Sex Recognition in Anole Lizards

Authors: Shelby Stavins and Dr. Matthew Lovern*

Abstract: Sexual selection is the process that furthers a species, and either improves the genetic variability or weakens it because the organisms go after appearance versus superior traits. In this experiment, the researchers studied the behavior between different sexes of juvenile brown Anole lizards to see if they displayed signs of sexual recognition. They hypothesized that there will be sexual recognition in the juvenile brown Anoles. The lizards were separated according to gender for the first couple of weeks so that they could mature to about 1 year old. Then, the lizards were housed one per cage and the trials were recorded. The trials consisted of 4 female v female, 5 male v male, and 7 female v male trials which were each 10 minutes long and each lizard only went one time per day. During these trials the lizards were numbered and were introduced into an existing lizard's cage. The researchers stood 3 feet from the cages to avoid interfering with the displays and recorded the results from the trials. The results suggested that the juvenile brown Anole lizards were either too young to care, or too young to be able to differentiate between the sexes of the lizards they were exposed to; this caused the researchers to rejected their hypothesis.

Keywords: Sexual selection juvenile Anole Lizards

Introduction

Evolution is the development of a species in which selective pressures either aid the spread or the decline of a population of animals (Kirkpatrick 1982). These selective pressures include, geographical barriers, gametic barriers, temporal barriers, behavioral barriers, and mechanical barriers.

An example of temporal barriers would be: the marine iguana in relation to the land iguana of the Galapagos islands. These animals are of the same species and would produce viable offspring, however, their mating seasons are different so therefore they do not recognize each other as possible mates (Christian and Tracy 1981).

An example of a gametic barrier would be, if a lion and a tiger attempted to mate. Lions and tigers are the in the same genus, but different species, so their genetic makeup is too different for successful breeding, which does not allow for viable offspring (Frazee and Masly 2015). Although there are Ligers in existence, this is because scientists will take the egg and sperm and force them to combine in a test tube: in vitro. The postzygotic result would be hybrid sterility, which is when the chromosomal numbers cause problems for meiosis, and therefore the offspring is infertile. Not only are lions and tigers not able to reproduce because of gametic barriers, they live halfway across the planet from each other. This causes the problem of geographical barriers and in the real world, outside of zoos and labs, tigers and lions would very rarely, if ever, come into contact with each other.

Many plant populations display a wide variety of plants and flower forms; because of this, pollination of certain species within the population may be difficult to complete due to mechanical barriers. An example of mechanical barriers would be in floral isolation. One species of flower may have a stem that is too long or too short, or

* Faculty Mentor, Department of Integrative Biology, Oklahoma State University

may have parts that limit access to the pollen itself so the vector species, or pollinator, will be unable to complete its job. The parts of the flower must fit the parts of the pollinator for pollination to occur (Levin 1971).

Sexual selection is a part of evolution that can either increase or decrease genetic variability in a population. For example, in brown-throated three-toed sloths, the female emits loud vocalizations repeatedly to attract the male (Bezerra et al. 2007). In this scenario, the male is not paying attention to how fit the female is, only to how "attractive" her mating call is. This will decrease genetic variability, because the female is not being chosen off superior traits, rather her mating displays. These vocalizations increase competition among the males for the females attention, again without taking into consideration how fit for survival the female is.

In this experiment, researchers will study brown Anole lizards, of the species *Anolis carolinensis*, for their behavior displays in recognizing the opposite sex, which in turn would lead to sexual selection. Although there are many species of lizards that live in North America, the Green Anole lizard is the only native species which dwells in trees and shrubs, so researchers will be using the invasive species (Bartlett and Bartlett 2001). Green and Brown Anole lizards can reach up to 12 inches in the wild, but usually will stay around 9 inches in captivity due to the conditions they are exposed to while captured such as: not having adequate lighting, food, and space (Bartlett and Bartlett 2001). The reason these lizards need adequate lighting is that there is only so much an artificial light can do for an organism. Non artificial light is extremely important in both plants and animals. For example, plants need sunlight to complete photosynthesis, producing oxygen. Also, sunlight helps organisms to produce vitamin D. If organisms are only exposed to artificial light, there will be a limited supply of vitamin D even with the UV lights that are used. This dilemma will cause the lizards to be shorter in length. Space and food would inhibit growth because in the wild there would be more food available and more space to live in. Space, food and artificial lighting would all be limiting factors in this experiment.

