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Effects of Gene Methylation on Behavior in Green Anole Lizards (Anolis carolinensis)

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Effects of Gene Methylation on Behavior in Green Anole Lizards (Anolis carolinensis)

An Honors Thesis

Submitted in Partial Fulfillment of the

Requirements for Graduation with

Undergraduate Research Honors

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Under the Direction of Walter Wilczynski, Ph.D

ABSTARCT

The green anole lizard (*Anolis carolinensis*) is a valuable animal model for examining social behaviors and aggression in lizards. These vetebraes have been widely used in studies to analyze behavior and aggression, however, new aims are developing that focus on epigenetic manipulation for gene methylation. Epigenetic changes in gene methylation shifts the availability of DNA transcription. In our study we aim to propose that a change in gene methylation will impact the social behaviors that green anole lizards use to establish dominant-subordinate relationships. This aim has two experiments. Experiment one uses the drug Zebularine which is a methylation blocker, while in experiment two the drug L-Methionine causes an increase in gene methylation. The overall results of this study show that changes in gene methylation impacts social behavior. An increase in gene methylation caused more aggressive bouts, while the preliminary results suggest that a decrease in gene methylation stimulated less aggression.

Index Key Words: Green Anole Lizards, Zebularine, L-Methionine, aggression, social behavior, *Anolis carolinensis*, epigenetic gene methylation

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DEDICATION

I dedicate this paper to my Mother, Mauverlyn M. Archer, who sprouted in me a love for science and has always been a continuing motivator in my life. I will never forget her saying… "When men on earth have done their best, God in heaven will do the rest" (Philippians 4:13).

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I would like to acknowledge Dr. Walter Wilczynski for the privileged of conducting this experiment in his lab and the guidance he has given me as a future researcher.

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Introduction

For several decades, the green anole lizard (*Anolis carolinensis*) has captured the interest of professionals from scientific researchers to sociologists. The popularity of these species are due to the complexity of their social aggressive interactions among males [2]. The aggressive behaviors of green anole lizards have been well documented and studied [2]. Characteristic aggressive behaviors increase during the breeding season, as male anoles attempt to dominant females and territories. As two males fight for territorial control, dominant and subordinate relationships were formed [2]. To achieve a dominant position, the males conduct characteristic displays that involve: push-up displays, dewlap extension, lateral compression of body, biting, and parallel advancement of the opponent [2], [6]. The push-up display is a vertical motion of the anterior body [6]. The dewlap extension is a quick augmentation of a red throat fan [6]. The lateral compression consists of lengthening the throat coupled with sagittal expansion of the body [6]. These displays allows the lizard to appear larger than normal and threaten the opponent [2]. Biting and parallel advancement are techniques intimidation and can escalate to the injury of one or both males [2]. An important note in analyzing aggressive behavior of anolian males is that the lateral compression is characteristic to aggressive encounters only [6]. The sequence of events that commence during an encounter usually involves matched males who assume the push-up, dewlap extension, and lateral compression demonstrations. The males advance towards each other in parallel formation about three inches in distance. This type of circling standoff can continue until one male attempts a retreat, followed by the other male in pursuit [2]. In addition to social actions conducted during aggressive confrontation, changes in the physiology of the lizard can exhibit

social status [4], [6]. The dominant male will exhibit certain characteristics like appearing a greenish color and occupying a high perched position, while the subordinate male will occupy a lower perched position, appear a brownish color, and display a low profile compared to the dominant male [4],[6]. These social behaviors establish dominant and subordinate statuses among green anole lizards and are useful in examining social positions.

Associations like dominant-subordinate relationships can form during social interactions stimulating changes in the physiology of an organism. Aggressive behavior in green anole lizard promotes the release of hormones and activates the sympathetic nervous system [4]. This concept is evident in lizards that accepts a subordinate position by the color of the skin changing from light green to dark brown. This physiological change is due to catecholamines which are hormones released form the adrenal gland [4], [6]. Furthermore, the color of a skin patch behind the eye, called the eyespot, will become darkened during aggression. The eyespot's change in color from light green to black is due to the activation of the sympathetic nervous system and plasma adrenal catecholamines [7]. Additionally, dominant males have a marked higher plasma testosterone concentration when compare to subordinate males. Conversely, subordinate males have a higher concentration of corticosterone, which is an adrenal stress hormone [4], [6], [7], [8]. Ultimately, the physiology of these lizards change in response to stressful stimuli.

