Evolutionary Persistence of Chemically Elicited Ophiophagous Antipredator Responses in Gartersnakes (*Thamnophis sirtalis*)

John S. Placyk, Jr.  
University of Texas at Tyler

Gordon M. Burghardt  
University of Tennessee

The ability to detect and respond to potential predators is key for the survival of individuals, but this ability is sometimes lost via relaxation of antipredator behavior when prey species are separated from predators. Adult and predator-naïve neonate gartersnakes (*Thamnophis sirtalis*) from mainland and insular sites where they do and do not occur with ophiophagous (snake-eating) snakes were tested to determine if responses to such predators have been lost, reduced, or retained, and what might be causing differences in such responses. Our data indicate that, overall, adult snakes from populations syntopic with ophiophagous milksnakes are more responsive to chemical stimuli from milksnakes than adults from areas where they are not syntopic with milksnakes, whereas there were few differences with neonate gartersnakes. Experiments with neonates with or without periodic ophiophagous snake chemical experience over several weeks showed that gartersnakes with such experience became more responsive to ophiophagous cues rather than habituating to them. Such evidence of both genetic and experiential factors underlying the antipredator responses to ophiophagous snakes indicates that antipredator responses have persisted despite separation of predator and prey.

Keywords: antipredator behavior, evolutionary persistence, ophiophagy, relaxed selection, gartersnakes

The inability to effectively detect and respond to predators may have significant consequences on evolutionary fitness. However, despite the strong selection pressures exerted on potential prey by their predators, antipredator behaviors may be lost when the two are separated and natural selection relaxed (Beauchamp, 2004; Blumstein & Daniel, 2005; Coss, 1999; Lahti et al., 2009; Orrock, 2010; Stankowich & Coss, 2007). This may result from the cost of maintaining unutilized behaviors (Blumstein, 2006; Magurran, 1999). While the loss of such behaviors due to the relaxation of selection pressures has been well-documented, the mechanism(s) underlying the maintenance and/or loss of these behaviors are less well known (i.e., evolutionary change vs. phenotypic plasticity vs. some combination of the two). Here, we present results of laboratory experiments using the common gartersnake (*Thamnophis sirtalis*) to (a) detect the retention and/or loss of antipredator behaviors and (b) begin to understand the mechanism(s) underlying these phenomena through experimentation on the role of phenotypic plasticity.

Snakes, in general, are excellent candidates for studying the role of genetics and environment on behavior, as they have highly precocial young that can be tested when naïve to external stimuli that elicit behavioral responses (e.g., Burghardt, 1993). In addition, many snakes have large litter/clutch sizes, which provide an opportunity to split litters/clutches among environmental treatments to study genotype-by-environment interactions and common family environment (including maternal) effects (e.g., Badyaev et al., 2002; Brodie & Garland, 1993; Burghardt & Schwartz, 1999; King, 2002; King et al., 2001). Gartersnakes (*Thamnophis* spp.) are especially fruitful model species for studying diverse areas of biology (Rossman et al., 1996), making *Thamnophis sirtalis* an ideal species for studying these phenomena through experimentation on the role of phenotypic plasticity.