The question that is going to be studied here is whether or not juvenile female and male Anole lizards demonstrate sex recognition. The researchers hypothesize that the lizards will demonstrate sex recognition at this age through behavioral displays and that these displays will occur the most during the male v male trials and the female v female trials (Lovern and Jenssen 2001) both from the female and male. These displays will not only signal if they are attracted, or aroused by the opposite sex, but also if they can identify competition between the males, which would lead to displays of aggression. The researchers intend to record all behaviors listed above. Since the resources are limited in each tank, while the lizards are not being watched for mating displays, it is possible for them to display acts of aggression, even though they are quite young (Greenberg and Crews 1990). The confounding variables in this experiment could be if the lizards display mating behaviors between the same sex or fight when not exposed to the opposite sex. Fighting when not exposed to the opposite

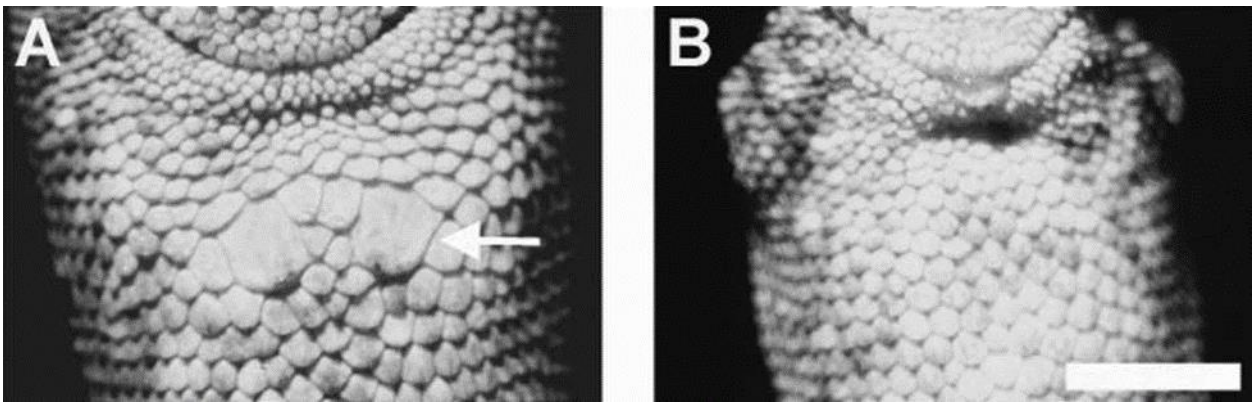

Figure 1 - Postanal scales on Anole lizards are shown above. Side A is a male anole because of the two larger postanal scales and B is a female anole because she does not have these enlarged postanal scales (Lovern, M. et al 2004).

sex may be due to fluctuating hormone levels in the lizard's endocrinology, or simply that they are transitioning from adolescence to adulthood. Females do not normally demonstrate aggression. If the lizards display mating behaviors between the same sex, then it will become apparent that they are either the outlier or that they can not determine the difference between male and female yet. The researchers expect that there will be demonstrations of sex recognition in this stage of adulthood in the female and male anole lizards.

The researchers expect the brown anole lizards to be able to demonstrate sex recognition at this age because they will be entering into adulthood. It is to be expected that the lizards will display signs of recognition of the opposite sex. If this occurs then that could suggest that they (the lizards) can recognize the coloration and body distinctions (Figure 1) and would therefore have an increased chance in reproduction which would ultimately favor them in spreading their DNA. This would decrease genetic variability within the population because the lizards able to recognize sex differences would become dominant within the ecosystem. Those lizards (male in this example) would be able to identify male competition and dominate to get the female's attention to spread "his" DNA. If the lizards are not able to demonstrate sex recognition then that would suggest that yearling lizards are too young to be able to reproduce and would need more time to mature.

Methods

Evolution is the development of a species in which selective pressures either aid the spread or the decline of a population of animals (Kirkpatrick 1982). These selective pressures include, geographical barriers, gametic barriers, temporal barriers, behavioral barriers, and mechanical barriers.

An example of temporal barriers would be: the marine iguana in relation to the land iguana of the Galapagos islands. These animals are of the same species and would produce viable offspring, however, their mating seasons are different so therefore they do not recognize each other as possible mates (Christian and Tracy 1981).

