model for transition probabilities as most appropriate (likelihood = -16.0334 , transition rate = 0.005406). We then applied the categorical approach to describing the behaviour of SSD on a given branch (described in detail above) to separate groups of arboreal and terrestrial lineages. Once again, we used chi-square goodness-of-fit tests to examine whether any one category of SSD behaviour occurs more frequently than predicted by a null model.

Results

Phylogeny and divergence times

The BEAST analysis converged quickly (ESS > 200 in < 5 million generations). The summarized topology is generally well supported (74.6% of nodes have $Pp > 0.95$), and the time-calibrated tree is shown in Appendix S3. Unfortunately, the nodes forming the back bone of the tree receive some of the weakest support values, and we are able to offer no additional

resolution regarding the sister relationships of the larger clades of NW crotalines (e.g. which clade represents the sister lineage of the *Bothrops* group). Weak support among the deeper nodes of the NW crotaline phylogeny is not uncommon (e.g. Castoe & Parkinson, 2006; Castoe *et al.*, 2009; Jadin *et al.*, 2011; Pyron *et al.*, 2013) and is reflected in the historical variability of hypothesized higher relationships in this group (Carrasco *et al.*, 2012). Divergence-time estimates give a mean age of 15.5 Ma to the MRCA of NW crotalines (HPD_{95%} = 13.2–18.2), which is in concordance with previous estimates (Wüster *et al.*, 2002; Castoe *et al.*, 2009). Although parts of the alignment contain relatively high amounts of missing data (e.g. 7% coverage for c-mos), simulation studies have indicated that reliable phylogenetic reconstruction can be performed despite missing data in the alignment [see Wiens & Morrill (2011) for review], and therefore, we are confident in this topology as a basis for phylogenetic comparative tests.

Evolution of SSD

Ancestral reconstruction of habitat onto the phylogeny indicates that the MRCA of the NW crotalines was a relatively small terrestrial species, approximately one metre in length (Boback & Guyer, 2003). This is congruent with previous hypotheses (Greene, 1992), and our data also support the inference that the ancestor of *Bothrops* was a small, stout, terrestrial species (Martins *et al.*, 2001). Our reconstructions indicate that arboreality has evolved at least three times within the NW crotalines: (i) in the lineage subtending *Bothriechis*; (ii) between the MRCA of the sister group comprising *Bothrops* and *Bothriopsis* and the MRCA of *Bothriopsis* alone, and (iii) in *Bothrops punctatus*, representing a recent transition to arboreality within *Bothrops*.

Overall, the average body size of the arboreal species was smaller than that of their terrestrial counterparts (logFTL = 4.506 in arboreal lineages, 4.529 in terrestrial; and logMTL = 4.408 in arboreal lineages, 4.514 in terrestrial). Statistical support for this pattern is weak, however, likely due to the large variance in body size within the NW crotalines. In the *Bothrops* group, a clade with both arboreal and terrestrial members, FTL of the terrestrial taxa was larger than that of the arboreal taxa, and there is marginally significant support ($F_{9,374} = -1.727$, $P = 0.058$) for a decrease in female body size associated with arboreality.

In terms of direction of SSD, the NW crotalines exhibit an approximately even split between male-biased (MBSSD) and female-biased (FBSSD) dimorphism across the group (Fig. 1). Taking each habitat in isolation, terrestrial species exhibit MBSSD in 52% of lineages, and FBSSD in 48% of lineages. In arboreal lineages, the proportion of FBSSD rises significantly to 70% ($Z = 1.849$, $P = 0.032$), suggesting a positive