*Thamnophis sirtalis* is the most wide-ranging snake in North America (Rossman et al., 1996) being found in almost all states in the United States and provinces in Canada and is considered the most common snake species in the Great Lakes region (Harding, 1996). The Beaver Archipelago is located in northeastern Lake Michigan and is ca. 25–30 km from both the lower and upper peninsulas of Michigan (see Figure 1). It is the largest group of islands in Lake Michigan, consisting of one main island (Beaver, 15130.0 hectares [ha]), three moderately large islands (Garden, High, and Hog, 1023.9 to 1989.0 ha), and six smaller islands (Hat, Pismire, Shoep, Squaw, Trout, and Whiskey, 1.0 to 52.3 ha). This archipelago is ideal for studies on geographic variation in pheno-
typic traits, as gartersnake prey and predator abundance and diversity vary from island to island and between the islands and the surrounding mainland roughly following island biogeography theory (Bowen & Gillingham, 2004; Hecnar, Casper, Russell, Hecnar, & Robinson, 2002). In addition, the archipelago has a well-documented geological history (Hough, 1958) and both the lower and upper peninsula of Michigan and all but the smallest islands (i.e., Hat, Pismire, and Shoe) support *T. sirtalis* populations (Hatt, Tyne, Stuart, Pope, & Grobman, 1948; Placyk & Gillingham, 2002). The origin and underlying genetics of the island populations were recently revealed through mtDNA studies (Placyk et al., 2007). We also know that the mainland populations from which island snakes are thought to have originated vary in antipredator behavior (Burghardt & Schwartz, 1999) and that gartersnake predators and prey vary from island to island as well as between the mainland populations (Placyk & Burghardt, 2005). Experience with predators also varies across populations, as shown by variation in the frequency of failed predatory attacks as measured by wounds and scars (Placyk & Burghardt, 2005).

For this study, we were specifically interested in the response of gartersnakes to ophiophagous snake predators. Antipredator responses to ophiophagous snakes are common among snakes that may become prey (e.g., Gutzke, Tucker, & Mason, 1993; Weldon, 1982; Weldon & Burghardt, 1979), and such responses have been found to result from both innate factors and postnatal experience (e.g., Weldon, 1982; Weldon & Burghardt, 1979). However, while responses to ophiophagous snakes appear to be maintained at birth in populations of snakes that no longer occur with such predators, there is some confusion as to how such responses may change over time (e.g., Gutzke et al., 1993; Weldon, 1982; Weldon & Burghardt, 1979). Some studies indicate that the behavior is maintained throughout the life of potential snake prey and change very little, while other studies indicate that regular exposure to ophiophagous snakes is required to maintain such responses (e.g., Gutzke et al., 1993; Weldon, 1982; Weldon & Burghardt, 1979).

We examined responses to ophiophagous snakes by populations of gartersnakes that occur with them (e.g., lower peninsula [LP] of Michigan), as well as populations that do not occur with them (e.g., upper peninsula [UP] of Michigan). In addition, mtDNA sequence data (Placyk et al., 2007) indicate that the island populations examined in our study are derived evolutionarily from two source populations, namely, the LP and UP of Michigan, and this may allow us to better tease apart the mechanism(s) underlying responses to ophiophagous snakes. To test various hypotheses on the evolutionary persistence of antiphage responses, we developed an antiphage antipredator test to survey adults and neonates from mainland and island sites. This allowed us to determine (a) if adults from the different populations vary in their responses to ophiophagous snakes and, if so, (b) would neonates also display population level differences in behavior at birth? A positive answer to the latter would indicate a genetic component to population differences and the operation of relaxed selection. This initial set of tests was then followed up by examining the role of phenotypic plasticity on antipredator responses to ophiophagous snakes by neonatal snakes. This combination of tests allowed us to better understand the role of genetics (evolutionary change) and environment (phenotypic plasticity) in producing population-level differences in gartersnake antipredator behavior.

