Chemical Preference Studies on Newborn Snakes of Three Sympatric Species of *Natrix*

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Newborn young of three sympatric species of water snakes (*Natrix*) were tested with water extracts of a variety of small animals including fish, earthworms, amphibians, slugs, crickets, baby mice, and crayfish. *N. s. sipedon* responded with increased tongue flicking only to fish and amphibian extracts. *N. grahamii* and *N. septemvittata* responded only to crayfish extracts, and most strongly to extracts prepared from newly molted crayfish.

INTRODUCTION

NEWBORN snakes will respond to water extracts prepared from various small animals with increased tongue flicking and prey-attack behavior (Burghardt, 1966, 1967). Different species, however, will respond to different classes of extracts (earthworms, fish, amphibians, slugs, insects, *etc*). These differences are closely related to the normal feeding behavior and ecological preferences of the parent. Within the garter snakes (genus *Thamnophis*) such differences have been found in newborn snakes neither previously fed nor in any other way exposed to the species' normal environment after birth (Burghardt, 1969). It seems highly probable, then, that such species differences and similarities in naive young are "innate" and dependent to a large degree upon genetic information. This being the case, it would follow that such perceptual characteristics can be acted upon by natural selection, and hence can be used in the study of ophidian evolution. The value of overt behavioral differences in the study of reptile relationships has already been shown (Auffenberg, 1965; Carpenter, 1963).

If inexperienced newborn snakes are presented with a series of extracts from a variety of different types of prey animals, it should be possible to determine the relative releasing value of each if the proper experimental precautions are taken, such as controlling for order of stimulus presentation, means of extract preparation, age of extract, etc. From the various releasing values obtained in this way, extract response profiles can be constituted for the groups of snakes under consideration. In this paper such profiles are presented and compared for newborn young of three species of water snakes (*Natrix*).

METHODS AND RESULTS

The details of housing, testing, and other procedural methods have been presented in other papers dealing with other colubrid snakes (Burghardt, 1967, 1969). Only a brief description will be given here; details concerning individual litters will be given under the treatment of that litter.

The newborn snakes were from litters borne by gravid females captured in or near the Palos Forest Preserve, Cook County, Illinois. The females were maintained in display cages at the Little Red Schoolhouse Nature Center in the preserve until parturition. Shortly after birth and before ever being fed, the young were removed to the laboratory, weighed, measured (Table 1), and then isolated in glass tanks measuring 23 $\times$ 14 $\times$ 17 cm. Each tank was placed on white shelf paper and the four outside walls were covered with white partitions. The floor of each tank was bare except for a small plastic petri dish containing water. Except for testing periods, the aquaria were covered with glass tops. The temperature of the room in which the snakes were housed always ranged between 22–26° C; during testing the temperature was maintained at 24–25° C.

Each member of the three litters of newborn snakes was tested only once on a series of extracts of the surface substances of potential prey such as fish, earthworms, amphibians, and crayfish. Distilled water was the control. The extracts were all made by the same procedure. One or more of the living intact animals was placed in 50° C distilled water for one min in the proportion of 10 ml of water/1.5 g of body weight. The water was stirred gently during this period. The animal was then removed and the resulting liguid centrifuged, and the

TABLE 1. WEIGHT AND LENGTH DATA OF NEWBORN WATER SNAKES, GENUS *Natrix*.

Species	Date Born (1965)	No. Born	No. Alive	No. Measured	Wt (mg)		Snout–vent Length (cm)	
					$\bar{x}$	Range	$\bar{x}$	Range
N. s. sipedon	5 Sept	14	14	14	4252	3960–4647	16.0	15.3–16.6
N. grahamii	1 Sept	14	13	13	3981	3720–4319	18.5	18.0–19.1
N. septemvittata	18 Aug	20	19	17	2937	2608–3294	16.1	15.4–17.2

supernatant fluid refrigerated until use. Extracts for a given test series were always prepared on the same day and testing with them was completed before they were 48 hr old.

The testing procedure consisted of dipping a 15 cm cotton swab into the extract or control at room temperature, slowly introducing it into the tank, and bringing it within about 2 cm of the snake's snout. If the swab was not attacked within 30 sec, it was moved closer until it gently touched the snout. If no attack was made at the end of one min, the swab was removed and the total number of tongue flicks emitted in the one-min interval recorded.

