

# A Role for microRNAs in the modulation of Rattlesnake Venom Expression

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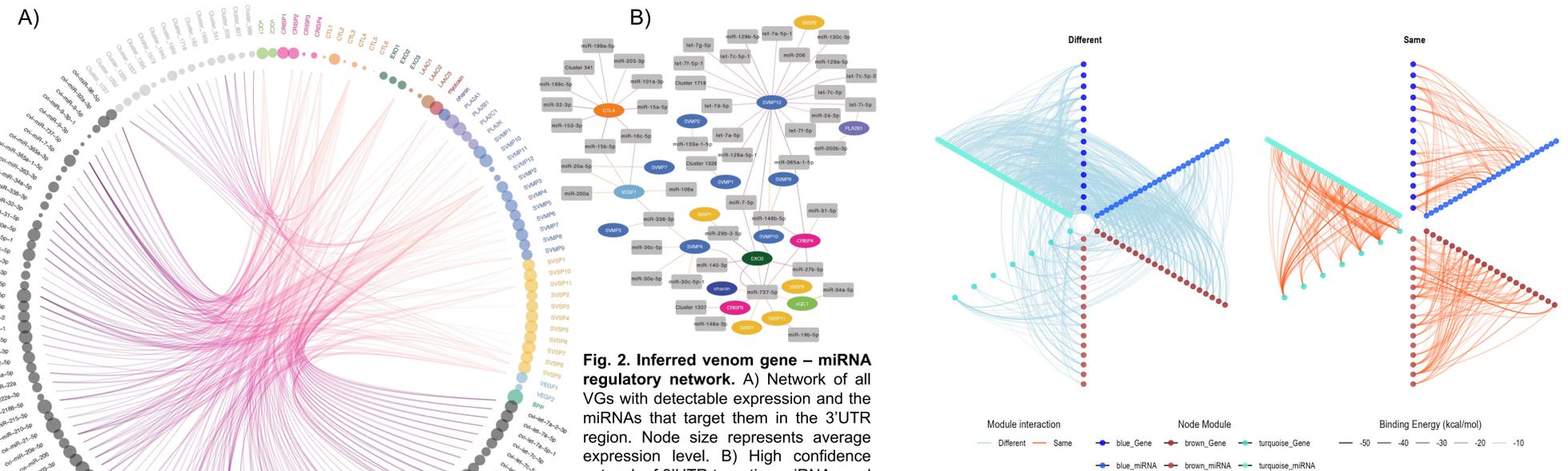
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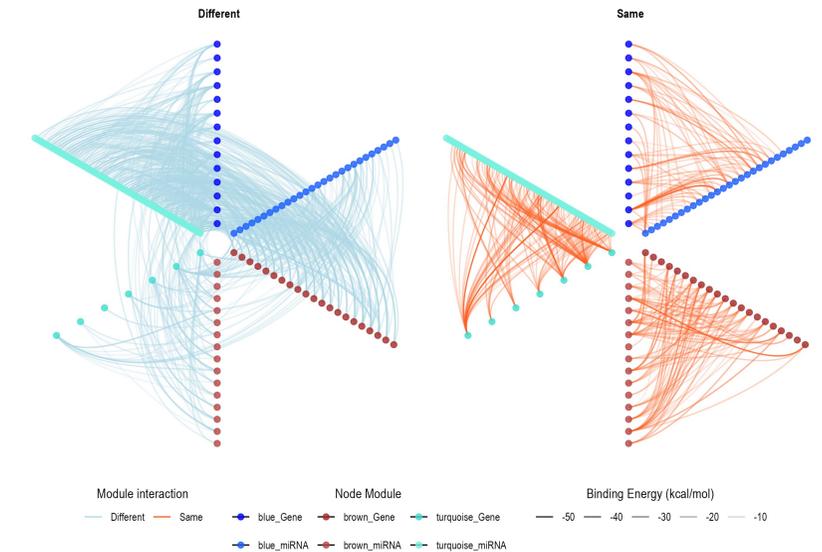