**General Method**

Gartersnakes were collected from field sites in both the LP and UP of Michigan and from the three largest islands located in the Beaver Archipelago (i.e., Beaver Island (BI), High Island (HI), and Garden Island (GI)). Given the size of Beaver Island, it was possible to sample two sites nearly 1 km apart (i.e., Miller’s Marsh.
Following capture, snakes were placed, by site, into separate 61 × 32 × 33 cm aquaria to reduce breeding and disease transmission between different populations. Aquaria were kept between 20 and 25 °C on a 16:8 Light:Dark cycle, to simulate northern Michigan summer conditions, with fresh water and shelter available at all times. Snakes were permitted to acclimate for 48 h, after which behavioral testing was conducted. Since T. sirtalis is diurnal, all behavioral tests were conducted during photophase. Following behavioral testing, nonpregnant females and males were scale-clipped for future identification (Brown & Parker, 1976), had tissue samples taken for genetic analyses (e.g., Placyk et al., 2007), and were released at their respective capture locations. Some pregnant females (determined via palpating for developing embryos) were held until parturition after which time they were released at their respective capture locations. Neonates were kept for additional testing and were maintained in 15 × 30 × 9 cm cages with shelter, offered water ad libitum, and fed chopped worms (Lumbricus spp.) three times per week. Following neonate behavioral testing, animals were released at the site of their mother’s capture, maintained for other experiments, or transferred to other research facilities. Prior to release, adult snakes held more than 72 h and neonates were submitted to a health screening carried out by veterinarians from the University of Tennessee, Knoxville College of Veterinary Medicine to ensure that they were disease free and in a condition suitable for release.

Adult snakes were typically tested at the Central Michigan University Biological Station on Beaver Island if they were caught in the LP or on any of the islands or at Northern Michigan University if they were caught in the UP of Michigan. All neonate testing took place at the University of Tennessee. Only snakes that appeared outwardly healthy and, for neonates, were eating on a regular basis were tested.

**Experiment I: Antipredator Responses to Ophiophagous Snakes**

To determine if adult and neonate snakes from our populations (a) responded to ophiophagous snakes and (b) expressed geographic variation in ophiophagous snake responses, behavior was observed under three conditions: (1) in a sanitized test arena (washed with distilled water and clinical detergent powder (Alconox) before each trial); (2) in the presence of ophiophage chemical cues; and (3) in the presence of nonophiophage chemical cues. Milksnakes (Lampropeltis triangulum) were used to provide ophiophagous snake chemical cues, as they are known to consume gartersnakes (Blanchard, 1921; Greene, Zimmerer, Palmer, & Bernard, 2010; Groves & Sach, 1973; McCauley, 1945; Williams, 1988), they elicit ophiophage antipredator responses from common gartersnakes (T. sirtalis) (Weldon & Burghardt, 1979; Weldon, 1982), and they occur over most of the Great Lakes Region (Harding, 1996). Thus, adult gartersnakes from areas where they are sympatric with milksnakes may have experience with them. Northern ringneck snakes (Diadophis punctatus edwardsii), sympatric with all populations of gartersnakes in this study, provided nonophiophagous snake chemical cues. Indeed, all three species are largely sympatric in that they are often found in the same fields under comparable cover objects. For example, although ringneck snakes throughout most of the Great Lakes region prey almost exclusively on salamanders, thus differing from common garter-snakes (Harding, 1996), they are frequently found sharing cover objects with them (Burghardt, personal observation, 1967-present; Placyk, personal observation, 2000-present), and gartersnakes apparently find them inoffensive. Milksnakes were maintained on a diet of various small snakes, including Thamnophis, and lab-bred mice, whereas the ring-necked snakes were maintained on a diet of eastern red-backed salamanders (Plethodon cinereus, Plethodontidae), their prey of choice in northern Michigan.

**Method**

A total of 274 adult gartersnakes were captured and tested. These consisted of 16 from LP, 3 from UP, 97 from MM, 40 from SM, 66 from GI, and 52 from HI. A total of 117 neonate gartersnakes were born in the laboratory from field-caught females and were tested. These consisted of 37 from LP (5 litters), 21 from UP (2 litters), 36 from MM (8 litters), 7 from SM (2 litters), 12 from GI (2 litters), and 4 from HI (2 litters). Each individual snake was subjected to each treatment with the order of treatments randomized for each snake and equal intervals between each treatment.