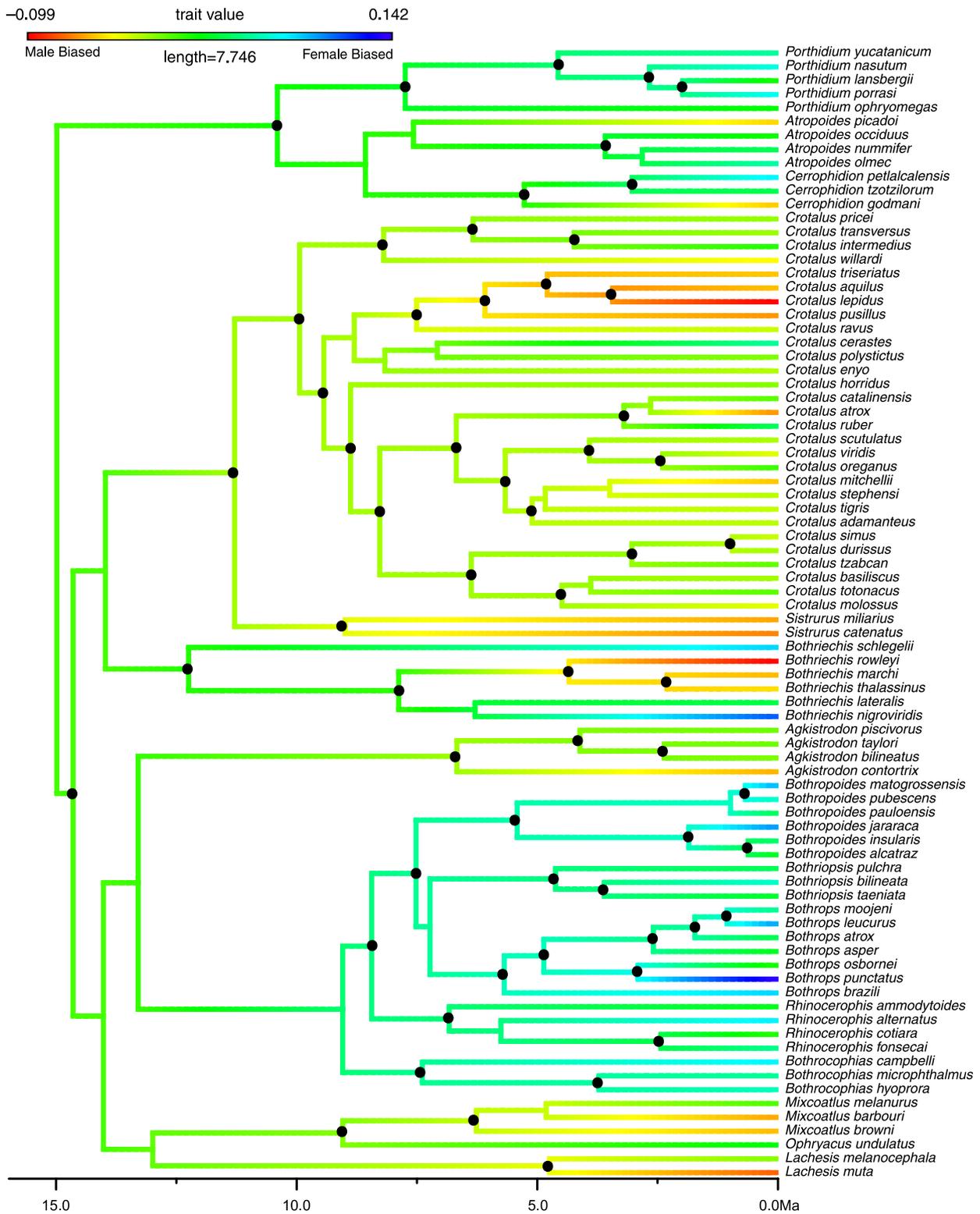


Fig. 1 Phylogeny of 82 species of NW crotalines, with branches coloured/shaded by ratio SSD values. SSD was reconstructed onto the phylogeny using the contMap function of the 'phytools' package (Revell, 2012) in R 2.15.2 (R Development Core Team, 2012). Red/white and blue/black coloration corresponds to the extremes of male-biased and female-biased SSD, respectively. Black circles indicate nodal support > 95% (see Appendix S3 for all support values).

correlation between a macrohabitat transition to arboreality and a shift towards female-larger dimorphism (Fig. 2). Continuous-trait mapping was used to visualize the distribution of SSD bias on the phylogeny (Fig. 1). Although lineages with MBSSD and FBSSD are dispersed throughout the phylogeny, clear predominance of MBSSD in *Crotalus* and FBSSD in the *Bothrops* group can be observed. Average SSD in each group deviates significantly from a null expectation of monomorphism (SSD = 0.0) when compared with a null data set of SSD values generated using the variance of the experimental data, but constrained to a mean of 0 ($F_{55,987} = 2.466$, $P = 0.017$ in *Crotalus*, $F_{50,427} = -6.627$, $P \leq 0.005$ in the *Bothrops* group).

