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COMPARATIVE PREY-ATTACK STUDIES IN NEWBORN SNAKES OF THE GENUS *THAMNOPHIS*

by

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(With 11 Figures)

(Rec. 11-VI-1968)

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I. INTRODUCTION

The relations between perception and overt behavior are central to the understanding of the physiological mechanisms and evolution of behavior. The study of the differences in the overt behavioral movements, especially fixed action patterns, of related species has often proved useful in evaluating hypotheses about the differentiation of motor patterns in the course of evolution (LORENZ, 1941; DILGER, 1960; HESS, 1962). The comparative study of the perceptual mechanisms leading to behavior has rarely been attempted in a manner analogous to the comparative study of movement patterns. Of course, there have been numerous studies on the sensory abilities of animals and comparative conclusions drawn (HESS, 1960). But studies of the absolute and relative sensory abilities of animals have a rather low resolving power when it comes to differentiating organisms within families or genera. If, however, we study the perceptual preferences of effective releasing stimuli in closely related species, we may find rather marked differences, and, if the effects of experience are controlled, it is possible to consider such perceptual differences as much evolved as different behavior patterns themselves. Indeed, the perceptual side of a behavior pattern may differentiate species clearer than a consideration of the behavior itself.

The lack of clear differences in the overt prey-attack response of newborn snakes, especially of the same genus, would appear to hold little promise for comparative studies. However, I have recently shown that on the perceptual side of this behavior there are dramatic species differences in naive newborn young, and that these seem closely related to their evolution and natural history (BURGHARDT, 1967). The newborn young selectively respond with attack behavior to water extracts of the skin substances of normally eaten animals. This chemically released response is probably mediated by Jacobson's organ, a receptor in the roof of the mouth which operates in association with the tongue and is phylogenetically related to olfaction (WILDE, 1938; NAULLEAU, 1966; BURGHARDT & HESS, 1968).

In this paper the responses of a number of species of garter snakes from the genus *Thamnophis* to a series of extracts from a variety of organisms will be presented and discussed. Newly born, inexperienced snakes were used

throughout. This genus is a relatively large one and is widespread over North America. Subspecies abound, and the relationships of its various members are complex and little understood. It is a very successful group of snakes: members have invaded almost all habitats with the exception of arid regions.

II. METHODS

The general experimental procedures will be given in this section. Details and minor variations concerning individual litters will be given in the results section, III, under the heading of the particular species involved. Even more details are presented in BURGHARDT (1966a).

a. Subjects.

Nine litters of newborn young from five species of garter snakes were tested in these experiments. The snakes were born in captivity to gravid wild-caught females. Young of the following forms were tested: the eastern garter snake, *Thamnophis sirtalis sirtalis*; the red-sided garter snake, *T. s. parietalis*; the Chicagoland garter snake, *T. s. semifasciata*; the eastern plains garter snake, *T. r. radix*; Butler's garter snake, *T. butleri*; the aquatic garter snake, *T. elegans aquaticus*; and the northwestern garter snake, *T. ordinoides*.

Information concerning the morphology, habits, and distribution of the species covered may be found in SMITH (1961), STEBBINS (1954), and WRIGHT & WRIGHT (1957). In the discussion following each kind of snake used, mention is made of the probable natural feeding habits of the species involved. There are, unfortunately, few extensive field studies which provide precise information. Feeding behavior in captivity has also not been very systematically investigated. The latter is important in showing what the animal *will* eat, although it does not determine what the snake *does* eat in the wild. The most complete reference is WRIGHT & WRIGHT (1957). Although this handbook is very useful, it should be noted that the authors uncritically list virtually every food item to be found in the literature. Many of these are based upon insufficient evidence and are probably erroneous, or constitute only a minute fraction of the diet.

b. Housing.

In most instances the gravid females were kept isolated in aquaria lined with newspaper and containing water dishes and bark. The ambient temperature was usually 25-30° C.

After birth, all of the young snakes were weighed and their snout-vent

TABLE I
Weight and length data of newborn snakes

Species	Date Born	Total #	# Alive	# Measured	Mean Weight	Range	Mean S-v Length	Range
<i>Thamnophis s. sirtalis</i>	7/31/65	19	19	19	1141 mg	976-1265 mg	13.0 cm	11.7-13.7 cm
<i>Thamnophis s. semifasciata</i>	7/14/65 (litter 1)	26	24	20	1306	1000-1445	12.8	12.2-13.4
<i>Thamnophis s. semifasciata</i>	7/26/65 (litter 2)	≥46	44	43	1784	1585-2003	14.9	14.2-15.5
<i>Thamnophis s. parietalis</i>	8/5/65 (litter 1)	12	12	12	1575	1437-1702	14.4	14.0-15.0
<i>Thamnophis s. parietalis</i>	7/8/65 (litter 2)	≥24	13	13	922	823-1060	11.9	11.3-12.3
<i>Thamnophis r. radix</i>	8/16/65	24	22	22	1429	938-1555	12.5	10.9-13.2
<i>Thamnophis butleri</i>	7/26/65	15	15	15	1343	701-1627	11.4	9.4-12.4
<i>Thamnophis elegans aquatics</i>	10/17/65	9	9	9	2135	1975-2331	15.1	14.4-16.2
<i>Thamnophis ordinoides</i>	7/31/65	12	12	12	1547	1364-1767	13.0	11.9-14.3

lengths measured (Table 1). They were then individually isolated in glass aquaria measuring $23 \times 14 \times 17$ cm. The aquaria were placed on white shelf paper and all four sides were covered with 8×5 in (20.3×12.7 cm) white cards. Covering each tank was a piece of plate glass. These glass covers were always in place except when testing the nimals or changing the water. The interior was bare except for a 60×15 cm plastic Petri dish or cover which contained water.

The newborn snakes were maintained and tested in an air conditioned room in which the temperature rarely varied more than between $22-26^\circ$ C. During testing, the temperature was always held between $24-25^\circ$ C. Illumination was via sunlight and incandescent ceiling fixtures.

c. Preparation of the test stimuli.

The basic method of preparing the chemical extracts began by rinsing off a small quantity of a given prey animal with tap water. This was then dried between absorbent paper and weighed to the nearest 0.1 gm. Distilled water in the proportion of 10 cc of water to 1.5 gm of the test organism was heated to 52° C so that the water would be at about 50° C after the organism was introduced. The animal was placed in the warm water and stirred gently for one minute. The animal was then removed and the resulting extract of its surface substances was centrifuged at 2500 rpm for 10 minutes. The supernatant liquid was poured into glass vials, stoppered with tight fitting plastic caps, and kept refrigerated until used. These extracts were quite clear and usually indistinguishable from water, both to the human eye and nose.

All extracts for any given experiment were prepared at the same time and on the day of, or the day prior to, the commencement of testing. An extract three days old has been shown to lose none of its effectiveness (BURGHARDT & HESS, 1968).

d. Presentation of the stimuli.

All the young snakes were tested before they had any feeding experience whatsoever. Since newborn garter snakes can go for several weeks before their first meal, the exact age at which they were tested is probably not too critical. However, all the testing reported here was completed before the snakes reached the age of one week.

The details of the sequence and timing of the testing procedure will be discussed under each individual litter, and even more procedural detail is recorded elsewhere (BURGHARDT, 1966a). The extracts were taken from

the refrigerator and placed on a table in the proper order in the testing room. About 20 minutes was allowed for them to warm up to room temperature. The glass tops were removed from the tanks and remained off until the end of the testing session.

The test solutions were presented to the snakes on commercial cotton swabs 15 cm long. The cap of the extract vial was removed briefly while the swab was dipped into the liquid. Excess liquid on the swab was removed with a quick shake. The swab was then slowly introduced into the home cage of the snake and moved to within 2 cm of its snout. If after 30 seconds no attack response was made, the swab was brought closer to the snake and gently touched its lips three times. If no prey-attack response was made within a total of 60 seconds, the swab was removed and the total number of tongue flicks emitted by the snake in the one minute interval was recorded. When a snake did attack, the number of elapsed seconds was recorded, measured to the nearest 0.1 second.

The response to an effective stimulus was very clearcut. The snake would increase its rate of tongue flicking and then lunge forward at the swab with its jaws wide open at about a 45° angle. Before introduction of the swab the snake was usually resting and emitting no tongue flicks. With a particularly potent extract, an attack would often occur almost immediately, without being preceded by many tongue flicks. An attack was always preceded by at least one flick, however. To prevent the snake from entangling his teeth in the cotton, the swab was removed at the moment of the strike.

Most of the litters were tested on a series of extracts prepared from the same 12 species of small animals. These 12 extracts usually employed will be referred to henceforth as the standard extract series. The 12 extracts were used most frequently in a predetermined order which will be referred to as the standard extract order. The standard extract series and order along with other extracts which were sometimes used is given in Table 2. The common names, scientific names, and symbols of the prey animals are also given.

During the early comparative tests the following procedure was utilized. A distilled water control swab was presented first, then 3 test extracts, another water control swab, 3 more extracts, and then another control. The following day this sequence was repeated (with the 6 remaining extracts) so a total of 6 controls and 12 experimental tests were run. This method is referred to as the 6-control method.

Later in the comparative testing this procedure was simplified and improved. In this method the control swab was only presented once to each animal. The position of the control swab in the sequence of extracts was

varied systematically for each individual snake. This removed the possibility of the control score being misleading low because of selective habituation to it over repeated presentations (TINBERGEN & PERDECK, 1951). This could only apply to the tongue flick scores since control swabs never released a prey-attack. This second procedure will be called the one-control method. Analysis of the data indicated that the specific control method used

TABLE 2

Animals used in preparation of extracts

Standard Extract Order

Symbol	Common Name	Latin Name
LW	leafworm	<i>Lumbricus rubellus</i>
G	guppy	<i>Lebistes reticulatus</i>
SA ^a	salamander adult	<i>Ambystoma texanum</i> or <i>jeffersonianum</i>
LE _p	turtle leech	<i>Placobdella parasitica</i>
M	minnow	<i>Notropis atherinoides acutus</i>
FR	cricket frog	<i>Acris crepitans blanchardi</i>
RW	redworm	<i>Eisenia fetida</i>
SL	slug	<i>Deroferas gracile</i>
SA ¹	salamander larva	<i>Ambystoma jeffersonianum</i>
NC	nightcrawler	<i>Lumbricus terrestris</i>
BM	baby mouse	<i>Mus musculus</i> , laboratory strains
GF	goldfish	<i>Carassius auratus</i>

Additional Extracts Tested

LE _m	giant leech	<i>Macrobdella decora</i>
CR	cricket	<i>Acheta domestica</i>
FR _x	clawed frog	<i>Xenopus laevis</i>
MT	horsemeat	<i>Equus caballus</i>

was not an important factor. A detailed treatment of the water control data will be given near the end of the results section.