Trials for the ophiophagous antipredator testing were conducted in 51 × 26.5 × 30.5 cm aquaria that had been sterilized or that previously housed either an ophiophagous snake or a nonophiophagous snake. Each test chamber had a corrugated cardboard cage liner substrate that had not previously been used as substrate for another test subject. The test chamber was blinded on all sides with cardboard to prevent test subjects from being exposed to unnecessary visual cues that may have confounded the results. The ophiophages and the nonophiophages were permitted 24 h to deposit chemical cues on the cardboard. Trials began with a 30 s undisturbed period followed by a 2-min period during which mobility duration was recorded using a stopwatch and number of tongue-flicks were recorded with a hand counter. The observer stood approximately 50 cm from the test chamber while recording observations and did not appear to be noticed by test subjects, although differences due to an observer effect were also controlled by monitoring snakes in a test chamber that did not contain snake chemical cues. The primary defense of gartersnakes is to attempt to flee from a potential predator, oftentimes until exhaustion (e.g., Arnold & Bennett, 1984; Herzog & Burghardt, 1986; Herzog, Bowers, & Burghardt, 1989; Herzog, Bowers, & Burghardt, 1992) before resorting to other antipredator displays. We used mobility duration to measure this early reactivity. Tongue-flicking is an appropriate measure of antipredator behavior in gartersnakes (e.g., Herzog & Burghardt, 1986), and Weldon (1982) found that Thamnophis emit significantly greater numbers of tongue-flicks in the presence of ophiophagous snake chemical cues than with nonophiophagous snake chemical cues. In addition, the reliability and repeatability of tongue-flick counts and antipredator behavior has been shown to be relatively stable over time, further supporting the utility of the measures used (e.g., Arnold & Bennett, 1984; Brodie, 1993; Burghardt, 1967; Burghardt, Layne, & Konigsberg, 2000; Herzog & Burghardt, 1988; King, 2002). Adults were tested 1 to 2 days after capture and neonates were always tested within 1–5 days following birth.
Statistical Analyses

Differences in either the number of tongue-flicks or mobility duration within a treatment (control, ringneck, milksnake) due to a site effect were assessed using one-way ANOVAs, while differences across treatments were detected using repeated-measures ANOVAs. Significant pairwise differences were detected with Tukey-Kramer multiple comparison tests. For adults, two-sample t tests and ANOVAs were used to test for effects of sex, reproductive state, or the presence of wounds from failed predatory attacks for every combination of treatment and behavior. Sex effects were examined for neonates and any litter effect was also tested and controlled for each treatment and behavior combination using a nested one-way ANOVA.

Results

When adult snakes were observed in test arenas that were sanitized (control) or that previously housed either an ophiophagous snake (milksnake) or a nonophiophagous snake (ringneck snake), both frequency of tongue-flicks ($F_{2,536} = 166.84, p < .001$; Figure 2a) and mobility duration ($F_{2,536} = 198.61, p < .001$; Figure 3a) significantly differed across the three conditions. In general, adults tongue-flicked and were more active in the presence of milksnake chemical cues than when in the presence of ringneck chemical cues ($p < .05$) or when in the control test arena ($p < .05$). Adult snakes also tongue-flicked more in the presence of ringneck chemical cues than when tested in the control treatment ($p < .05$), but this difference did not extend to duration of mobility ($p > .05$).

In addition to these general responses, site differences, both within and across treatments, in tongue-flicking (Figure 2a) and mobility duration (Figure 3a) were also detected. Specifically, adult UP animals did not differ in amount of tongue-flicking observed across the three treatments and adult LP animals did not tongue-flick more in the ringneck condition than in the control treatment ($p > .05$; Figure 2a). Adult animals from HI and LP tongue-flicked significantly more in the presence of milksnake chemical cues than adults from MM, SM, GI, or the UP ($p < .05$; Figure 2a), and HI adults tongue-flicked more than MM adults under the control treatment ($p < .05$; Figure 2a). In terms of mobility duration ($F_{10,536} = 2.50, p < .01$; Figure 3a), LP, GI, and HI adults moved more than MM adults under the milksnake treatment, SM adults moved more than MM and HI adults under the ringneck treatment, and GI and HI adults moved more than MM adults under the control treatment ($p < .05$). Within treatments, differences due to sex, reproductive state, or the presence of wounds from failed predatory attacks did not influence site effects for either adult tongue-flicking or mobility duration ($p > .05$).

