Unique physiological and regulatory activity drives divergent toxin and non-toxin gene expression in rattlesnake accessory venom glands

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Abstract: Snake venom glands are an valuable system to test hypotheses related to the evolution and specialization of novel physiological function, as these modified salivary glands have evolved over ~60 MY to synthesize and store venom. Front-fanged venomous snakes (elapids and viperids) possess two types of venom glands: the main and accessory glands. The larger main gland produces greater quantities of venom toxins and has been studied extensively, while the smaller accessory gland has received less attention. Here, we explore gene expression differences between main and accessory venom glands across three rattlesnake species (*Crotalus cerberus*, *C. oreganus concolor* and *C. viridis*). Our findings indicate that accessory glands express most venom genes at significantly lower levels than the main gland, with a few exceptions that may represent biologically relevant contributions of accessory glands to venom. The two glands also exhibit distinct trans-regulatory environments that we link to key differences in their underlying physiology and secretory roles. Our results further suggest that two signaling pathways that regulate venom, the unfolded protein response (UPR) and extracellular signal-regulated kinase (ERK), show significantly lower activation in the accessory gland. These findings provide insight into the physiological and functional diversification of snake venom systems, highlighting how distinct glandular systems have evolved contrasting and complementary roles driven by distinct physiological and regulatory mechanisms.

Introduction and Rationale

While previous studies have examined the physiology and venom expression of the accessory venom gland (AVG; Kerkkamp et al., 2017; Perry et al., 2022; Schield et al., 2019; Valente et al., 2018; Vonk et al., 2013) , the gene regulatory architecture underlying venom variation in this tissue remains largely unstudied. By comparing differential expression of venom genes and transcription factors between the AVG and the main venom gland (MVG), we hypothesized that these glands are governed by distinct regulatory networks. Our pathway results further support this, revealing enrichment of the unfolded protein response (UPR) and extracellular signal-regulated kinase (ERK)—both of which are critical for the upregulation of venom gene expression.

Approach

We collected the right and left primary venom gland and the right accessory venom gland from three species: *Crotalus cerberus* (1), *C. o. lutosus* (1), and *C. viridis* (8), and prepared a poly-A selected mRNA library which was sequenced on the Illumina NovaSeq6000 (Fig. 1). This design represents a highly controlled experiment incorporating three distinct gland types from a total of ten individuals. RNA-seq data were analyzed in R using a various packages (e.g., DeSeq2, Pearson Correlation) to identify differences in both gene expression and transcription factor activity. For transcription factor analysis, we focused on those previously implicated in venom production, including factors involved in key pathways such as the unfolded protein response (UPR) and ERK signaling (Perry et al. 2022), to compare regulatory enrichment activity in the main versus accessory venom glands.

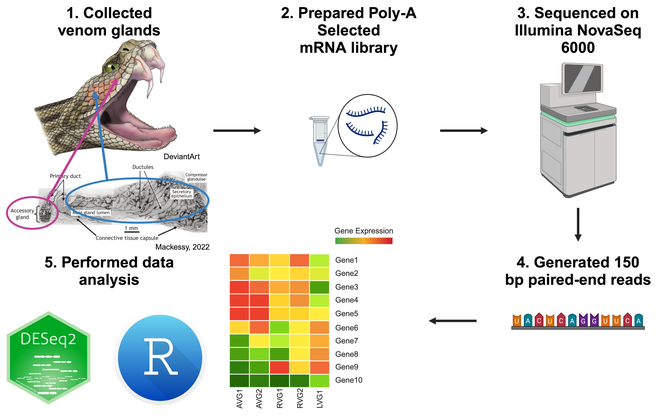


Fig. 1. mRNA library preparation for main and accessory venom glands.

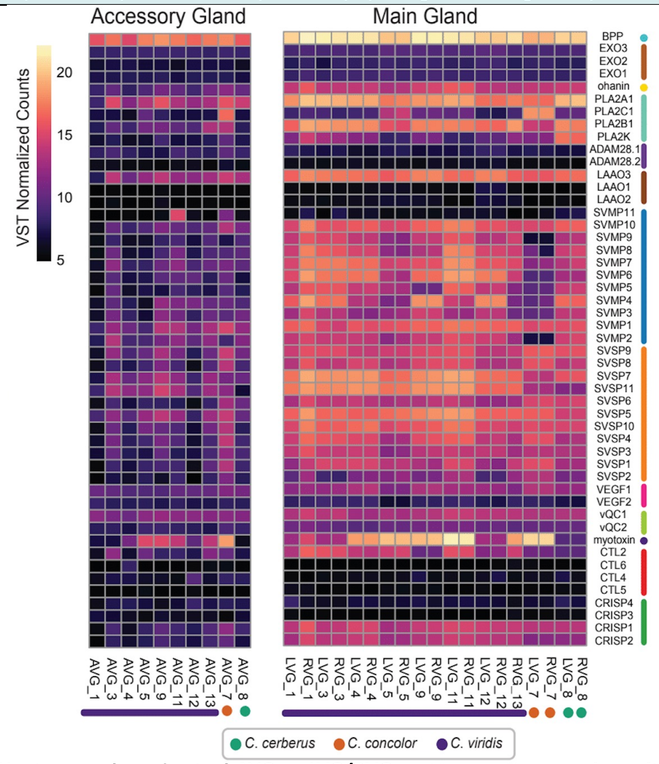


Fig. 2. Heat map of a subset of AVG vs LVG/RVG venom gene expression within and across species. Venom gene families are grouped by colored dots on the left. MVG shows higher expression of venom genes when compared to AVG