The order of the test extracts was determined independently of the control method employed. The standard order or sequence has already been given. This was predetermined in such a way that extracts representing prey animals from the same class (*e.g.* worms or fish) were not next to each other. With different individuals from the litter the same order was used except that it began with different extracts. For example, Subject 1 would get the order as given, Subject 2 would start with the second extract (guppy) and the first extract would drop to the twelfth position, and so on. This is referred to as the rotation of the standard sequence of extracts. Variations due to the number of animals in the litter and other factors will be discussed with the litters concerned.

e. Scoring procedure.

In the first study (BURGHARDT, 1966b) tongue flick data were not utilized. However, it was noted that the amount of flicking was correlated with the apparent intensity of interest shown in the swab. Some snakes which did not actually attack a swab would give a large number of tongue flicks to it over and above that elicited by an identical swab dipped in distilled water. This led to the idea that a more quantitative representation between the response to controls and that to attack-releasing stimuli could be measured by considering the tongue flick data as well as that concerning the frequency and latency of attacks. A formula was devised to incorporate these data, resulting in one score.

The basic unit was the maximum number of tongue flicks given by any individual of the litter tested to any of the test stimuli (the maximum was invariably to a non-control swab). For instance, if snake No. 4 gave 60 flicks to the minnow extract but did not attack, and this was the highest number emitted by any snake in the group, then 60 was the basic unit for this group. The scoring for each test was straight forward. A snake which did not attack was given a score identical with the number of flicks it emitted toward the swab in the one minute test period. Since an attack was considered more definitive than any number of tongue flicks, every attack was worth at least the maximum number of flicks given by any snake not attacking (even though the attacking snake may not have emitted many tongue flicks before attacking). In addition, each attacking snake received 1 point or fraction for every second or fraction less than one minute that the snake responded. It was assumed that a more potent stimulus would lead to an attack with a shorter latency than would a weak stimulus. Note that this method for combining attack latencies and tongue flicks, although arbitrary, allows for a most conservative distinction between actual attacks and tongue flicks. The formula for attacking snakes (unit of measure, seconds) can be represented by:

$$\text{Score} = \text{base unit} + (60 - \text{response latency})$$

While a separate base unit score for each subject would have certain advantages, they were not used here because of the wide range of individual variation. For instance, a snake which attacked all or most effective extracts would often have a lower maximum tongue flick score than one which attacked none or only a few extracts. In combining scores for a group of snakes this would result in some scores for attacking snake to be less than some tongue flick scores for non-attackers, a situation I wanted to avoid.

Although the combination of a behavioral (frequency) score and a latency measure is not ideal statistically, it does make use of more data than either score alone.

f. Analysis of the results.

A score for each test extract and control given each snake was calculated according to the procedure outlined in the preceding section.

Most of the results and the statistical treatment of the data for each litter are presented simultaneously in graphical form. The graphical technique is based on the rationale of the t-test when the variance is unknown but considered equal for all groups of observations. For each litter of snakes, the standard deviation (SD) and mean was computed for each test extract and control; Each SD was then multiplied by a factor $\sqrt{2/N}$ where N is the number of snakes in the particular litter. On the graph, the short mark in the center of the bar represents the mean score. The extension of the bar to both sides of the mean is equal to the mean \pm SD $\sqrt{2/N}$. At the point of no overlap between any 2 bars the difference is significant at the .05 level. Bars separated further are significantly different to a greater extent.

III. COMPARATIVE TESTS AND RESULTS

A. EASTERN GARTER SNAKE

This is the common garter snake (*Thamnophis s. sirtalis*) which is found almost everywhere in the eastern half of the United States. It lives in a wide variety of habitats and is perhaps the most adaptable snake in America — the ophidian house sparrow. Perhaps more is known about the general behavior and biology of this snake than any other species.

S u b j e c t s.

The mother was captured on June 23, 1965 in Porter county, Indiana. She would occasionally eat minnows and nightcrawlers. She gave birth to 19 young on July 31, 1965. The babies were tested on the fourth and fifth days after birth.

M e t h o d.

The standard 12 extracts were tested. The rotation method of the standard sequence of extracts was used for the first 12 snakes. The 6-control method was used so there were 4 blocks of three extracts each. In order to arrange a different sequence for the remaining 7 snakes, each block of 3 extracts as appearing in subject 1 were internally rearranged. This new sequence was then followed for snakes 13-19 with each one beginning the sequence with a different extract.

The standard testing procedure was employed. About 60 minutes separated trials on

both days. A litter of 12 *Thamnophis ordinoides*, born on the same date, was tested along with the present litter on the same series of 12 extracts and using identical orders, snake for snake, as the first 12 subjects of the present litter.

Results and Discussion.

These results are presented in Figure 1. Note that all the test extracts gave a significantly higher score than the water controls with the exception of the adult salamander, slug, and baby mouse. One attack, however, was made to the adult salamander extract. Since attacks were never made to the controls, it is reasonable to assume that there was something in the adult salamander extract which elicited a prey attack. It is interesting that

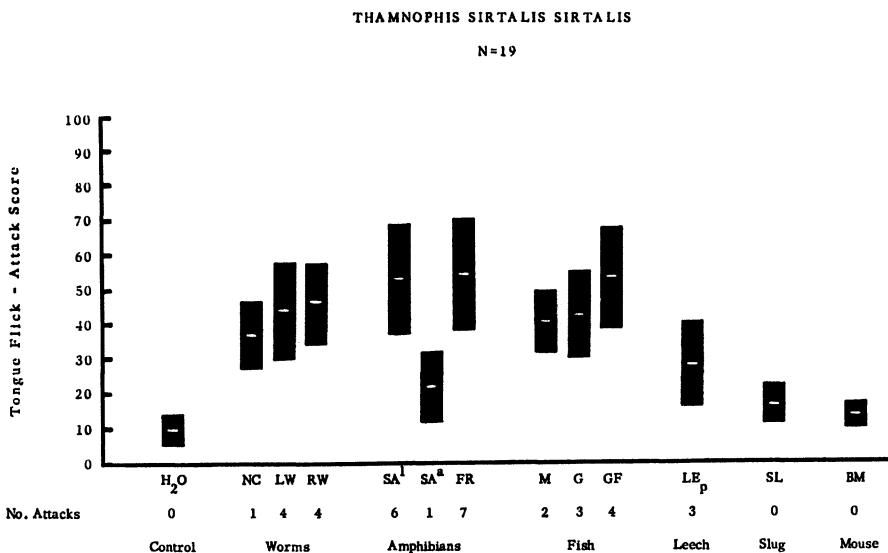


Fig. 1. Extract response profile of the eastern garter snake, *T. sirtalis sirtalis*.

the larval salamander extract was significantly more potent than the metamorphosed adult extract.

SMITH (1961) lists the following animals as being eaten by this species: earthworms, frogs, toads, salamanders, fish, young birds, and leeches. Extracts from these animals included in the standard 12 all elicited attacks. It is clear, therefore, that naive young can recognize, by chemical means alone, a large number of the food items utilized by this species in nature. CARPENTER (1952), in a very thorough field study, found that a few soft bodied caterpillars were taken and that in adult snakes voles constituted 2 percent of the diet.

B. CHICAGOLAND GARTER SNAKE

This subspecies (*Thamnophis sirtalis semifasciata*) is not recognized by WRIGHT & WRIGHT (1957) but is recognized by SMITH (1961). Its range is limited to the Chicago area, hence its name. In habitat preference and feeding habits it seems identical with the common garter snake. It is distinguished from the common or eastern garter snake by having the anterior part of the lateral stripes broken by black blotches. Two litters of this form, totalling 35, were tested.

Litter 1.

Subjects.

The mother was caught in June of 1965 at the Palos Forest Preserve in Cook County, Illinois and was maintained there, being fed local earthworms and perhaps other food until it gave birth to 26 young on July 14th. Of the 26 young, 2 died during parturition, and one was born with deformed and greatly enlarged eyes. Twenty of the normal young were tested on the second and third days after birth.

Method.

The standard 12 extracts were used with the exception that *Xenopus*, the South African clawed frog, was substituted for the slug. This extract was prepared by placing the live frog in distilled water at 28°C. for five minutes.

The sequence of the 12 extracts differed from the standard one. It was as follows: LW, G, LEP, SA¹, NC, M, FR, BM, RW, SA^a, GF, and FR_x. The rotation method was employed with this sequence. The orders beginning with the first 8 extracts were repeated twice, only one snake was run on each order beginning with the last 4 extracts.

The 6-control method was used. About 40 minutes elapsed between tests.

Results and Discussion.

The results are shown in Figure 2. They are generally similar to those for the preceding subspecies. The worms as a group, however, were much more effective than the fish as a group. Although no attacks were made to the adult salamander, its relationship to the salamander larva and cricket frog is very similar to *Thamnophis s. sirtalis*. The response to the clawed frog extract prepared at room temperature was practically identical with that to water. Since this frog is eaten readily by garter snakes, it may be concluded that the warm water is necessary to leech out the effective chemicals with the method used. Unfortunately, no extract of this animal prepared in the standard fashion was used to conclusively prove this point.

Litter 2.

Subjects.

