SEXUAL DIMORPHISM IN NEURON COUNT AND DENSITY IN *ANOLIS CRISTATELLUS*

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Background

- Adaptive radiation of the *Anolis* in the Greater Antilles has resulted in many different *Anolis* species that are morphologically and behaviorally differentiated based on the environment that they occupy (1). It has been suggested that sexual dimorphism plays an important role in ecomorphological diversity due to sexual variation in morphology, habitat preference, diet and behavior (2).
- Previous studies of *Anolis* brain have primarily focused on the relationship between relative brain size in relation to structural habitat (3, 4). However, the link between sexual size dimorphism and neuronal density is poorly understood, even though there is a distinct difference in brain size.

Objective

Evaluate if sexual size dimorphism impacts neuronal density in *Anolis cristatellus.*

Methods

- Anoles were intracardially perfused using PBS, followed by 4% paraformaldehyde.
- The brains were dissected into three regions (cerebellum, telencephalon and rest of brain), and weighed.
  - The rest of brain consists of the diencephalon, mesencephalon, pons and myelencephalon.

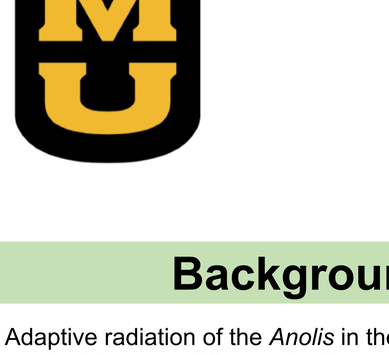

Figure 1: comparison of female (left) and male *Anolis cristatellus brains.*

- Each brain region was put into a 1mL tissue grinder with dissociation solution (1% Triton-X + 40mM sodium citrate) and homogenized for at least 15 minutes.
- The homogenate was collected using a Pasteur pipette into a 15mL centrifuge tube after repeated rinsing.
- The homogenized tissue was centrifuged to isolate the nuclei pellet and remove supernatant. The tube volume was adjusted with PBS to 10^6 nuclei/mL.
- DAPI was added to the tubes at a 20x dilution (2).
- The tube was inverted, and samples were aliquoted onto the hemocytometer and counted under the Leica DM5500 fluorescence microscope.
  - Four or more samples were counted or until coefficient of variance was 0.15 or lower to ensure consistency.
  - Average of counts was calculated from these four values.
  - Erythrocyte nuclei were excluded from count by their shape and their autofluorescence.

Figure 2: Image showcasing size difference between adult male (upper) and female (lower) *Anolis cristatellus*

Results

Table 1: Cell counts and region mass across the brain of female and male *Anolis cristatellus*

Sex:	Mean Body Mass (g)	Region:	Mean Region Mass (mg):	Mean N x10^6:	Mean DN (cells/mg) x10^6:
A. cristatellus male	4.6 ± 0.346	BR (n=9)	0.0457 ± 0.00154	4.87 ± 0.299	0.107 ± 0.007
		TEL (n=10)	0.0168 ± 0.0071	1.83 ± 0.080	0.11 ± 0.0069
		CER (n=9)	0.0017 ± 0.00007	0.753 ± 0.073	0.450 ± 0.038
		ROB (n=10)	0.0269 ± 0.00075	2.26 ± 0.178	0.084 ± 0.0068
A. cristatellus female	1.67 ± 0.133	BR (n=6)	0.0314 ± 0.00181	4.03 ± 0.159	0.131 ± 0.012
		TEL (n=6)	0.0117 ± 0.00094	1.37 ± 0.097	0.122 ± 0.016
		CER (n=6)	0.0015 ± 0.00009	0.659 ± 0.039	0.454 ± 0.047
		ROB (n=6)	0.0182 ± 0.00080	2.00 ± 0.100	0.111 ± 0.0084