In contrast to newborn *Thamnophis*, *Storeria*, and *Opheodrys* (Burghardt, 1967), prey-attack behavior by young *Natrix* was rare; some possible reasons for this are discussed below. Consequently, the results are based almost entirely upon tongue flick frequencies. Scores for snakes attacking the swab were derived using a formula described elsewhere (Burghardt, 1967).

Natrix sipedon sipedon

The northern banded water snake is the most widespread of a number of subspecies of *N. sipedon* found in North America. According to Smith (1961), this snake is essentially a stream species, although it is also found near lakes and ponds.

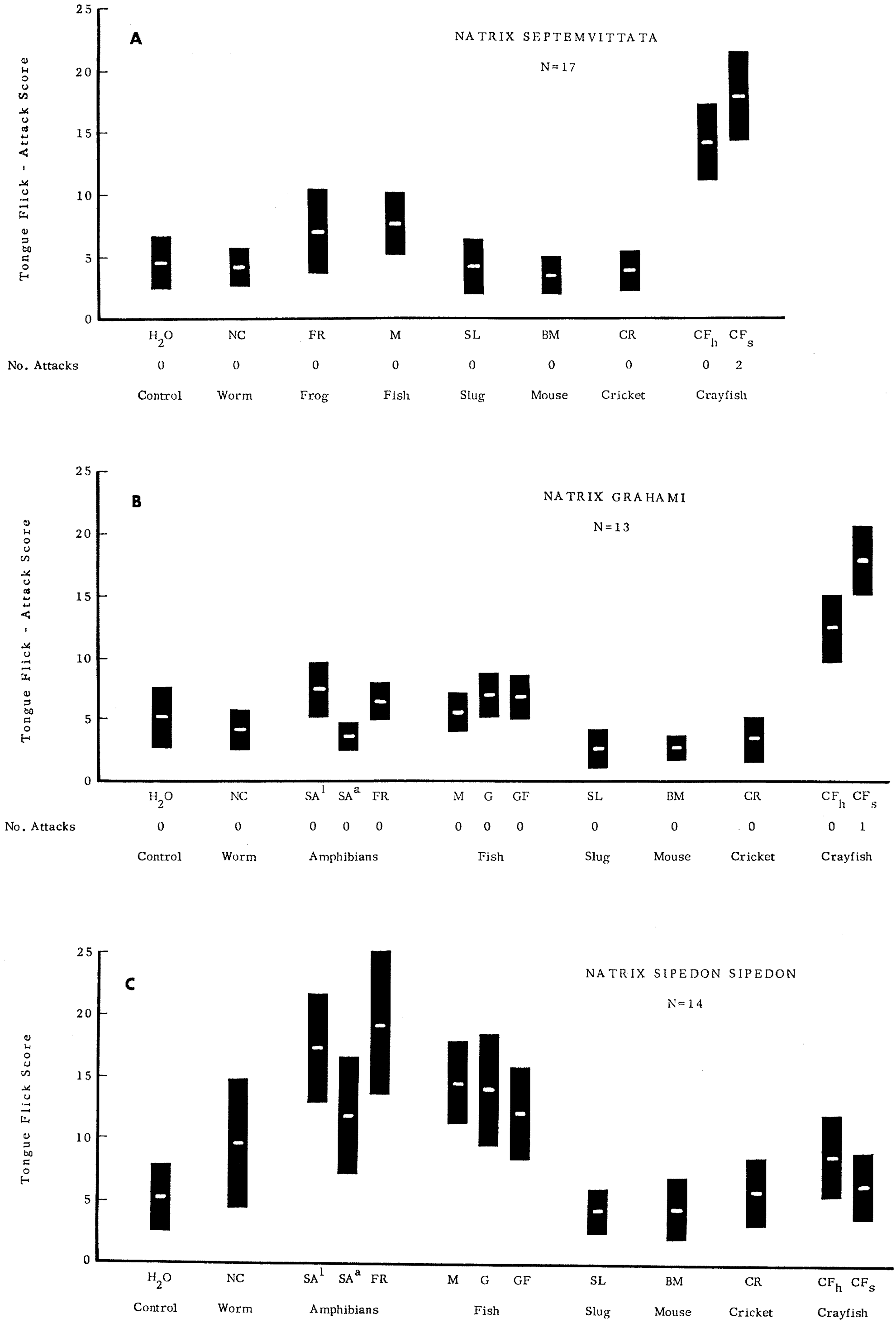
The mother was caught at the Palos Forest Preserve in early summer and kept on display, being fed fish. She gave birth to 14 live young on 5 September 1965. They were tested on the third and fourth days after birth.