A variety of studies have explored anole male behavior ranging from stress manipulations to habituation enforcement of aggression. Such studies analyze physiological changes, stress response, and development of dominant-subordinate relationships [4], [6]. For example, in the study conducted by Plavicki, Yang, and Wilczynski, changes in body color and

eyespot were used to determine dominant and subordinate male's statuses in green anole lizards [4]. However, few scientists have attempted to explore the role of epigenetic methylation manipulations in social behavior and aggression. Epigenetic is the change of gene activity without the alteration of the DNA code [5]. DNA methylation is a biological process in which a methyl group is added to an adenine or cytocine DNA nucleotide [5]. Methylation plays a crucial role in gene expression by controlling the access of transcription factors to promoters on the DNA strand [5]. This concept means that as more methyl groups are added to the DNA, the DNA becomes less available to protein expression. Additionally, the reverse is true that if less methylations occurs in the genome, the DNA is more prone to protein binding of transcription factors. This reaction leads to more DNA expression [5].

Researchers have been interested in the effects of epigenetic methylation in many species such as plants. In the study by Sushil Kumar et al, environmental stresses were shown to stimulate changes in DNA methylation in plants [3]. For example, when the plant *Phoenix dactylifera* was submitted to biological stress hypo-methylation resulted causing an increase production of branches. The phenomena of less methylation caused the genome to become more permeable to transcription factor which lead to increased protein production to form more branches [3]. Similarly, the genome of green anole lizards can be manipulated by subjecting the DNA to specific drugs that will cause an increase or decrease in gene methylation. Zebularine is a common cytidine analogue used for decreasing gene methylation in DNA [9]. This drug is very stable and must be used in low concentrations due to its toxicity [9]. A drug that can cause increase methylation is L-methionine [1].

In this study, green anole lizards were paired based on weight and size and housed in a ten gallon aquarium tank for ten days. Each pair was subjected to two drugs: Zebularine and L-methionine and then allowed to establish dominant and subordinate relationships. Based on the male's social behavior responses and the types of social statuses each male acquired we determined if epigenetic changes to behavior influenced social interactions.

Methods

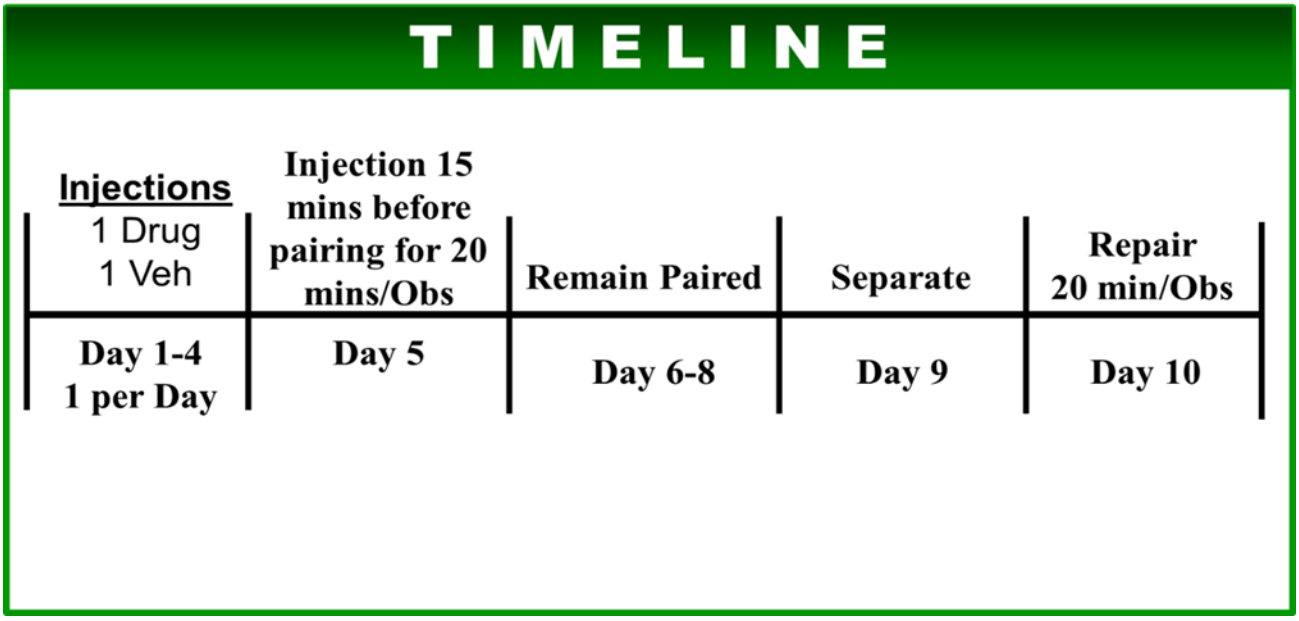
Experiment one and two:

There is one aim for this research with two experiments. Experiment one determines if blocking gene methylation increase or decrease the probability of a male becoming dominant, while experiment two examined whether enhancing gene methylation at the first social encounter affects the possibility of dominance or subordinance. For experiment one, 18 male lizards were matched according to size and paired forming a total of 9 pairs. Each pair was housed in ten gallon aquariums and separated by a barrier for five days. During this time, one lizard in each pair received a 0.5 mg in 50 µl IP injection of Zebularine (Zeb), a methylation blocker, while the other receives a vehicle daily. On the fifth day, the barrier was removed 30 minutes after administering the injections, allowing the pairs to interact. The first 20 minutes was video recorded. The pairs remained together for four more days. On the ninth day, the pairs were separated and reintroduced on the tenth day for another 20 minute video recording. For experiment two, the same procedure was used as in experiment one except 33 mmol in a 50 µl IP injection of L-Methionine (Met), a methylation enhancer, was given instead. At the conclusion of the second pairing, animals were sacrificed. Plasma and brains were collected for future analyses (Fig. 1). Nine pairs of males were used for experiment one, while three pairs were used for experiment two. Experiment two consisted of fewer males due to the low availability of lizards at the end of their normal activity season. Consequently, experiment tow should be considered as a preliminary study until more lizards are tested.

Behavioral Scoring:

Each 20 minute video was scored using StopWatch+. The latency to begin behavior and number of head bobs and dewlap extensions were noted. Head bobs and dewlap extensions rarely occur in isolation but rather in bouts of 5-10 bobs with brief pauses between bouts. This number was recorded as bout number seems to be a more salient measure of behavior as opposed to raw bob/dewlap number.

Figure 1. Timeline demonstrates the procedure over a ten day period.



Results

Zebularine injected lizards vs. Vehicle

Paired t-tests demonstrated that Zebularine injected lizards performed significantly more aggressive behavior bouts than their Vehicle (Veh) injected partners during the first 20 minutes of pairing (t= -2.556; p=0.034) (Fig. 2). This difference was not observed when animals were paired a second time following group housing (t= -1.531, p=0.164) (Fig. 2.). Seven Zebularine (Zeb) males were dominant and two were subordinate. The standard deviation for experiment one first and second pairing was Zeb: 22.22, Veh: 2.78; Zeb: 10.11, Veh: 1.16, respectively. The standard error margin for the first and second pairing was Zeb: 7.41, Veh: 0.93; Zeb: 3.37, Veh: 0.38, respectively.

L-methionine injected lizards vs. Vehicle

Paired t-test demonstrated that L-Methionine (L-Meth) injection did not significantly affect aggressive behavior during either the initial (t=-1.031; p=0.411) or second pairing (t= -0.866; p=0.478) (Fig. 3). Two L-Metionine males were subordinate and one dominant. The standard deviation for experiment two first and second pairing was L-Meth: 3.464, Veh: 26.963; L-Meth: 2.0, Veh: 5.292, respectively. The standard error margin for the first and second pairing was L-Meth: 2.0, Veh: 15.56; L-Meth: 1.15, Veh: 3.05, respectively.

Figure2. The first grouping of pairs showed significantly more aggressive bouts compared to vehicle, in contrast, the second grouping of pairs were not significantly different then the vehicle.

