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Ontogenetic co-option of myotoxin expression variation in island Eastern Diamondback Rattlesnake (*Crotalus adamanteus*) venoms

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ABSTRACT

Rapid adaptive evolution often arises from standing genetic variation within populations because of the rarity of *de novo* beneficial mutations and the time needed for their fixation. Eastern Diamondback Rattlesnakes (*Crotalus adamanteus*), a model species in the study of venom evolution, frequently inhabit islands, providing a useful model for studying the types of genetic variation leading to rapid venom evolution. In *C. adamanteus*, the primary axes of venom expression variation across the range are ontogeny (i.e., adult-juvenile) and regional differences in myotoxin (crotamine) expression; for the latter, myotoxin expression generally increases with latitude independent of age class due to differences in gene copy number. Prior work, however, found that on Caladesi Island (Florida), a juvenile and adult *C. adamanteus* exhibited expression patterns inconsistent with those observed across the species' range; here, the juvenile expressed high levels of myotoxin which was largely absent in the adult. To determine whether this variation reflected the co-option of existing axes of variation (i.e, myotoxin being integrated into the ontogenetic network) or standing polymorphism in myotoxin expression in the island population, we analyzed venom protein expression in 19 *C. adamanteus* of varying body sizes across three young islands (<5000 years old), including Caladesi Island, off the west coast of Florida. We found clear ontogenetic differences in venom expression across all three islands, including a decline in myotoxin expression with age. The incorporation of myotoxin expression into ontogeny has not been observed elsewhere within *C. adamanteus*. The novel integration of the two largest axes of variation via co-option of myotoxin into the ontogenetic venom expression network likely facilitated rapid adaptation in these island populations. Overall, rapid venom evolution on these islands appeared to be biased not only towards standing genetic variation but large, existing axes of variation previously subjected to selection.

1. Introduction

Populations colonizing new environments often experience strong and novel selective pressures that induce rapid adaptation, typically occurring through standing genetic variation (Barrett and Schluter, 2008; Kersten et al., 2023). Soft selective sweeps occur more rapidly than hard sweeps from *de novo* mutations because existing beneficial alleles are immediately available for selection to act on and can quickly fix within a population (Innan and Kim, 2004). Since these alleles are already present, they often have contributed to phenotypes previously favored by selection, suggesting a history of conditional advantage that enables these traits to reemerge and spread rapidly when similar selective pressures recur (Rieseberg et al., 2003). Because species are more frequently encountering altered environmental conditions and novel selective pressures in the Anthropocene, understanding the mechanisms enabling rapid adaptation are increasingly important for conservation (Franks et al., 2007).

Snake venoms are model systems for studying rapid adaptive evolution in response to novel environments. Snake venoms are a polygenic trait composed of multiple proteinaceous toxins (Daltry et al., 1996; Mackessy et al., 2006). Venom is subject to strong directional, diversifying, and/or balancing selection, depending on ecological context, due to its function in feeding and defense (Daltry et al., 1996; Margres et al., 2017b; Holding et al., 2016b; Mackessy et al., 2018; Schield et al., 2022). Venom has been shown to vary across species (Casewell et al., 2013, 2020), populations within species (Rokyta et al., 2015b; Holding et al., 2016b; Barlow et al., 2009; Gibbs et al., 2009), and within individuals across life history (Mackessy, 1988; Wray et al., 2015; Mackessy et al., 2018; Hirst et al., 2024). The Eastern Diamondback