The 14 young were tested along with the 13 newborn inexperienced Graham's water snakes discussed below. The following 12 animal extracts were tested on the snakes using a rotating sequence based on this order: CF_h—hard-shelled crayfish (*Cambarus*), G—Guppy (*Lebistes reticulatus*), SA^a—salamander adult (*Ambystoma jeffersonianum*), CR—cricket (*Acheta domestica*), M—minnow (*Notropis atherinoides acutus*), FR—cricket frog (*Acris crepitans blanchardi*), CF_s—soft-shelled crayfish (*Cambarus*, same species as CF_h), SL—slug (*Deroceras gracile*), SA^l—salamander larvae (*A. jeffersonianum*), NC—nightcrawler (*Lumbricus terrestris*), GF—goldfish (*Carassius auratus*), and BM—baby lab mouse (*Mus musculus*). Each of the first 13 snakes was tested in this order, beginning with a different extract. A water control was introduced systematically into the rotating extract series at different positions so that a total of 13 tests was given to each snake. Snake 14 was given a rearranged version of this standard order. Approximately 60 min elapsed between tests. On the first day of testing 7 tests were run, and on the second day 6 tests were run.

The results are shown in Fig. 1C. Although no prey attacks were made, significantly higher tongue flick scores were obtained with salamander larva, frog, and all fish extracts. This fits the general opinion of the feeding habits of this species. Smith (1961:259) stated that their natural diet "consists primarily of fish and amphibians." However, he lists no other items. A recent study of adults and newborn young by Dix (1968) supports this conclusion.

This species is rather stocky at birth, and it might therefore be expected that they have more food reserves than newborn *Thamnophis sirtalis* and need not eat as shortly after birth. Indeed, none of the 14 water snakes would eat a guppy offered immediately after the testing was finished. It may be concluded that no actual prey attacks were elicited because the snakes were not sufficiently motivated. These snakes did begin to feed at about 3 weeks of age.

The baby water snakes were advanced, however, in other forms of behavior. They would strike out defensively at large or quick moving objects brought into their immediate vicinity from the time of birth. It was necessary to bring the test swabs to the snakes very slowly or they would assume the coiled defensive posture, flatten the head


A
NATRIX SEPTEMVITTATA
N=17
Tongue Flick - Attack Score
25
20
15
10
5
0
H_2O NC FR M SL BM CR CF_h CF_s
No. Attacks 0 0 0 0 0 0 0 0 2
Control Worm Frog Fish Slug Mouse Cricket Crayfish
B
NATRIX GRAHAMI
N=13
Tongue Flick - Attack Score
25
20
15
10
5
0
H_2O NC SA^l SA^a FR M G GF SL BM CR CF_h CF_s
No. Attacks 0 0 0 0 0 0 0 0 0 0 0 0 1
Control Worm Amphibians Fish Slug Mouse Cricket Crayfish
C
NATRIX SIPEDON SIPEDON
N=14
Tongue Flick Score
25
20
15
10
5
0
H_2O NC SA^l SA^a FR M G GF SL BM CR CF_h CF_s
Control Worm Amphibians Fish Slug Mouse Cricket Crayfish

and neck region, hiss, and strike out "blindly." By moving the swabs slowly it was possible to eliminate this response more than 95% of the time. When it did occur, the snake was re-tested after at least a 15 min delay.

Natrix grahamii

This serpent is mainly found in the belt of states west of the Mississippi, although it overlaps with the queen snake in Illinois. Sluggish water areas are its habitat (Smith, 1961).

The mother was caught at the Palos Forest Preserve in July and kept on display. Although offered fish and frogs, it would only eat freshly molted crayfish. She gave birth to 13 live and one dead young on 1 September 1965. They were tested on days 7 and 8 after birth, concurrently with the 14 banded water snakes. They were tested with the same extract orders as the first 13 banded water snakes.

The results are shown in Fig. 1B. Only the two crayfish extracts had significantly higher scores than the controls. A swab dipped in the soft-shelled newly molted crayfish extract elicited a prey-attack. In fact, the score for the soft-shelled crayfish was significantly higher than that for the hard-shelled crayfish extract at the .05 level of significance.

