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Research

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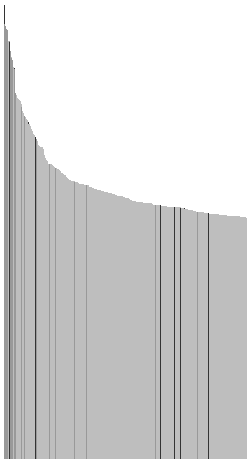
Venom proteins evolve rapidly, and as a trophic adaptation are excellent models for predator–prey evolutionary studies. The key to a deeper under-

three lizards were injected with only 0.9% saline as controls. Doses were adjusted to individual animal body masses, with lethality expressed as mg venom or toxin per gram body mass producing 50% mortality after 24 h [42]. The nonparametric Spearman-Kärber method was used for LD_{50} value estimations and determination of 95% confidence intervals [43]. All procedures were reviewed and approved by the UNC IACUC (protocol 1504D-SM-SMLBirds-18).

(e) Prey-specific toxin evolution in rear-fanged venomous snakes

Prey-specific monomeric 3FTxs from O. fulgidus (C0HJD3), Boiga dendrophila (Q06ZW0) and sulmotoxin 1 from S. sulphureus (this study) were submitted to the Phyre2 server [44] for modelling based on homology detection methods using server default parameters. To determine 3FTx residues available for binding, modelling was restricted to monomeric 3FTxs because the dimerization between the sulditoxin 3FTx subunits might result in different modes of receptor binding. These three models were over-