The mother was caught at the Palos Forest Preserve in the early part of July 1965. It was kept at the nature center and fed at least earthworms until parturition. On

July 26, 1965, it gave birth to 46 young. Two were born dead and one died the following day. The mother died immediately after giving birth. Fifteen of the 43 surviving young were selected at random to undergo the comparative test series. The rest were utilized in a deprivation study reported elsewhere (BURGHARDT & HESS, 1968). Testing was on the fourth and fifth days after birth.

Method.

The standard 12 extracts were used and prepared in the standard manner. The rotation of the standard order was used for the first 12 snakes. However, the goldfish and baby mouse extracts were interchanged in the basic sequence. Slightly altered orders were used for the remaining 3 snakes. The 6-control method was used with about 60 minutes between tests on individual snakes. Fifteen *Thamnophis butleri* born on the same date, were tested at the same time with the identical series of extracts presented in identical orders, snake for snake, as the present species.

THAMNOphis SIRTALIS SEMIFASCIATA

N=20

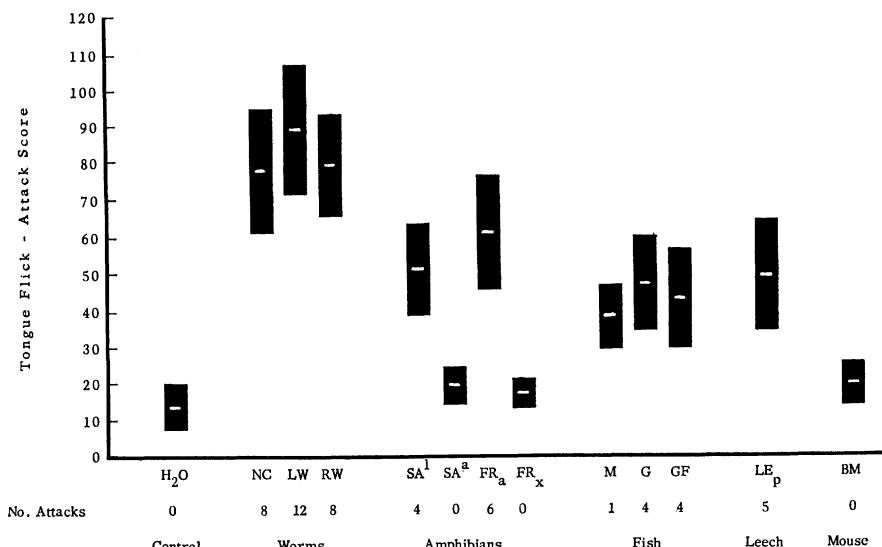


Fig. 2. Extract response profile of the Chicagoland garter snake, *T. sirtalis semifasciata*, litter 1.

Results and Discussion.

The results for the 15 newborn young are presented in Figure 3. They are quite similar to those obtained with the first litter of this subspecies tested with the exception that the worms are less clearly superior to fish. However, the same 9 extracts are significantly higher than the controls. In fact, the rank correlation coefficient, r_s , for the 12 stimuli common to

the two litters is .85 ($p < .01$). This includes the controls but not the FR_x or SL extracts which were only given the first and second litters respectively.

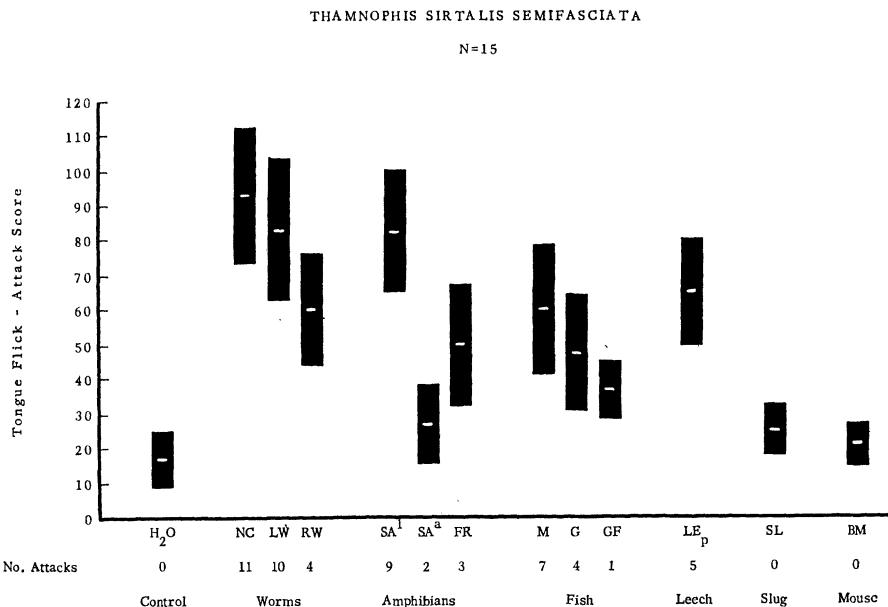


Fig. 3. Extract response profile of the Chicagoland garter snake, *T. sirtalis semifasciata*, litter 2.

C. RED-SIDED GARTER SNAKE

This is commonly known as the red-sided garter snake because the skin between the scales near and including the lateral stripe is colored red. This subspecies (*Thamnophis sirtalis parietalis*) is recognized by WRIGHT & WRIGHT (1957), but not by SMITH (1961) for Illinois. No one disputes the fact that it intergrades readily with other *sirtalis* subspecies. It is most common west of the Mississippi River, but both litters used here were from females caught east of the Mississippi. The amount of red is slight compared to the middle of the range of *Thamnophis s. parietalis*, but is separated here from *Thamnophis s. sirtalis* because both forms are found in the same area in Indiana, and the red form is generally much darker in coloration — easily recognizable at a glance.

Litter I.

Subjects.

The mother was caught near Waterloo in southwestern Illinois, on May 2, 1965, and both she and the young were given to me the day after parturition. The date of birth

was July 8, 1965. During her sojourn in captivity prior to giving birth, the female had no more than a few insects and worms offered as food. It is presumed she ate the latter. The mother ate several adult *Ambystoma* salamanders and baby mice in my laboratory, as well as fish and worms.

The 13 live babies were tested on the second through fifth days after birth.

Method.

This was the first litter to be tested and, at the time, the full details of the subsequent methodology had not been completely worked out. Twenty-two tests were run on the four days of testing. During the first 5 tests (which included 2 controls) no tongue flick records were made. A correction for this missing data was made later in analyzing the results.

On July 10-12, the following extracts were run in a varied, although not completely balanced order (in the sense that each test extract occupied every position in the sequence an equal number of times): RW, (prepared on 7/10), M, SA¹, NC, FR, NC

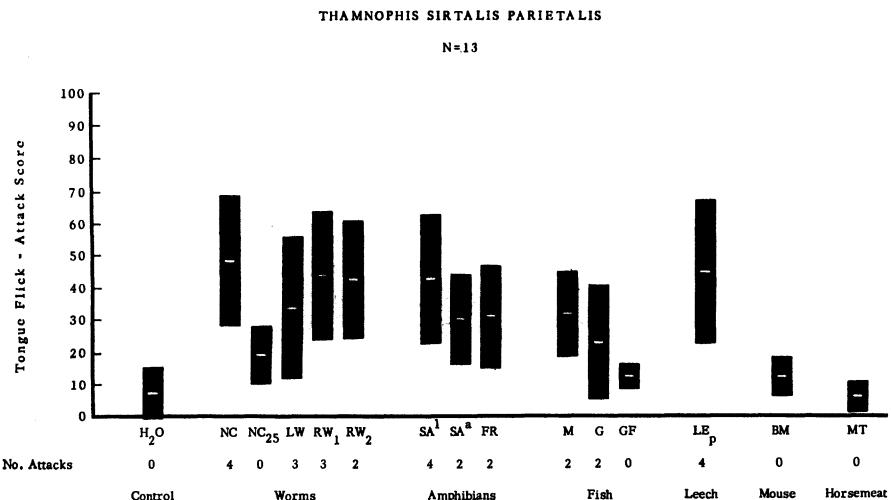


Fig. 4. Extract response profile of the red-sided garter snake, *T. sirtalis parietalis*, litter 1. NC₂₅ prepared at 25°C.; RW₁ and RW₂ prepared on days 1 and 2 of testing, respectively.

(prepared at 25°C), GF, (all prepared on 7/11). Water control swabs were run at constant positions in the order (1, 3, 8, 9, 12). Two tests were made on the first day of testing, 7 on the second day, and 5 on the third. The solutions were made and presented in the standard manner.

On July 13, six new extracts were made and tested. The rotation of a basic order was used with controls in positions 1, 5, and 9. The extracts in their basic order were LE_p, G, SA^a, LW, BM, MT. Two snakes were run on each of the 6 orders generated by rotating the starting extract. Subject 13 was run on a variation of the standard order.

Results and Discussion.

The results for all 13 snakes are shown in Figure 4. A correction method was used to fill the empty cells with the exception of the controls, since

6 other control scores were available for each snake. Where there was a score missing for a given snake on a certain extract, the sum of that snake's scores to all other *test* extracts (not including control scores) was multiplied by the sum of all scores given by other snakes to that extract and divided by the grand sum of all scores for all snakes and extracts. The resulting number was used to fill in the missing cell.