Neonates were also observed to differ in their frequency of tongue-flicking ($F_{2,180} = 109.04, p < .001$; Figure 2b) and amount of mobility ($F_{2,180} = 110.35, p < .001$; Figure 3b) as a result of the test treatments. As with adults, neonates, in general, moved and tongue-flicked more in the presence of milksnake chemical cues than when observed in either a control treatment or in the presence of nonophiophagous snake chemical cues ($p < .05$) and this held for neonates from all sites except those from HI, which did not vary in mobility duration between any of the treatments ($p > .05$; Figure 3b). In addition, only GI neonates tongue-flicked more in the presence of nonophiophagous chemical cues than when tested

![Figure 2.](image-url) Mean number of tongue-flicks (±95% CI) exhibited by (a) adult ($n = 274$) and (b) neonate ($n = 117$) common gartersnakes, Thamnophis sirtalis, from the lower (LP) and upper (UP) peninsulas of Michigan, and Miller’s Marsh (MM) and the Sawmill (SM) from Beaver Island, High Island (HI), and Garden Island (GI) of the Beaver Archipelago when tested in either a sanitized test arena or in the presence of chemical cues from either ophiophagous eastern milksnakes, Lampropeltis t. triangulum, or nonophiophagous northern ring-necked snakes, Diadophis punctatus edwardsii. Different letters indicate significantly different means ($p < .05$) within each treatment within each age class. Litter means, not individual means, are shown for neonates.
under the control treatment \((p < .05)\) and only UP neonates moved more under the ringneck treatment than in the control treatment \((p < .05; \text{Figure 3b})\). Differences in tongue-flicking \(\left( F_{10,180} = 2.25, p < .05; \text{Figure 2b} \right)\) and mobility duration \(\left( F_{10,180} = 2.12, p < .05; \text{Figure 3b} \right)\) among sites within each treatment also varied significantly for neonates. Specifically, LP and GI neonates tongue-flicked more than MM neonates, LP neonates tongue-flicked more than UP neonates in the milksnake condition \((p < .05; \text{Figure 2b})\), and UP neonates tongue-flicked less than GI neonates in the ringneck condition \((p < .05; \text{Figure 2b})\). As for mobility duration, LP neonates spent more time moving than UP and HI neonates in the milksnake condition, GI neonates spent more time moving than HI and MM neonates in the ringneck condition, and UP neonates spent more time moving than GI and LP neonates in the control condition \((p < .05)\). As with the adults, sex differences for each site within treatments were not detected \((p > .05)\). When litter was nested within site, a significant litter effect was detected for neonate mobility duration \(\left( F_{15,90} = 1.91, p = .03 \right)\). No treatment by litter effect was detected \(\left( F_{30,180} = 0.76, p = .82 \right)\) and no litter effect was detected for neonate tongue-flicking \(\left( F_{15,90} = 1.00, p = .47 \right)\).

**Discussion**

Responses of snakes to the chemical cues of ophiophagous snakes (milksnakes) versus nonophiophagous snakes (ringnecks) showed the same trend for neonates and adults from most collection sites. Snakes tongue-flicked and moved more in the presence of milksnake chemical cues than in the presence of ringneck chemical cues or a sanitized control treatment. While adult snakes from the UP did not tongue-flick more in the milksnake condition than in the other two conditions, they did move more in the milksnake condition and neonates born to UP mothers moved and tongue-flicked more in the milksnake condition than in the other two conditions, despite being from populations not syntopic with milksnakes.

