Active foraging for toxic prey and chemical protection of offspring by females of *Rhabdophis tigrinus*

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Introduction

Many animals sequester dietary defensive compounds and incorporate them into offspring, which protects their offspring against predation. One possible but poorly investigated question is whether females of such species actively prey upon a toxic diet to acquire defensive chemicals for endowment to offspring. An Asian amphibian-eating snake *Rhabdophis tigrinus* sequesters defensive steroids from toads that are consumed as prey. Females produce chemically armed offspring in direct proportion to their own level of toad-derived toxins by provisioning the toxins to their eggs. Therefore, it is predicted that gravid females of *R. tigrinus* actively forage for toads because of the need for a sufficient amount of sequestered toxins for provisioning. To test this hypothesis, I investigated the ecology and behavior of *R. tigrinus* by a series of field researches and a laboratory experiment.

Materials and methods

My study was conducted in the Ashiu Forest Research Station of the Field Science Education and Research Center, Kyoto University, Japan. Habitats of the study site include forests, grasslands, open riverbanks, and a small paddy field. Most areas of the study site are covered with forests. *Rhabdophis tigrinus* is an active forager that primarily feeds on anuran amphibians. In the study site, *R. tigrinus* has been shown to feed mainly on *Rhacophorus arboreus* and *R. schlegelii*, which are locally abundant, non-toxic frogs. The Japanese common toad (*Bufo japonicus formosus*) inhabits the study site, but it has been reported to comprise only 0.9% of the snake's diet. I investigated movements and habitat use of *R. tigrinus* by radio-tracking 11 females and 13 males for up to 13 months each between June and November in 2009 and between April and November in 2010. I also investigated habitat use of toads and relative abundance of anuran species in each habitat by census surveys. I radio-tracked another set of nine gravid *R. tigrinus* from 16 April to 16 July in 2013 to clarify their foraging behavior and diet. To elucidate preference for toads, I conducted Y maze experiments in which I gave a choice of a trail with chemical cues of the toad and that of *Rhacophorus arboreus* to field-collected *R. tigrinus*.

Results

Rhabdophis tigrinus exhibited seasonal migrations. From May to June, *R. tigrinus* tended to aggregate in relatively small areas along a river, although females, which were gravid in these months, sometimes performed movements away from the river. Grasslands scattered along the river were frequently used by both sexes throughout the warmer period of the year despite the small total area of grasslands. In particular, males predominantly used grasslands from May to June, whereas gravid females used both grasslands and forests at a similar frequency. Toads were found solely in forests and were much rarer than other anuran species, such as *Rana tagoi* and *Rhacophorus arboreus*. Gravid snakes were more likely to exhibit foraging behavior when they were in forests than when they were in other habitats. Gravid females mainly ate toads in forests and *Rhacophorus* spp. in grasslands. Five of the nine gravid females monitored daily exhibited foraging behavior in forests, whereas the remaining four females never showed foraging behavior in that habitat. The former snakes mainly fed on toads, whereas the latter snakes were never found to eat toads and mainly fed on *Rhacophorus* frogs. In the Y maze experiment, gravid females were more likely to choose the trail of toads than were males or non-gravid females.

Discussion and conclusion

The results demonstrated the active foraging for toxic prey by gravid females of *R. tigrinus* by showing that (1) gravid females use forests, which are the habitat of toads, more frequently than do males; (2) gravid females exhibit active foraging behavior while they are in forests; (3) gravid females frequently eat toads even though they are a scarce resource; and (4) when given a choice of a toad and a non-toxic frog, gravid females are more likely to choose a toad than are males or non-gravid females. Sexual differences in the forest use pattern and the preference for toads in the Y maze experiments were detected specifically during the gestation season. This suggests that the active toad feeding by females is related to gestation and thus supports the hypothesis that females actively acquire defensive toxins for provisioning to offspring. The maternal provisioning of diet-acquired toxins to offspring and the associated active foraging for toxic prey may be tandem adaptations to reduce predation on an early life stage. One unexpected finding is the variation in foraging behavior and diet among individual gravid snakes. Such individual variation could influence local predator-prey dynamics by mediating variation in chemical defense among members of the local community. In conclusion, the present study provides a unique example of a complex system of offspring protection in snakes, involving active feeding upon toxic prey by gravid females.