It can be seen from the graph that although the same extracts were responded to as with the preceding and following litters, the overall scores were not as high and not as many reach significance over the controls. Part of this seemed due to the variability in the responsiveness of the young. Subsequent to this experiment, the young were utilized in feeding behavior tests and 5 of the young refused to eat any type of food until they were

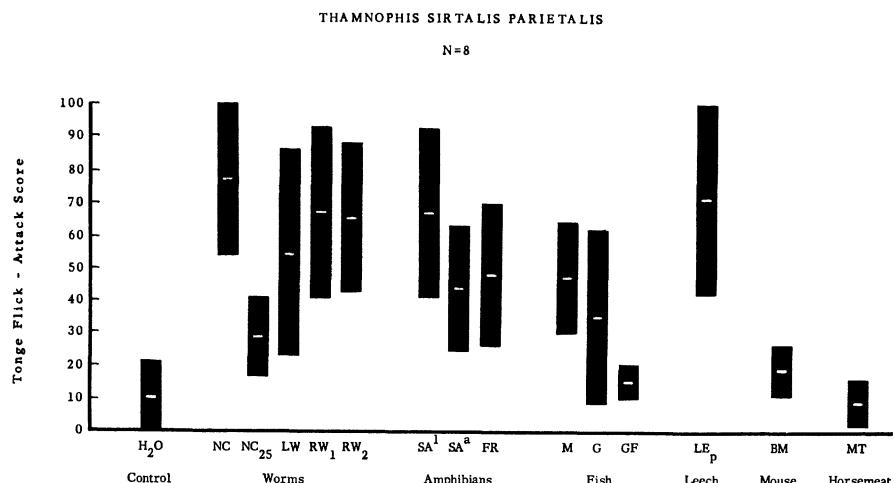


Figure 5. Extract response profile of the red-sided garter snake, *T. sirtalis parietalis*, litter 1. Same as Figure 4 except noneaters omitted.

several weeks of age. These 5 noneaters were also those which did not show any interest in the test extracts. Since it seems clear that at the time of testing they were not motivated by hunger, their lack of responsiveness is accounted for and it is justifiable to eliminate them from consideration here. After these snakes began to eat, they would also respond to extracts. Figure 5 shows the results for this litter when the 5 nonresponders are eliminated.

The worms as a group were superior to the fish, the exception being the nightcrawler extract prepared at 25°C. This again shows that the warm water is necessary to leech out the relevant substances. The 2 redworm extracts prepared a day apart showed almost identical scores. The goldfish

showed a surprisingly low score. This may or may not be meaningful since the goldfish extract was run early with the unbalanced orders.

FITCH (1965) has reported a field study on *parietalis* found in Kansas. There the problem of intergradation with *sirtalis* is not present. He found that these snakes, even as young, took a much larger percentage of amphibians and a much lower percentage of earthworms compared to more northern *sirtalis*. Since different populations of his snakes in Kansas showed different overall distributions of specific food items, it is possible that this difference is based on the availability of prey. It would be interesting to test an unquestioned "pure" *parietalis* on the series of extracts.

Litter 2.

Subjects.

The mother was caught in Porter County, Indiana, on June 3, 1965. This was the same locale where the *Thamnophis s. sirtalis* was found, although morphologically both the mother and young were quite different, being darker and with an indistinct dorsal stripe. It gave birth to 12 young on August 5, 1965. Before parturition its main diet was minnows, although it also had occasional nightcrawlers and one turtle leech and one cricket frog. The young were tested on the fourth and fifth days after birth.

Method.

The standard 12 extracts were presented in the standard rotating order. Since there were exactly 12 young, the order was completely balanced. A litter of midland brown snakes (*Storeria dekayi wrightorum*) born on the same date were tested on the same days with the same extracts. The 6-control method was used. Approximately 40 minutes elapsed between trials for individual snakes.

Results and Discussion.

The results are shown in Figure 6. The typical *sirtalis* pattern is seen with the following exception: an attack was made to the slug extract. This unusual finding led to a subsequent experiment which demonstrated a rapidly learned inhibition of the attack response to slugs. The mean tongue flick-attack score to the slug extract was not significantly higher than the controls, however. Likewise, although an attack was given to the leech solution it was not significantly higher than the control solution. The worms as a group were more potent than fish as a group.

A rapidly learned response inhibition.

One of the snakes tested above attacked the swab dipped in slug extract. This species of snake has never been reported to eat slugs in the field nor

in captivity, and the mother of the litter ignored the slugs. Outside of the one attack, an inordinate amount of tongue flicking was not seen to the slug extract. It was decided to look at the snakes' behavior toward live slugs on a systematic basis.

M e t h o d.

On the sixth day after the comparative testing the snakes were studied while still individually isolated in the standard test aquaria. They were unfed. On the morning of August 17th, a live slug was placed in the tank of each of the 12 snakes and allowed

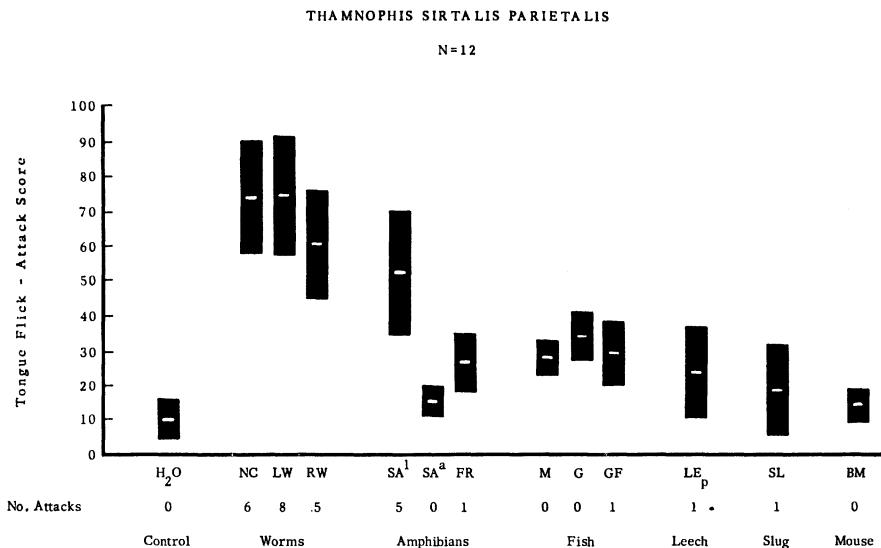


Figure 6. Extract response profile of the red-sided garter snake, *T. sirtalis parietalis*, litter 2.

to remain for one minute unless it was eaten or in the snake's mouth. The reactions of the snakes were noted. On the morning of the following day this procedure was repeated. After all the snakes had been tested on the slug, each snake was presented with a dead redworm in the same manner and the behavior towards it also noted. The worms were killed by immersion in hot water.

R e s u l t s.

Table 3 gives the protocols for the 12 snakes. Interest is defined by orientation toward the stimulus object and flicking the tongue. Note that on the first day 7 snakes attacked the slug but only one actually ate it. For six of the snakes, attacks were followed by rejection. This rejection took two forms: either the snake actively rejected the slug by backing away from it or by rubbing his head against the glass bottom of the tank, or it stopped the usual behavior pattern of ingestion which flows on smoothly after an attack

TABLE 3

*Responses of *T. s. parietalis*, litter 2, to slugs and redworms*
Stimulus

Subject	Live Slug, Day 1	Live Slug, Day 2	Dead Worm, Day 2
1	mild interest	no interest	ate worm
2	attacked and ate	twice attacked	ate worm
	slug	and rejected	
3	one attack and	no interest	ate worm
	rejection		
4	no interest	mild interest	mild interest
5	mild interest	mild interest	ate worm
6	one attack and	one quick attack	ate worm
	rejection	and rejection	
7	no interest	no interest	mild interest
8	two attacks and	mild interest	ate worm
	rejections		
9	one attack and	mild interest	ate worm
	rejection		
10	mild interest	no interest	ate worm
11	one attack and	no interest	mild interest
	rejection		
12	two attacks and	mild interest	mild interest
	rejection		
Totals	7 attacked	2 attacked	8 ate worm
	1 ate slug	0 ate slug	

and remained motionless with its jaws over the slug. In this situation the slug would proceed to crawl out of the snake's mouth to safety. The one snake who actually ate the slug seemed to do so by "mistake". By this is meant the fact that the behavior was neither as intense nor as qualitatively the same as when taking a food these animals are known to eat such as fish or worms. It seemed to swallow the slug in spite of its rejection movements. Subsequent tests support this conclusion.

The reactions to the slug on the following day were quite different. Only two snakes attacked the prey object and none ate it. One of the animals who attacked the slug was the snake who ate one the preceding day. However, after attacking and rejecting the slug twice he ignored it.

The test with the dead redworm immediately following the second slug test showed that the snakes were indeed hungry in that 8 of them ate the worm in one minute, including the snake which had eaten the slug the preceding day but ignored the slug the second day. All in all, 5 of the 7 snakes who attacked and rejected the slug on either day ate the redworm.

Discussion.

It can be concluded from this experiment that the snakes rapidly learned

not to attack the slugs. Hunger is removed as a variable because the majority would eat other food (redworms). This rapid learning to inhibit a response to certain stimuli has been noted in a variety of other animals, usually involving visual cues. A well known example is that of the toad and the bee (BROWER, BROWER & WESTCOTT, 1960).

A most interesting problem is *why* the slug should be an aversive stimulus to this species of snake. The case is far from analogous, in this respect, to the toad or chicken which learns to avoid insects which sting. Other species from the same genus eat slugs readily, not only in captivity, but also in the field where they may form the bulk of the snakes' normal diet (Fox, 1952). It seems easier to understand species differences in what releases the prey-attack than in why some objects which innately release the attack are subsequently rejected.

In evolutionary terms, it can be said that the potential to attack and eat prey which the species no longer encounters in nature is a character which at one time in the past history of these snakes possessed adaptive value. But it must be admitted that there is no apparent explanation why, in this case, the attack should have remained but not the ingestion. Perhaps the chemical stimuli emanating from the slug are, in one critical aspect, very similar to that of a prey object the species normally eats. The taste of the slug, however, is immediately recognized as not corresponding to the innate schema and causes rejection. The rapid learning to inhibit the attack response (via exteroceptive chemical perception) is then seen as an evolved adaptive mechanism designed to rectify matters.

However, more experiments need to be performed before the ecological and perceptual aspects are clear. Nonetheless, this seems to be an unquestionable example of ophidian learning — a process which has proved difficult to demonstrate (KELLOGG & POMEROY, 1936; WOLFE & BROWN, 1940; CRAWFORD & BARTLETT, 1966; CRAWFORD & HOLMES, 1966).

D. EASTERN PLAINS GARTER SNAKE

This species (*Thamnophis r. radix*) is common throughout the midwestern United States. Its build is superficially similar to *sirtalis*, although the stripes are more orange-colored. The preferred habitat is variable and, although similar to *sirtalis*, is slightly more aquatic. According to WRIGHT & WRIGHT (1957), and SMITH (1961) the feeding habits of this species are similar to *sirtalis*, although no extensive field study has been performed.