An example of a gametic barrier would be, if a lion and a tiger attempted to mate. Lions and tigers are the in the same genus, but different species, so their genetic

makeup is too different for successful breeding, which does not allow for viable offspring (Frazee and Masly 2015). Although there are Ligers in existence, this is because scientists will take the egg and sperm and force them to combine in a test tube: in vitro. The postzygotic result would be hybrid sterility, which is when the chromosomal numbers cause problems for meiosis, and therefore the offspring is infertile. Not only are lions and tigers not able to reproduce because of gametic barriers, they live halfway across the planet from each other. This causes the problem of geographical barriers and in the real world, outside of zoos and labs, tigers and lions would very rarely, if ever, come into contact with each other.

Many plant populations display a wide variety of plants and flower forms; because of this, pollination of certain species within the population may be difficult to complete due to mechanical barriers. An example of mechanical barriers would be in floral isolation. One species of flower may have a stem that is too long or too short, or may have parts that limit access to the pollen itself so the vector species, or pollinator, will be unable to complete its job. The parts of the flower must fit the parts of the pollinator for pollination to occur (Levin 1971).

Sexual selection is a part of evolution that can either increase or decrease genetic variability in a population. For example, in brown-throated three-toed sloths, the female emits loud vocalizations repeatedly to attract the male (Bezerra et al. 2007). In this scenario, the male is not paying attention to how fit the female is, only to how "attractive" her mating call is. This will decrease genetic variability, because the female is not being chosen off superior traits, rather her mating displays. These vocalizations increase competition among the males for the females attention, again without taking into consideration how fit for survival the female is.

In this experiment, researchers will study brown Anole lizards, of the species *Anolis carolinensis*, for their behavior displays in recognizing the opposite sex, which in turn would lead to sexual selection. Although there are many species of lizards that live in North America, the Green Anole lizard is the only native species which dwells in trees and shrubs, so researchers will be using the invasive species (Bartlett and Bartlett 2001). Green and Brown Anole lizards can reach up to 12 inches in the wild, but usually will stay around 9 inches in captivity due to the conditions they are exposed to while captured such as: not having adequate lighting, food, and space (Bartlett and Bartlett 2001). The reason these lizards need adequate lighting is that there is only so much an artificial light can do for an organism. Non artificial light is extremely important in both plants and animals. For example, plants need sunlight to complete photosynthesis, producing oxygen. Also, sunlight helps organisms to produce vitamin D. If organisms are only exposed to artificial light, there will be a limited supply of vitamin D even with the UV lights that are used. This dilemma will cause the lizards to be shorter in length. Space and food would inhibit growth because in the wild there would be more food available and more space to live in. Space, food and artificial lighting would all be limiting factors in this experiment.

The question that is going to be studied here is whether or not juvenile female and male Anole lizards demonstrate sex recognition. The researchers hypothesize

Table 1 - Table that allows researchers to assign point values to displays shown by the anole lizards, this graph will be used for each trial. The point values for displays made by the lizards are as follows: Head bobbing is 4 points, Approach/retreat is 8 points, Perch shift is 1 point, Tongue touch is 5 points, Engorged throat is 3 points, Sagittal expansion is 2 points, Dewlap Expansion is 6 points and Attack is 10 points.

Display:	Minute 1	Minute 2	Minute 3	Minute 4	Minute 5	Minute 6	Minute 7	Minute 8	Minute 9	Minute 10
Head Bobbing										
Approach/retreat										
Perch Shift										
Tongue Touch										
Engorged Throat										
Sagittal Expansion										
Attack										
Dewlap Expansion										
Total displays:										
Total points:										

that the lizards will demonstrate sex recognition at this age through behavioral displays and that these displays will occur the most during the male v male trials and the female v female trials (Lovern and Jenssen 2001) both from the female and male. These displays will not only signal if they are attracted, or aroused by the opposite sex, but also if they can identify competition between the males, which would lead to displays of aggression. The researchers intend to record all behaviors listed above. Since the resources are limited in each tank, while the lizards are not being watched for mating displays, it is possible for them to display acts of aggression, even though they are quite young (Greenberg and Crews 1990). The confounding variables in this experiment could be if the lizards display mating behaviors between the same sex or fight when not exposed to the opposite sex. Fighting when not exposed to the opposite sex may be due to fluctuating hormone levels in the lizard's endocrinology, or simply that they are transitioning from adolescence to adulthood. Females do not normally demonstrate aggression. If the lizards display mating behaviors between the same sex, then it will become apparent that they are either the outlier or that they can not determine the difference between male and female yet. The researchers expect that there will be demonstrations of sex recognition in this stage of adulthood in the female and male anole lizards.

