The Effect of Dewlap Size on Combat Trial Performance in *Anolis sagrei*

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ABSTRACT

Anolis sagrei, or brown anoles, often display their dewlaps during combat with other males. These dewlaps are brightly-colored tissue under their chin that can be expanded by the anole. We sought out to see if the size of these dewlaps was associated with the anole's aggression or dominance during combat. We also wondered if the size of an anole's dewlap could point toward other factors such as their bite force or chance of winning a fight. We tested these ideas by performing a series of tests. We measured bite force on a force transducer, and we determined the area of the anoles' dewlaps by outlining them in an image processing software. We then examined combat trials between similarly-sized sexually mature male brown anoles. Our results showed that dewlap size was positively correlated with both maximum bite force and body length. However, dewlap size was not correlated with the anole's chance of winning or their levels of aggression.

Keywords: Anoles, dewlap size, combat performance, behavior, bite force, body size, dominance, aggression, *Anolis sagrei*

INTRODUCTION

We performed this experiment to determine if dewlap size in *Anolis sagrei*, or brown anoles, was correlated with their aggression or dominance in any way. One study of green anoles showed a correlation between an anole's bite force and the size of its dewlap. Therefore, there is a possibility that an anole's dewlap serves as a signal to other males that they have a strong bite allowing them to assert their dominance (Henningsen and Irschick, 2012). Another study also showed that the dewlap was likely used primarily for signaling threat during territorial and aggressive interactions between males (Ord et al., 2015). Additionally, a study found that males with larger dewlaps tend to exhibit more boldness and thus more dominance (Putman et al., 2019). This study was done to find out if the size of an anole's dewlap was correlated with how much they displayed dominance or how strong their bite was. We hypothesized that the dewlap size would likely be correlated to the behavior and bite force of the anole. This hypothesis was tested extensively. We measured the anole's body and dewlap size, performed bite force tests to measure the maximum force exerted, and had size-matched competitions between the male anoles to examine their aggressive behavior. After these tests were performed, the behaviors studied in the anoles were given numerical values to create an ethogram score that gave a quantitative value to their level of aggression. The higher the anole's ethogram score, the more aggression they showed during combat. In order for the hypothesis to be supported, the anoles overall needed to have consistent changes in behavior and bite force as their dewlap size changed as well.

METHODS

All research was approved under IACUC protocol AS-19-8. For this experiment, we examined the morphology and behavior of sexually mature brown anoles. 63 anoles were

shipped to us. After arrival, we gave the anoles one week to adjust and recover from the shipping process. We began our measurements and tests after this week to ensure the animals had time to recover from stress, poor nutrition, or any other factors. The brown anoles' lengths were measured in millimeters from the tip of their snout to the vent at the base of their tail. They were also weighed to obtain their mass in grams. After these measurements were taken, we took steps to determine the bite force of the anoles. Each anole had their bite force tested three times. Before each bite test, the anole was incubated at 30°C to ensure the anoles' core body temperatures were kept the same and to prevent any other confounding variables. We then had the anoles bite on a custom-built machine that was hooked up to a force transducer and would measure the force applied (Image 1). Each anole performed all three of their bites on the same day, and the anole would spend time in the incubator before each bite test. The peak force measured from each bite was recorded. After all three bites were measured, only the strongest bite recorded was kept.

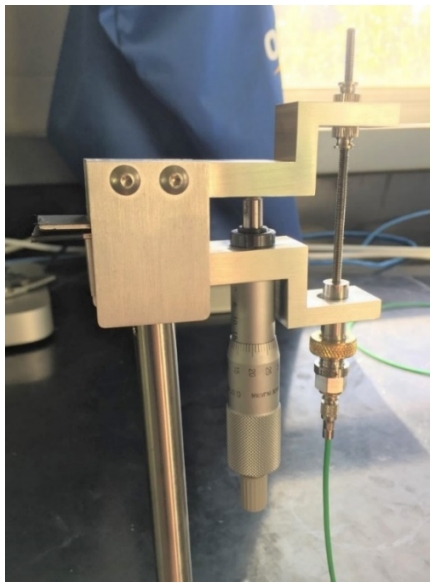


Image 1: This image shows the device used to measure the bite force of the anoles. The anoles would bite the metal piece on the left of the image, and the force was relayed to the transducer.