Subjects.

The mother was caught at the Palos Forest Preserve in the middle of June. She was

fed mainly minnows but also a few nightcrawlers. She gave birth to 24 young on August 16, 1965, but two of the young were born dead. The 22 live young were tested on the fourth, fifth, and sixth days after birth.

M e t h o d.

The standard 12 extracts were used with the exception that the giant leech replaced the turtle leech in the basic series and a cricket extract was added to the end of the sequence. The latter was done because green snakes (*Opheodrys vernalis blanchardi*) were tested simultaneously with the present litter, (BURGHARDT, 1967). The one-control method was utilized. On the second day of testing, a turtle leech was located so that it was possible to reinsert it into the sequence for half the snakes on the second day. Since the two species of leeches seem to elicit quite different responses on the part of the snakes, the third day each snake was given a test on the type of leech extract it was not exposed to during the regular testing.

The first 13 snakes were given the normal rotating series of extracts. For animals 14-22 the basic sequence was reserved. The reversed sequence was also rotated for each individual. Approximately 60 minutes elapsed between successive tests.

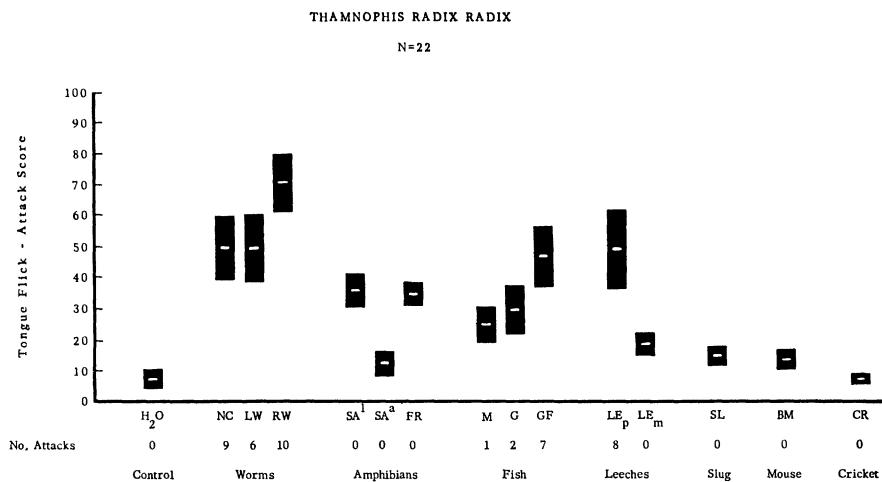


Fig. 7. Extract response profile of the eastern plains garter snake, *T. radix radix*.

R e s u l t s a n d D i s c u s s i o n .

The results are shown in Figure 7. No great differences from *sirtalis* are evident. Although no attacks were given any of the amphibian extracts, highly significant increased tongue flicking was seen to the frog and larval salamander extracts. What is surprising is the difference between the two species of leeches. No attacks at all were given to the giant leech, but 8 were given to the turtle leech. Tests with the deprived *sirtalis* (BURGHARDT & HESS, 1968) demonstrated that they will attack giant leech extract. Perhaps, this is a real species difference. Adult plains garter snakes would not eat giant leeches, but readily ate turtle leeches. As with most *sirtalis*, worms as a class were superior to fish as a class.

E. BUTLER'S GARTER SNAKE

This species, *Thamnophis butleri*, is locally common in certain areas in the midwest, especially Michigan and southeastern Wisconsin. It has morphological characteristics quite distinctive from the previous *Thamnophis*. The most obvious are the stocky body and the relatively small head. Behaviorally, it is remarkably even tempered and rarely attempts to bite. According to CARPENTER (1952) it is basically a grassland form like *Thamnophis radix*, although also usually found close to water. WRIGHT & WRIGHT (1957), in fact, consider *butleri* merely as a subspecies of *radix*. Other authorities do not accept this view (SCHMIDT, 1953; CONANT, 1958). SCHMIDT (1938), however, advanced the view that *butleri* is a descendant of *radix*. RUTHVEN (1904), also thought that *butleri* and *radix* were closely related.

Subjects.

The parent was caught in southern Michigan on May 16, 1965. She was fed minnows and nightcrawlers. She gave birth to 15 young on July 26. One of the young was born not only very small but also quite weak. All 15 were tested on the fourth and fifth days after birth.

Method.

These snakes were born on the same date as the second litter of *Thamnophis s. semifasciatus* and were tested simultaneously with them and with the same extracts. Since there were the same number tested from each litter, the ordering of the extracts for different individuals was the same for both species.

Results and Discussion.

The results are shown in Figure 8. The same general picture of effective extracts is seen as in the prior garter snakes. According to CARPENTER (1952) this species eats virtually nothing but worms and leeches in nature, but will take fish and amphibians in captivity. The naive young responded to the fish and amphibians as well as earthworms and leeches. Indeed, the scores for the worms as a group were not consistently higher than the fish as a group, although twice as many attacks were made. This implies that the feeding habits of this species in nature do not reflect the range of classes of prey animals to which the naive young will respond. Since these other classes of animals are found in the snake's habitat it is possible that factors other than preference are involved. The species' stocky build, for instance may render it incapable of catching quick moving prey, or at least less efficient than competitors.

F. AQUATIC GARTER SNAKE

This snake, *Thamnophis elegans aquaticus*, is a member of the taxonomically confused West Coast *elegans*-group of garter snakes. It was first

described by Fox in 1951. Little is known about its habitat except that it is at least semiaquatic. STEBBINS (1966) has revised this group of snakes and calls this form *Thamnophis couchi aquaticus*.

Subjects.

The mother was caught on June 14, 1965, in Sonoma County, California. She readily ate minnows but refused worms, although after repeatedly offering her nightcrawlers she eventually began to take them. Nine young were born on October 10, 1965. They were tested on the third and fourth day after birth. These young were unusual in that they would sometimes strike out defensively at strange objects, even immediately after birth. This behavior involved flattening the neck and body, coiling, and striking out vigorously, but somewhat undirected, in the general direction of the offending object.

THAMNOphis BUTLERI

N=15

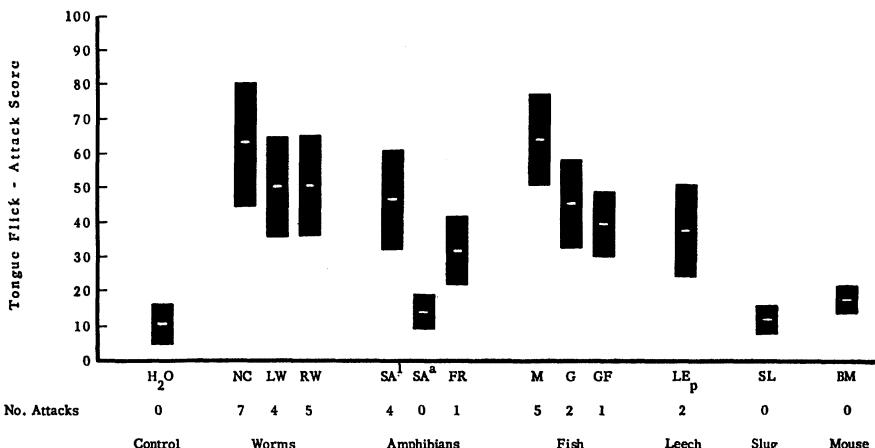


Figure 8. Extract response profile of Butler's garter snake, *T. butleri*.

Method.

The standard 12 extracts were used with the exception that the giant leech was substituted for the turtle leech. A cricket solution was also used. The standard sequence was used in rotating fashion with the cricket extract added to the end of the standard sequence. Extracts 1, 2, 3, 4, 5, 6, 8, 10, or 12 began the order for the 9 snakes. The one-control method was used. Approximately 20 minutes elapsed between tests.

Results and Discussion.

The results are shown in Figure 9. It is quite clear that this species differs substantially from the preceding *Thamnophis*. Only fish and amphibians were attacked, and all scored much higher than the control or other extracts. Contrary to the preceding forms, the worm extracts were little different from the controls. Since this species responds to fish and not to worms, it is

reasonable to assume that the effective substances responsible for the prey attack to fish are different from those involved in the prey attack to worms, even in snakes responding to both classes of prey objects.

STEBBINS (1954), lists fish, frogs, and tadpoles as being eaten by this species. The present results are in accord with this, especially if one makes the reasonable assumption that larval frogs and salamanders are similar types of prey. FOX (1952) states that fish or frogs are preferred in both the wild and captivity. He implies that salamanders (adult) are not readily eaten by this species. The present results show that while a significant increased amount of tongue flicking was shown to adult salamander extract, no attacks to it were made and the other amphibians and fish were all significantly higher than it.

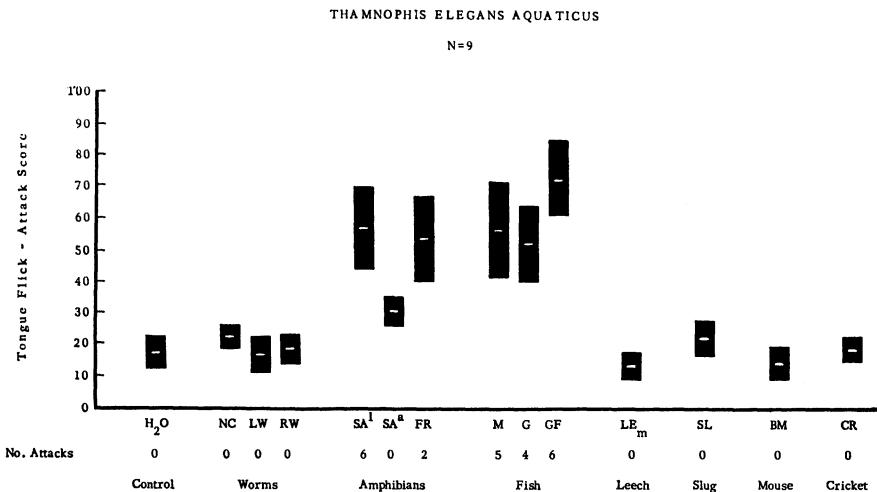


Fig. 9. Extract response profile of the aquatic garter snake, *T. elegans aquaticus*.

