**Into a rabbit hole: Brain evolution in leporids and statistical artifacts of small sample size in phylogenetic comparative studies.**

**Abstract**

**Introduction**

A paragraph on Leporids in general.

Brain evolution (from vertebrates to leporids) – explain the main hypothesis tested here in this order: seasonality, maternal investment, cognitive, foraging/ecological.

Why olfactory bulb along whole brain.

A few words about stats – Bayesian vs frequentist, imputations, lambda estimates

**Material and Methods**

**Study animals**

Morphometric and ecological data were collected from 61 leporid skulls spanning 18 species within all extant genera of Leporidae housed in the American Museum of Natural History (AMNH), the Los Angeles County Museum of Natural History (LACM) PLUS a bunch of other museums! Information is on Veras computers, not mine! I think I removed Museum info from my files but I have no idea why – there’s a note I’ve made from summer semester mentioning all the other museums my samples were from. NEED TO MAKE TABLE OF ALL INFORMATION I CAN FIND FOR EACH SPECIMEN, could just be a text file for supplementary information!). The samples reflect the phylogenetic coverage in the Kraatz and Sherratt (2016) study, based off the Matthee et al. (2004) leporid phylogeny. All speciemens used were adults, characterised by fully fused occipital sutures (Hoffmeister & Zimmerman 1967).

**Ecological data**

10 ecological and life history variables were collated from existing literature including: activity cycle (diurnal, nocturnal, creposcular), locomotor mode (generalised, saltatorial), diet breadth (three categories), gestation length, home range, geographic range, litter size, burrowing behaviour and seasonality in precipitation and temperature (Kraatz et al. 2015). Pantheria (Jones et al. 2009) was used to source the following variables: adult body mass (grams); diet breadth (defined as the number of host plants used and assigned to three different categories); gestation length (days); home range (km2); litter size (individuals), and geographic range (total extent of species range with a global equal-area projection). Average temperature and precipitation seasonality were compiled for each leporid species within its range. For species with contiguous geographic distributions, at least 10 years of data for monthly temperature and precipitation were sampled from KNMI Climate Explorer database (climexp.knmi.nl) using every available weather station within minimum and maximum latitudes and longitudes within each species geographic range. For species with discontiguous ranges or low/no weather stations in the KNMI Climate Explorer database, monthly temperature and precipitation were sampled manually from weather stations within the species geographic range from Weatherbase (www.weatherbase.com). The average annual seasonality for combined years and all sampled weather stations for both temperature and precipitation within a given species range was defined as:

Where within the geographic range of a given species, *S* is the annual seasonality of temperature or precipitation, *SD* is the standard deviation of mean monthly temperature (oC) or precipitation (mm), and *M* is the mean monthly temperature (oC) or precipitation (mm).  
Temperature values were first converted to absolute temperature by adding 273.15 and after calculation the seasonality using the above formula the results were multiplied by 100. (Source: Dr Peter D Wilson in the public Maxent google group - https://groups.google.com/forum/#!topic/maxent/GyvpH1DlnVA)

**3D reconstruction of endocasts**

Virtual reconstructions of endocasts from [name types of scans here i.e. μct scans] from skulls of the leporid sample (REF) were prepared in Mimics (version number+ref) – check that this is how Brian did the scans! Brain endocasts were prepared through “flood-filling” the cranial cavities of scanned specimens in Mimics and 3Matic (Version number) – again double check that this is true.

**Endocranial volume measurements**

Three metrics of brain volume were used for analyses: Total brain volume; olfactory bulb volume (OB); and total minus olfactory bulb volume (ROB – Rest of Brain). To compartmentalise the brain into OB and ROB, brain endocasts were virtually segmented using the software Mimics (version number - REF). First, the brain stem was digitally removed by placing an arbitrary line along the axial plane from the dorsal cerebellum to the point which removed most of the brain stem without cutting any other brain compartments. This was done to standardise brain stem sizes between endocasts, which may have otherwise confounded volumetric analyses. OBs were digitally separated by the placement of a coronal plane line immediately after the proximal point of the olfactory bulb. Partition volume for the OB and ROB endocast were calculated using Mimics (version number REF).

**Statistical analyses**

All analyses were conducted in the R statistical environment (R Core team 2019) using the following packages for the analysis: ape, MCMCglmm, MulTree, phytools, mice and phylomice, and ggplot2, bayesplot, hdrcde and performance for plotting.

**Phylogeny**

We used a tree provided by Matthee et al. 2004 including the 18 species studied here. The tree had 2 branches with 0 length which had to be resolved due to the requirements of some of the analysis. We did so by adding 0.01% of the median branch length. Subsequently the three was ultrametricized using extension, from the package phytools (citation).

**Imputations**

The dataset contained 3.3% of missing data (with Home Range and Diet Breadth containing 22% missing values and Gestation Length containing 5.5%). No phylogenetic signal in the missingness pattern was detected and detailed description of the missingness pattern is included in the supplementary material.

We imputed 5 datasets using the phylogenetic predictive means matching algorithm in phylomice and the multiple imputed sets were used in models including variables with missing values (Home Range, Diet Breadth, and Gestation Length). For details regarding the imputation algorithm and protocol see (REFS).

