Exploratory and defensive behaviours change with sex and size in eastern garter snakes (*Thamnophis sirtalis)*

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1. ABSTRACT

Behavioural syndromes are important to consider when examining behaviour in animals since they can help explain the persistence of maladaptive behaviours. These suites of correlated behaviours may be governed by underlying factors such as body size or sex. Behaviours such as exploration, boldness, and defense may vary with body size and sex because different intensities of a behaviour may be advantageous for males versus females, or at different body sizes. I tested the hypothesis that there is a behavioural syndrome related to body size and sex in *Thamnophis sirtalis*. I conducted three behavioural trials to elicit three separate responses on wild snakes from eastern Ontario, Canada. I measured exploratory, boldness, and defensive behaviour to investigate whether a syndrome between these behaviours existed in garter snakes. Although the presence of a behavioural syndrome was not found, exploratory and defensive behaviours were related to body size and sex.The relationship between exploratory behaviour and size differed between males and females. Defensive score was lower in males than in females, meaning that males were more likely to flee when faced with an attack. I also tested the assumption of individual consistency in behaviour and found that the measured behaviours were consistent through time. Future studies should further investigate whether snakes have behavioural syndromes, while taking into account the individual consistency of behaviours.

2. ACKNOWLEDGEMENTS

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6. INTRODUCTION

Organisms often display groups of related behaviours that occur across various situations. These related behaviours are known as behavioural syndromes (Sih et al. 2004). Behavioural syndromes can have important ecological implications such as limitations to distribution and abundance, effects on species interactions, population dynamics, response to novel environments and environmental change, and ecological invasions (Sih et al. 2004; Sih et al. 2012). Behavioural syndromes can also help explain the maintenance of maladaptive behaviours such as overexposure under predation risk (Bell and Sih 2007). There are various types of behavioural syndromes, but one of the more commonly studied is the aggression-boldness syndrome. As shown by Riechert and Hedrick (1993) using the desert grass spider (*Agelenopsis aperta*), aggressive females displayed more boldness following a simulated predatory attack. A similar result was also found in sticklebacks, with high activity scores for individuals who were bolder when faced with a predator (Bell and Stamps 2004).

Several proximate mechanisms can underly behavioural syndromes. Factors such as body size (Herzog et al. 1992; Krause et al. 1998; Bell and Stamps 2004; Glaudas et al. 2005) or sex (Scudder and Burghardt 1983; Herzog and Burghardt 1986; Shine et al. 2000; van Oers et al. 2005) can predict an individual's relative placement on a behavioural axis. For example, a study exploring cottonmouth (*Agkistrodon piscivorus*) reactions to predatory stimulation, supported the idea that there are differences in antipredator response between neonates and adult snakes (Glaudas et al. 2005). In addition, Scudder and Burghardt (1983) found that in banded water snakes (*Nerodia fasciata*), males responded more aggressively to predator stimulation than females. Thus, it seems plausible that sex and body size play an important role in determining an individual's behavioural syndrome.

For a behavioural syndrome to be considered present in an individual, it must be consistent through time and across situations (Jacobs 2009). Behavioural syndromes will have a particularly large impact to an organism's fitness if they are stable over long periods of time (Sih et al. 2004). Many behavioural studies fail to address the assumption of individual consistency (e.g., Scudder and Burghardt 1983; Riechert and Hedrick 1993; van Oers et al. 2005; Bell and Sih 2007) because difficulties arise when testing for a behaviour multiple times on one individual. For instance, behavioural results on subsequent trials may differ due to environmental conditions during the tests (Archer 1973; Mettke-Hoffmann et al. 2006). In addition, performing the same standardised test on an individual to determine whether the behaviour is consistent may result in habituation, therefore producing different results through time (Groves and Thompson 1970; Archer 1973; Herzog et al. 1989; van Oers et al. 2005). Habituation is also important in the context of behavioural syndromes because greater ability to habituate may be associated with improved fitness (Rodriguez-Prieto et al. 2010).