G. NORTHWESTERN GARTER SNAKE

This species (*Thamnophis ordinoides*) is one of the smaller garter snakes and is a North Pacific Coast species. It is usually found in openings of dense undercover near humid forests (WRIGHT & WRIGHT, 1957).

Subjects.

The parent was purchased from a dealer in California, and was said to be from Oregon. It did not eat too readily, but would take minnows and once ate a nightcrawler very hesitatingly after much testing with the tongue. On July 31, 12 young were born. They were tested on the fourth and fifth days after birth.

Method.

The snakes were born on the same date as the litter of *T. s. sirtalis* and were tested simultaneously with them and with the same extracts. The extract orders were the same as the first 12 *sirtalis*.

Results and Discussion.

The results are presented in Figure 10. Only one attack was made, and that was to the slug extract. According to FOX (1952), slugs would seem to be a preferred food in both captivity and nature. WRIGHT & WRIGHT (1957), also list frogs and salamanders as being eaten. Although some of the other extracts elicited significantly increased tongue flicking over and above the

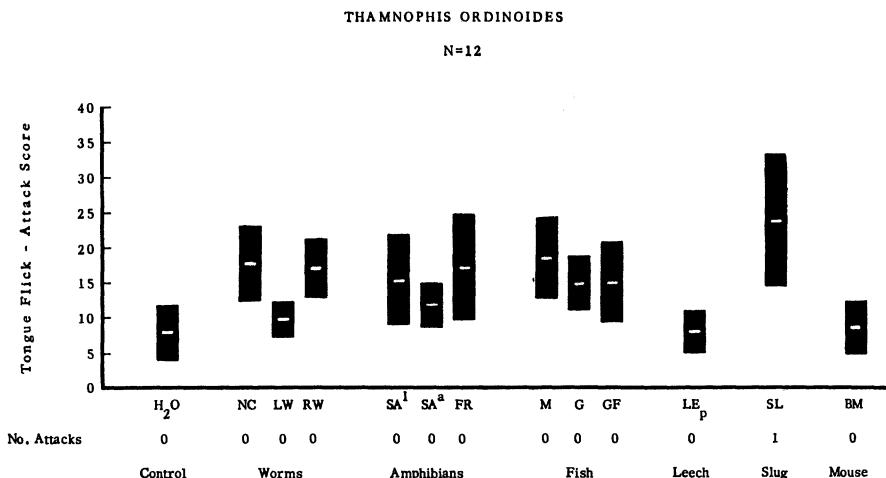


Fig. 10. Extract response profile of the northwestern garter snake, *T. ordinoides*.

controls, definite conclusions should perhaps not be drawn. Immediately after the testing was completed, the 12 snakes were offered small leeches, red-worms, guppies and slugs. Only three ate at all and these took slugs. Two of the three also ate the guppy offered but none took the leech or worm. It can be concluded that at the age tested the young were not as food motivated as the other forms of *Thamnophis* tested. Future studies with this species should be performed at an older age. In general, the behavior of this species was quite slow and deliberate and their responses to stimuli of all types were of low intensity.

H. RESPONSES TO THE WATER CONTROLS

Do the responses to the water control swabs alone tell us anything of interest about the phenomenon studied here? It is clear that a swab dipped

in distilled water never elicited a prey-attack response in the naive snakes. But tongue flicking did occur in the presence of control swabs and non-attack eliciting extracts such as baby mouse extract. Since with a number of the litters tested the control swabs were presented a total of 6 times to each subject, the question arises as to whether the mean control swab scores are misleadingly low due to selective habituation. In Figure 11 the results for each control run are shown for the 6 litters in which this method was systematically used. A definite, although by no means highly consistent decrease is apparent. The most dramatic decrease, however, occurs after the first control test. This was the first swab of any type presented to the snakes

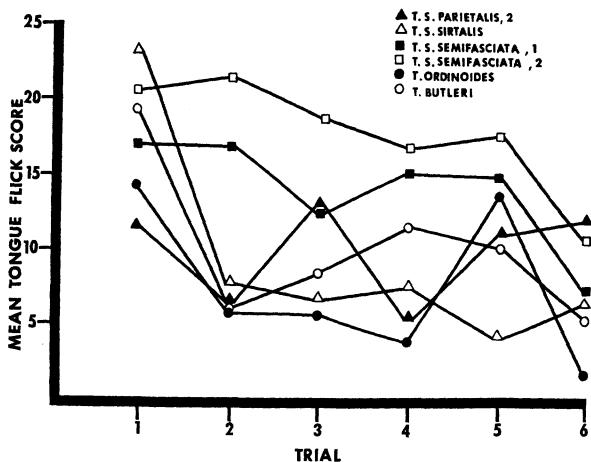


Fig. 11. Tongue flick scores to the 6 control swabs for the litters with which the 6-control method was used. Controls 1 and 4 were the first swabs to be presented on test days 1 and 2 respectively.

and an initial "novelty" effect due to the presentation of the swab and its visual properties might have been responsible for the high tongue flick scores. In subsequent testing where only one control swab was presented, all the extracts had an equal chance of starting the test series. This had the effect of nullifying any biasing of the results in favor of extracts appearing first. With the 6-control method, however, the higher rate of flicking on the first test serves to help balance out the habituation effect with subsequent tests. It should be noted that the control scores with litters tested on the one-control method were similar to the mean values arrived at using the 6-control method.

It will be recalled that control trial 4 was the first test on the second day. With every litter this control trial led to a lower rate of flicking than on the

TABLE 4

Mean tongue flick scores to successive water control swabs in snakes which attacked at least one extract vs snakes which attacked no extracts

Litter	<i>T. s. sirtalis</i>		<i>T. s. semifasciata</i>		<i>T. s. semifasciata</i>	
	Attackers	non- attackers	Attackers	non- attackers	Attackers	non- attackers
Number	11	8	14	6	14	1
Control						
1	22.6	24.8	19.1	14.2	20.7	17
2	12.4	3.5	15.4	20.3	21.6	10
3	9.9	2.9	13.8	8.0	18.3	16
4	12.4	3.2	15.7	13.0	16.6	12
5	7.2	1.4	14.1	15.3	17.1	1
6	11.8	1.4	9.9	1.8	12.5	2
Litter	<i>T. s. parietalis</i>		<i>T. butleri</i>		<i>T. ordinoides</i>	
	Litter 2					
	Attackers	non- attackers	Attackers	non- attackers	Attackers	non- attackers
Number	8	4	12	3	1	11
Control						
1	13.0	9.0	18.2	11.7	5	15.4
2	7.8	6.8	8.0	4.0	18	6.2
3	15.8	9.8	9.6	3.3	10	4.9
4	4.8	7.2	13.9	5.3	6	4.1
5	14.5	6.2	12.0	8.0	10	14.4
6	15.4	5.5	5.8	9.3	0	2.4

first day. Hence, complete spontaneous recovery did not occur. Since half of the litters showed a decrease from the last test given the first day (control trial 3) evidence for even a partial recovery of control swab responsivity is lacking.

Another point of interest in Figure 11 is the similarity of control swab scores shown by the two litter of the same subspecies, *Thamnophis s. semifasciata*.

If tongue flicking is related to the search for food, it might be expected that snakes "interested" in food would explore introduced objects more than less food motivated individuals, even in the absence of chemical stimuli from potential prey. By considering snakes which attacked at least one extract as hungrier than those which did not attack a single extracts it is possible to compare the tongue flick responses of each group to successive control swabs. This information is shown in Table 4. The attacking snakes gave a larger mean number of tongue flicks to the first control swab (before any other swabs were presented) in 4 of the 6 litters. This difference became

even more pronounced as successive controls were tested, indicating that habituation of tongue flicking to water control swabs proceeds faster in non-hungry snakes. However, after the first control swab other stimuli were presented. If we just consider snakes which gave at least one prey-attack, and look at the number of tongue-flicks given to a control swab in relation to the effectiveness of the preceding extract, a clear relationship is seen (Table 5). The first control scores on each of the two test days are omitted,

TABLE 5

Relation of control scores to effectiveness of preceding extract in snakes attacking extracts at least once.

Litter	Mean control scores following an unattacked extract	Mean control scores following at least 1 attacked extract	Mean control scores following 2 attacked extracts
<i>T. s. sirtalis</i>	8.6	16.4 **	25.5 ***
<i>T. s. semifasciata</i> , 1	11.7	16.5 *	19.5
<i>T. s. semifasciata</i> , 2	11.0	27.0 ***	33.2 **
<i>T. s. parietalis</i> , 1	4.0	26.4 ***	27.2 **
<i>T. s. parietalis</i> , 2	11.3	17.2	29.7 **
<i>T. r. radix</i>	4.6	25.5 **	—
<i>T. butleri</i>	8.3	11.7	—
<i>T. elegans aquaticus</i>	16.7	21.5	—

* $p < .1$ Mann-Whitney test, one-tailed test of significance vs

** $p < .01$ controls following unattacked extracts

*** $p < .001$

(more in the case of *Thamnophis s. parietalis*, litter 1). In every instance, the mean control score following an attack eliciting extract was higher than the mean score following an ineffective extract. If the preceding two extracts elicited attacks an even higher rate of tongue flicking to the water swab occurred. Sometimes, however, the difference was not significant as so few scores were involved (2 or 3 in several cases).

This increase in flicking to control swabs after effective extract swabs would serve to counter any habituation to the control swabs. Therefore, it can not be concluded that in the absence of effective extracts habituation to controls in hungry snakes is slower than in less motivated snakes. Does the facilitation to controls following attacks represent a conditioning phenomenon or does the snake just become more sensitive to environmental stimuli after an attack which leads to no food reward? Further experiments are needed to determine which is operating here.