The researchers expect the brown anole lizards to be able to demonstrate sex recognition at this age because they will be entering into adulthood. It is to be expected that the lizards will display signs of recognition of the opposite sex. If this occurs then that could suggest that they (the lizards) can recognize the coloration and body distinctions (Figure 1) and would therefore have an increased chance in reproduction which would ultimately favor them in spreading their DNA. This would decrease genetic variability within the population because the lizards able to

Table 2 - Contains the trial points from each trial according to Table 1's description on the point system

Female v. Female

Female 1 v Female 2 total points:	**0**
Female 3 v Female 4 total points:	**207***
Female 1 v Female 3 total points:	**90**
Female 2 v Female 4 total points:	**77**

Table 3 - Contains the trial points from each trial according to Table 1's description on the point system

Male v. Male

Male 2 v Male 1 total points:	**20**
Male 3 v Male 1 total points:	**214***
Male 2 v Male 4 total points:	**46**
Male 6 v Male 5 total points:	**55**
Male 2 v Male 5 total points:	**20**

*= trial stopped early **Male v. Female**

Table 4 - Contains the trial points from each trial according to Table 1's description on the point system

Male 6 v Female 1 total points:	**141**
Male 5 v Female 2 total points:	**20**
Male 4 v Female 3 total points:	**233***
Male 3 v Female 4 total points:	**48**
Male 6 v Female 2 total points:	**59**
Male 1 v Female 1 total points:	**61**
Male 4 v Female 4 total points:	**186***

*=trial stopped early

recognize sex differences would become dominant within the ecosystem. Those lizards (male in this example) would be able to identify male competition and dominate to get the female's attention to spread "his" DNA. If the lizards are not able to demonstrate sex recognition then that would suggest that yearling lizards are too young to be able to reproduce and would need more time to mature.

Results

The trials with the asterisks beside the total points (Tables 2-4) had attacks and the points were altered to reflect the aggressiveness of the trial; altered in this sense meaning that the researchers took the trial that had the most point without attacks which was Male 1 v Female 1 (Table 4). However the averages on the error bar graph (Figure 2) are not statistically different (p = 0.55). Overall there are more displays of aggression between male and female lizards than in trials of the same sex (Table 5).

Discussion

Researchers mimicked a natural environment for the Brown Anole lizards, modifying the lighting and timing to what it would be in their home. The researchers separated the lizards a

month before the trials took place, which ensured that the lizards would not recognize each other from the past tank they were kept in, and made sure that the displays elicited by these lizards were genuine. The displays made by these lizards that would be consistent with courtship would be dewlap expansion which was only present in 2 out of the 16 trials completed making the 2 trials where dewlap expansion occurred statistically obsolete. Also, during the trials where dewlap expansion was observed, one had an attack which caused the trial to end early and the other had more aggression displays than courtship throughout the entire trial. The aggression shown in these trials outshines the courtship displays that were most likely done to size the other lizard up or to show that the lizard recognized the other's dominance and territory.

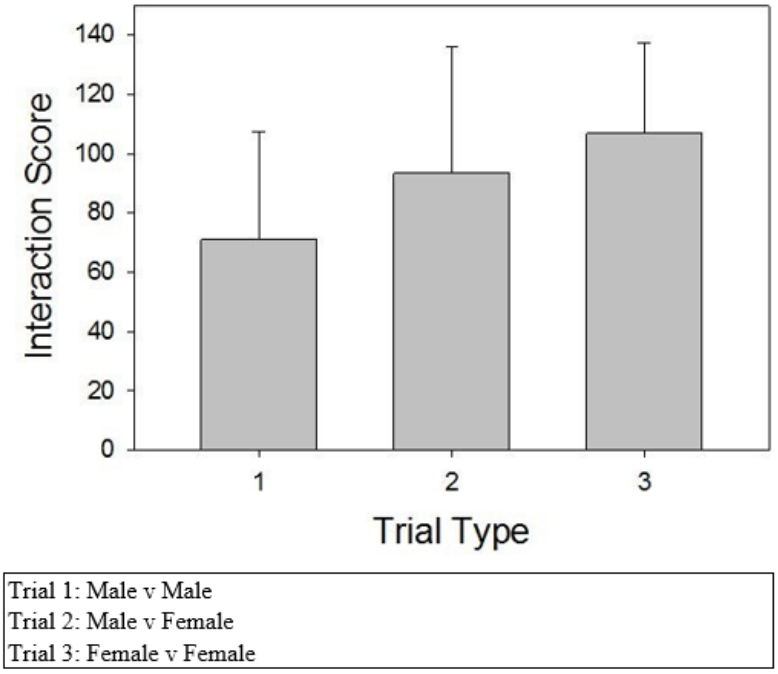

Figure 2 - Demonstrates the correlation between the three different types of trials in the experiment