Drivers of SSD

There is a significant relationship between male body size and SSD ($R^2 = 0.194$, $F_{2,77} = 19.76$, $P \leq 0.005$), as expected if sexual selection drives dimorphism, but no relationship between female size and SSD, suggesting fecundity selection does not contribute significantly to increased SSD overall. With respect to the ecological drivers of SSD, the area-rates tests recovered no significant geographical patterns in SSD, and there was no elevational gradient in SSD when corrected for phylogeny. In contrast, SSD (ratio) was found to vary significantly between macrohabitats both in terms of

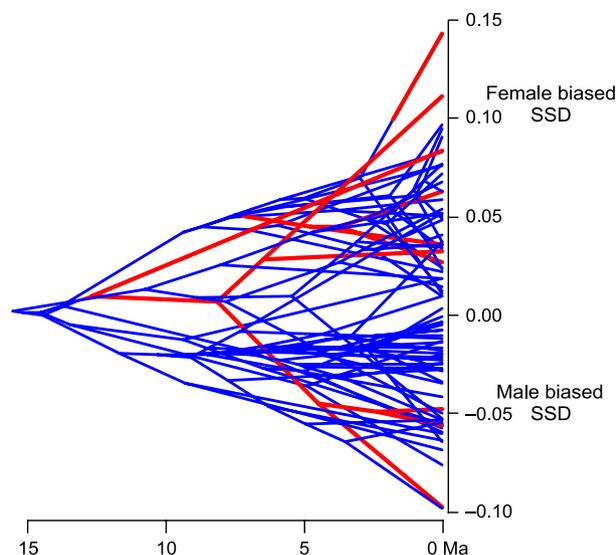


Fig. 2 Traitgram illustrating distribution of lineages along a ratio SSD axis. Lineages with MBSSD have negative values, with positive values of ratio SSD indicating FBSSD. Isometry is encountered at 0.0. Branches are coloured/shaded by macrohabitat; blue/light grey indicates terrestrial lineages, whereas thicker, red/black lines denote arboreal lineages. 70% of reconstructed arboreal lineages fall on the FBSSD side of the continuum.

rates of evolution of SSD and mean dimorphism, with increased rates ($\sigma^2 = 8.13e^{-04}$ and $3.4e^{-04}$, in Arboreal and Terrestrial lineages, respectively) and higher mean dimorphism (Arboreal = $4.514e^{-02}$ log units, Terrestrial = $3.217e^{-07}$ log units) in arboreal lineages than in their terrestrial counterparts, with significantly better fit of a habitat-specific model ($\Delta AICc = 4.0568$) over a single-rate model.

Allometry of SSD

We found no support for Rensch's rule in NW crotalines. The maximum likelihood values for the branch length parameters of the PGLS regression were optimized as follows; delta = 0.97956, max. log-likelihood = 24.13773; lambda = 0.87755, max. log-likelihood = 24.48964; kappa = 0.97956, max. log-likelihood = 24.14322. With a regression slope of 0.924 ($CI_{95\%} = 0.813-1.036$, $r^2 = 0.764$), there is no significant deviation from an isometric relationship between body size and SSD in this group.

The Fairbairn–Preziosi hypothesis predicts that the pattern of allometry in SSD is caused by directional selection towards increases in male body size. In order for this to be true for any particular lineage, a certain combination of attributes of SSD must occur in that lineage. In NW crotalines, none of the combinations of attributes predicted under the Fairbairn–Preziosi hypothesis occurred on more branches than expected under a null model (chi-square tests, $P \geq 0.05$ in all cases). Specifically, in lineages with FBSSD, there was no evidence that changes in male body size act to reduce SSD ($\chi^2_1 = 0.0041$, $P = 0.9489$), and in lineages exhibiting MBSSD, no support was found for the expectation that greater change in male size is responsible for increasing dimorphism ($\chi^2_1 = 0.9259$, $P = 0.3359$). Therefore, although we find that crotalines exhibit sexual selection on male body size, we reject the Fairbairn–Preziosi hypothesis as the causative mechanism of the patterns observed. Directional selection towards larger male size cannot be implicated as the sole driving mechanism for the evolution of SSD in the group.