IV. GENERAL DISCUSSION

A. INTER AND INTRA SPECIFIC COMPARISONS

Although the different species could be compared in great detail, only a brief discussion of the major differences and similarities will be presented here.

Nine litters of snakes from the genus *Thamnophis* were tested. They represent 5 species. The majority of snakes were in the subspecies of *Thamnophis sirtalis* tested. All 5 litters tested responded to the same classes of prey objects: worms, amphibians, fish, and leeches. One *Thamnophis sirtalis parietalis* attacked a slug extract. This exception has already been discussed. The four classes of prey objects are in this species' diet in nature.

Thamnophis r. radix responded to the same classes of prey objects as did *sirtalis*, although no actual attacks were made to the amphibian extracts. In the field, *radix* has habitat and feeding preferences similar to *sirtalis*.

Thamnophis butleri also responded to the same four classes of prey, even though in the field it seems never to eat fish or amphibians (CARPENTER, 1952). In captivity the species readily eats fish and amphibians. A possible explanation of this phenomenon is presented below.

A California species, *Thamnophis elegans aquaticus*, known for its aquatic habits responded only to fish and amphibian extracts. Surprisingly, it did not respond to the aquatic giant leech. However, this species is known to eat mainly fish and amphibians (STEBBINS, 1954).

Thamnophis ordinoides, although apparently not very hungry when tested, attacked the slug extract. Slugs probably constitute a major portion of the diet of this species in nature.

In Table 6 the mean latencies of the attacks given to the various extracts by the litters are presented. Tables 7 and 8 give all the possible intercorrelations between the various litters for all the extracts common to any particular pair (11-14). Table 7 gives the computed Spearman rank correlations (r_s) and Table 8 gives the computed Pearson correlations. The latter makes use of the magnitude of the differences between various extracts as well as their rank ordering. Both tables are reproduced as some large discrepancies occur. However, the general picture shown by the tables is clear and quite consistent with the more gross comparisons made earlier.

A few of the results will be pointed out here. The 2 litters of *Thamnophis s. semifasciata* are highly correlated by both measures. The 2 litters from the same locality in Indiana, referred to here as *T. s. sirtalis* and *T. s. parietalis*, litter 2 are correlated to a rather lower degree (.57 and .67) than each is with a number of other litters, including *T. butleri* and *T. radix*. On the

TABLE 6
Attack latency data 1

Species	NC	LW	RW	SA ¹	SA ^a	FR	M	G	GF	LE _p	SL
<i>T. s. sirtalis</i>	27.1	23.6	38.2	25.1	6.3	28.8	52.5	25.9	14.2	34.8	(—)
<i>T. s. semifasciata</i> (litter 1)	14.4	18.9	24.5	28.2	(—)	18.9	36.0	34.1	30.0	27.8	
<i>T. s. semifasciata</i> (litter 2)	19.7	23.1	25.6	25.5	43.7	10.0	32.0	24.4	41.2	26.2	(—)
<i>T. s. parietalis</i> (litter 1)	24.8	8.6	14.4	26.7	37.9	16.2	50.1	16.5	(—)	14.6	
<i>T. s. parietalis</i> (litter 2)	20.4	31.8	27.6	30.9	(—)	57.5	(—)	(—)	43.6	16.2	8.2
<i>T. r. radix</i>	38.4	23.0	22.8	(—)	(—)	(—)	36.8	32.7	35.6	25.1	(—)
<i>T. butleri</i>	29.6	29.5	37.8	33.6	(—)	36.6	34.0	25.2	53.9	19.5	(—)
<i>T. elegans aquaticus</i>	(—)	(—)	(—)	41.3	(—)	10.3	30.8	37.6	26.7	(—)	
<i>T. ordinoides</i>	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	(—)	19.0

1. See appropriate graphs for number of attacks involved. Only extracts eliciting attacks are listed.
 A (—) signifies no attacks given to that extract by that litter. A blank means that the extract was not presented to that litter.
 Unit of measure — seconds.

TABLE 7

Spearman rank correlations of the extract response profile in the 9 litters of newborn snakes

	<i>T. s. semifasciata, 2</i>	<i>T. s. sirtalis</i>	<i>T. s. parietalis, 1</i>	<i>T. s. parietalis, 2</i>	<i>T. r. radix</i>	<i>T. r. butleri</i>	<i>T. elegans</i>	<i>T. aquaticus ordinoides</i>
<i>T. s. semifasciata, 1</i>	.85 **	.52 *	.73 **	.85 **	.90 **	.63 *	-.04	.40
<i>T. s. semifasciata, 2</i>		.85 **	.90 **	.85 **	.83 **	.80 **	-.22	.25
<i>T. s. sirtalis</i>			.19	.67 *	.61 *	.52 *	.62 *	.36
<i>T. s. parietalis, 1</i>				.60 *	.72 **	.64 *	-.12	.41
<i>T. s. parietalis, 2</i>					.85 **	.84 **	.15	.35
<i>T. r. radix</i>						.71 **	.26	.23
<i>T. r. butleri</i>							.25	.41
<i>T. e. aquaticus</i>								.45

* $p < .05$ one-tailed test.

** $p < .01$

Standard 12 extracts plus H₂O for all litters except those involving *T. s. semifasciata*, 1 (no SL); *T. s. parietalis*, 1 (no SL); *T. r. radix* (added LE_m and CR); *T. elegans aquaticus* (no LE_p but tested on LE_m and CR).

TABLE 8

Pearson rank correlations of the extract response profile in the 9 litters of newborn snakes

	<i>T. s. semifasciata, 2</i>	<i>T. s. sirtalis</i>	<i>T. s. parietalis, 1</i>	<i>T. s. parietalis, 2</i>	<i>T. r. radix</i>	<i>T. r. butleri</i>	<i>T. elegans</i>	<i>T. aquaticus ordinoides</i>
<i>T. s. semifasciata, 1</i>	.83 **	.66 **	.68 **	.91 **	.87 **	.72 **	-.09	.40
<i>T. s. semifasciata, 2</i>		.62 *	.84 **	.88 **	.73 **	.85 **	.12 **	-.24
<i>T. s. sirtalis</i>			.40	.57 *	.68 **	.70 **	.68 **	-.08
<i>T. s. parietalis, 1</i>				.66 **	.64 *	.64 *	-.06	-.09
<i>T. s. parietalis, 2</i>					.76 **	.75 **	-.12	-.11
<i>T. r. radix</i>						.70 **	.22	-.20
<i>T. r. butleri</i>							.32	-.04
<i>T. e. aquaticus</i>								.40

* $p < .05$ one-tailed test

** $p < .01$

other hand the *T. s. parietalis* litter from Indiana is not any more highly correlated with the *T. s. parietalis* from Illinois (litter 1). It is clear that intraspecific differences may occur in chemical cue preferences just as Dix (1968) has recently shown geographical food preference differences in newborn *Thamnophis sirtalis*. All the litters except *T. elegans aquaticus* and *T. ordinoides* responded to similar classes of prey extracts and large differences are not to be expected until a more extensive series of extracts are tested under more standardized procedures. However, all these litters do show a markedly lower similarity with *T. elegans aquaticus* and *T. ordinoides*, many of the correlations even being negative. Some of the correlations are not strictly comparable because of the different extracts involved. For instance, the correlation between *T. s. semifasciata*, litter 1, and *T. ordinoides* is higher than might be expected because the slug extract, which was most effective with *ordinoides*, was not presented to *T. s. semifasciata*, litter 1.

B. THE EVOLUTION OF RELEASING MECHANISMS

These comparative findings can best be discussed by invoking evolutionary principles. That is, by the process of natural selection, each species of newborn snake has come to recognize, by chemical cues, the type of prey that is proper for it to eat. The fact that exceptions to this can occur can be treated the same as vestigial structures. These may support the notion that the perceptual responses studied here have not only evolved, but have taxonomic importance for the study of snake relationships.

The prey-attack response of snakes may be suitable material for studies on the evolution of releasing mechanisms for several advantages present themselves. The naive young can be tested shortly after birth in a situation where all but the critical sensory cues (chemical) are either eliminated or easily controlled for. Since closely related species (and subspecies) of snakes show both similarities and differences in their feeding habits and chemical preferences, it is probable that the releasing mechanisms involved have evolved at neither too conservative nor too rapid a rate to be of taxonomic value.

Although the comparative studies did not involve enough species to make definite taxonomic statements, enough was found out to be able to indicate the lines an evolutionary analysis would take. For instance, in the genus *Thamnophis*, *sirtalis* is known to eat worms, leeches, fish, and amphibians in nature, *butleri* eats worms and leeches in the field, and *elegans aquaticus* eats only fish and amphibians. From this information alone no conclusions

can be reached, except possibly that *butleri* and *elegans aquaticus* are both more similar to *sirtalis* than to each other. However, newborn *butleri* will respond to chemical cues from the same four prey classes that *sirtalis* will respond to, whereas newborn *elegans aquaticus* respond only to chemical extracts from fish and amphibians. This leads to the conclusion that *butleri* is much more closely related to an ancestral form which, like *sirtalis*, ate worms, fish, and amphibians, than is *elegans aquaticus*. In other words, *butleri* retains the perceptual side of a releasing mechanism that appears to be of no selective advantage in its present mode of life. In fact, SCHMIDT (1938) concluded that *butleri* is derived from *radix*, which like *sirtalis* responds to all four classes of prey. In following this approach, one must always be sure to have data from naive newborn young. Feeding experiences may subsequently alter the responses given by naive snakes to extracts. Data not reported here suggests that this can indeed occur (FUCHS & BURGHARDT, in preparation). Feeding habits in nature and in captivity are good sources for supplementary information but can not replace testing naive young.

The possibility of convergent as well as homologous releasing mechanisms must be considered when trying to determine evolutionary relationships. For this reason, anatomical and physiological evidence must also be used. The chemical bioassay of the extracts themselves may be of value. Perhaps attacks to the same extract by widely divergent species are mediated by different chemical substances.