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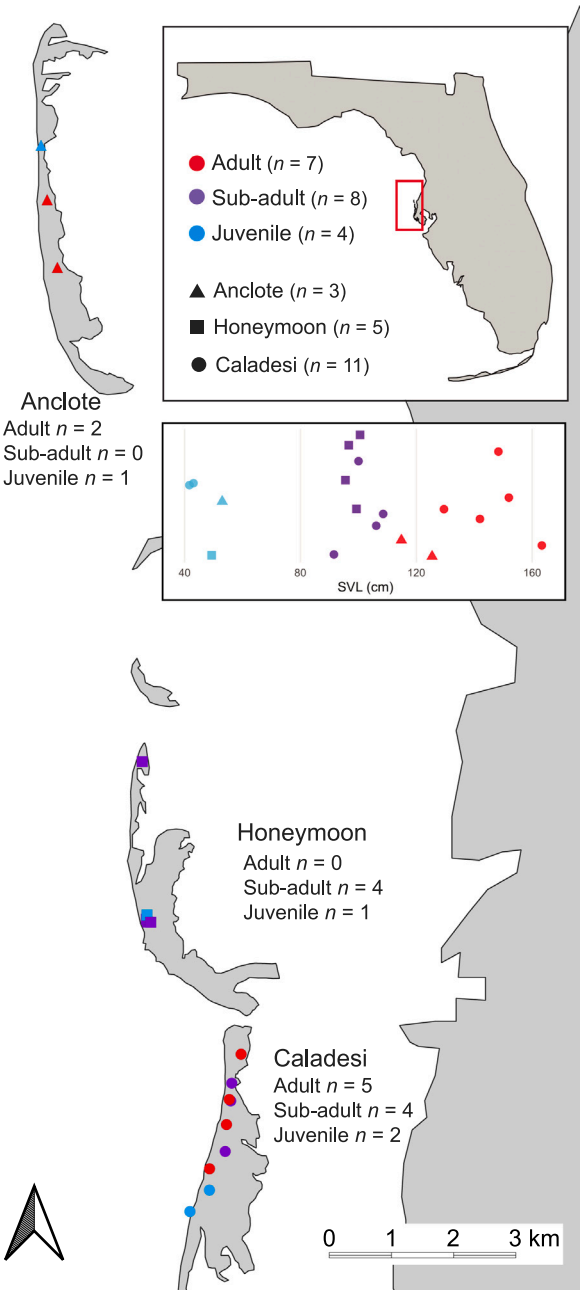

Fig. 1. Map of sampling locations consisting of Anclote Key, Honeymoon Island, and Caladesi Island. Collection sites are marked, with colors indicating juveniles (≤ 90.0 cm), sub-adults (90.1–109.9 cm), and adults (≥ 110.0 cm) collected at each site (shape). The distribution of snake size (SVL: Snout-vent length) is also shown.

Rattlesnake (*Crotalus adamanteus*) is one of the most comprehensively studied venomous snake species, with a chromosome-level genome assembly, detailed analysis of range-wide venom expression variation, and the identification of putative regulatory elements and dietary differences across populations (Margres et al., 2014, 2015b,a, 2016, 2017b; Rokyta et al., 2011, 2012, 2015a, 2017; Hogan et al., 2024; Nachtigall et al., 2025). The two largest axes of venom variation in *C. adamanteus* across the range are life history (i.e., ontogeny; Margres et al., 2015b) and myotoxin expression (Margres et al., 2017a).

Ontogenetic shifts in toxin expression represent the primary axis of venom variation in *C. adamanteus*, reflecting dietary changes across life history stages (Margres et al., 2015b). As snakes grow, expression of key venom components gradually shift to match developmental and ecological demands, like changes in prey size and/or species (Margres et al., 2015a; Schonour et al., 2020). Juvenile *C. adamanteus* venoms, for example, have a higher toxicity to mice than adults, supporting toxin specificity towards small mammals as prey shifts from small mice and cotton rats as juveniles to squirrels and rabbits as adults (Rokyta et al., 2017; Means, 2017; Schonour et al., 2020). Juveniles express most adult-biased toxins, though expression is upregulated in adults, resulting in a more functionally complex venom phenotype (Rokyta et al., 2017; Margres et al., 2015b; Wray et al., 2015; Hogan et al., 2024). The second largest axis of variation is in the expression of myotoxin Margres et al. (2017a). Myotoxin, or crotamine, is often the most abundant venom component in populations north of the Suwannee River while mostly (and often entirely) absent in southern populations, regardless of life stage (Margres et al., 2017a, 2019). Myotoxin is a small, basic protein that aids in prey capture via muscle breakdown and paralysis of hind limbs (Peigneur et al., 2012; de Oliveira et al., 2003). Geographic divergence in myotoxin expression is due to variation in copy number and may represent local adaptation to distinct prey communities (Margres et al., 2015b, 2016, 2017b). Together, these patterns of venom variation underscore how ecological pressures, such as prey availability and life stage, can shape venom phenotypes along predictable axes. Such axes may facilitate rapid adaptation when populations face similar selective pressures, particularly in novel environments.

Crotalus adamanteus are abundant on islands, where novel ecological conditions such as altered prey composition, competition, and/or abiotic factors can drive rapid adaptation (Millien, 2006; Blondel, 2000; Hirst et al., 2025). For example, previous work in *C. adamanteus* showed that venom adaptation in an island population occurred exclusively through regulatory changes rather than coding sequence evolution in less than 5000 years (Margres et al., 2017b). However, whether these regulatory shifts follow known axes of expression variation, such as ontogenetic patterns or range-wide geographic differences, remains unknown.

