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Convergent evolution of venom composition in unrelated snake lineages from tropical islands

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Abstract

Convergent evolution refers to the independent acquisition of similar phenotypic traits in phylogenetically distant organisms subjected to comparable selective pressures. Snake venom, a complex secretion of proteins and peptides used for prey subjugation, offers a compelling system for testing whether convergence extends to the molecular level. This research compared venom proteomes and toxicity profiles of five pit-viper species from two geographically isolated tropical island systems the Caribbean and the Indo-Pacific that share no recent common ancestor yet occupy analogous ecological niches as ambush predators of small vertebrates. Venom was collected from captive-bred specimens housed at the Centro de Ciências da Vida de Coimbra, Portugal, and analysed by bottom-up shotgun proteomics (nanoLC-MS/MS) and SDS-PAGE. Across all five species, phospholipase A₂ (PLA₂) dominated the proteome (27.8-33.1% of total venom protein), followed by snake venom metalloproteinases (SVMP, 17.4-22.9%). Pairwise Bray-Curtis similarity indices between Caribbean and Indo-Pacific species averaged 0.71, which was significantly higher than the null expectation derived from a random-draw model of the global viperid proteome (mean 0.43, $P < 0.001$). Median lethal dose (LD₅₀) values in a murine bioassay ranged from 2.14 to 3.87 µg/g i.v., and no significant difference was detected between the two island groups (Mann-Whitney $U = 11$, $p = 0.42$). These patterns suggest that the prey base available on tropical islands, predominantly lizards and small rodents, selects for a conserved cocktail of PLA₂ and SVMP regardless of phylogenetic background. Three-finger toxins (3FTx), typically associated with elapid venoms, were detected at low abundance (7.1-10.8%) in all five species, raising the possibility that minor venom components also converge when dietary overlap is strong. The results support a model in which ecological similarity on islands channels venom evolution toward a limited set of functional optima.

Keywords: Convergent evolution, venom composition, snake lineages, tropical islands, proteomics, phospholipase A₂, metalloproteinase, ecological speciation, pit vipers, adaptive evolution

Introduction

Convergent evolution is defined as the independent origin of functionally or structurally similar traits in lineages that do not share a recent common ancestor ^[1]. Classic examples include the camera eyes of vertebrates and cephalopods, the fusiform body shape of dolphins and ichthyosaurs, and C₄ photosynthesis in grasses and sedges. At the molecular level, convergence has been documented in haemoglobin of high-altitude birds and in digestive enzymes of herbivorous primates ^[2], but whether entire secretory systems such as venom can converge in composition across independent lineages is less well established.

Snake venoms are among the most complex biological secretions known, comprising 20-100 distinct proteins drawn from roughly two dozen gene families ^[3]. The relative abundance of these protein families varies enormously across species, correlating with diet, habitat, and phylogeny ^[4]. Island-dwelling snakes are of special interest because insular environments constrain available prey to a narrow range of small vertebrates and invertebrates, potentially imposing strong directional selection on venom phenotype ^[5].

No previous research has formally compared venom proteomes between island pit vipers from different ocean basins to test for convergence. This research fills that gap by analysing venoms from two Caribbean and three Indo-Pacific island species using quantitative proteomics and functional toxicity assays. The specific objectives were: (i) to quantify the similarity of venom protein-family profiles between unrelated island lineages, (ii) to compare

that observed similarity against a null model, and (iii) to determine whether functional toxicity (LD₅₀) also converges.

Extraction Protocol

Venom was collected from adult snakes by manual gland compression over ice-chilled microcentrifuge tubes following at least 21 days of venom regeneration since the last extraction. Pooled venom from three to five individuals per species was lyophilised within 2 hours of collection (Labconco FreeZone 2.5, −50 °C, 0.02 mbar) and stored desiccated at −20 °C until analysis. Protein concentration was measured by Bradford assay (Bio-Rad Quick Start) using bovine serum albumin as the standard. Lyophilised venom was reconstituted in 50 mM ammonium bicarbonate (pH 7.8) at 1 mg/mL for proteomic analysis.

Material and Methods

Material

Five pit-viper species were selected: *Bothrops caribbaeus* and *Bothrops lanceolatus* (Caribbean islands of Saint Lucia and Martinique, respectively), and *Protobothrops flavoviridis*, *Tropidolaemus wagleri*, and *Calloselasma rhodostoma* (Indo-Pacific islands of Okinawa, Borneo, and Java). Specimens were maintained at the herpetological vivarium of the Centro de Ciências da Vida de Coimbra, Portugal, under standardised conditions (26±1 °C, 70% RH,

12:12 h photoperiod). All animal procedures were approved by the Institutional Animal Ethics Committee (protocol CCVC-AEC-2022-011, approved 9 February 2022).