An unexpected pattern emerges during the analysis of the distribution of shifts in body size and dimorphism: when SSD is increasing in arboreal lineages with FBSSD, decreases in male body size occur more often than predicted by a null model (average male body size change = -0.22939 log units, $\chi^2_1 = 4.667$, $P = 0.0308$). This indicates that a decrease in male body size has a significant contribution to increasing SSD in arboreal lineages.

Speciation and SSD

The most appropriate model of trait evolution for SSD was the Ornstein–Uhlenbeck (OU) model with an

AICc value of -279.142 ($\Delta\text{AICc} = 0.879$), and therefore, we transformed the branch lengths accordingly for subsequent analyses. With regards to the relationship between SSD and speciation rate in crotalines, the hump-shaped (modal) model of diversification was found to be the best-fit using QuaSSE ($P = 0.03507$, $\Delta\text{AIC} = 2.603$). The highest speciation rates are estimated to occur between ratio SSD values of 0.0051 and 0.0127 ($\text{CI}_{95\%}$, mean = $8.91e^{-03}$, var = $3.099e^{-04}$). This indicates that lineages with intermediate values of ratio SSD experience the highest rates of diversification, which then decreases towards the extremes of the range. Within NW crotalines, ratio SSD ranges from -0.099 in lineages with MBSSD to 0.142 in lineages with FBSSD. Intermediate values of SSD thus represent the lineages that are the closest to monomorphism. There is no influence of the direction of SSD bias on speciation rate, as indicated by the hump-shaped model selection in QuaSSE over a directional or invariant model.

Discussion

Origins of SSD

We find strong support for the effect of sexual selection on male size driving SSD patterns in crotaline snakes, although not in accordance with the expectations of the Fairbairn–Preziosi hypothesis. Conversely, there is no direct relationship between female size and SSD, suggesting that direct selection on fecundity is not a primary driver of SSD in the group. There is no latitudinal gradient in SSD, suggesting that neither latitudinal variation in body size or climate, nor differential diversification patterns on a latitudinal axis are responsible for driving SSD in this group. Additionally, SSD does not vary with elevation, indicating that ecological pressures across latitudinal or elevational scales are not driving patterns in sexual selection.

Our data indicate that macrohabitat use has the strongest effect on SSD in crotalines, with higher rates of SSD evolution in arboreal lineages, as well as an increased degree of dimorphism. Macrohabitat has been shown to be correlated with degree of SSD in anoles and turtles (Butler *et al.*, 2000; Stephens & Wiens, 2009); however, this study provides the first empirical evidence for such a relationship in snakes. A shift in macrohabitat use from terrestriality to arboreality may be expected to affect body size as different physiological and functional demands between the habitats may be expected to generate different selective peaks (Collar *et al.*, 2011). We find a general decrease in average body size with the transition to arboreality, a pattern also observed by Martins *et al.* (2001), and observe that the maximum length in arboreal crotalines is approximately 1 m, in accordance with the predictions of Lillywhite & Henderson (1993). Adaptive explanations for

this upper limit in body size in arboreal species include i) cardiovascular restrictions on length and width imposed by gravity (Lillywhite & Smits, 1992) and ii) physiological constraints on maximum size imposed by vertical posturing and cantilevering among branches (Lillywhite & Henderson, 1993).

While these factors may account for the overall reduction in body size, sex-independent ecological pressure must act equally on both sexes, and therefore, we propose that differential sexual selection, in combination with increased sources of ecological opportunity, is responsible for the evolutionary pattern of SSD in arboreal crotalines. This is thus an extension of the secondary ecological dimorphism hypothesis (Shine, 1989); ecological divergence exaggerates SSD present through sexual or fecundity selection due to the physiological constraints imposed by the underlying ecomorphological divergence between or among the sexes.