To understand how venom expression rapidly evolves following island colonization and determine whether such rapid evolution is biased towards existing axes of variation, we investigated a system of recently colonized barrier islands off the Gulf Coast of central Florida. The island chain, comprising of Anclote Key and Caladesi and Honeymoon Islands (Fig. 1), is ~3000–5000 years old. *Crotalus adamanteus* may have colonized the islands as early as 2500 years ago, following vegetation establishment and geomorphological stabilization, although the exact timing of introduction remains uncertain Kimble (2012), Davis Jr. (1994), Davis and Elko (2003). In a range-wide study of *C. adamanteus*, Rokyta et al. (2017) reported toxin expression profiles from an adult and a juvenile sampled on Caladesi Island, the southernmost island in the chain. Here, the juvenile expressed >5x more myotoxin transcripts per million than the adult (see Table 3 in Rokyta et al., 2017), a pattern that contrasts with previous findings showing that myotoxin expression is generally stable across life stages and only weakly expressed in southern populations, including Caladesi Island Margres et al. (2017a). Given the limited sample size ($n = 2$) and representation from only a single island in the region (Rokyta et al., 2017), it remains unclear whether these observations reflect polymorphism, where myotoxin presence is variable among individuals within the island population regardless of life history stage, or incorporation into the ontogenetic regulatory network, in which myotoxin expression becomes developmentally regulated. In this study, we investigated how *C. adamanteus* may have leveraged existing axes of expression variation to rapidly adapt following island colonization.

2. Materials and methods

2.1. Sampling

We collected 19 *C. adamanteus* across Anclote Key ($n = 3$), Caladesi Island ($n = 11$), and Honeymoon Island ($n = 5$) off the west coast of

central Florida, USA (Fig. 1). Venom was collected and lyophilized, and snout-vent length (SVL) was recorded for each individual before release at their original capture site. We classified snakes with a SVL of ≤ 90.0 cm as juveniles, 90.1–109.9 cm as sub-adults, and ≥ 110.0 cm as adults (Margres et al., 2015b; Waldron et al., 2013) to determine how venom expression varies across life history. Samples were collected under Florida Fish and Wildlife Conservation Commission Scientific Collecting Permit LSSC-23-00332 and Florida Department of Environmental Protection/Florida Park Service Scientific Collecting Permit 10182414. Procedures were approved by the University of South Florida Institutional Animal Care and Use Committee (IACUC) under protocol IS000012403.

2.2. Venom proteomics

2.2.1. Reverse-phase high performance liquid chromatography

To generate venom profiles of adult, sub-adult, and juvenile *C. adamanteus*, we performed reversed-phase high performance liquid chromatography (RP-HPLC) on venom samples from three representative individuals. The adult snake (SVL = 125.5 cm) was sampled from Anclote Key, and the sub-adult (SVL = 96.5 cm) and juvenile (SVL = 49.5 cm) snakes were sampled from Honeymoon Island. Prior to analysis, venom samples were lyophilized and resuspended in HPLC-grade water. Samples were run on a Dionex ultimate 3000 UHPLC DAD (Thermo Fisher Scientific, Waltham, MA) and a BeckmanSystem Gold HPLC (BeckmanCoulter) at the Chemical Purification Analysis and Screening Core Facility at the University of South Florida. We injected 50 μg of total venom protein onto a Jupiter 5 μm C18 300 Å, LC 250 × 2 mm, Ea column using the conditions and methodology described in Hirst et al. (2024) and Margres et al. (2014).

2.2.2. Quantitative mass spectrometry

To identify and quantify specific venom components, quantitative mass spectrometry was performed on all 19 venom samples at the BioMS Facility at the University of South Florida. Lyophilized venom was solubilized with 5% SDS, 50 mM triethyl ammonium bicarbonate (TEAB) (pH 7.6), incubated at 95 °C for 5 min, and sonicated at 20% amplitude. Using the Pierce 660 Assay (Thermo Scientific), we determined protein concentrations and digested 100 μg using S-traps (Protifi). Proteins were briefly reduced with dithiothreitol (DTT), alkylated with iodoacetamide (IAA), acidified using phosphoric acid, and combined with S-trap loading buffer (90% methanol, 100 mM TEAB). Proteins were loaded onto S-traps, washed, and digested with Trypsin/Lys-C overnight at 37 °C. Peptides were eluted and dried with a vacuum concentrator. Peptides were resuspended in H_2O/0.1% formic acid for LC-MS/MS analysis. Peptides were separated using a 75 μm × 50 cm C18 reversed-phase-HPLC column (Thermo Scientific) on a Vanquish Neo UHPLC (Thermo Scientific) with a 120 min gradient (2%–32% ACN with 0.1% formic acid) and analyzed on a hybrid quadrupole-Orbitrap instrument (Q Exactive Plus, Thermo Fisher Scientific). Each sample was run in triplicate. Full MS survey scans were acquired at 70,000 resolution. Data were acquired with data-dependent acquisition, selecting the top 10 most abundant ions for MS/MS analysis. Raw data files were processed in MaxQuant (www.maxquant.org) and searched against annotated coding-sequences from the reference genome Cadamanteus_3dDNAHiC_1.2 (Hogan et al., 2024). Proteins were identified using the filtering criteria of 1% protein and peptide false discovery rate. Label-free quantification (LFQ) intensity values, reflecting relative protein abundance based on peptide ion signal intensity (Cox and Mann, 2008; Cox et al., 2014), were used for downstream analysis as described below.