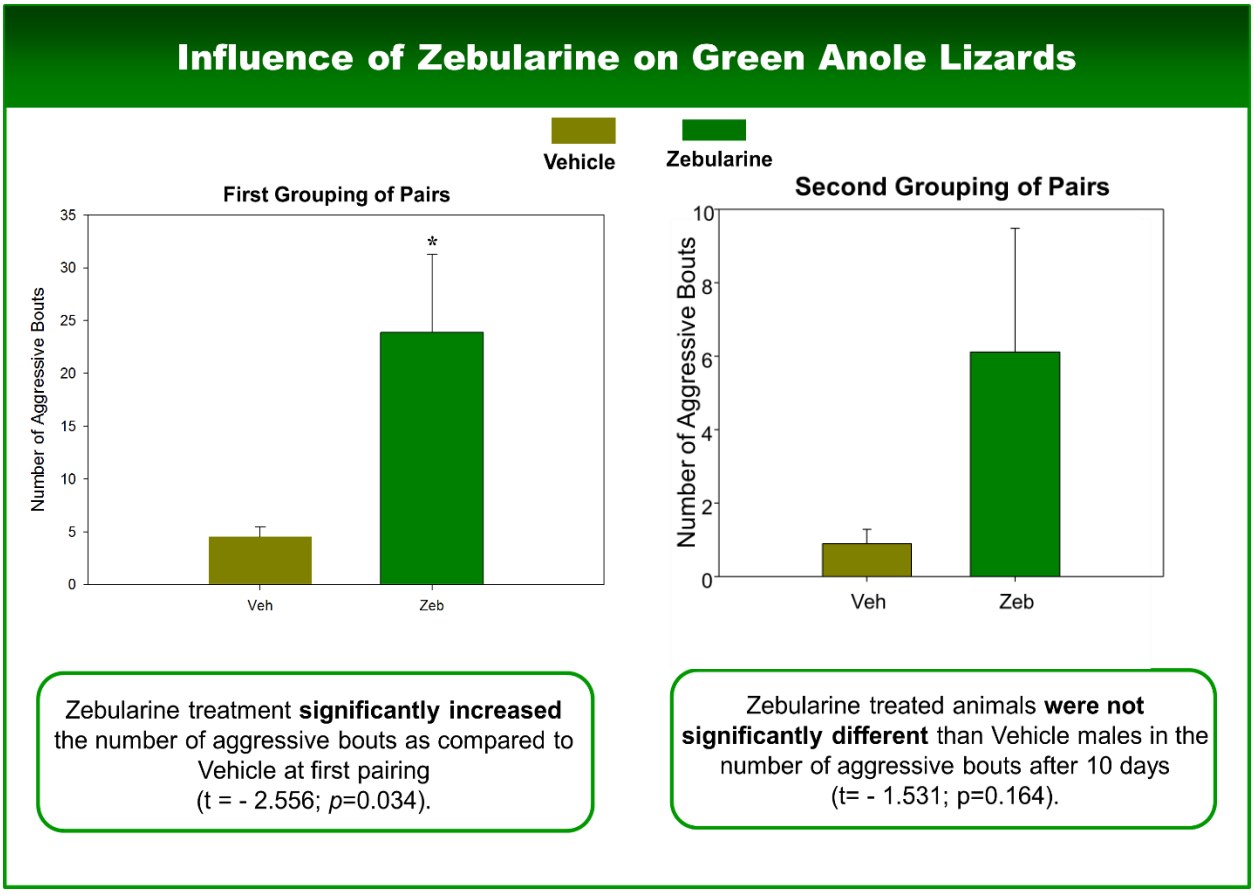
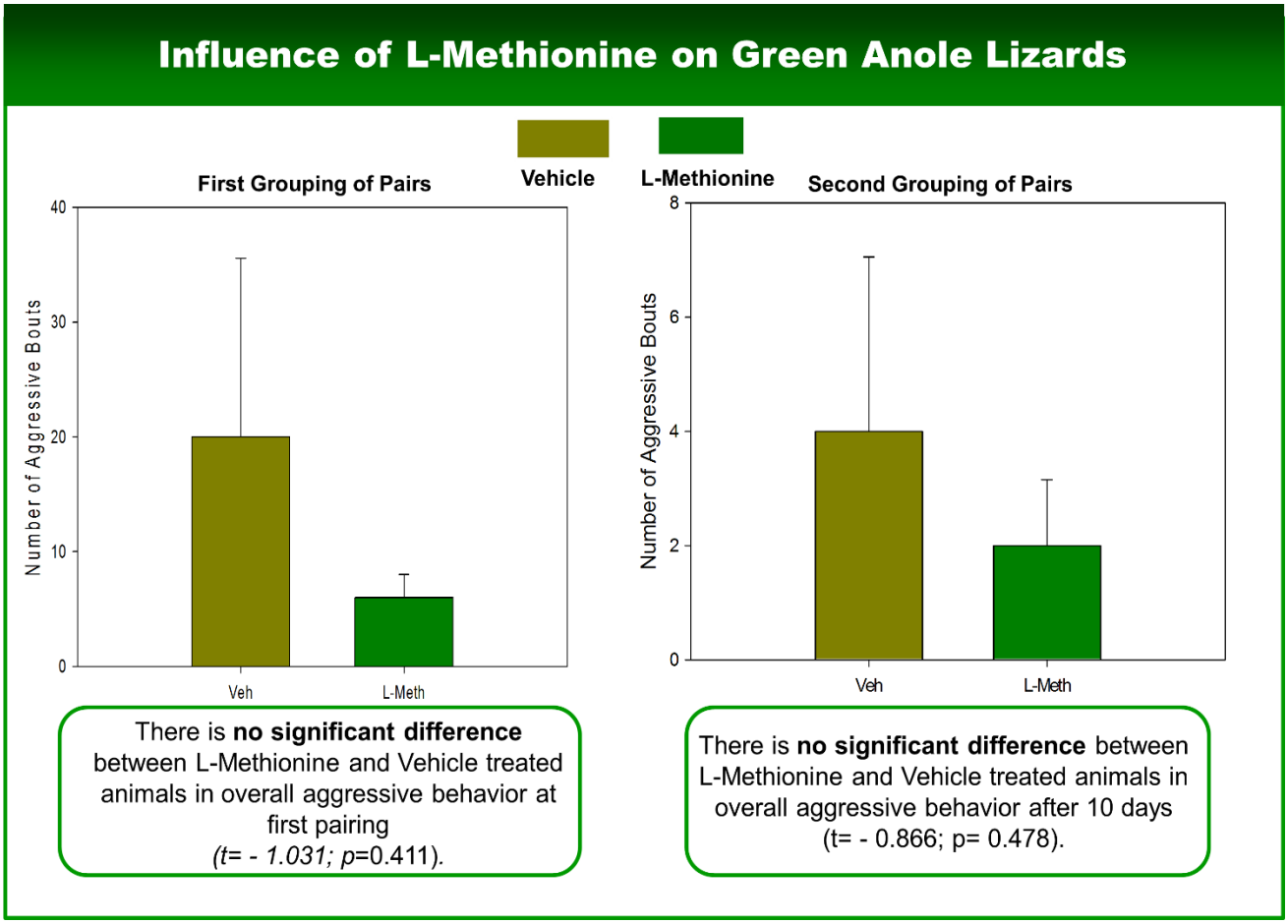