The graph also shows that the responses to the salamander larvae, frog, and three fish extracts are higher than the control, though not significantly so. According to Smith (1961), newly molted crayfish are the major food item, although fish and amphibians are also eaten. The mother of the present litter would eat only the crayfish.

Natrix septemvittata

The queen snake is found east of the Mississippi River and prefers fast moving streams in forested regions (Smith, 1961).

The parent was caught in June at the Palos Forest Preserve. She would only eat soft-shelled crayfish. On 18 August 1965, 19 live and one dead young were born. Seventeen of the live young were received on the day of birth and tested 11 days later.

A reduced number of extracts were run in one day of testing. The basic series in order were: minnow; hard-shelled crayfish; cricket frog; slug; nightcrawler; baby mouse; soft-shelled crayfish; and cricket. This series was rotated in the usual manner for the first nine snakes. Only one control was used. It was in position 1 for subject 1, position 2 for subject 2 and so on. For snakes 10–17 the reverse order was used and rotated in the usual manner. About 50 min elapsed between tests.

The results are shown in Fig. 1A. They are very similar to those for Graham's water snake in that only the crayfish scores are significantly higher than the controls and attacks were made only to newly molted crayfish extract. The difference between the two crayfish extracts, although not significant by the procedure used in the figure, is significant if the differential response of an individual snake to the two extracts is considered. ($P < 0.05$, $x = 4$, one-tailed Sign test). The amphibian and fish extracts are higher than the controls but not significantly. Smith (1961) stated that newly molted crayfish are the chief diet, but added that newly captured individuals sometimes regurgitate small fish.

Discussion

The results show that clear differences exist within the genus *Natrix* in the perceptual schema of newborn snakes. Fig. 2 compares the three species with respect to certain of the extracts. The species responding to crayfish, *N. grahamii* and *N. septemvittata*, are evidently much more similar to each other than either is to *N. s. sipedon*. Thus, the chemical preferences of the newborn young correspond to the species-characteristic food preferences. *N. grahamii* and *N. septemvittata*, both in the Regina group

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Fig. 1. A, Extract response profile for 17 queen snakes (*Natrix septemvittata*). The middle of each bar represents the mean response and the extension of each bar is the mean SD $\sqrt{2/N}$ where SD is the standard deviation and N is the number of subjects. Any two bars that do not overlap are significantly different at least at the .05 level. Abbreviations for food items are explained in text. Crayfish extracts were the only ones that were effective and newly molted crayfish extracts were most effective. B, Extract response profile for 13 Graham's water snakes (*Natrix grahamii*). Results were very similar to those for *N. septemvittata*. C, Extract response profile for 14 inexperienced northern banded water snakes (*Natrix s. sipedon*). Only fish and amphibian extracts were effective.

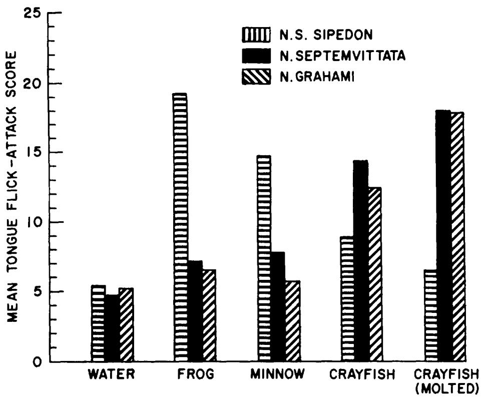

Fig. 2. Selected food extract scores for three species of *Natrix* showing the divergence of *N. s. sipedon* from *N. grahamii* and *N. septemvittata.*

of the genus *Natrix*, are indeed more closely related to each other than either is to *N. s. sipedon*, and Rossman (1963) has proposed that they be placed in a separate genus (*Regina*).

Both *N. grahamii* and *N. septemvittata* gave more tongue flicks to the fish and amphibian extracts than to distilled water. While this increase over the control was in no case significant, testing of more individuals or the use of stronger extracts or more highly motivated subjects might lead to the substantiation of a significant difference. Such a difference would be compatible with the scattered reports of these two species feeding on fish and amphibians (Smith, 1961; Wright and Wright, 1957). While crayfish undoubtedly are the main diet in both forms, a slight responsitivity to fish and amphibians could be either a vestigial character or a mechanism guarding against the ecological hazards of specializing on one food source. We need much more information on the habits of these species in the field and in captivity.