2.3. Multivariate analysis of venom qMS data

To address the dependence of raw LFQ intensity values generated from the qMS runs, we transformed each replicate using the centered log ratio (CLR) as described in Rokyta et al. (2015a) and Aitchison (1982). Proteins absent in >80% of samples (i.e., zero LFQ intensity values) were identified and removed during the multiplicative zero-replacement step implemented in the zCompositions package (Palarea-Albaladejo and Martín-Fernández, 2015) before further analyses (Table S1). This filtering step was necessary to reduce analytical noise and ensure appropriate application of the CLR transformation. The mean and standard error of the replicates for each individual were calculated following transformation. We ran a PERMANOVA (Anderson, 2001) on the CLR transformed qMS data to determine differences in venom protein expression related to ontogeny (SVL) and population (island) using the "strata" parameter to constrain the permutations within each replicate. Data dispersions were visualized using the vegan package in R. We then conducted a linear regression across PCoA1 scores, the axis explaining the most variation, and SVL to test for effects of ontogeny on venom protein expression. To determine whether specific toxins were significantly differentially expressed across juveniles (n = 4) and adults (n = 7), we used the limma package (Ritchie et al., 2015) in R (R Core Team, 2024); note that we excluded sub-adults (n = 8) from this analysis to avoid introducing intermediate expression patterns, confounding the ontogenetic transition between juvenile and adult venom components if present. Differential expression significance was determined in limma using the FDR-adjusted p-value (Padj) at an α < 0.05 threshold and a log2 fold change (LFC) cutoff of ≥ 2. All analyses were performed in R (R Core Team, 2024). qMS data are available in Supplementary Data File 1.

2.4. Myotoxin expression modeling

To explore how myotoxin expression varied across life history, we generated multiple models comparing size (i.e., SVL) and mean myotoxin expression and performed model selection. First, we calculated mean percent myotoxin expression. Here, expression levels for all venom proteins were estimated as the percentage of raw LFQ intensity values from the qMS data; from these values, the mean and standard error were calculated across all replicates for each individual. Similar to the approach described above, proteins with >80% zeros were removed for consistency. Then, to determine the nature of the relationship between mean myotoxin expression and SVL, we performed model selection for both linear and nonlinear models. Specifically, we compared a linear model, generalized additive model (GAM; Wood, 2003), and nonlinear least squares (NLS; Pinheiro and Bates, 2000) power and exponential models. The best performing model was selected using ΔAIC and plotted using ggplot2 (Wickham, 2016) in R (R Core Team, 2024).

3. Results

3.1. Venom expression differentiation across life history and populations

We compared venom proteomic expression data from 19 *C. adamanteus* across three islands and found significant differences across SVL (p < 0.001, R^2 = 0.224), population (p < 0.001, R^2 = 0.117), and the interaction between the two (p < 0.001, R^2 = 0.106). The PCoA dispersion plot showed evidence of clear ontogenetic variation in venom expression, although population differences were less clear (Fig. 2A). The SVL~PCoA1 regression showed a significant and largely continuous ontogenetic shift in venom expression across SVL (p < 0.001; R^2 = 0.687; Fig. 2B).

We next performed differential expression analyses across age classes and found that 13 toxins were significantly biased towards juveniles and 14 toxins were significantly biased towards adults (Padj < 0.05,