Conclusion

- Snake main versus accessory venom glands demonstrate substantial differential expression of almost all venom genes, with higher overall expression in the main venom gland.
- This distinction between main and accessory gland venom gene expression is consistent across populations and species.
- For venom-associated transcription factors, accessory venom glands have higher expression in TFs correlated with venom gene repression.
- The accessory venom gland is less enriched for UPR and ERK pathways explaining why we see less venom gene expression.
- Together our results suggest that divergent gene regulatory network activation (TFs and pathway activation) contributes to the venom expression divergence between main and accessory venom glands.
- These two venom glands are an interesting comparative model for understanding the origins and divergence of gene regulatory networks, and how that drives phenotypic variation.

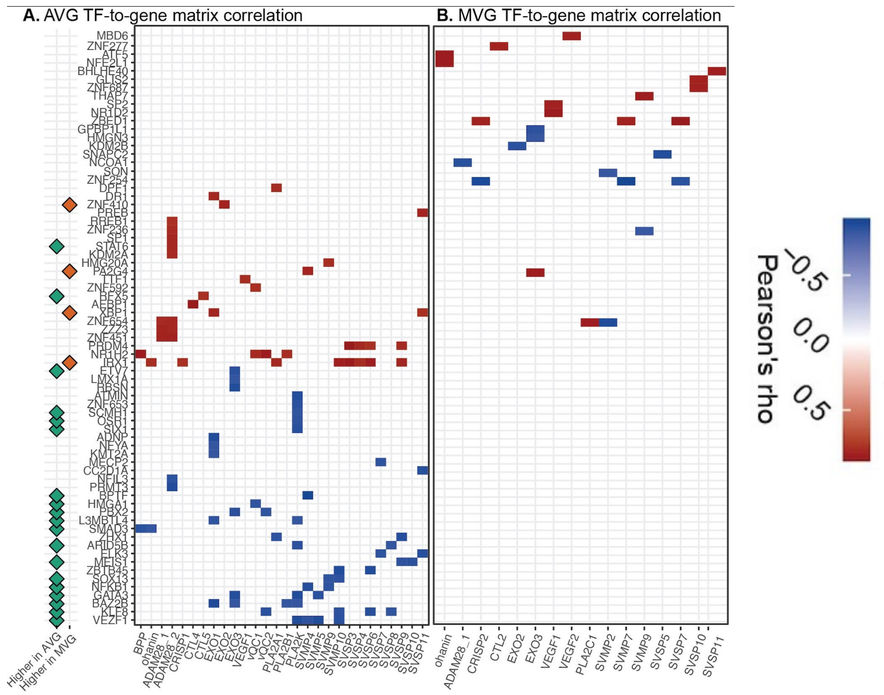


Fig. 3. TFs correlations that act as repressors and or activators. AVG has the pattern of being more highly expressed for TFS correlated with venom gene repression while MVG has the opposite correlation.

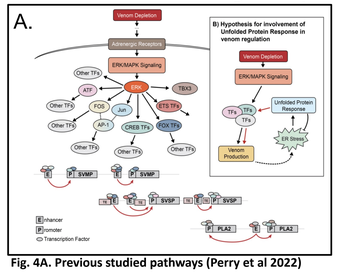
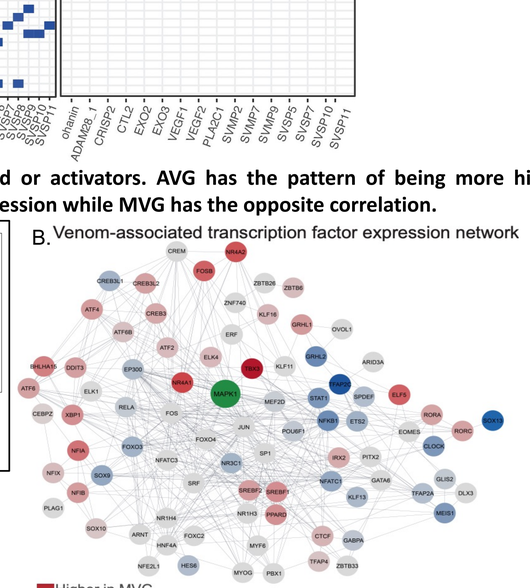


Fig. 4A. Previous studied pathways (Perry et al 2022)



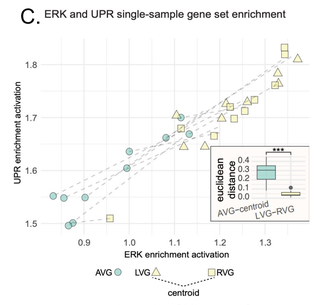


Fig. 4B. Cytoscape networks of differentially expressed transcription factors relating to venom within the AVG vs MVG previously implicated in other paper (Westfall et al 2023). Fig. 4C. Correlations showing MVG has more ERK enrichment compared to the MVG

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