In this study, I will examine whether there is a behavioural syndrome between exploratory, boldness, and defensive behaviours in eastern garter snakes (*Thamnophis sirtalis*). While eastern garter snakes have been shown to have consistent behaviours (e.g., Herzog and Burghardt 1986, Shine et al. 2000), these studies have not examined whether a syndrome is present. In eastern garter snakes, some individuals are more likely to strike than others (Shine et al. 2000), but it remains unclear whether this is one component of a behavioural syndrome. I will examine whether proximate factors, such as body size or sex, are driving exploratory, boldness, and defensive behaviours. Garter snakes are ovoviviparous; therefore gravid females carrying developing young may defend themselves and forage differently than males due to their reproductive state (Graves 1989; Brown and Shine 2004; Sperry and Weatherhead 2011). Also, Shine et al. (2000) found that smaller male garter snakes were more likely to flee from a predatory stimulus than larger females. It is possible that larger individuals are more inclined to defend themselves because they may have a better chance of successfully dissuading a predator compared to a smaller snake (Glaudas et al. 2005). Larger snakes may also exhibit higher activity due to an increased endurance over smaller snakes (Pough 1977). Based on these findings, I tested the hypothesis that the behavioural syndrome of garter snakes is a function of their body size and sex. More specifically, I tested the predictions that larger snakes will be more exploratory, bolder, and more defensive than smaller snakes. Similarly, females will exhibit more exploratory, bolder, and more defensive behaviours when compared to males. I will use condition, along with snout-vent length (SVL), because condition may be a better indicator of an individual's health. To examine the assumption of consistency, I also tested whether the behavioural syndrome is consistent through time, and whether the individually measured exploratory, boldness, and defensive behaviours change in subsequent trials.

7. METHODS

7.1 Study system

The species used in this study is the eastern garter snake, *Thamnophis sirtalis*. Snakes are ideal candidates for studying behaviours because they exhibit a variety of defensive behaviours and are among the most precocial animals, meaning that behaviour in younger snakes can be compared easily to older snakes (Herzog and Burghardt 1986). This study was conducted over the summer and fall of 2013 using 42 snakes for the behavioural trials and 19 other snakes for the consistency trials. I collected snakes for the behavioural trials opportunistically between May and July at the Queen’s University Biological Station (QUBS), approximately 100 km southwest of Ottawa, Ontario, Canada. Two of the snakes for the consistency trials were caught in August in Gatineau, Québec, while the remaining 17 were caught at hibernacula in October in areas surrounding Ottawa, Ontario and Gatineau, Québec. Snakes were caught by hand with the help of cover boards made of tin set out to attract snakes.

Snakes were kept in captivity for a maximum of two weeks while conducting trials. The snakes were housed in bins (30.5 × 48.5 × 31.5 cm) and provided with water, and newspaper as substrate. Snakes were fed earthworms once per week if they were kept in captivity for more than one week.

7.2 Behavioural Trials

To test for the presence of a behavioural syndrome, I conducted three groups of trials, each with the purpose of eliciting a different type of behavioural response. I conducted these trials twice for each individual to test for individual consistency: once after a minimum of one day acclimation to captivity, and the second occurring two days later. To further test for individual consistency, I conducted a separate set of trials using one behavioural trial multiple times, over a longer duration (see next section: *Consistency Trials)*. I covered the sides of a 60 × 30.5 × 31.5 cm terrarium with paper to block all visual stimuli outside of the terrarium, and I recorded all trials with a video camera

I examined exploratory behaviour by placing the snake in the middle of the terrarium and recorded the time spent exploring and the area explored over five minutes. I placed a grid of 5 x 5 cm squares on the bottom of the terrarium to quantify the area explored. Using this grid, I counted the total number of squares that the head of the snake passed over during the trial, and calculated the total percentage of squares that the snake passed over. I converted the measurement to a percentage of the terrarium floor explored to allow for easier interpretation of my results.

Second, I evaluated boldness by placing the snake in a refuge (half of a PVC pipe: L = 25.5 cm, H = 9.5 cm) in the terrarium. I left the snake in the refuge for a thirty second acclimation period, and then uncovered the entrance to determine how long it takes for the snake to exit the refuge (latency to leave), for a maximum of five minutes. I determined latency to leave as the time it took for the snake's entire head to be exposed for a minimum of ten seconds.