## Introduction

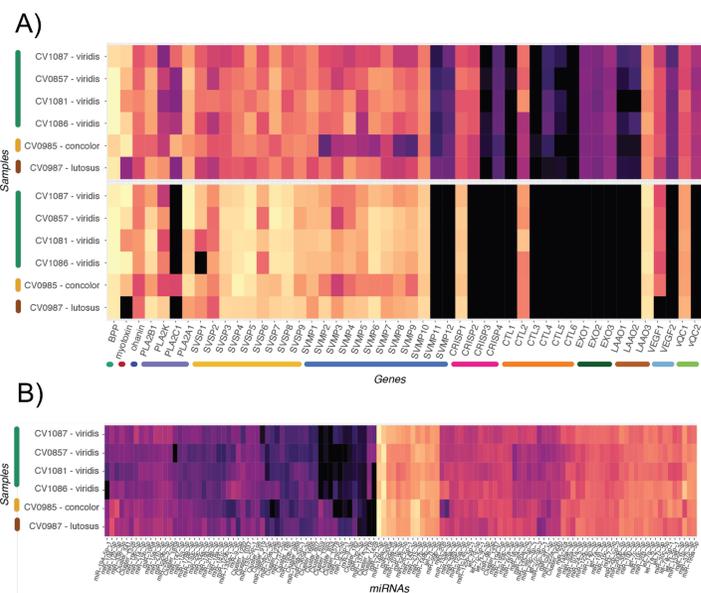
- Venom represents a powerful model for studying the evolution of novel organismal traits due to its tractable number of gene families that have a direct effect on fitness<sup>1-4</sup>.
- Snake venom has been the subject of considerable study in recent years<sup>4,5</sup>, with recent work finding that the unfolded protein response (UPR) and the extracellular signal-regulated kinase (ERK/MAPK) pathways have been co-opted to regulate venom gene (VG) expression pre-transcriptionally<sup>5</sup>.
- There is also evidence that VGs are post-transcriptionally regulated through microRNA (miRNA) binding and degradation<sup>6-9</sup>.
  - miRNAs are small RNA molecules ~22 nucleotides long that are known to act as post-transcriptional repressors<sup>10</sup>.
- Conversely, other work has indicated that post-transcriptional regulation has little effect on protein abundance<sup>3</sup>.



**Fig. 2. Inferred venom gene – miRNA regulatory network.** A) Network of all VGs with detectable expression and the miRNAs that target them in the 3'UTR region. Node size represents average expression level. B) High confidence network of 3'UTR targeting miRNAs and the venom genes they target (binding score  $\geq 155$ ; binding energy  $\leq -7$ ).



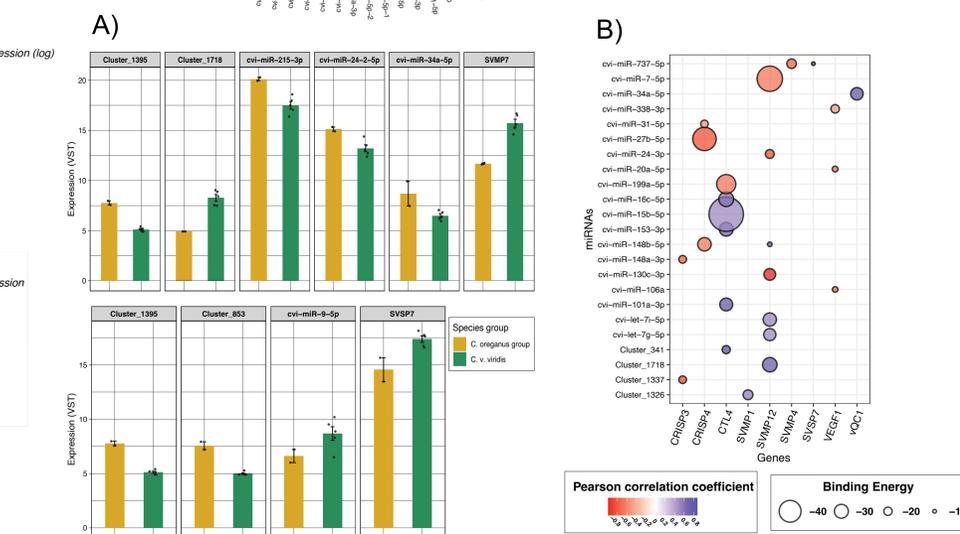
**Fig. 4. Hive plot of WGCNA modules containing both miRNAs and VGs.** miRNAs and VGs WGCNA modules, with miRNAs and VGs split between tracks to show intra-module targeting relationships. On average, intra-module putative target relationships are more numerous than inter-module target relationships (correct for module size).