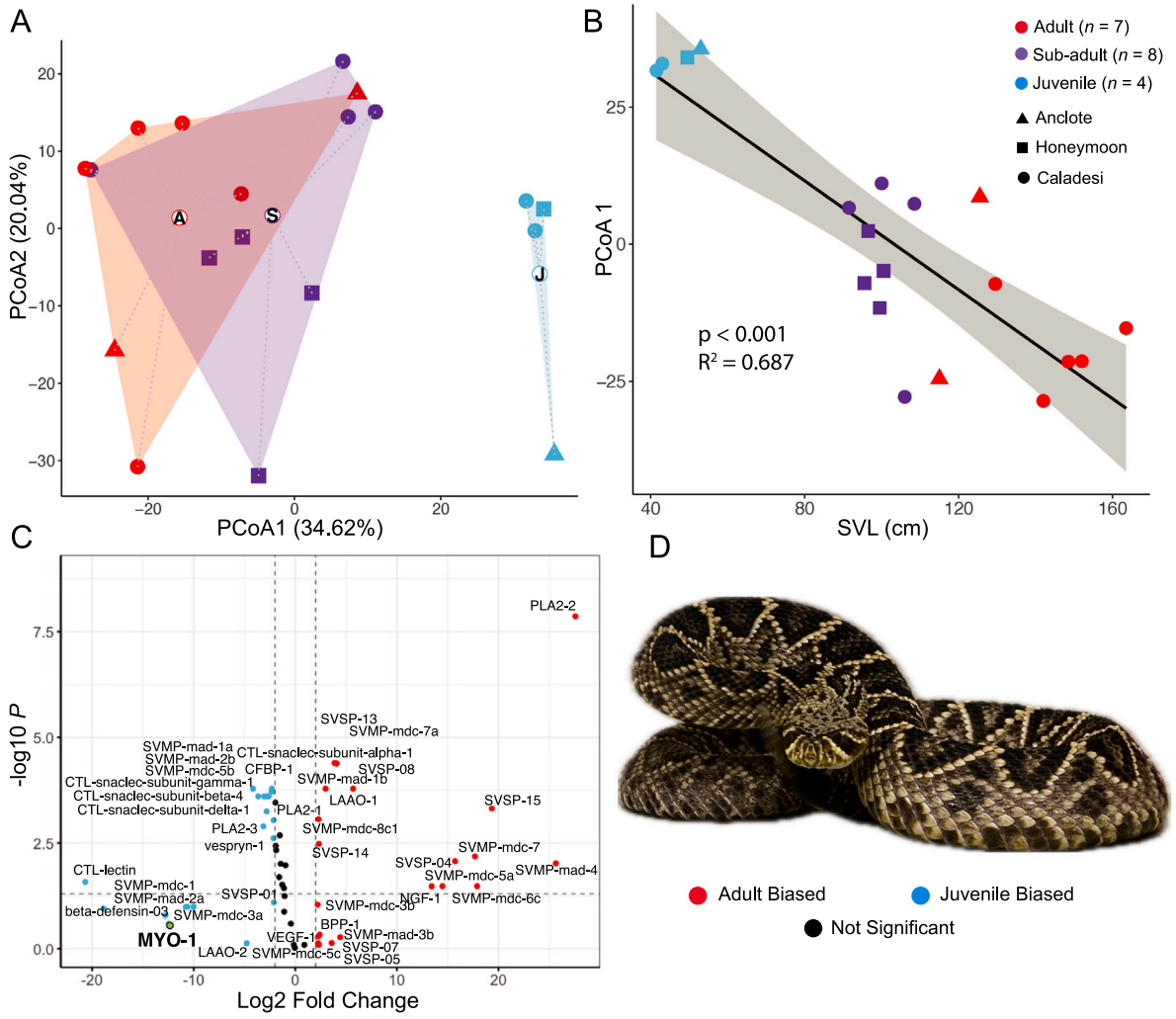

Fig. 2. Venom expression differs in *Crotalus adamanteus* across life history. (A) Dispersion plot highlights the clear separation in venom expression between juveniles and adults/subadults along PCoA1. Variation explained by each PCoA is indicated along the axis. (B) Linear regression between PCoA1 from (A) and SVL shows venom expression significantly changes with snake size. Age class is indicated by color, and island is represented by shape in both panels. Juveniles were classified as ≤ 90.0 cm, sub-adults as 90.1– 109.9 cm, and adults as ≥ 110.0 cm. (C) Volcano plot of differential expression calculated from limma between adults (red; $n = 7$) and juveniles (blue; $n = 4$). Toxins are color-coded as biased if Log2 fold change (LFC) ≥ 2 regardless of FDR. Vertical dotted lines represent LFC ≥ 2, and horizontal dotted lines represent $\alpha \leq 0.05$. (D) Eastern diamondback rattlesnake. Abbreviations: BPP, Bradykinin-potentiating peptides; CFBP, C-type lectin-like fibrinogen-related protein binding; CTL, C-type lectins; LAAO, L-amino acid oxidase; MYO, Myotoxin (bolded); NGF, Nerve growth factor; PLA2, Phospholipase A2; SVL, Snout-vent length; SVMP, Snake venom metalloproteinase; SVSP, snake venom serine protease; VEGF, Vascular endothelial growth factor.