Figure 3. The first and second group of pairs show no significant overall aggressive behavior.



Summary

These results show that a change in gene methylation causes an alteration in social behavior. Over the five day period, one of the males in each pair was injected with a drug, while the opponent was given a vehicle. Although the plasma hormone levels were not recorded, the physical and aggressive behaviors were substantial to evaluate and confirm the dominant-subordinate relationships between each male. In the zebularine pairs, seven out of the nine males that were treated with the drug exemplified aggressive behavior. The initial pairing has a low p value of 0.034. This value indicates that there is a significant difference between the

numbers of aggressive bout conducted by the zebularine treated males in contrast to the vehicles. On the other hand, the second pairing p valve is high and signifies no significant difference between treated and non-treated males. The same is true for the L-methionine cases in both the first and second pairings, however, the results were not significant.

Discussion

The results support the hypothesis that a change in gene methylation causes an alteration in behavior. In experiment one, the drug zebularine is document to cause less gene methylation. The higher the availability of the genome to allow binding of proteins, directly influences the amount of protein products that are produced. It was speculated that the drug affected the genes that control the amount of plasma testosterone and catecholamines, causing an increase that directly stimulated aggressive behavior. These findings imply that a daily injection of zebularine into the abdomen caused a significant change in behavior. The initial pairing demonstrated that the zebularine injected males performed more aggressive bouts than the vehicles.