**Fig. 1. Variation in venom gene expression in both the transcriptome and proteome and variation in miRNAs that target them.** A) Variation in mRNA and protein expression for all paired samples. B) Variation in expression for miRNAs that target the above VGs.

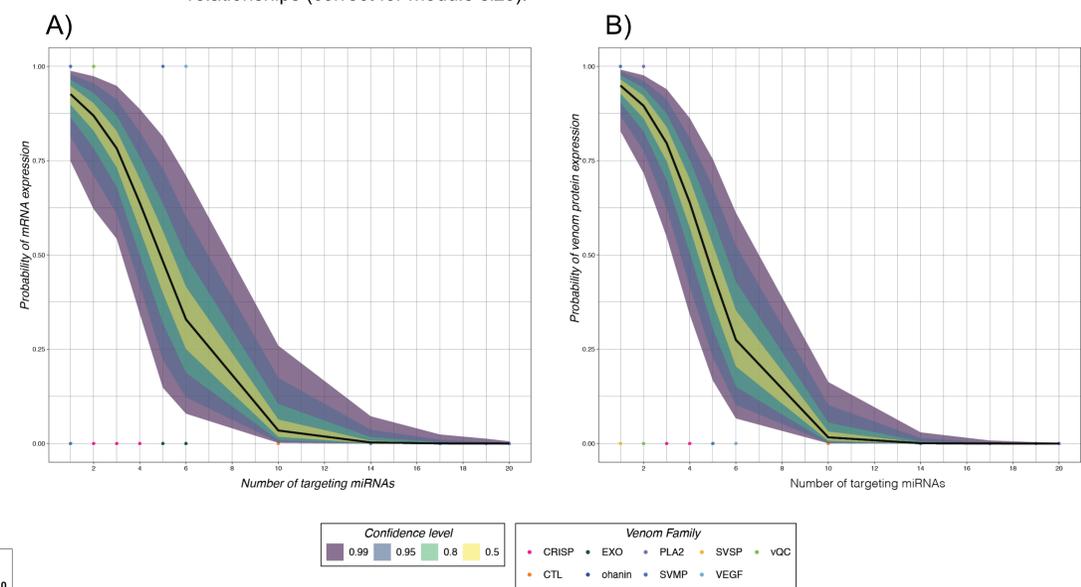
## Methods

- We collected seven snakes from three different species (*Crotalus viridis viridis*, *C. oreganus lutosus*, and *C. o. concolor*), gathering RNA-Seq, small RNA-Seq (smRNA-Seq), and protein mass spectroscopy data from each.
- We normalized both the smRNA-Seq and RNA-Seq using DESeq2<sup>10</sup> for downstream analysis.
- Using ShortStack<sup>11</sup> and miRanda<sup>12</sup> we quantified miRNA expression and putative targets.
- We used these results to generate inferences about how miRNAs affect venom composition within and between species.



**Fig. 3. Subset of venom genes and miRNAs predicted to interact that have strong evidence for expression modulation.** A) Two VGs (SVMP7 and SVSP7) and the miRNAs that target them. Results show a general pattern of higher expression of miRNAs in samples with lower venom expression. All loci present were significantly differentially expressed. B) VGs with venom protein residuals that were strongly correlated ( $r \geq \pm 0.5$ ) with miRNAs that target them.

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**Fig. 5. Probability of mRNA and protein expression vs. the number of miRNAs that target a given VG.** A) Probability that an mRNA is expressed (expression threshold  $> 1000$  raw counts) against the number of miRNAs that target a given gene. B) Probability that a VG protein is expressed against the number of miRNAs that target it.

## Conclusions

- Venom gene expression and venom targeting miRNAs vary considerably within and between species (Fig. 1).
- We find an extremely complex network between miRNAs and target loci (Fig. 2), with relationships being highly variable within and between venom gene families.
- There is a subset of venom genes that have considerable evidence for miRNAs modulating expression levels for at both post- and pre-transcriptionally (Fig. 3).
- Venom genes and the miRNAs that target are generally co-expressed, and we were able to detect this despite small sample size (Fig. 4).
- The likelihood that a venom gene is expressed, in either the transcriptome or proteome, decreases as the number of miRNAs that target it increases (Fig. 5).
- In totality of the evidence available, it is likely that miRNAs play notable role in the modulation of venom gene expression, despite previous work indicating post-transcriptional control mechanisms contributed little to venom variation<sup>3</sup>.