LFC ≥ 2; Fig. 2C). Most toxins biased towards juveniles belonged to the C-type lectin (CTL; n= 5) and Phospholipase A_2 (PLA_2; n = 2) protein families, with five of nine CTL and two of three PLA_2 proteins more abundantly expressed in juveniles. Most adult-biased toxins belonged to the snake venom metalloproteinase (SVMP; n = 6) and snake venom serine protease (SVSP; n = 5) toxin families, including SVMP-mdc-5a, SVMP-mad-4, SVSP-08, and SVSP-13. When considering only the LFC ≥ 2 regardless of FDR, 22 toxins were biased towards juveniles and 21 toxins were biased towards adults (Fig. 2C). Here, an additional CTL (CTL-snaclec-subunit-beta-3), four additional SVMPs, SVSP-01, L-amino-acid-oxidase-2 (LAAO), myotoxin (MYO), and beta-defensin-03 were biased towards juveniles. In adults, three additional SVMPs, two additional SVSPs, Bradykinin-potentiating peptide-1 (BPP), and Vascular endothelial growth factor-1 (VEGF) were significantly biased. Of note, myotoxin exhibited one of the largest LFC differences (−12.38), with a clear bias towards juveniles, despite a non-significant adjusted p-value (0.269; Fig. 2C). Full differential expression results are included in Supplementary Data File 2.

3.2. Myotoxin expression variation across life history

To determine the relationship between myotoxin expression and SVL, we generated linear, GAM, and NLS power and exponential models. All models revealed a significant, negative relationship between SVL and myotoxin expression (Table 1). Model comparison using AIC identified the NLS power model as the best-supported model, though the NLS exponential model performed comparably (ΔAIC <2; Table 1); both NLS models performed significantly better than the GAM and linear models and were therefore retained for visualization and interpretation (Fig. 3D).

4. Discussion

Island populations of *C. adamanteus* exhibited clear and significant ontogenetic variation in venom expression, including in myotoxin expression, consistent with evolutionary co-option, where an existing trait or developmental program is adapted for a new function or environment (Gould and Vrba, 1982). Our findings suggested that

