Factors involved in the evolution of firefly toxin utilization in natricine snakes genus *Rhabdophis*: approaches from chemical recognition, morphology, and feeding behavior

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Introduction

Toxin sequestration is widely known in invertebrates and vertebrates. Several Asian natricine snakes of the genus *Rhabdophis* feed on toads and sequester steroidal cardiac toxins known as bufadienolides (BDs) from them. A recent study revealed that species of the *R. nuchalis* Group ingest lampyrine fireflies to sequester BDs. As amphibians are considered the plesiomorphic diet of *Rhabdophis*, it is presumed that a dietary transition of toxin source from toads to fireflies has occurred within the radiation of *Rhabdophis*. There are at least three possible factors that would facilitate the evolutionary acquisition of this novel prey: chemical similarity, habitat similarity, and morphological similarity. In the present study, I focused on the chemical and morphological similarity hypotheses to investigate how the firefly toxin utilization has evolved in the *R. nuchalis* Group.

Materials and Methods

I conducted four experimental studies including chemical recognition, feeding morphology, and prey-handling behavior to investigate how the toxin change has occurred. In the first study, I examined whether *R. chiwen* distinguishes lampyrine firefly larvae that have BDs from other species of firefly larvae. I conducted chemical preference tests, feeding tests, and Y-maze tests to examine the snakes' preference toward these larvae. In the second study, I conducted chemical preference experiments

using *R. tigrinus* to identify the chemical cues that the snake utilizes to recognize toads. In the third study, I examined morphological differences among four species of *Rhabdophis* (*R. tigrinus*, *R. leonardi*, *R. nuchalis*, and *R. chiwen*). I provided a detailed structural comparison of the cranial design and the feeding apparatus among the four species. In the fourth study, I described and compared prey handling behavior of *R. chiwen* feeding on earthworms and firefly larvae.

Results and Discussion

In the first and second studies, *R. chiwen* distinguished lampyrine fireflies from other fireflies that do not possess BDs. Both *R. chiwen* and *R. tigrinus* showed a high chemical preference to their respective toxin source, but neither species showed a high chemical preference toward a BD (cinobufagin). A subsequent experiment suggested that *R. tigrinus* recognizes toads by detecting the non-volatile and hydrophobic chemical components contained in the toad skin. These results suggest that the chemical cue that species of *Rhabdophis* utilize to recognize toxin source would be a combination of BDs or their precursors. In the third study, I found several differences in the cranial morphology among the four species of *Rhadophis*. For instance, frog-eating species possessed a larger head size compared to earthworm-eating species. These data on cranial morphology, along with the dietary information, suggest that, within the genus *Rhabdophis*, the reduction of head size has occurred. In the fourth study, *R. chiwen* took a longer time in handling firefly larvae than earthworms. A possible reason for this is that the exploitation of fireflies has occurred recently, and the behavioral specialization to firefly larvae has not yet evolved extensively in *R. chiwen*.

Conclusion

In the present study, I provided the basic dietary and morphological information of the species of *Rhabdophis*, especially the *R. nuchalis* Group. Although further investigation is necessary for confirmation, species of *Rhabdophis* would have an ability to recognize toxin source by detecting the chemical components related to BDs. Along with the dietary change, species of the *R. nuchalis* Group have reduced gape size and cranial morphology, suggesting that the adaptation to smaller prey has evolved.