Lastly, I recorded defensive behaviour by placing the snake in the terrarium for a one minute acclimation period before dropping a model predator claw (shaped like an osprey claw: 19.3 cm long, 5.3 cm wide at the top, 2,1 cm wide at the base with four 6.7 cm talons coming out from the base; Figure 1) above the snake (modelled after Jonsson et al. 1996). I dropped the model from approximately 50 cm above the bottom of the terrarium and allowed it to stop approximately 10 cm above the middle of the snake's body. The talon model was attached to one end of a stick and with the opposite end held by the researcher. I attached padding to the top of the terrarium to allow the talon model to be dropped at a constant speed until it contacted the padding. I recorded activity for a full minute following the predatory attack to compare activity between the first and second minute. I categorized defensive behaviour as one of six responses with their corresponding defensive score in parentheses: flee (-2), aversion (-1), no reaction (0), tense (1), S-pose (2), or strike (3). I defined S-pose as the shape of the snake when it is partially coiled in an "S" shape and is ready to strike [see Shine et al. 2000, and Hailey and Davies's (1986) "viperine display"]. The scores were determined through pilot tests that indicated the presence of these different reactions. The scores are arranged in a scale from least defensive (flee) to most defensive (strike), similar to the scale used by Shine et al (2000). However, no snakes struck the model claw, so the highest score achieved was 2. If a snake demonstrated multiple behaviours, then I added the scores from each behaviour together. For example, if a snake went into an S-pose and then quickly fled, its final score was 0 because of the +2 for S-pose and the -2 for fleeing. I also recorded movement for a full minute following the predatory attack to compare activity between the first and second minute. Movement was recorded as the number of seconds the snake was moving; the timer started whenever the snake was moving, and stopped whenever the snake was still.

I examined the correlation between the five measured variables using Spearman's rank correlations (package: stats; function: cor.test) to determine whether a behavioural syndrome was present. I used a Spearman's rank correlation test because my variables are not measured on the same scale, and Spearman's correlations are widely used in behavioural syndrome studies (e.g., Bell and Sih 2007; Höjesjö et al. 2011; Nyqvist et al. 2013).

I then used simple regressions (package: stats; function: lm, R Core Team 2013) to examine the relationship between the measured behaviours and sex, condition, and SVL. I used SVL rather than mass in my analyses because SVL is more linear than mass. I calculated condition as the residuals from a linear regression of log10(SVL) to log10(Mass) (Green 2001). I log-transformed SVL and mass to achieve a linear relationship between them. I calculated condition separately for males and females because some of the females were gravid, therefore females could on average have higher conditions than males. I also tested for seasonal effects due to gravidity in females occurring in July.

I used paired t-tests in R(package: stats; function: t.test) to assess the consistency between the two sets of behavioural trials (e.g., latency to emerge in trial A versus trial B conducted two days later).

7.3 Consistency trials

To further examine consistency, I put a separate group of snakes through the defensive behaviour trial every other day for 10 days following a 1 day acclimation period, for a total of 5 trials per snake. I used a repeated measures ordinal logistic regression (package: MASS; function: polr; Venables and Ripley 2002) to examine the consistency between the 5 trials. The ordinal logistic regression suggests that the snakes achieving the two most extreme scores (flee and S-pose) were not consistent through time, but that snakes consistently had a negative score or a positive score through time. I tested for this difference using two separate mixed effects models (package: lme4; function: lmer; family: binomial; Bates et al. 2004) with trial number as a categorical fixed effect, and with individual as a random effect to control for individual variation in habituation. In the first model, I grouped scores of 0, -1, and -2 together, and scores of 1 and 2 together. The scores of 0 were grouped down because of a smaller difference between 0 and the negative scores in the ordinal logistic regression. For the second model, I removed scores of 0 to compare snakes with negative defense scores to positive defense scores.

8. RESULTS

8.1 Behavioural Trials

The Spearman's rank correlation test indicated that percentage explored and time spent exploring were highly correlated (P = 0.61, $p < 0.001$), but no other correlations were present

(Figure 2). I eliminated the time spent exploring variable from the subsequent analyses because percentage explored and time spent exploring are correlated and most likely represent a very similar behaviour. I chose to use percentage explored instead of time spent exploring because it is a better indicator of exploratory behaviour, rather than activity. Gravidity of females had no effect on any of the relationships found.