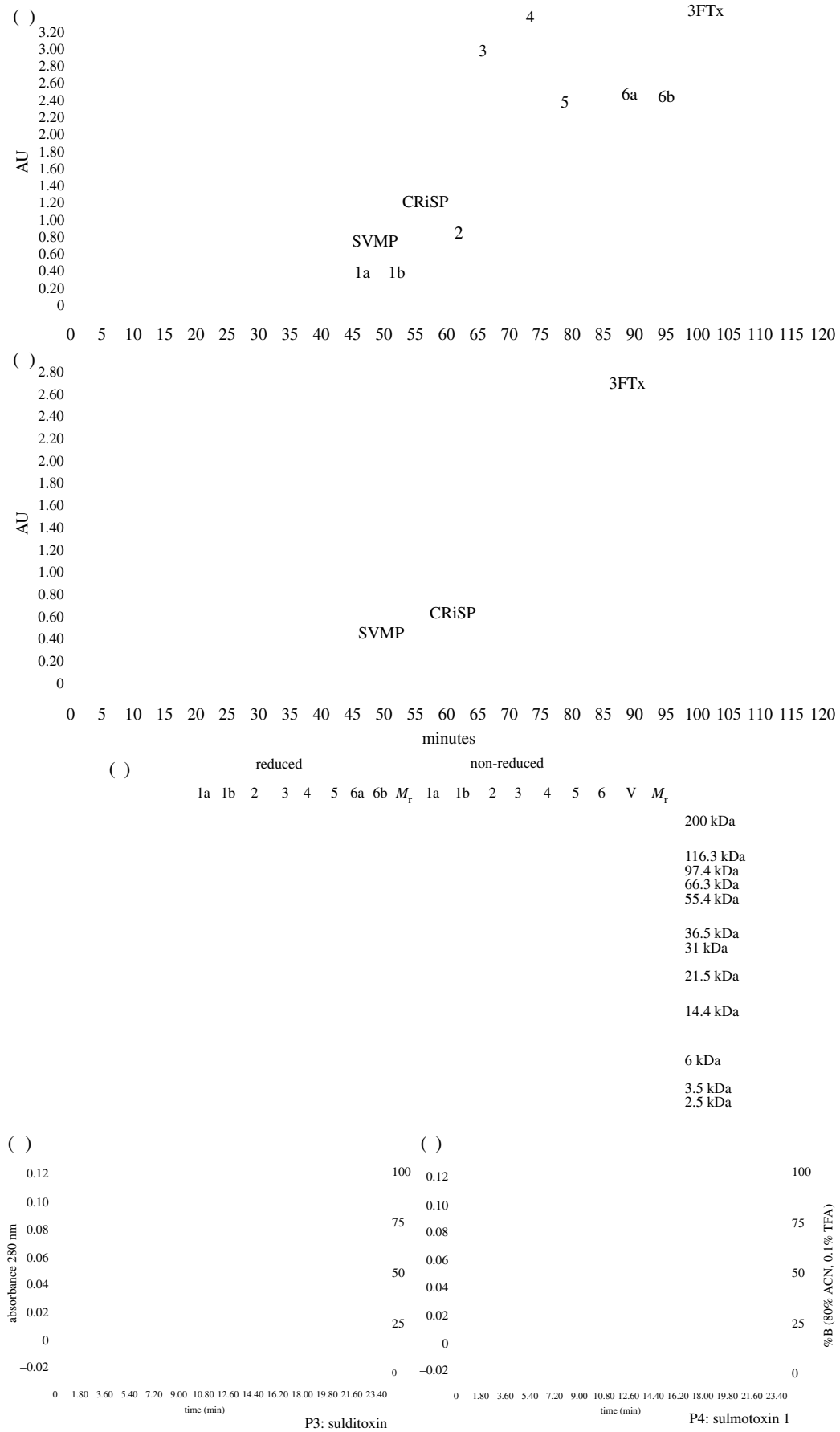

( )
3FTx
4
3
5
6a
6b
AU
CRiSP
2
SVMP
1a
1b
minutes
( )
reduced
non-reduced
1a 1b 2 3 4 5 6a 6b M_r
1a 1b 2 3 4 5 6 V M_r
200 kDa
116.3 kDa
97.4 kDa
66.3 kDa
55.4 kDa
36.5 kDa
31 kDa
21.5 kDa
14.4 kDa
6 kDa
3.5 kDa
2.5 kDa
( )
absorbance 280 nm
%B (80% ACN, 0.1% TFA)
time (min)
P3: sulditoxin
( )
time (min)
P4: sulmotoxin 1

Figure 2.

rodents. Unexplored venoms and venom gland transcriptomes from rear-fanged snakes are probably rich sources in which to discover functional diversification of toxin proteins. We find that the venom of *S. sulphureus* is unique, as two distinct toxins that target very different prey taxa have evolved in this species. This is, to our knowledge, the first instance in which this dual phenomenon has been discovered in any snake species.

Venom gene expression within the venom gland of *S. sulphureus* is similar to the 3FTx-dominated elapid-like venom gland transcriptome of the rear-fanged brown treesnake *B. irregularis* [34,35]. However, the presence of many fewer 3FTx transcript isoforms in *S. sulphureus* (16) compared to *B. irregularis* (65) [35] indicates that gene duplication is much less extensive and that *S. sulphureus* venom composition is considerably less complex, and this is also corroborated by the presence of only five 3FTxs highly expressed in the venom. Fifteen of the 37 full-length venom proteins in *S. sulphureus* were detected via LC-MS/MS

across numerous species of *Boiga*. The lack of toxic venom components specifically targeting mammalian prey, as demonstrated by previous studies [47], does not restrict the trophic ecology of *B. irregularis* owing to the use of differing predatory strategies when subduing prey: envenomation without constriction to subdue bird and lizard prey (primarily via irditoxin), and constriction alone to subdue mammalian prey [47]. *Spilotes sulphureus*, on the other hand, produces venom containing both lizard- and mammal-specific toxins, and adult *S. sulphureus* do not constrict mammalian prey, but use venom and body pinning to subdue both lizard and mammal prey (C.M. Modahl 2014, personal observation). The efficacy of this mode of feeding is greatly facilitated by the presence of different taxon-specific toxins that affect both prey types. The occurrence of prey-specific 3FTxs in the venom of *S. sulphureus*, a snake species native to the western hemisphere and evolutionarily distinct from Asian *Boiga*, emphasizes the significance of diet and predatory strategies in snake toxin evolution: both species preferentially use bird and lizard prey, but both can also take mammalian prey using different strategies (chemical versus mechanical subjugation). Sulditoxin and irditoxin therefore probably represent convergence upon a common structural solution (heterodimerism) to produce a lizard/bird-specific toxin, and the lack of close sequence identity between them supports the interpretation of functional convergence.

Relatively simple venoms with a small number of highly abundant toxins have been suggested to be characteristic of snakes with simple diets, such as sea snakes feeding on teleost

Ethics. All experiments involving animals were subjected to prior review by the University of Northern Colorado Institutional Animal Care and Use Committee (UNC-IACUC), and protocols 9204 and 1504D-SM-SMLBirds-18 were approved before any work was initiated. All work conforms to the ethics guidelines of the University of Northern Colorado, the International Society on Toxinology and the Society for the Study of Amphibians and Reptiles.

Data accessibility. Additional details of the analyses can be found in the electronic supplementary material, appendices S1 and S2. All toxin transcript sequences were submitted to GenBank and are available at NCBI under Bioproject PRJNA448354 and accession nos MH232964–MH233012.

Authors' contributions. C.M.M. and S.P.M. jointly conceived of and designed the study. C.M.M. and S.F. conducted all transcriptome and biochemical/biological characterization experiments. C.M.M., M. and S.F. conducted all bioinformatic analyses. C.M.M. and S.P.M. jointly wrote the original draft of the manuscript, and all authors edited the final manuscript and gave final approval for publication.

Competing interests. We have no competing interests.

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