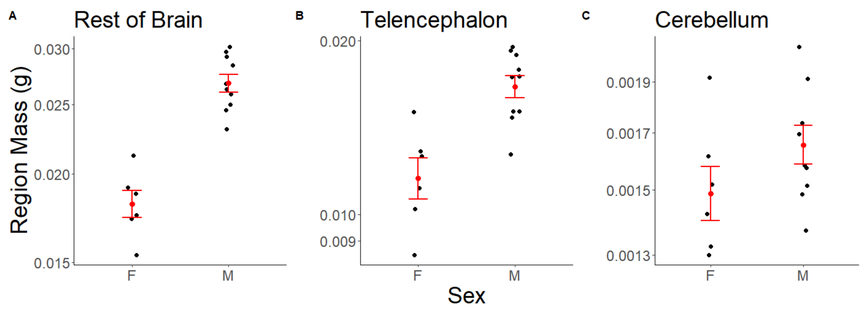

Figure 3: Males and females exhibited differences in region mass in the rest of brain ($p>1.68 \times 10^{-6}$), telencephalon ($p>0.00075$), but not the cerebellum ($p<0.15$). The red dot represents the mean value. The bars represent standard error. The data points are jittered.

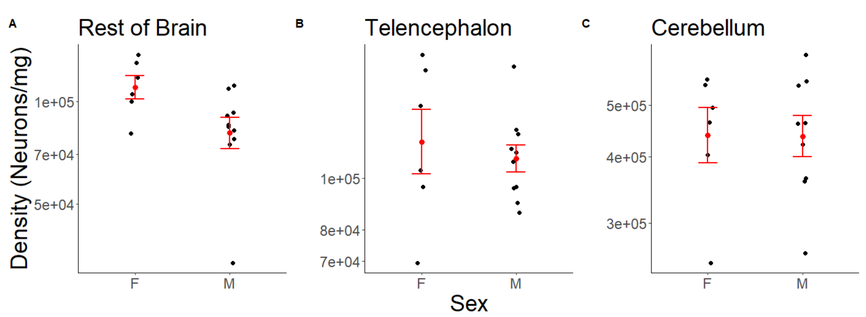

Figure 4: Males and females trended toward significance in neuronal density in the rest of brain ($p<0.06$) but not in the telencephalon ($p<0.59$) and cerebellum ($p<0.98$). The red dot represents the mean value. The bars represent standard error. The data points are jittered.

Methods Continued

- The total number of cells in the solution was then calculated

$$\frac{Cells\ counted}{0.0001\ mL} \text{ x total volume of solution (mL)} = \text{total cells in solution}$$

- The samples were immunoreacted to label neuronal nuclei (NeuN; ABN78C3) and then counted.
  - We counted the proportion of labeled neurons out of 500 nuclei.
- Once the percent of neurons was obtained, that number was multiplied by the total number of cells
  - Values were compared between males and females using ANOVA

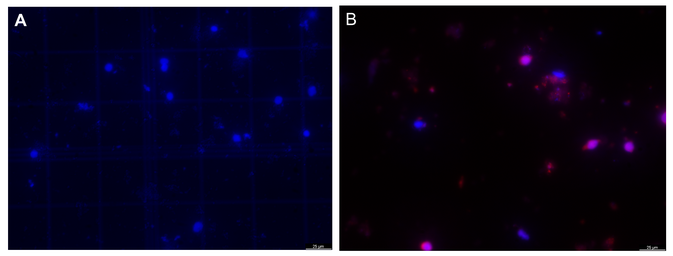

Figure 5: A) Image at 400x magnification showing brain nuclei stained with DAPI within the gridlines of the hemocytometer. B) Image at 400x magnification showing brain nuclei double labeled with DAPI (blue) neuronal nuclei antibody (red)

Conclusion

- Our results show that there is a difference in total brain mass, telencephalon and rest of brain region mass, but not in the cerebellum between male and female *Anolis cristatellus.*
  - The differences in brain mass between sexes is likely due to the differences in body mass.
  - There is not a significant difference in cerebellar mass between males and females
    - Could be due to less variation in cerebellum size as they get bigger in body size
- There is no significant difference between neuronal density in male and female *Anolis* in the telencephalon or cerebellum. However, there is a trend toward significant difference in neuronal density in the rest of brain.
  - Males and females might require the same density of neurons in the telencephalon and cerebellum
  - Females might require larger neuronal density in rest of brain than males

Next Steps

- Collecting more brains to increase our sample size and see if the same trends hold true with more data.
- Looking at the neuronal density of different *Anolis* ecomorphs, and see if different degrees of sexual size dimorphism influences neuronal density

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