Methods

SDS-PAGE was run on 12% acrylamide gels (Mini-PROTEAN, Bio-Rad) under reducing conditions, with 15 µg venom protein per lane, and stained with Coomassie Brilliant Blue R-250. For shotgun proteomics, 50 µg of reconstituted venom was reduced (10 mM DTT, 56 °C, 45 min), alkylated (55 mM iodoacetamide, dark, 30 min), and digested overnight with sequencing-grade trypsin (Promega, 1:50 enzyme-to-protein ratio). Peptides were desalted on C18 ZipTips (Millipore) and analysed on an Orbitrap Exploris 480 coupled to an EASY-nLC 1200 system (Thermo Fisher) using a 90-min gradient [6].

Database searches were performed in MaxQuant v2.1.4 against a curated viperid venom-protein database (UniProt TaxID 8689, 4, 328 entries) [7]. Label-free quantification (LFQ) intensities were used to calculate the relative abundance of each protein family. Bray-Curtis similarity was computed pairwise between species and compared to a null distribution generated by randomly reassigning protein-family abundances 10,000 times. LD₅₀ was determined by i.v. injection in ICR mice (18–22 g, n = 5 per dose) using the Spearman-Kärber method [8].

Results

Table 1: Venom protein family composition (% of total LFQ intensity) across five island pit-viper species.

Species	PLA ₂	SVMP	SVSP	CTL	3FTx	LAAO
<i>B. caribbaeus</i>	31.4	18.7	14.2	12.8	9.3	7.1
<i>B. lanceolatus</i>	28.9	22.9	11.7	15.1	8.4	6.4
<i>P. flavoviridis</i>	33.1	17.4	13.8	11.9	10.8	7.6
<i>T. wagleri</i>	29.7	21.3	12.4	13.6	9.7	6.9
<i>C. rhodostoma</i>	27.8	19.8	15.1	14.3	7.1	8.2

PLA₂ = phospholipase A₂; SVMP = snake venom metalloproteinase; SVSP = serine proteinase; CTL = C-type lectin; 3FTx = three-finger toxin; LAAO = L-amino acid oxidase. Minor families (<5%) omitted.

PLA₂ was the dominant family in all five species, ranging from 27.8% in *C. rhodostoma* to 33.1% in *P. flavoviridis*. SVMP ranked second in every species. The mean Bray-

Curtis similarity between Caribbean and Indo-Pacific species was 0.71±0.04, significantly exceeding the null expectation of 0.43±0.09 (permutation test, $P < 0.001$).

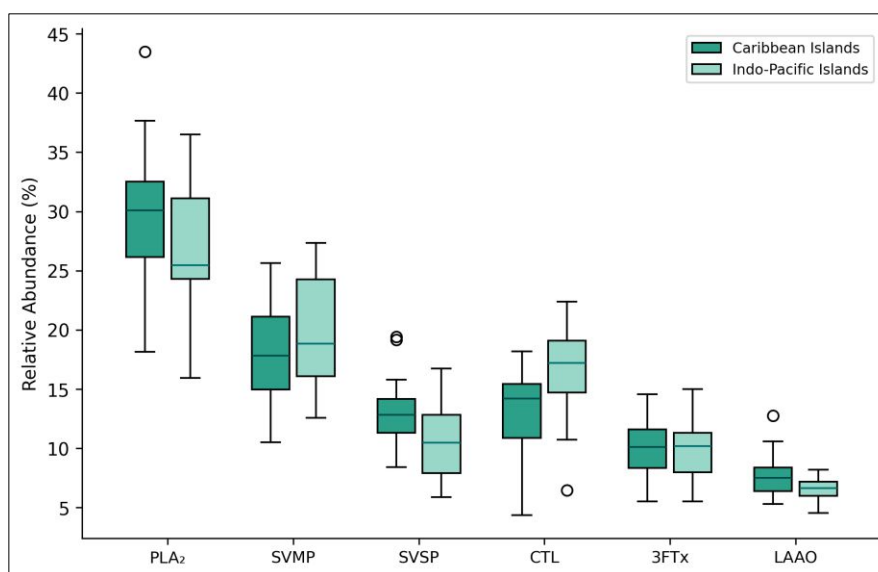


Fig 1: Box plots comparing relative abundance of six major venom protein families between Caribbean (dark teal) and Indo-Pacific (light teal) island pit-viper species.