Variability within litters should be investigated also. There undoubtedly are motivational differences among young born at the same time. Usually some individuals would not respond to any extracts (or live food) at the time of testing, but would respond and eat at a later date. Holding age constant, therefore, is not the best way to remove variability due to motivational differences. The occurrence of perceptual preference "polymorphism" within litters is also a possibility.

One point should be discussed. By considering the chemical cue recognition of snakes as evolved adaptations, nothing is implied about the mechanisms by which the newborn snake acquires this ability except that inheritance is important somewhere in the system. My use of the term "innate" is meant to cover those aspects of the snake's behavior and perceptual selectivity present at birth before the snake has ever engaged in feeding behavior. Subsequent experiences with prey and feeding will interact dynamically with those factors present at birth to determine the behavior at a later time in a constantly interacting manner.

An another level, however, we can inquire into the source of the information which makes species A respond to different extracts than species B.

This is rather specific information of the type discussed by LORENZ (1965). The only two ultimate sources of this information that appear capable of structuring the Extract Response Profile into the developing embryo are influences transmitted from what the mother eats and specific genetically based information which is translated into the embryo during the course of development. While both sources of information may be involved, it is hard to see how maternal feeding could be the exclusive source of information. First of all, the young responded to extracts not fed the mother in captivity such as frogs or leeches, often even at a higher level than extracts fed the parent. In addition, the young respond to extracts from animals it is hardly possible the mother ever encountered in the field. Moreover, since naive snakes from an oviparous species (*Ophiodrys vernalis blanchardi*, the western smooth green snake) also show an Extract Profile (BURGHARDT, 1967), it is clear that maternal feeding during embryonic development is not crucial — not to mention the fact that adults often feed on different prey than newborn young (FITCH, 1965). Whether the feeding history of the mother is a factor can, of course, be experimentally determined and such investigations are planned.

C. FEEDING BEHAVIOR IN THE NEWBORN SNAKE

The newly born snake is alone. Although often entering the world in the presence of its mother, there is no evidence that she cares for or guides the young in any way. In addition, the young themselves scatter and adaptive social interactions between them have not been demonstrated. Nonetheless, the young snake must seek shelter, find food, and protect itself from predators.

The behavior pattern most carefully studied has been feeding. The most important sense organs involved are eye, nose, and Jacobson's organ. In vipers, the relative roles of these senses has been well worked out as far as the adults are concerned (NAULLEAU, 1966). Visual stimuli release the killing strike but chemical stimuli are most important for the instigation of actual ingestion. In garter snakes, which are nonpoisonous, feeding behavior is less complex and the visual sense is less important. Visual stimuli, especially movement, are involved in attracting the attention of a newborn garter snake toward small objects (BURGHARDT, 1966 b) but unlike animals such as common toads (*Bufo*) or snapping turtles, visual stimuli alone are insufficient to elicit the ingestive feeding response. Chemical cues from the prey object must be received by Jacobson's organ,

normally via the tongue, for the prey-attack response to occur (WILDE, 1938; BURGHARDT & HESS, 1968) although in adult garter snakes there is some evidence that olfaction can take over the role of a destroyed Jacobson's organ (NOBLE & CLAUSEN, 1936). The importance of olfactory stimuli received through the external nares would seem to be the conveying of information from a distance. Air passed over prey and introduced into snake cages resulted in increased locomotion and "searching" (Fox, 1952).

A typical sequence of events in the normal hungry snake might be as follows. Since garter snakes live near the ground in vegetated areas, the first indication it receives of a potential prey animal in the vicinity will be through chemical stimuli sensed via olfaction. This results in increased locomotion and tongue flicking in the general direction of the source. At this time the threshold of responsiveness to movements of small objects may be lowered. As the snake comes nearer to the prey, the latter may appear in its visual field and attract the snake by its movement. However, no prey attack response will occur unless the chemical cue is recognized as a proper one by Jacobson's organ. This necessitates tongue flicking in the immediate vicinity of the prey. The tongue tips must actually touch the surface of the animal (SHEFFIELD, LAW & BURGHARDT, 1968). If the prey is attacked and inside the mouth purely local senses such as gustation and touch may determine whether the organism is actually swallowed. This may be of most importance in the laboratory situation where an object normally not eaten but coated with an extract from a normally eaten organism is attacked but often not swallowed. Fox (1952) and I have noted the subsequent regurgitation of animals which the snake has been decoyed into eating with chemical stimuli.

If, however, the object which originally attracted the snake's attention visually is not deemed appropriate by Jacobson's organ, the interest in it rapidly wanes and he searches elsewhere. It may also happen that while approaching the area from where the olfactory stimuli are emanating, the prey has moved on. When the snake comes to the area he most likely will find and use the trail scent left by the recently departed animal. Experiments by a number of investigators have shown that snakes readily use trails in preference to airborne cues and are quite accurate in following them (BAUMANN, 1929; NAULLEAU, 1966; NOBLE & CLAUSEN, 1936). Disagreement exists over the relative roles of olfaction and Jacobson's organ, but the latter is more likely to be important since trails are made by body contact with the substrate similar to that which will induce an anosmic snake to recognize and attack a cotton swab (BURGHARDT & HESS, 1968).

We are faced, then, with a series of responses in which the different

receptors have varying functions and importance depending on the phase involved. Of course, as already implied, the sequence is not a rigid one and may be started at any point. For voluntary feeding, vision and olfaction are not needed in the newborn garter snake, but in free ranging conditions the snake would probably not survive without them.

The above should be applied to other than garter snakes with caution, and even with some caution to them. In addition the distinction between naive young and the experienced adult should be realized. It is hard to believe that a snake living on quickly moving prey such as frogs must always get chemical feedback via the tongue from the immediate vicinity of the prey before attempting to capture it in its mouth. Perhaps, appropriate chemical stimuli in the general area are eventually sufficient to elicit an attack at a visually perceived object. Or the association of food reinforcement with visual characteristics of the prey such as color, form, size, and type of movement pattern may eventually result in the visual cue alone having attack releasing ability. Certainly, however, the probability of specific innate visual recognition of prey is slight (BURGHARDT, 1966 b). A third possibility is that the snake might learn that in specific localities all objects with certain gross visual characteristics are edible. Other possibilities might also exist and the interaction of two or more may occur. We need more observations of hunting and feeding behavior of snakes under naturalistic or at least semi-free ranging conditions, even though the secretive nature of serpents makes this difficult.

The experiments presented here have dealt in a comparative manner with the chemical recognition of food items in newborn garter snakes. Their ability to recognize those animals which both young and adult snakes feed upon in the wild is remarkable. It has been noted that the feeding habits of snakes in the field change with age and also with season of the year and locality (FITCH, 1965). Availability probably accounts for most of the differences between time of year and locality, although innate intraspecific differences have been found (DIX, 1968). The size correlated differences seem most likely due to niche splitting between adult and young and size limitations upon what can be swallowed. Nightcrawler extract was very effective, yet no newborn garter snake could swallow nightcrawlers from which the extracts were made. On the other hand, efficiency considerations could lead to the abandonment of very small food items in the diet of adults and their concentration upon larger species never eaten by young. Hence, the postulation of a maturational change in feeding preferences is unnecessary. Indeed, captive adults would eat all food items responded to by the inexperienced young and they, in turn, seem to respond to chemical stimuli from most, if not all, organisms eaten by adults.

SUMMARY

Garter snakes (*Thamnophis*) from five species (including three subspecies of one form) were tested several days after birth with water extracts of at least 12 small animals (1.5 g animal to 10 ccm warm distilled water). The 12 prey animals included three species of earthworms and three of fish, a salamander and its larva, a frog, a leech, a slug and a baby mouse. A distilled water swab elicited tongue flicking only, while certain extract swabs resulted in actual prey-attack behavior after at least one tongue flick. A score was given to each extract test using a simple formula based upon tongue flick frequency and attack latency.

Differences and similarities between the species were found and are discussed in relation to the actual feeding preferences in nature and captivity. For example, the aquatic *Thamnophis elegans aquaticus* attacked only the extracts made from the salamander larva, the frog, and the three fish; *Thamnophis sirtalis* also attacked the leech and the three earthworm extracts. It is suggested that the perceptual selectivity shown by naive snakes is an evolutionary response to present and past ecological conditions. The ability of newborn snakes to rapidly acquire a food avoidance response was also demonstrated.

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ZUSAMMENFASSUNG

Strumpfbandnattern (*Thamnophis*) von fünf Arten, deren einer in drei Unterarten, bot man wenige Tage nach ihrer Geburt, nachweislich vor jeder Nahrungsaufnahme, auf Wattebüschchen Extrakte (1.5 g Substanz auf 10 ccm warmdestilliertes Wasser) von mindestens 12 Beutetieren, nämlich von je drei Arten Regenwürmern und Fischen, einem Molch und seiner Larve, einem Frosch, einem Egel, einer Wegschnecke und einer Jungmaus. An extraktfreien Wattebüschchen züngelten die Jungschlangen nur,

nach den Extraktbüschchen schnappten sie auch, nach mindestens einmaligem Zungeln. Aus der gesamten Züngelzahl und den Schnappern wurden nach einer einfachen Formel Werte gemittelt, die sich für dasselbe Extrakt je nach der Schlangenart bzw. Unterart unterscheiden; z.B. schnappte die im Wasser lebende *Thamnophis elegans aquaticus* nur nach den Extrakten aus der Molchlarve, dem Frosch und den drei Fischen; *Thamnophis sirtalis* schnappte auch nach Extrakten aus dem Egel und den drei Regenwürmern. Es wurden Unterschiede und Übereinstimmungen zwischen den Arten gefunden, die im Zusammenhang zu der tatsächlichen Futterwahl unter natürlichen- und Gefangenschaftsbedingungen besprochen wurden. Die von naiven Schlangen gezeigte Wahrnehmungsselektivität wurde als eine im Laufe der Evolution entstandene Anpassung an vergangene sowie gegenwärtige ökologische Bedingungen gedeutet. Doch können auch schon junge Schlangen rasch eine Abneigung gegen bestimmte Nahrung erwerben.