These results indicate recognition and behavioral discrimination of ophiophagous snakes is present at birth. Site differences in adult snake behavioral responses may be the result of not encountering milksnakes or their odors on a regular basis. However, since mobility duration of adults is still higher in the milksnake condition then in the other two conditions, there could also be a maturation effect. The finding that both ophiophage antipredator behaviors are present in neonates of UP populations suggests that (a) the cost of maintaining such a behavior may be less than the benefit; (b) UP populations have not been separated long enough from other populations that still occur with milksnakes to have lost the behavior; (c) gene flow between populations that occur with milksnakes and those that do not is maintaining the behavior; or (d) that some combination of these three hypotheses may be at play, as they may not be mutually exclusive. The fact that neonates, in general, responded similarly to adults in this test is not surprising, as there is an obvious advantage to expressing such behaviors given that the first encounter with an ophiophagous snake could be the last, especially for smaller individuals. Our data correspond with past research showing that naïve neonate garter-snakes, as well as naïve neonates of other species of snakes (e.g., Cooper, Burghardt, & Brown, 2000), respond to ophiophagous snakes (Weldon & Burghardt, 1979) even when they no longer occur with them. In addition, some antipredator behaviors of snakes that may be ineffective may persist in some populations...
(e.g., Mori & Burghardt, 2000). For example, Japanese natricine snakes, *Rhabdophis tigrinus* (Colubridae), which sequester toxins from their toad prey in special nuchal glands on their necks (Hutchinson et al., 2007), still display toxic nuchal gland specific behaviors (e.g., neck arching, neck butting, Mori & Burghardt, 2008), even when the source of the toxins in those nuchal glands (i.e., toads) is absent from their diets (Mori & Burghardt, 2000). The behaviors are, however, reduced in frequency and intensity. In fact, innate behavioral responses and relaxed selective pressure from predators are known from a wide variety of animal taxa, not only from reptiles (see review in Coss, 1999). While snakes from our study tended to respond more strongly to milksnake chemical cues than controls regardless of the population they were from, the response of neonates within the island populations to milksnake chemical cues does not appear to be explainable without taking into account their evolutionary history on the islands, as shown by our previous genetic studies (Placyk et al., 2007).

Specifically, island populations of gartersnakes appear to be the result of mixed ancestry from both the UP and LP with gene flow currently being maintained between the islands and both mainland sources; however, island populations show a greater genetic influence from UP populations than from LP populations. These underlying genetic signatures appear to be reflected in the data collected here from neonates exposed to milksnake chemical cues. As discussed above, for both tongue-flicking and mobility duration, UP neonates were always less reactive than LP neonates. Evidence for mixed ancestry on the islands may be seen in the tongue-flicking data, in which individuals from three of the four island populations exhibited intermediate levels of tongue-flicking that were not significantly different from the responses seen from either the UP or LP. Conversely, our mobility duration data set show that neonates from three of the four islands (individuals from the fourth island site exhibited an intermediate response) did not differ in responses from UP neonates, indicating that UP ancestry may be influencing that behavior. Only one island population for one of the two behaviors mimicked the LP population (MM for tongue-flicking). Therefore, our first experiment supports a genetic component to the behaviors exhibited, but the role of phenotypic plasticity cannot be discounted and requires further examination to better understand the behaviors exhibited by adult snakes.

### Experiment II: Plasticity of Responses to Ophiophagous Snakes

The fact that UP adults tongue-flicked less than adults from any other site suggests that some “relaxation” of the selection pressure from predation may be occurring (e.g., Coss, 1999). The difference in response to ophiophages between adult and neonate UP snakes, as supported by t tests adjusted for unequal variances (*t* = 2.83, *df* = 6.22, *p* = .03 for tongue-flicking; *t* = −2.70, *df* = 21.9, *p* = .01 for mobility duration) may indicate that (a) there may be a maturational shift in the behavior, or (b) some environmental influence is shaping the responses of the adults. To further investigate the cause of this potential loss of antipredator behavior, a second set of experiments, using a method modified from Herzog (1990), was conducted with neonates to evaluate the plasticity of the behavior.