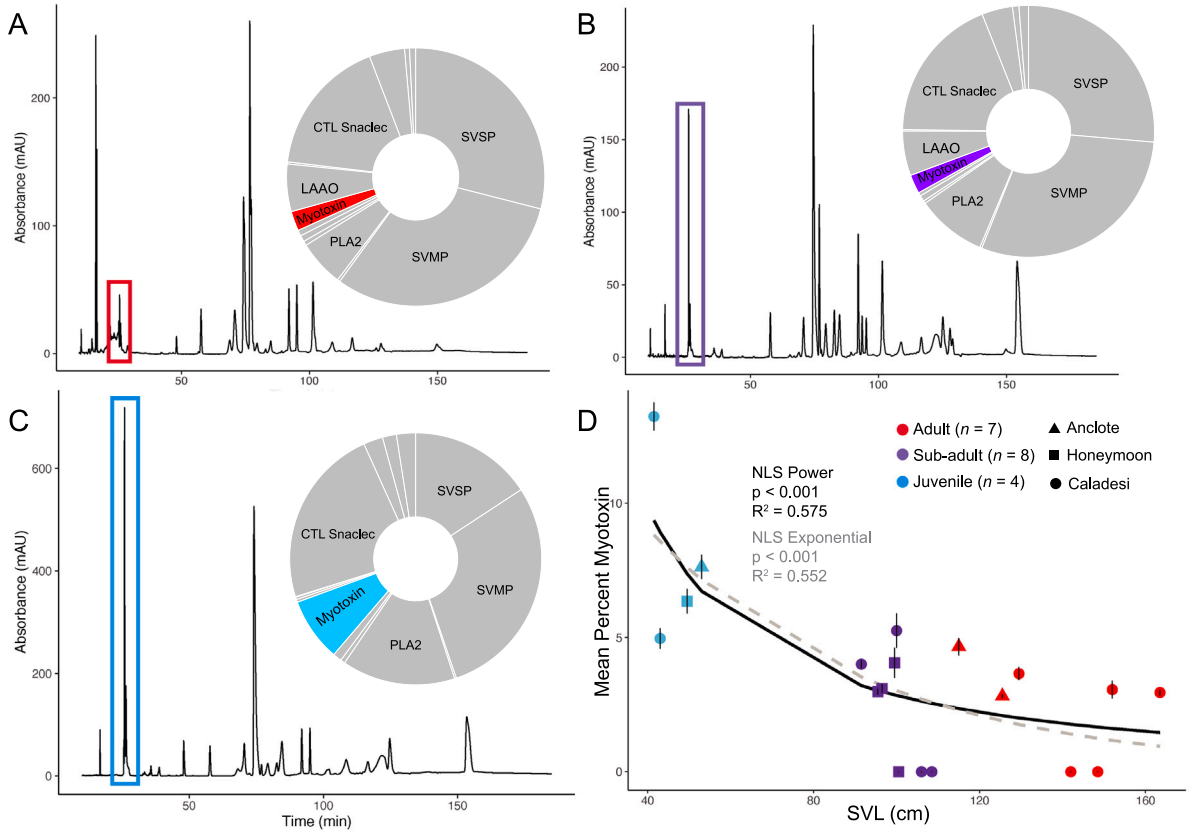

Fig. 3. Expression of myotoxin decreased across life history. Representative RP-HPLC chromatograms and associated pie charts of qMS-generated venom data of (A) adult (SVL 125.5 cm), (B) sub-adult (SVL 96.5 cm), and (C) juvenile (SVL 49.5 cm) snakes. Myotoxin is highlighted in each chromatogram and pie chart. Pie charts display the percentage makeup of each venom class. (D) Regressions showing the significant, non-linear negative relationship between myotoxin expression and SVL across all 19 individuals. Both NLS Power and Exponential models were significantly better than linear and GAM models, with NLS power being the best model. Both NLS models are shown. Age class is indicated by color, and island is represented by shape. Abbreviations: CTL, C-type lectins; GAM, generalized additive models; LAAO, L-amino acid oxidase; NLS, Non-linear least square; PLA2, Phospholipase A2; qMS, quantitative mass spectrometry; RP-HPLC, reversed-phased high-performance liquid chromatography; SVL, snout-vent length; SVMP, Snake venom metalloproteinase; SVSP, snake venom serine protease.

Table 1
Results of linear and nonlinear regression models evaluating the relationship between mean percent myotoxin expression and snout–vent length (SVL). Model selection using Akaike Information Criterion (AIC) was performed. Abbreviations: GAM, generalized additive model; NLS, nonlinear least squares.

Model	*p*-value	R^2	ΔAIC
NLS Power	0.00025 (b)	0.58	0.00
NLS Exponental	0.0006 (b)	0.55	0.98
GAM	<0.001	0.55	2.54
Linear	0.0018	0.44	5.11

myotoxin, previously known only as a geographically variable toxin in *C. adamanteus* (Margres et al., 2017a), was exapted to be under ontogenetic regulation in these island populations (Fig. 3), representing a novel integration of spatial and life history axes of venom evolution.

As expected, we found significant differences in venom expression between age classes, though the interaction between island population and SVL was also significant. The significant interaction term was unsurprising given the sampling bias across islands; only three snakes in total were sampled from Anclote Key, and juveniles were scarce on Honeymoon (one juvenile, four (sub)adults) and Caladesi (two juveniles, 9 (sub)adults) Islands (Fig. 1). Consequently, much of the observed inter-island variation likely reflected age-related differences from uneven sampling. Our PCOA1~SVL regression showed that ontogenetic venom expression differences represented the largest axis of variation within this island system, and differential expression analyses identified particular toxins biased towards each age class (Fig. 2C), consistent with range-wide patterns (Margres et al., 2015b; Hogan et al., 2024). For example, Hogan et al. (2024) reported CTL-lectins were biased towards juvenile *C. adamanteus*, and SVMP and SVSP paralogs, including SVMP-mdc-5a and SVSP-08, respectively, were biased towards adults, consistent with our results. Here, myotoxin showed a strong bias towards juveniles (LFC >12), though it did not meet the FDR significance threshold, likely reflecting reduced statistical power due to the small juvenile sample size ($n = 4$). Regardless, all regression models showed a significant decline in myotoxin abundance throughout life history (Fig. 3), demonstrating that myotoxin expression is ontogenetically-regulated in these island populations.

Patterns of *C. adamanteus* venom expression within this island system united the two major axes of known variation: ontogenetic shifts in venom expression and north–south population differences in myotoxin expression (Margres et al., 2015b,a, 2017a). These axes appear to be integrated through the co-option of myotoxin into the ontogenetic venom expression network, a process that may have facilitated the rapid adaptation of island populations to novel ecological conditions. To date, myotoxin had not been shown to be ontogenetically regulated elsewhere within the range of *C. adamanteus*, though this pattern has been observed in other rattlesnake species (Borja et al., 2018; Lourenco Jr. et al., 2013; Durban et al., 2017). While our results clearly demonstrate an ontogenetic regulation of myotoxin in island populations, future work incorporating mainland individuals across various life stages would allow us to understand whether the observed pattern is unique to this island system or reflects broader,