The next step we performed was photographing the anoles' dewlaps for measuring and processing. We held the anole and used forceps to fully extend the dewlap. The forceps grabbed the cartilage rod on the anole and then pulled up to get this full extension. We took three photographs of the anole's dewlap, and we allowed the dewlap to fully collapse before reextending it. A tripod camera was used to take the pictures, and it was placed 13 inches away from the animal. An example of these images may be seen in Image 2. We placed a 10-millimeter box grid behind the anole to use as a reference when measuring with the computer software, ImageJ. For each image, we set the scale in ImageJ by zooming in on the grid behind the anole until about four or five boxes filled the screen. We then used the line tool to draw a straight line across the four or five boxes. After creating this line, we would select "Analyze" and then click on "Set Scale" from the dropdown menu. Each box on the grid was 10 millimeters, so our line would be either 40 or 50 millimeters depending on if we used four or five boxes respectively. We would type the number "40" or "50" into the "Known Distance" field and type "millimeters" or "mm" for the "Unit Measured" field. Lastly, we would click "OK" to set this as the scale for the image. Next, we selected the polygon tool and outlined the edge of the dewlap. This outline included the yellow margins on the edges. After outlining, we clicked "M" on the keyboard to get the results of the measurement. Next, we repeated these steps to outline the inner red part of the dewlap excluding the yellow margin. After getting both results, we saved the measurements to later be further processed. During this later processing, the area of the inner red part of the dewlap was subtracted from the overall dewlap area. This processing gave us the area of the yellow margin of the dewlap. Three different researchers helped analyze these images to determine the anole's dewlap size. We each measured a different image, and the average of the

three measurements was recorded as the anole's dewlap size. This helped to balance out any slight variations between how we performed our measurements.

Image 2: This is an example of one of the images we used to analyze the anoles' dewlap sizes.

The last test performed on the male brown anoles for this study was a combat trial. These fights occurred in a 10-gallon aquarium with a dowel rod placed in the exact middle of the tank leaning against the back wall (Image 3). Opponents were size-matched to ensure that there was no more than a two-millimeter difference in length between the males. Cardboard dividers were placed in the aquarium to separate it into 3 areas. The day before the combat trials, the males were randomly assigned to start in the left or right end of the tank. Males who were assigned to the left end were marked with a red paint marker, while males on the right end were painted white. This marking was placed dorsally over their pelvis to help with identification during the trial. The markings were also placed the day before combat trials to ensure the human interaction or paint fumes did not affect their performance. Once it was time for combat trials to begin, combatants were placed in the left and right ends of the tank for 15 minutes to reduce the effect of handling on their performance. The dividers were then removed, and the trial began when the

camera started recording. The camera recorded for 15 minutes. Multiple researchers watched these recordings to analyze the behavior of each anole during the trial. Several behaviors were measured and recorded. We counted the number of times each anole did a push-up, dewlap extension, combined push-up with dewlap extension, advanced toward their opponent, retreated from their opponent, bit their opponent, and was bitten by their opponent. Each of these behaviors were given a numerical value and added together to create an ethogram score. The ethogram score rated the level of aggression in the anole. The values given to each behavior for use in calculating the ethogram score may be seen in Table 1. An advance was defined as moving a full-body length toward their opponent, while a retreat was a full-body length move away from their opponent. Each of these movements had to occur in the same plane as the opponent, and it also had to be viewed as a purposeful event rather than just mindless movement in the given direction. A dewlap extension occurred when the throat fan was pushed out to reveal its colors. Push-ups were determined by the anole lifting the front half of its body from neutral and then quickly lowering it. Head bobs were also considered as a push-up. This occurred when the male moved its head in a similar manner to the push-up but did not move its body. A joint push-up and dewlap extension was counted when the male extended the dewlap during a push-up. A bite was defined when an anole put its open mouth on some part of its opponent, and being bitten would occur when the opponent's open mouth contacted the anole's body. Some other behaviors that were recorded, but they did not affect the anole's ethogram score, were the time it took for the anole to display a behavior, how much time the anole spent on the dowel rod, which anole was first on the dowel, and which one was the last anole on the dowel. An anole was considered to be on the dowel rod if they had at least one limb on the dowel. The winner of the

combat trial was determined by whichever anole had the higher ethogram score at the end of the trial.