An increased proportion of FBSSD in arboreal lineages is thus likely due to fecundity selection imposing a lower bound on female size that does not necessarily apply to male body size. NW crotalines are almost exclusively viviparous (with the exception of the four *Lachesis* species), and it is known that abdominal volume limits fecundity in viviparous species (Shine, 1988; Lourdaix *et al.*, 2002). Therefore, we find that fecundity selection is not a primary driving factor in SSD in NW crotalines at the upper bounds of female size, which does not rule out that there may be lower bounds on female size imposed by fecundity selection. Maternal fecundity in arboreal snakes is also affected by altered positioning of the ovaries, with anterior movement resulting in a nonoverlapping position to minimize girth during vitellogenesis (Pizzatto *et al.*, 2007). In a system in which ecological selection pressures cause a reduction in body size, it follows that females will remain as large as possible within the constraints of the environment to maximize fecundity, creating a lower bound of female size approximately equal to the maximum size physiologically viable for arboreal habitat use.

It should be noted that although FBSSD is more prevalent in arboreal lineages than MBSSD, that is not to say that there are no arboreal lineages in which MBSSD is observed. Bias of SSD in arboreal pitvipers of the genus *Bothriechis* is evenly split between male-biased and female-biased (Fig. 1) and illustrated phenotypically by a branch which falls on the male-biased side of the SSD continuum, despite an arboreal habitat reconstruction (Fig. 2). The species exhibiting MBSSD (*B. marchi*, *B. rowleyi* and *B. thalassinus*) are all members of the Nuclear Central America group of palm pitvipers (Townsend *et al.*, 2013), although they share no clear pattern in behaviour of SSD. This suggests that the interplay of underlying evolutionary selective forces might be particularly complex in this group of species, likely with some as-yet undetermined factor having an influential role in the evolution of male body size.

Patterns of SSD

We find no support for the validity of Rensch's rule in NW crotalines, in that there is no significant hyperallometric scaling of male body size to female body size. We are able to reject the Fairbairn–Preziosi hypothesis for the relationship between male body size and SSD and conclude that while shifts in male body size certainly occur within the group, a consistent trend towards positive change in male body size is not a significant driving force in the evolution and maintenance of SSD. Our findings indicate that the mechanisms driving the evolution of SSD in NW crotalines cannot be explained by allometry alone.

Our data indicate that increasing magnitude of SSD in arboreal lineages is driven by decreases in male body size, implying that males continue to respond to ecological selection for smaller size past the point where fecundity selection constrains lower female size. While this may represent solely ecological pressures, it is likely that sexual selection has an additive effect. The Ghiselin–Reiss hypothesis predicts that the trade-off between reproduction and foraging will result in selection for reduced male size (Blanckenhorn *et al.*, 1995). Males of species which do not engage in combat will increase reproductive success by actively searching for as many mates as possible (Duvall *et al.*, 1992), and whereas combat is common in terrestrial crotalines (Shaw, 1948; Klauber, 1972; Shine, 1978), it is less likely in arboreal species (Shine, 1993).

Although male–male combat is well known in species of *Crotalus* and *Agkistrodon*, there is no clear evidence of corresponding territoriality in the mating system. Thus, the smaller optimum body size for males observed in arboreal species seems unlikely to be advantageous in terrestrial species, as there is no potential for the sneak-competitor approach to reproductive success that is often evolved when males defend a fixed breeding territory (Gross, 1996; Sinervo & Lively, 1996). Smaller male body size is likely reinforced as a selective optimum by the demands of successful foraging in an arboreal environment. Smaller and lighter snakes are better able to maximize their use of the canopy, accessing narrower perches which may have a high availability of roosting prey (Lillywhite & Henderson, 1993).