The trials that were stopped before the 10 minutes were up, was due to an attack made by either the male or female. In the Male 2 v Male 1 trial, male 2 was introduced into Male 1's tank and almost immediately Male 2 hid underneath the rod. Male 1 was alert the whole 10 minute trial and actively searched for Male 2 which was an aggression avoidance. Male 2 recognized Male 1 as an aggressor and backed off to avoid aggression and a possible attack, which suggests that the juveniles in this trial did not in fact recognize each other for the gender they were, but recognized that they were in another lizards territory by the initial responses shown when Male 2 was introduced into the tank. This territorial aggression is consistent throughout all trials and multiple attacks were displayed causing the trials to be stopped early. The attacks happened in all 3 different types of trials which suggests to the researchers that the lizards are not responding to the gender as a stimulus but to the aggression shown from lizards protecting their

Table 4 - The amount of aggressive displays made in each type of trial

	Male v Male	Male v Female	Female v Female
Displays:			
Headbobbing	20	32	15
Engorged Throat	2	3	0
Attack	1	3	1
Sagittal Expansion	7	4	1

territory. Many instances throughout the Female v Male trials, the females would camouflage themselves against the dirt or the top of the cage possibly recognizing the male as a threat.

One reason researchers believe that the lizards were responding to the territorial aggression signs was during Male 4 v Male 2's trial on March 29th, it was noted that the day before during the Male 4 v Female 3, Male 4 attacked the female when she was introduced into his cage, however, during the Male 4 v Male 2's trial when Male 4 was introduced into Male 2's tank, there was very little aggression shown and no attack was made by Male 4. One other trial that was consistent with territorial aggression was Male 4 v Female 4. The female was introduced into the tank and immediately moved to the perch rock where male 4 was sitting.

Male 4 sagittally expanded and then attacked the female twice before the researchers could intervene. This trial was stopped before minute 2 had started which further suggests to the researchers that these lizards have become territorial over their tanks and when an "intruder" comes into the tank, they are responding aggressively to protect "what is theirs." These findings have lead the researchers to reject their original hypothesis that these juvenile lizards would be able to tell the difference between female and male anole lizard and if they are able to, then the sex of the lizards does not matter to them yet. The displays shown are more about protecting their resources and space than courtship (Figure 2).

A follow up study could be done taking out the confounding variable of territorial aggression, by still housing the lizards in the different tanks like the researchers originally did, but when the trials are conducted, the lizards will be placed in a completely new tank identical to the tank they were originally in.

Literature Cited

Bartlett, R.D., and P.P. Bartlett. 2001. Anoles: Barron's Educational Series Hauppauge, NY.

Bezerra, B.M., A.D.S. Souto, L.G. Halsey, and N. Schiel. 2007. Observation of brown-throated three-toed sloths: mating behaviour and the simultaneous nurturing of two young. Journal of Ethology 26: 175-178.

Christian, K.A., and C.R. Tracy. 1981. The effect of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. Oecologia 49 :218-223.

Frazee, S.R., and J.P. Masly. 2015. Multiple sexual selection pressures drive the rapid evolution of complex morphology in a male secondary genital structure. Ecology and Evolution 5: 4437-4450.

Greenberg, N., and D. Crews. 1990. Endocrine and behavioral responses to aggression and social dominance in the green anole lizard, *Anolis carolinensis*. General and Comparative Endocrinology 77: 246-255.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution 36: 1-12.

Levin, D.A. 1971. The Origin of Reproductive Isolating Mechanisms in Flowering Plants. International Association for Plant Taxonomy (IAPT).

Lovern, M.B., M.M. Holmes, and J. Wade. 2004. The green anole (*Anolis carolinensis*): a reptilian model for laboratory studies of reproductive morphology and behavior. ILAR Journal 45: 54-64.

Lovern, M., and T. Jenssen. 2001. The effects of context, sex, and body size on staged social interactions in juvenile male and female green anoles (*Anolis Carolinensis*). Behaviour, 138: 1117-1135.

Rush, M., M. Nightengale, S. Pogue, R. Rogers, and T. Richardson. 2014. Growing plants in artificial lighting conditions: photosynthetic rates of *Dracaena godseffiana* when exposed to various wavelengths. Journal of Introductory Biology Investigations 1.