Image 3a and 3b: 3a.) This image shows the camera set up to record the combat trials between male brown anoles. 3b.) This image shows a close up of the tank where the combat trials occurred.

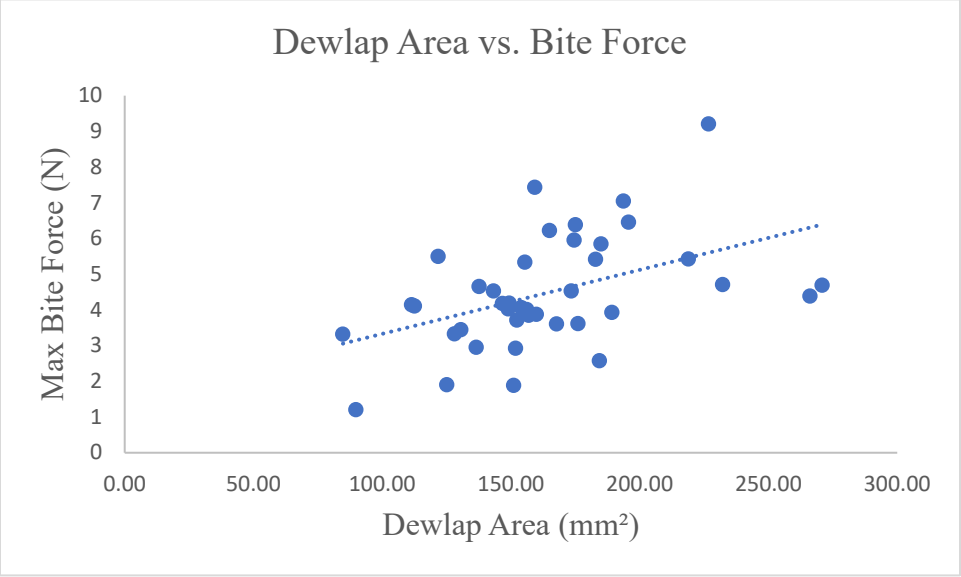
Behavior	Score
Push-Up	+0.5
Dewlap Extension	+0.5
Push-Up with Dewlap Extension	+0.5
Advance Toward Opponent	+1

Retreat From Opponent	-1
Biting Their Opponent	+1
Being Bitten By Their Opponent	-1

Table 1: This table lists the behaviors used to create an anole's ethogram score and their value in calculating the ethogram score.