Thus, SSD initially established by fecundity selection can be maintained and exaggerated by intersexual resource partitioning once dimorphism reaches a degree at which different resources are available to the sexes (Shine, 1993). Intersexual differentiation of resource use in diet is known to occur in lizards (Cox *et al.*, 2007), and more infrequently in snakes (Luiselli *et al.*, 1997, 2005). Although resource partitioning has not been documented for pitvipers, one could speculate that this is likely as a driver of SSD in arboreal snakes in particular for a number of reasons: (i) the complexity

of the habitat lends itself to differential foraging strategies, (ii) arboreal snakes have been noted as being trophically specialized (Lillywhite & Henderson, 1993) and (iii) intersexual differences in diet may be an inevitable consequence of SSD in gape limited predators (Shine, 1993).

SSD and diversification

We find a relationship between SSD and speciation rates such that the highest rates of speciation are observed in lineages with minimal dimorphism, essentially ruling out a role of SSD as a diversification mechanism for adaptive radiation in NW crotalines. Lineages at the extremes of the SSD observed in this group (both male-biased and female-biased) experience lower rates of speciation. Large magnitudes of SSD can reduce the amount of resource competition between the sexes by allowing intersexual differentiation in resource use (Bolnick & Doebeli, 2003). Each species is therefore able to utilize a larger proportion of a given resource axis, allowing for differentiation along a resource axis to be maintained intraspecifically as sexual dimorphism instead of manifesting as interspecific divergence.

Future directions

Why might crotalines shift to arboreal lifestyles and develop SSD to begin with? This study finds 3 independent origins of arboreality in NW crotalines; however, no common causal factor can be identified that may have been responsible for this shift. In all cases, the arboreal taxa are dietary generalists, preying on mammals, amphibians and reptiles (Campbell & Lamar, 2002), and so dietary specialization seems unlikely to be the cause of the shift to arboreal habitat use. The shifts to arboreality occur at intervals throughout geologic time, from relatively old transition in the lineage subtending *Bothriechis*, to a more recent shift in *Bothrops punctatus*. This means that any one climatic or geologic event in the history of South America cannot be inferred as a trigger for expansion into novel habitats. It does seem intuitive, however, that a transition in habitat use might occur for one of two primary reasons; first, shifts in vegetation zones in response to climatic oscillations may provide a new source of ecological opportunity, or second, competition for resources among multiple species in an ancestral area may have provided the catalyst needed for the development of novel habitat use. The elucidation of the causal factors in ancestral habitat use transitions is certainly an area that warrants further study.

Conclusions

The evolution and contemporary pattern of SSD in NW crotalines is complex. There is no support for Rensch's

rule to describe the allometric scaling of SSD, nor for the Fairbairn–Preziosi hypothesis to explain the selective forces acting on male body size within the group. The evolution of SSD in NW crotalines is neither linked to elevation, nor determined by region, but is significantly impacted by shifts in macrohabitat use. Both rates and magnitude of SSD are higher in arboreal species than in terrestrial species, and there is a pronounced shift to a dominance of female-biased SSD in arboreal lineages. In the light of the relationship observed between SSD and speciation rate, this differential pattern of SSD across macrohabitat hints at a potential relationship between shifts in macrohabitat use and changes in speciation rate that certainly warrants future investigation. We find evidence that in NW crotalines, morphological disparity resulting from ecological selection on body size is maintained intraspecifically in the form of increased rates and magnitude of SSD, likely due to the effects of interspecific sexual and fecundity selection and the differential phenotypic plasticity of the sexes with regards to maximum body size. This is corroborated by rates of speciation in NW crotalines being highest at the lowest point of the dimorphism spectrum. The impact of ecological divergence on SSD in other groups will be an important area of future study, as physiological constraints on reproduction and sexual and fecundity selection have rarely been examined in a comparative phylogenetic context, but may provide an important avenue for understanding ecomorphological divergence in many taxa.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 GenBank Accession numbers for all mitochondrial and nuclear sequence data used in this analysis.

Appendix S2 All morphological and ecological data used in this analysis, including references for all data.

Appendix S3 Time-calibrated Bayesian phylogeny of 115 species of pitvipers (104 NW crotalines & 11 out-group taxa) generated using the full concatenation of 8 genes (7 mitochondrial & 1 nuclear) in BEASTv1.6.1.

Data deposited at Dryad: doi:10.5061/dryad.rf18s

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