429.29 Allometric Scaling of the Number and Size of Purkinje Cells in Galliform Birds

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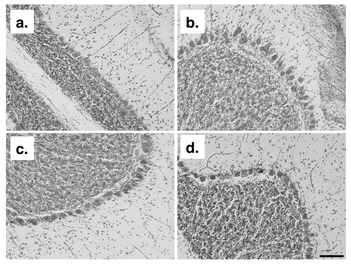
Introduction

Although the neural circuitry of the cerebellum is highly conserved within and among vertebrate groups, the size and shape of the cerebellum varies widely. Across different avian species, there is significant variation in cerebellar size, degree of folding of the cerebellar cortex (foliation) and proportional size of different folia. Among different species, the interspecific variation in foliation is significantly correlated with body size, brain size, and even tool use and nest construction. Having a higher degree of foliation in the cerebellar cortex is correlated with an increase in the number of Purkinje cells, which consequentially results in an increase in processing capacity of the cerebellar cortex. The degree to which body size, brain size and other variables vary with the number or size of Purkinje cells has, however, remained largely uninvestigated. Here, we specifically test whether soma size and number of Purkinje cells varies with the size of the body, brain and cerebellum of seven species of galliforms.

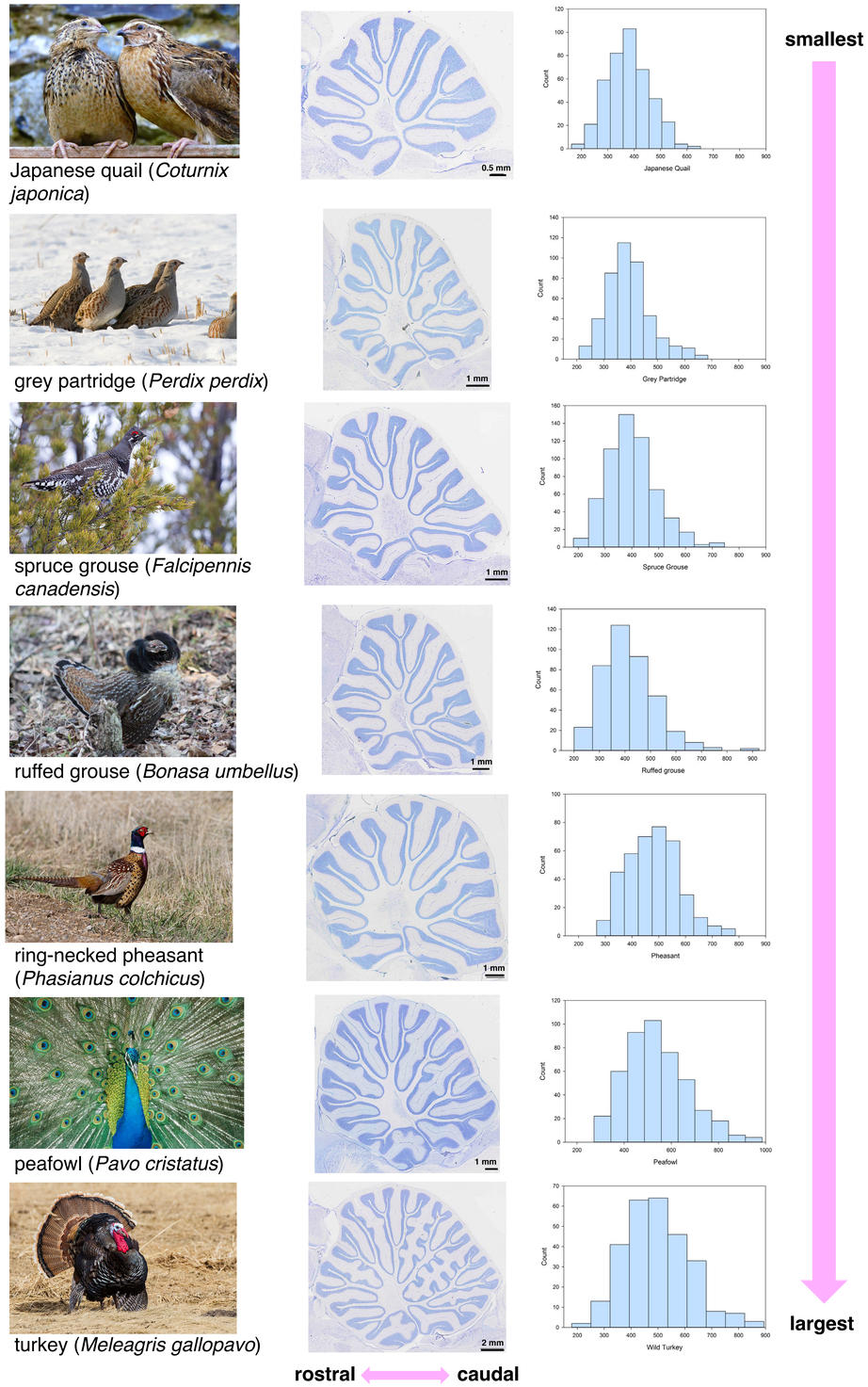
Methods

21 specimens representing 7 species were examined: wild turkey (*Meleagris gallopavo*, n = 2), peafowl (*Pavo cristatus*, n = 3), pheasant (*Phasianus colchicus*, n = 3), ruffed grouse (*Bonasa umbellus*, n = 3), spruce grouse (*Falcipennis canadensis*, n = 4), grey partridge (*Perdix perdix*, n = 3) and Japanese quail (*Coturnix japonica*, n = 3). Each specimen was immersion fixed in paraformaldehyde and the brains sectioned sagittally at 40 microns, and stained for Nissl substance. To measure cell sizes and measure volumes of whole brain and the cerebellum, all slides were then viewed as virtual slides created from z-stacks obtained on an Olympus VS120 slide scanner using a 40x lens. For each specimen, we measured the soma size of 130-150 Purkinje cells throughout the medio-lateral extent of the cerebellum using the interpolated polygon tool in VS-ASW FL Olympus software. We then used the optical fractionator, as implemented in Stereoinvestigator, to estimate the total number of Purkinje cells for each specimen with a 40x lens on a Zeiss Axiocam MRm microscope. Frame size was set at 80x80 microns and grid size changed with size of the species so that a minimum of 800 sampling sites were used per specimen (all CEs were ≤ 0.05).