The relationship between percentage explored (henceforth referred to as exploratory behaviour) and size differed between males and females ($t = 2.410$, $p = 0.02$). As females grow longer they score lower in exploratory behaviour, while males score higher as they grow longer (Figure 3). I found no relationship between the latency to emerge, a representation of boldness, and size or sex. On the other hand, defense score (henceforth referred to as defensive behaviour) differed between males and females, with males being more likely to flee than females ($t = -2.154$, $p = 0.04$; Figure 4). I also found that the difference in activity following a predatory attack (henceforth referred to as defense activity) was unrelated to body size or sex.

There was no difference between scores in the first trial and in the second trial (Table I), suggesting that these behaviours are consistent through time.

8.2 Consistency Trials

The ordinal logistic regression yielded differences in consistency between defense scores of -2 and -1 ($t = -2.6985$, $p = 0.0194$), and between 1 and 2 ($t = 2.8013$, $p = 0.0160$). Regardless of how defense score was grouped into 0 and 1, it did not change significantly between the 5 trials in either the first model, nor the second model, meaning that the recorded behaviours were stable.

9. DISCUSSION

My findings do not support the presence of a behavioural syndrome in garter snakes; however from this we cannot conclude that snakes do not have behavioural syndromes. The lack of syndrome present in this study supports the idea that the exploratory and defensive behaviour axes are unlinked in garter snakes, or that the methodology used in this study did not successfully measure these behaviours. Yet, the presence of the two separate behaviours could provide a starting point for future exploration into the occurrence of behavioural syndromes in snakes. Perhaps these behaviours may be linked to other axes that were not investigated within this study.

The fact that males and females differ in terms of their relationship with exploratory behaviour and defensive behaviour, provides support that sex and SVL are underlying factors that can explain these behaviours. First, the variation in exploratory scores associated with length suggest that different intensities of exploration could be favoured at different ages. These differences could be explained by varying levels of hormones (e.g., Bell and Stamps 2004; Dammhahn and Almeling 2012) associated with maturation. Moreover, the variation in exploration between males and females may be related to the fact that males will maintain larger home range sizes that females. This trend has been found by Hyslop et al. (2014) where male eastern indigo snakes will maintain larger home ranges than females. In a study done by Blouin-Demers and Weatherhead (2002), they found that male black rat snakes were more mobile than females during the mating season, most likely to increase their access to sexually viable females.

The relation between defense behaviour and sex shows that males are more likely to flee than females. My findings corroborate well with those of Shine et al. (2000), in which male snakes showed a higher proportion of fleeing compared to female snakes. This difference between males and females is most likely linked to the difference in testosterone levels. King (2002) found that females struck more frequently than males, but when injected with testosterone, would strike less frequently. Furthermore, females also may be more reluctant to flee due to locomotory costs associated with carrying developing young while they are gravid (Kissner et al. 1997). Brodie (1989) also suggested that defensive behaviour may not only be a result of the decrease in locomotory function, but rather a result of changes in physiology during pregnancy.

A main assumption of behavioural syndromes is that they are consistent through time (Jacobs 2009). The results from the repeated trials support the claim that the behaviours that I measured are consistent through time. This is an important assumption to test because if the behaviours were found to be inconsistent, we would not be able to conclude the presence of a behavioural syndrome, or personalities. Inconsistency could be a result of the snakes behaving randomly during each trial, rather than behaving according to underlying mechanisms related to body size or sex.