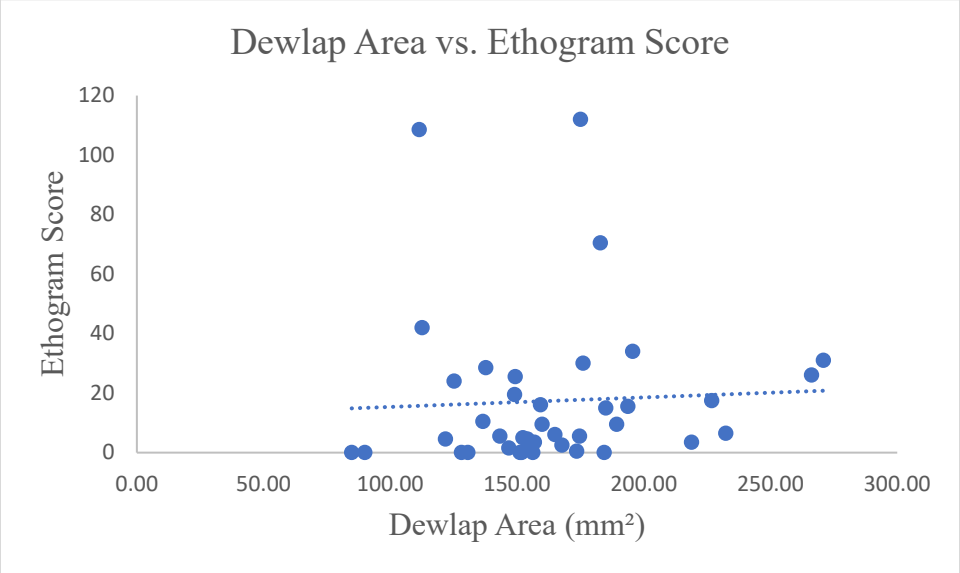
RESULTS

We began our study with 63 sexually mature, male anoles. However, we only got results from 43 anoles. Correlation between dewlap area and maximum bite force had a P-value of 0.003. Thus, it is statistically significant. Pearson's *r* value to show the strength for this relationship is 0.462.



Graph 1: This scatter plot shows the correlation between an anole's overall dewlap area and its maximum bite force.

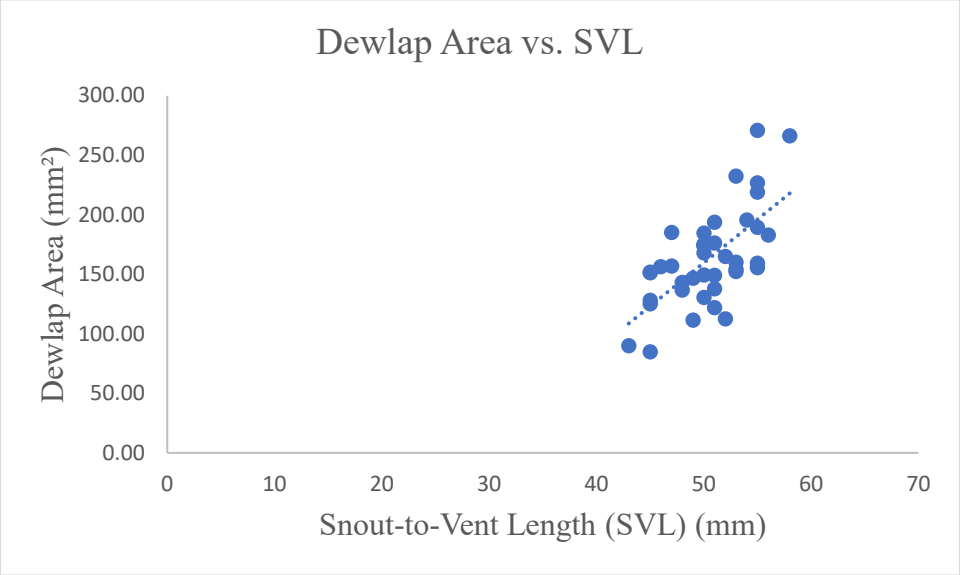
Correlation between dewlap area and ethogram score had a P-value of 0.094. Therefore, it is not statistically significant. Dewlap area and number of push-ups performed by an anole had a P-value of 0.077, and it is not significant either. Dewlap area and number of dewlap extensions also was not statistically significant with a P-value of 0.292. Push-ups and dewlap extensions had a P-value of 0.000 showing a statistically significant correlation.



Graph 2: This scatter plot shows no correlation between an anole's overall dewlap area and its ethogram score.

Dewlap area and snout-to-vent length (SVL) had a correlation P-value of 0.000. This correlation is statistically significant. This relationship had a strength of 0.659 for Pearson's *r*. Snout-to-vent length also had a P-value of 0.000 with the maximum bite force. This relationship had a Pearson's correlation *r* of 0.611. The correlation between snout-to-vent length and ethogram score was statistically significant with a P-value of 0.001 and a Spearman's rho correlation coefficient of 0.483. Ethogram score was also significantly correlated with latency to

display and time on dowel with P-values of 0.006 and 0.000 respectively. The relationship strength for these correlations was -0.419 and 0.643 respectively.



Graph 3: This scatter plot shows the correlation between an anole's snout-to-vent length and its overall dewlap area.

Correlation between winners of combat trials and their ethogram scores had a P-value of 0.000. Winners and the number of push-ups performed had a P-value of 0.001. The number of dewlap extensions correlated with winners with a P-value of 0.001. These results can be seen in Table 2.