### Method

Sixty neonate snakes were systematically balanced with respect to site, litter, and sex to one of two treatment groups of 30 snakes each. Eight to 10 days following the initial testing described in Experiment I, one group of 30 was exposed to chemical cues from ophiophagous snakes on a weekly basis and the other group of 30 was not. After 6 weeks of treatment, the two groups were tested again during week 7 using the ophiophagous antipredator test used in Experiment I. Four snakes from the exposed group and 7 snakes from the not-exposed group died or escaped before the end of the 42 days treatment period leaving samples sizes of 26 and 23, respectively. The final composition of the groups consisted of 7 males and 3 females from 5 LP litters, 5 males and 7 females from 8 MM litters, 1 female from 1 SM litter, 1 male and 1 female from 1 GI litter, and 1 female from 1 HI litter for the exposed group and 4 males and 7 females from 5 LP litters, 6 males and 3 females from 8 MM litters, 1 female from 1 SM litter, and 1 male and 1 female from 1 GI litter for the unexposed group. Exposure to chemical cues from ophiophagous snakes entailed placing snakes from the exposed treatment group into a 15 × 30 × 9 cm holding tank (not their home cage) for 15 min, once a week, with a corrugated cardboard substrate that had been placed in a holding tank with one of two milksnakes, *L. t. triangulum*, for a minimum of 12 h. Snakes from the unexposed treatment group were placed in a similar holding cage with a corrugated cardboard substrate that had not previously been used as substrate for another animal.

### Statistical Analyses

Differences in responses both within and between groups across the two testing periods were compared using repeated-measures ANOVAs with group and testing period as independent factors. Post hoc tests consisted of Tukey-Kramer tests.

### Results

When compared to their responses before the 42 days treatment period, neonates in the treatment group that were not exposed to milksnake chemical cues showed a significant decrease in the number of tongue-flicks exhibited (*Z* = 3.92, *p* < .0001; Figure 4a) and in the time spent moving (*Z* = 3.88, *p* < .001; Figure 4b) when tested in the presence of milksnake chemical cues after the treatment period. No other differences were detected when comparing the number of tongue-flicks and the time spent moving for any other condition (control, ringneck, milksnake) for either treatment group (*p* > .05). However, despite the difference in behavior following treatment, the treatment group that was not exposed to milksnake chemical cues continued to tongue-flick more (*F*<sub>2,69</sub> = 4.81; *p* = .01; Figure 4a) and move more (*F*<sub>2,69</sub> = 4.84; *p* = .01; Figure 4b) when tested in the presence of milksnake chemical cues than when tested in either in the control condition or in the presence of ringneck chemical cues (*p* < .05).

### Discussion

After the 42 days treatment period, neonates exposed to milksnake chemical cues responded at levels similar to their initial responses. They also clearly responded to such cues with greater tongue-flicking and movement than they did to control or ringneck...
snake conditions. Those snakes not exposed to milksnake chemical cues still responded more strongly to milksnake cues than to nonophiophagous or control cues, but the number and frequency of tongue-flicks and mobility duration significantly decreased. This supports the hypothesis that adult responses to ophiophagous snakes may be shaped by environmental influences; however, innate ontogenetic shifts in which antipredator behaviors are relaxed might also be expected, as milksnakes are presumably less of a threat to adult gartersnakes than to neonate gartersnakes. In addition, the higher responses to the ophiophagous eastern milksnakes, Lampropeltis t. triangulum. Neonates not exposed to L. t. triangulum chemical cues during the treatment period showed a significant decrease in both number of tongue-flicks and mobility duration (s) as indicated by asterisks (*) (p < .05).