The chemical discrimination of the newly molted crayfish from hard-shelled crayfish by naive snakes is certainly a surprising result, and indicates that the physiological and behavioral correlates of such abilities need study. While the preference of some water snakes for molted crayfish has been in the literature for a long time, the reasons for such a discrimination remain uninvestigated. In the absence of such studies, the preference for molted crayfish could be interpreted as a conditioned discrimination involving not only chemical cues, but perhaps also visual and behavioral differences from the hard-shelled stage. Clearly, it is not hard to sympathize with the snake's partiality to swallowing a softer object rather than a hard one, especially a crayfish. In addition, some nutritional differences could give the two stages differential reinforcement value. Whatever the role of such factors, however, the present results show that they can at most interact with an unlearned chemical preference which, of course, entails a discriminative capacity at birth.

Unlike garter snakes, it appears that *Natrix* should be tested at several weeks of age rather than several days after birth, since the low rate of actual prey-attacks in comparison to *Thamnophis* may represent a motivational difference. On the other hand, they may need stronger extracts, or respond best to chemical cues not extracted with water, or sensory cues other than chemical ones may play an important role in prey-attack. Perhaps changes in the testing procedure are also needed, but that significant differences in tongue flicking occurred with different extracts demonstrates that differences in chemical preference exist. Since Jacobson's organ, which operates in conjunction with the tongue, is the most important chemoreceptor involved in food recognition as studied with extracts presented on swabs (Wilde, 1938; Burghardt and Hess, 1968) the use of tongue flick rates are meaningful by themselves.

Whether the use of perceptual schemata will help elucidate the systematics of various groups of snakes is an interesting but presently unanswerable question. That species differences exist within the same genus is clear. Only newborn snakes, however, can be utilized in the study of evolutionary relationships since feeding experience may alter the relative "releasing values" of extracts (Fuchs and Burghardt, unpubl. data). There is no reason why perceptual differences, if carefully analyzed, cannot be used along with morphological and behavioral differences in the study of serpent evolution.

Acknowledgments

Supported, in part, by National Institute of Mental Health grant MH-766 awarded to Eckhard H. Hess and by NIMH grants MH-13375 and MH-15707 to the author. I thank Edward Lace and the other naturalists at the Palos Forest Preserve for providing the gravid female snakes, Robert Jenkins for his statistical advice, and Thomas Uzzell for providing the salamanders.

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Intraspecific Comparison of the Blood Properties of the Side-blotched Lizard, *Uta stansburiana*

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Hemoglobin and hematocrit values, and erythrocyte shapes and sizes were compared in the three subspecies of the side-blotched lizard, *Uta stansburiana*. Significantly higher hemoglobin values and corresponding hematocrits were found for the Colorado population, *U. s. stansburiana*. The lowest values were found in the Arizona-Mexico population, *U. s. stejnegeri*, while intermediate values were obtained from the California population, *U. s. hesperis*. Erythrocyte measurements were similar in all the populations sampled. The *Uta* blood values are within the ranges for other iguanid lizards. Variation in hemoglobin parallels altitudinal differences in the populations.

Introduction

LIZARD blood properties have received limited attention since the pioneer studies of oxygen dissociation curves of the chuckwalla, *Sauromalus obesus* (Dill *et al.*, 1935) and the gila monster, *Heloderma suspectum* (Edwards and Dill, 1935). Knowledge of oxygen capacity of lizard blood was expanded by Dawson and Poulson (1962) to include values from 19 species representing eight genera and three families. Erythrocyte measurements were reported for several lizards by Hartman and Lessler (1964). Haggag *et al.* (1966) correlated changes in blood glucose, hemoglobin, red blood cell numbers, and blood nitrogen with hibernation in the lizard, *Varanus griseus*. Additional reports on blood properties of lizards are in the literature (Prosser, 1950; Hernandez and Coulson, 1951; Dessauer, 1952; Bentley, 1959; Zarafonetis and Kalas, 1960).

This paper reports hemoglobin and hematocrit values, and erythrocyte shapes and sizes for the side-blotched lizard, *Uta stansburiana*. Populations of the three recognized subspecies were investigated.