previously undetected ontogenetic variation across their range. Additionally, geographic variation in myotoxin expression was shown to be predominantly driven by variation in copy number (Margres et al., 2017a). Our work demonstrates that myotoxin expression variation is not solely driven by variation in copy number in *C. adamanteus* but also through the regulation of existing paralogs across life history, highlighting how multiple genetic mechanisms can alter expression levels within a single gene family over relatively short timescales. Future work integrating epigenomic approaches (Hogan et al., 2024), such as Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) (Buenrostro et al., 2015) or Cleavage Under Targeted Accessible Chromatin (CUTAC) (Henikoff et al., 2021), could identify the regulatory mechanisms underlying the ontogenetic regulation of myotoxin expression in these island populations. Although we cannot definitively distinguish between regulatory differences and plasticity with our data, venom composition in *C. adamanteus* (Margres et al., 2015b) and other rattlesnakes (Gibbs et al., 2009) has been shown to be genetically determined, suggesting the observed patterns are very unlikely to be driven by plasticity. Common garden experiments could further clarify whether myotoxin expression is a plastic trait or developmentally regulated, but such experiments were outside the scope of this study.

Ontogenetic differences in venom expression are typically driven by dietary changes across life history, as gape limitation constrains snake diet (Margres et al., 2015b; Mackessy, 1988; Shine and Sun, 2003; Saviola et al., 2012; Madrigal et al., 2012; Durban et al., 2013). Geographic or population-level variation in venom expression, such as the near absence of myotoxin in the southern population of snakes, may reflect dietary divergence between populations, driven by differences in prey species and/or prey resistance to specific toxins (Daltry et al., 1996; Barlow et al., 2009; Biardi et al., 2006; Holding et al., 2016a). In these specific island populations, myotoxin may be utilized in the predation of small mammals, such as rodents (e.g., *Sigmodon, Peromyscus*), by juveniles but not in the predation of lagomorphs (e.g., *Sylvilagus*) in adult snakes. Adult snakes exhibit increased expression of specific SVMP paralogs, such as SVMP-mdc-7 or SVMP-mdc-5a (Fig. 2C), larger and more metabolically costly proteins, which may contribute to prey immobilization and digestion as dietary targets shift towards larger or more resistant animals (Rokyta et al., 2017; Mackessy, 1988; Fox and Serrano, 2005; Serrano and Maroun, 2005; Phillips et al., 2010). Future toxicity assays across age classes and prey species are needed to confirm these relationships. Myotoxin expression in island juvenile snakes suggests a functional similarity with northern populations, potentially reflecting shared ecologies, such as reliance on small mammalian prey (Margres et al., 2017a) relative to southern populations, or similar resistance phenotypes between northern and island prey.

Overall, island populations of *C. adamanteus* co-opted existing axes of variation to enable venom divergence in ≤2,500 years, providing a valuable model for investigating how adaptive traits such as venom evolve rapidly in response to local selective pressures. As a species currently under consideration for federal protection under the Endangered Species Act (Fish and Service, 2012), continued loss of rattlesnakes from these islands would eliminate a distinct venom phenotype. Due to their isolation and ecological constraints, islands often impose strong and novel selection pressures, and increasingly resemble the fragmented, human-altered landscapes that many species now face. Such systems offer important opportunities to study the mechanisms driving rapid evolutionary responses of venom to environmental change (Hirst et al., 2025). Further insights are critical for conservation efforts that prioritize not only genetic diversity but also functional diversity (Petchey and Gaston, 2006). Our results suggest that rapid adaptation on these islands was biased towards pre-existing axes of variation already shaped by selective forces, emphasizing the need to conserve such standing functional variation, as it increases the evolutionary capacity of populations facing environmental change.

CRediT authorship contribution statement

Ella G. Guedouar: Writing – original draft, Investigation, Formal analysis. **Samuel R. Hirst:** Writing – review & editing, Investigation. **Shantal S. Solis:** Investigation. **Dale Chaput:** Investigation. **Mark J. Margres:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethical statement

Samples were collected under Florida Fish and Wildlife Conservation Commission Scientific Collecting Permit LSSC-23-00332 and Florida Department of Environmental Protection/Florida Park Service Scientific Collecting Permit 10182414. Animal handling procedures were approved by the University of South Florida Institutional Animal Care and Use Committee (IACUC) under protocol IS000012403.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.toxicon.2026.109094.

Data availability

All raw qMS data are provided as Supplementary Data File 1 (‘Supplementary Data File 1.csv’). Differential expression data are available as Supplementary Data File 2 (‘Supplementary Data File 2.xlsx’).

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