The results must be considered while taking into account a few study limitations. Firstly, trap bias is a factor that can impact results in behavioural studies (Carter et al. 2012). The fact that the snakes were caught by hand, rather than using a trap, avoids some of this trap bias. By catching the snakes by hand, I avoided the issue that traps would catch the most exploratory and boldest individuals who would enter a completely novel object. However, it is still possible that the snakes caught were slightly more exploratory, bolder individuals that would bask in areas more easily seen by researchers, or were more likely to hide under the tin covers which could be considered as novel objects. Another limitation to consider is whether trait assays predict scores under natural conditions. By capturing the snakes and forcing them into novel, and consequently stressful situations, the snakes may behave differently than they would in natural contexts (Biro 2012). The snakes were allowed a one day acclimation period between catching and testing to relieve this trapping stress, however the acclimation response may differ between individuals, resulting in some individuals still behaving unnaturally (Biro 2012). Biro’s suggestion to overcome this limitation by measuring each behaviour 10 times per individual is not appropriate due to the results being confounded by possible habituation effects through associative learning (Edwards et al. 2013). However, my results are similar to those found by Shine et al. (2000), which were conducted under natural conditions, therefore my results were likely not overly biased by the artificial nature of the behavioural assays.

Ultimately, my findings do not support the presence of a behavioural syndrome; however I have found size and sex relationships with exploratory and defensive behaviours. Further investigation into whether snakes exhibit behavioural syndromes will give us a more holistic perspective on snake behaviour. By connecting these behaviours to underlying factors, we can more fully understand what drives behaviour.

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11. APPENDIX

Figure 1. A model claw used to elicite defensive behaviours in eastern garter snakes.

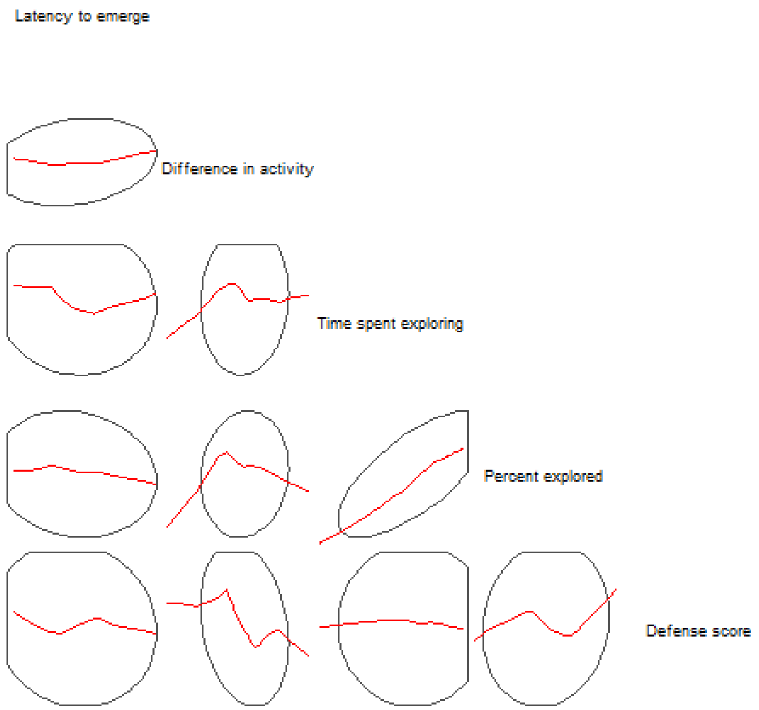

Figure 2. A correlation matrix between the five measured variables representing exploration, boldness, and defensive behaviour in eastern garter snakes. The percentage explored and time spent exploring are correlated, while correlations between other variables were not found.