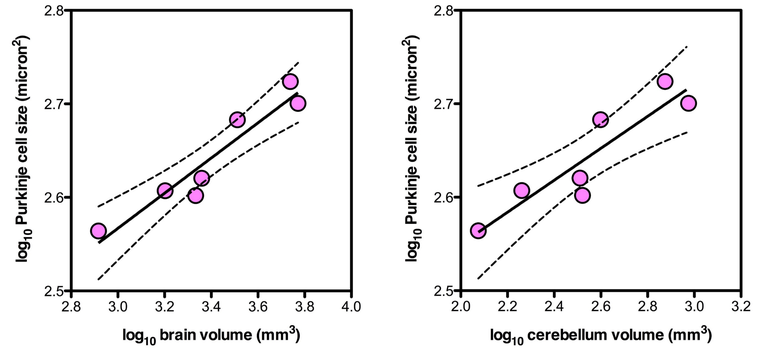
Right: Photomicrographs of the Purkinje cell layer of 4 species: **a.** Japanese quail, **b.** spruce grouse, **c.** ruffed grouse and **d.** turkey. Scale bar = 100 microns.

We used an ANOVA to test for species differences in soma sizes and cell counts. To test for allometric scaling patterns, we performed least-squares linear regressions of average species values for the number and size of Purkinje cells related to brain and cerebellar volume, as measured from the specimens.

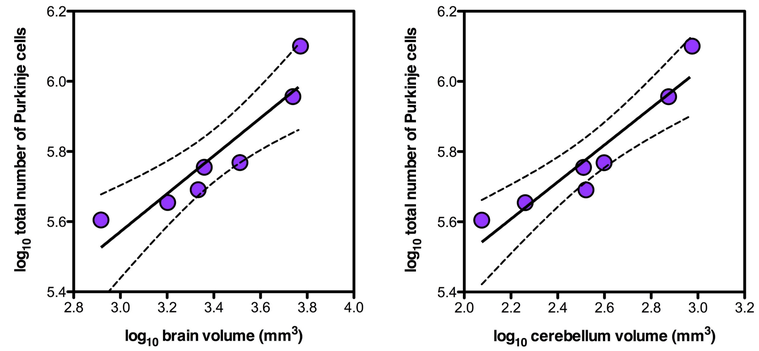


Left: all seven species in order of increasing body/brain size. **Middle:** midsagittal sections through the cerebellum of a representative specimen of each species. **Right:** frequency histograms of Purkinje cell size across specimens for each species. Average cell size varied significantly among species ($F_{6,14}$ = 7.84, $p < 0.01$), with peafowl having the largest cells and Japanese quail having the smallest cells.

Allometric Scaling



The size of Purkinje cell somata increased significantly with both brain (r^2 = 0.91) and cerebellar (r^2 = 0.83) volume across the 7 species. The slopes of the regression lines were both very similar to one another (brain = 0.171 ± 0.035, cerebellum = 0.188 ± 0.027). The dotted lines indicated the 95% confidence interval.



The number of Purkinje cells also increased significantly with brain (r^2 = 0.84) and cerebellar (r^2 = 0.89) volume. The slopes of the regression lines were similar to one another, but were much steeper than that observed for cell size (brain = 0.541 ± 0.105, cerebellum = 0.528 ± 0.084). This indicates that the number of Purkinje cells increases more rapidly than cell size in relation to brain size.

Conclusions and Future Directions

- **both the size and number of Purkinje cells vary with brain and cerebellum size**
- **Purkinje cell size changes at a much slower rate than the number of Purkinje cells**
- **but do these same patterns hold across other birds?**

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We thank Jeremy Corfield and Justin Krilow for assistance with histology and specimen collection, Breck and Caillie for providing specimens and CRC, CFI and NSERC for supporting our research.



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