Seven of nine males that were injected with zebularine displayed more aggressive bouts than their opponents. The behavior performed by these aggressive males involve push-ups, dewlap extension, and lateral compressions. A similar fight style was recorded of the *Leiocephalus schreibersii*, which swells their throat and expands their body linearly. Furthermore, the *Chalarodon madagascroriensis* swells their throat and extends their dorsal crest during aggression [2]. Physiological changes of the dominant male is the male remaining green with darken eyespots while the subordinate male is brown [4].

The zebularine injected males in the second pair conducted significantly lower aggressive bouts than in their first pairing. This result caused a higher p score valve of no significance. However, the quantity of the vehicle's aggressive decreased from the first to the second pairing and remained lower than the zebularine male's bouts. This trend in data shows

to some degree that lizards remembered their social status within the day break because the dominant-subordinate relationships were maintained. The results could also imply that the drug's affects lasted long enough to influence the social behavior of the males.

The low sample sizes of the experiment two makes any conclusion for this experiment tentative. With this qualification in mind, the mean differences between the L-Methionine males and the vehicle injected lizards suggest that L-Methionine has the opposite effect as Zebularine. The drug L-Methionine functioned as a methyl enhancer, causing the DNA to become more methylated. As a result, less genes were available for transcription. It was speculated that a higher level of methylation, promoted by the drug L-Methionine, lead to less production of hormones such as testosterone and catecholamines. Fewer aggressive hormones leads to less aggressive bouts. This may be why the males in experiment two that were injected with L-Methionine, displayed fewer aggressive bouts.

The sample size of lizards in experiment two were fewer than in experiment one. This difference in subjects may have impacted the p values, causing them to be higher than 0.05. Additionally, a trend of lower aggressive bouts can be observed in the first and second pairing of the L-Methionine injected males when compared to the vehicles. This result may be due to the lizards remembering the dominant-subordinate relationships that were established and/or the prolonged effects of the drug L-Methionine.

Overall, this study indicated that epigenetic changes in methylation to the genome of green anole lizards significantly altered social behaviors.

FUTURE DIRECTIVES

At the present time, little is known concerning the genes being affected by the drugs because this was a systematic treatment. However, it can be speculated that since the drugs were injected systematically, they targeted specific genes that regulate testosterone, stress responses, and significant neurotransmitters in the brain. Future studies will examine the brains collected from the males to verify changes in methylation from drug treatment.

REFERENCES

1. Batra, V., & Verma, P. (2014). Dietary l-methionine supplementation mitigates gamma-radiation induced global DNA hypomethylation: Enhanced metabolic flux towards S-adenosyl-l-methionine (SAM) biosynthesis increases genomic methylation potential. *Food And Chemical Toxicology*, *69*46-54.
2. Greenberg, B. & Noble, K. Social Behavior of the American Chameleon (Anolis carolinensis). *Physiology Zoology*, 393-400.
3. KUMAR, S., KUMARI, R., SHARMA, V., & SHARMA, V. (2013). Roles, and establishment, maintenance and erasing of the epigenetic cytosine methylation marks in plants. *Journal Of Genetics*, *92*(3), 629-666.
4. Plavicki, J., Yang, E., & Wilczynski, W. (n.d). Dominance status predicts response to nonsocial forced movement stress in the green anole lizard (Anolis carolinensis). *Physiology & Behavior*, *80*(4), 547-555.
5. Phillips, T. (2008) The role of methylation in gene expression. Nature Education 1(1):116
6. Yang, E., Phelps, S. M., Crews, D., & Wilczynski, W. (2001). The Effects of Social Experience on Aggressive Behavior in the Green Anole Lizard (Anolis carolinensis). *Ethology*, *107*(9), 777-793.
7. Yang, E., & Wilczynski, W. (2002). Relationships between Hormones and Aggressive Behavior in Green Anole Lizards: An Analysis Using Structural Equation Modeling. *Hormones & Behavior*, *42*(2), 192.
8. Yang, E., & Wilczynski, W. (n.d). Interaction effects of corticosterone and experience on aggressive behavior in the green anole lizard. *Hormones And Behavior*, *44*(3), 281-292.
9. Yoo, C., Cheng, J., & Jones, P. (2004). Zebularine: a new drug for epigenetic therapy. *Biochemical Society Transactions*, *32*(Pt 6), 910-912.