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Time on Dowel (s) is the same across categories of Winner?.	Independent-Samples Mann-Whitney U Test	.258[1]	Retain the null hypothesis.
2	The distribution of Latency to Display (s) is the same across categories of Winner?.	Independent-Samples Mann-Whitney U Test	.212[1]	Retain the null hypothesis.
3	The distribution of Ethogram Score is the same across categories of Winner?.	Independent-Samples Mann-Whitney U Test	.000[1]	Reject the null hypothesis.
4	The distribution of PercentCenter is the same across categories of Winner?.	Independent-Samples Mann-Whitney U Test	.285[1]	Retain the null hypothesis.
5	The distribution of # Push-Ups is the same across categories of Winner?.	Independent-Samples Mann-Whitney U Test	.001[1]	Reject the null hypothesis.
6	The distribution of # Dewlap Extensions is the same across categories of Winner?.	Independent-Samples Mann-Whitney U Test	.001[1]	Reject the null hypothesis.
7	The distribution of # Push-Ups & Dewlap is the same across categories of Winner?.	Independent-Samples Mann-Whitney U Test	.146[1]	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

[1]Exact significance is displayed for this test.

Table 2: This table shows the P-values of different variables in winners of combat trials and whether the given P-value rejects or supports the null hypothesis.

DISCUSSION

The results found in the experiment support the hypothesis that the anole's overall dewlap size and its maximum bite force are correlated. Since the P-value for this correlation is statistically significant, it is likely that these values are predictors of each other. The results show that as an anole's overall dewlap size increases, its maximum bite force also increases. However,

the results of the experiment do not support the hypothesis that the dewlap size is predictive of the anole's behavior. We did find that males with larger dewlaps tend to have longer bodies. Males with longer bodies also tend to have a stronger maximum bite force. Additionally, longer males had a higher ethogram score which means they showed more aggressive or dominant behavior. The results also showed that males with a lower ethogram score took longer to display any aggressive behaviors. These results could imply that a male showing off its dewlap size may be signaling to other males how big it is and how hard it can bite as well. The results showed that the winners of combat trials did more push-ups and more dewlap extensions, but they did not do more combination push-ups with dewlap extensions. Additionally, winners and losers did not differ in their latency to display one of the given behaviors, their time on the dowel rod, their bite force, or their overall dewlap size. The fact that winners did not have significantly larger dewlaps supports the idea that the dewlap may be more of a signaling mechanism than a display of dominance or aggression.

Another study had results that support the findings of our study. In the study by Ingram et al., they found that larger dewlap signals did not support speciation (2016). This supports our study's findings that larger dewlaps did not have any correlation with increased ethogram scores or the anole's ability to win a fight. These findings agree with each other because if a trait does not help in combat, or survival, then it is unlikely to have an evolutionary advantage and encourage speciation. A different study supported some aspects of our study and disagreed with others. Vanhooydonck et al. studied *Anolis carolinensis*, or green anoles, which are a relative to *Anolis sagrei*, the brown anoles from our study. In their study, they found that dewlap size and body size were positively correlated in small mature males, but there was no correlation in large mature males. Additionally, their study showed that dewlap size and bite force were correlated in

small mature males, but, again, there was no correlation in large mature males (Vanhooydonck et al., 2005). These differences between Vanhooydonck et al.'s study and our study may be due to species variation. Our study found that dewlap size correlated with both body size and bite force overall regardless of the male's size. It could prove valuable to look deeper into these differences.

Our study had some areas where it could improve. The sample size of 43 male brown anoles could be larger to ensure accurate and precise results across all categories being tested. It would also make some of the results clearer that are on the border of being significant or not. Our study could also be improved by ensuring combat trials occurred in the same size tank for each trial. In our study, some of the tanks varied slightly in size. This slight variation may have allowed an anole more space to be away from their opponent and discouraged them from engaging in more aggressive behavior than if they were more confined. Further studies could be done to investigate more deeply into this topic. One thing that could be researched is if a male's ethogram score during combat with another male is affected by the presence of a female. Additionally, more research could be directed toward other aspects of the male's dewlap and his personality such as dewlap coloration or levels of shyness. Further research could also be done to look at dewlaps and behavior in different life stages and sexes of the anoles like in Vanhooydonck et al.'s study (2005).

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