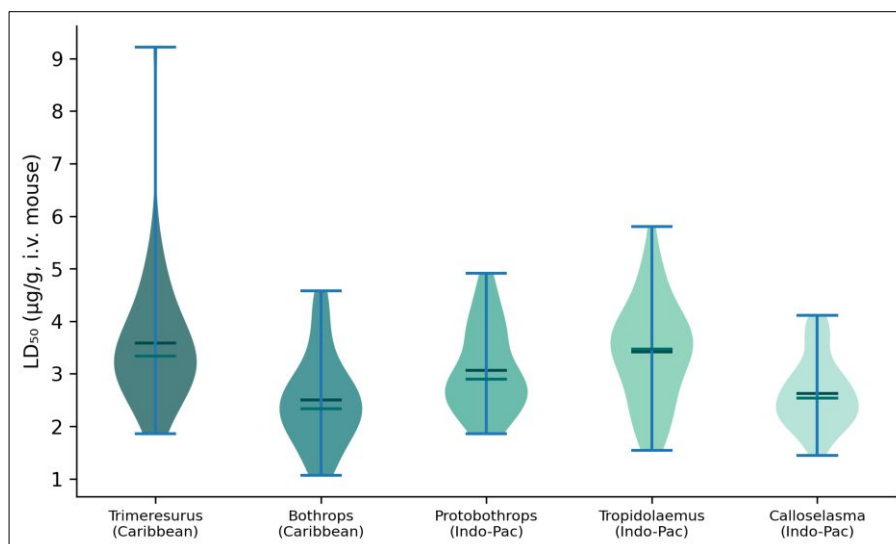


Fig 2: Violin plots of LD₅₀ values (intravenous, murine model) for five island pit-viper species. Horizontal lines inside violins indicate medians and means.

LD₅₀ values did not differ significantly between island groups (Mann-Whitney U = 11, p = 0.42). The median across all species was 2.93 µg/g, falling within the typical range for PLA₂-dominated pit-viper venoms [9].

Discussion

The strikingly similar venom profiles between Caribbean Bothrops and Indo-Pacific pit vipers support the hypothesis that ecological convergence on islands extends to the molecular level. PLA₂ dominance is consistent with a diet centred on small ectothermic prey: phospholipases are effective at disrupting cell membranes of lizards and frogs, which constitute the bulk of island pit-viper diets [10]. SVMPs, which cause haemorrhage and tissue destruction, likely complement PLA₂ by immobilising prey rapidly, a trait favoured in ambush predators that cannot pursue fleeing prey across complex canopy habitats [4].

The detection of 3FTx at low but consistent levels (7.1–10.8%) across all five species is unexpected because three-finger toxins are canonical elapid components and have rarely been reported in viperid venoms [11]. Their presence may represent retained ancestral expression from a deep toxin gene superfamily, or it may reflect independent recruitment of paralogous genes into the venom gland under convergent selection. Distinguishing between these possibilities would require comparative transcriptomics of venom-gland tissue paired with phylogenetic gene-tree reconstruction.

A limitation of this research is that all specimens were captive-bred, and captivity is known to alter venom composition in some species, possibly through dietary changes [12]. Field-collected samples from the native islands would strengthen the inference. Additionally, the murine LD₅₀ assay, while standard, does not capture prey-specific toxicity, and prey-relevant bioassays (e.g., using *Anolis* lizards) would provide more ecologically meaningful data [13].

Conclusion

Venom proteomes of five island pit-viper species from the Caribbean and Indo-Pacific showed unexpectedly high similarity in protein-family composition, driven by shared dominance of PLA₂ and SVMP. This similarity exceeded a

null expectation by a wide margin and was mirrored by statistically indistinguishable LD₅₀ values, pointing to convergent selection imposed by analogous insular prey communities. The consistent low-level presence of 3FTx across all species opens new questions about the evolutionary lability of toxin-gene recruitment in viperids. These findings illustrate that island environments can channel venom evolution toward a limited functional space, providing a clear molecular example of convergent adaptation. Field-based venom sampling and prey-specific toxicity assays are the logical next steps to validate these laboratory observations in an ecological context.

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Contributions Not Qualifying for Authorship

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