Figure 4. (a) Mean number of tongue-flicks (±95% CI) and (b) mean mobility duration, s (±95% CI) exhibited by neonate common gartersnakes, Thamnophis sirtalis, before and after treatments (not exposed to milksnake chemical cues [n = 23], exposed to milksnake chemical cues [n = 26]) designed to examine plasticity of response to ophiophagous eastern milksnakes, Lampropeltis t. triangulum. Neonates not exposed to L. t. triangulum chemical cues during the treatment period showed a significant decrease in both number of tongue-flicks and mobility duration (s) as indicated by asterisks (*) (p < .05).

Once heavily glaciated area. The results of our plasticity experiment do not completely correspond with those of Gutzke et al. (1993), who found that, in crotaline snakes, if snakes are not periodically confronted by ophiophagous snakes, they stop responding. Our snakes continued to respond to ophiophagous snakes, but responses were less intense. It is not clear how long the snakes from the Gutzke et al. (1993) study were not permitted to come in contact with ophiophagous snakes, so it is possible that if our treatment period were extended, snakes from our populations may have stopped responding to ophiophagous snakes altogether. On the other hand, crotaline snakes (Viperidae) are from an entirely different family of snakes than gartersnakes (Colubridae), which may also influence differences between the two data sets.
General Discussion

The differences in antipredator behavior exhibited by our populations are most likely the result of both environmental and genetic influences. Neonates from all sites and adults from most sites responded similarly to the presence of a potential predator despite the predator not occurring at some sites, indicating some genetic component to the behavior. In fact, Placyk et al. (2007) found that gene flow among the sites used for this study was common, possibly negating the effects of relaxed predator pressures and that behaviors exhibited by neonates may be explained by examining the evolutionary history of their source populations; however, adult snakes from at least one site where the predator is absent exhibited more subdued responses, indicating either a maturation shift, changes in responses due to environmental influences, or some combination of the two. While our work suggests that phenotypic plasticity may be involved in the observed differences between neonates and adults, maturational shifts cannot be totally ruled out and require additional long-term investigation.

Overall, at sites where milksnakes have been absent for thousands of years (Hough, 1958), we saw retention of antipredator responses by one of their potential ophidian prey, the common gartersnake, *Thamnophis sirtalis*. The multipredator hypothesis (Blumstein, 2006) suggests that antipredator adaptations evolve together and thus prey may respond to extinct predators as long as they have experience with other predators (Blumstein, 2006; Blumstein, Ferando, & Stankowich, 2009). Placyk and Burghardt (2005) documented additional potential predators at every site sampled for this study. In addition, differences in responses to milksnakes may be negated by ongoing gene flow between the sites used in this study (Placyk et al., 2007). The decrease in responses to milksnakes that we observed in our laboratory experimentation may also result from snakes not being exposed to any predators, let alone milksnakes, which may be why responses of our laboratory-reared snakes differed from those exhibited by wild-caught adults.

Future studies should more thoroughly examine more general antipredator responses of snakes from this system to determine if the overall relaxation of predator pressures at the various sites has a more overarching effect on such behavior, as the presence or absence of one type of behavior may or may not be related to other behaviors (Blumstein & Daniel, 2005). In addition, adult behaviors need to be more closely examined with an emphasis on population-level differences resulting from gene-environment interactions, developmental differences resulting from organism-environment interactions (Michel & Moore, 1995), and the multipredator hypothesis.

While the information provided by this study shows the complexity of potential genetic and environmental components in influencing population-level behavioral differences, it may also have a more practical component. Specifically, this information is relevant to plans to reintroduce captive born animals into the wild (Lahti et al., 2009). If animals are held in captivity for any period of time, they may become desensitized to potential predator pressures resulting in a disadvantage upon release. This information may be especially vital in the realm of conservation sciences (Lahti et al., 2009).

References


Hansman, A. K. (2003). Thermal and temporal aspects of cold-water foraging by the northern water snake (*Nerodia sipedon sipedon*) (Un-
published master’s thesis). Central Michigan University, Mount Pleasant, MI.


Received August 3, 2010
Revision received December 22, 2010
Accepted January 6, 2011