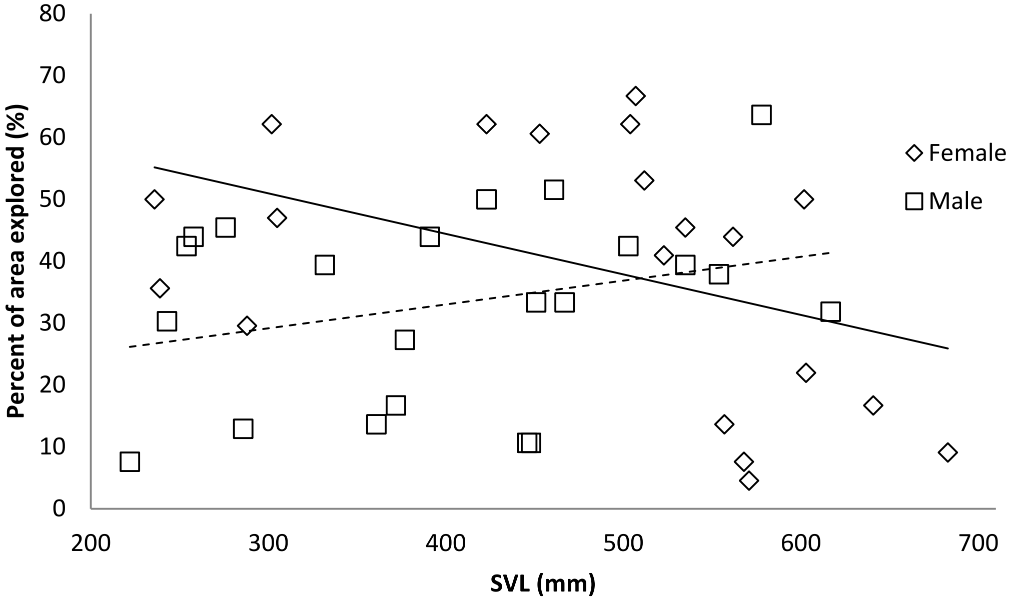

Figure 3. The exploratory behaviour of male (n=22) and female (n=20) eastern garter snakes are represented separately to display the differences in relationships between sexes. As males grew longer, they became more exploratory, while females became less exploratory as they grew longer.

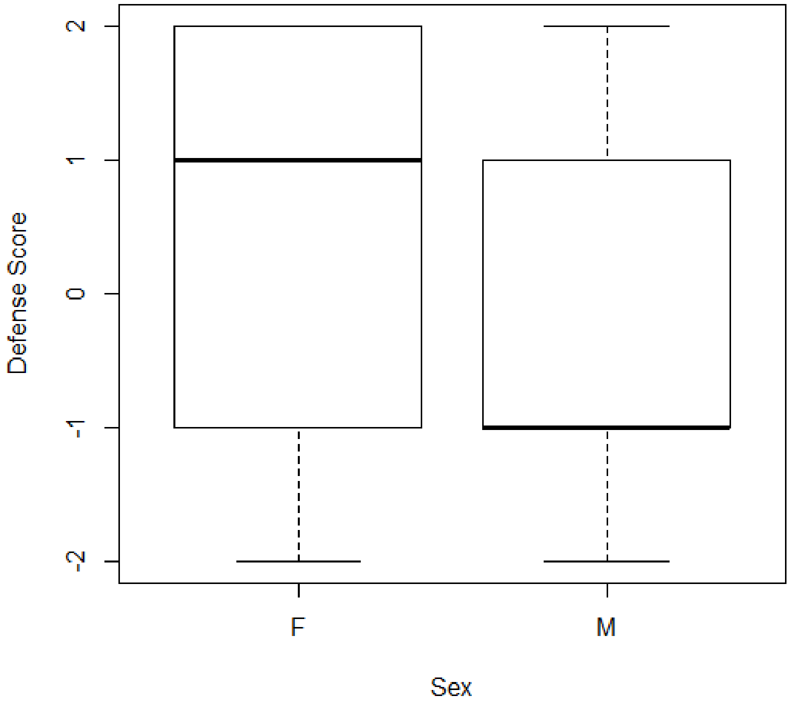

Figure 4. A box plot showing the defense scores (n = 42) of male and female easter garter snakes. This figure shows how males were more likely to flee than females.

Table I. Exploratory, boldness, and defensive behaviour were examined in eastern garter snakes. A paired t-tests between the 5 measured behavioural variables show that they are consistent between trials. df = 41. Trial A refers to the mean ± S.E. for that variable in the first trial, and Trial B refers to the same in the second trial.

Variable	Trial A	Trial B	t value	p value
Percentage explored	35.97 ± 2.79	35.41 ± 3.21	0.1658	0.8691
Time spent exploring	197.55 ± 15.88	194.88 ± 16.63	0.1303	0.897
Latency to emerge	135.50 ± 16.85	143.02 ± 18.17	0.5175	0.6076
Defense score	0.10 ± 0.23	0.21 ± 0.24	-0.3719	0.7118
Difference in activity	1.05 ± 2.89	6.67 ± 3.73	-1.4217	0.1627