**Modelling**

We tested 6 different models described in Table 1.

|  |  |
| --- | --- |
| Model name | Variables |
| Seasonality | Seasonality in precipitation and temperature |
| Maternal investment | Litter size, Gestation length\* |
| Spatial | Home range\*, Geographic area |
| Diet | Diet breadth\* |
| Behavioural (activity) | Locomotor mode, Activity cycle, Burrow |
| Burrowing | Burrow |

*Table 1: Variables included in the models. Each model included body mass as a covariate, and olfactory bulb or rest of the brain were used as dependent variables. Models containing variables with missing data (marked with asterisk) were run on multiple imputed datasets.*

All models were analysed using MCMCglmm and pGLS. Full description of the models tested is included in the supplementary material. Each model was assessed using the performance package (REF) and all met the assumptions for linear regression (see the supplementary material).

For the MCMCglmm models we used uniform and uninformative priors, which assume that all values of the parameters are equally likely. We run the MCMCglmm for 1 000 042 iterations with burn in of the first 100 000 iterations and sampling rate of 300. Each model was run on two chains and resulted in effective sample size of at least 2000. All models converged successfully - Gelman-Rubin criterion < 1.1.

The results from models based on the multiple imputed datasets (5 datasets on two chains) were pooled using Rubin's rules (REF). The fit of all models was compared using the deviance information criterion (DIC). It is an estimator of prediction error, similar to AIC, where the estimate is based on the posterior mean. Only models with substantial posterior distribution above 0, defined as at least 95% above or below 0, were selected as being significant.

For running pGLS we used the package ape, using Pagel correlation structure and omitting all cases with missing values.

**Results**

We set out to test the effect of seasonality on leporid brain evolution, and more specifically, on the variation of OB and ROB volumes. We did not find any support for such effect (see Table 2). We tested 5 other models related to hypotheses explaining the evolution of brain size variation (shown in Table 1) and we only found support for two of them: the pGLS models indicated that leporids with larger home ranges and such that exhibit burrowing behaviour (Figure 1) have larger brains than non-burrowers or species with smaller home ranges. These results included lambda values (indicating the strength of phylogenetic signal in the residuals) that were negative or higher than 1, which violates the definition of lambda per se (see Discussion). Moreover, these results could not be replicated using MCMCglmm modelling.

Figure 1. ANCOVA of total brain volume plotted against body weight of all specimens in the sample (n=61). Different species are indicted in different colours (listed in the legend in increasing order related to body size). Red line indicates burrowing species, and green line non-burrowing. Red X-s mark the mean value per species used in the pGLS and MCMCglmm regressions.



|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **MCMCglmm** | | | | **pGLS** | | | |
| **Model** | **β** | **SE** | **Posterior distribution > 0 (%)** | **DIC** | **t** | **p** | **λ** | **DF** |
| Seasonality  **ROB**  Temperature  Precipitation  **OB**  Temperature  Precipitation | 0.0140  0.0001  0.0527  0.0039 | 0.0544  0.0050  0.0801  0.0075 | 61  51  76  71 | -19.8  -17.1 | 0.48  1.05  0.07  0.01 | 0.63  0.31  0.49  0.59 | -0.33\*  0.58 | 18 |
| Maternal investment  **ROB**  Litter Size  Gestation Length  **OB**  Litter Size  Gestation Length | 0.0833  -0.0069  0.1313  -0.1612 | |  | | --- | |  | |  |   0.0871  0.2757  0.1325  0.3674 | 84  48  85  35 | -19.8  -16.2 | 1.05  -1.80  0.05  -0.29 | 0.31  0.1  0.32  0.78 | -0.38\*  0.58 | 17 |
| Spatial  **ROB**  Home Range  Geographic Area  **OB**  Home Range  Geographic Area | 0.0243  -0.004  0.0093  -0.0145 | 0.0167  0.0142  0.0256  0.0232 | 94  38  66  25 | -28.7  -21.6 | 7.42  -0.1  1.56  -0.55 | **<0.001**  0.85  0.15  0.6 | 1.12\*  0.93 | 14 |
|  |  |  |  |  |  |  |  |  |
| **Model name** | **Beta** | **SE** | **Posterior distribution > 0 (%)** | **DIC** | **t-value** | **p-value** | **Lambda** | **DF** |
| Diet  ***ROB***  Diet Breadth  ***OB***  Diet Breadth | 0.0159  -0.0106 | 0.0939  0.1347 | 58  47 | -21.5  -19.8 | -0.91  -0.76 | 0.39  0.46 | 1.05\*  0.72 | 14 |
| Behavioural (activity)  ***ROB***  Locomotor mode Generalised  Locomotor mode Saltatorial  Activity cycle  Burrow  ***OB***  Locomotor mode Generalised  Locomotor mode Saltatorial  Activity cycle  Burrow | 0.0527  0.0507  -0.1682  -0.0520  -0.0179  0.0816  0.1460  0.0424 | 0.1595  0.1156  0.1396  0.0788  0.2518  0.1842  0.2234  0.1281 | 64  68  11  24  47  68  24  65 | -21.7  -13.0 | 0.25  0.71  1.25  -0.44  -0.26  0.28  1.02  -0.29 | 0.81  0.49  0.24  0.67  0.8  0.79  0.33  0.78 | -0.27\*  0.55 | 18 |
| Burrowing  ***ROB***  Burrow  ***OB***  Burrow | 0.0258  0.0458 | 0.0684  0.1007 | 65  69 | -20.1  -20.7 | 2.57  1.4 | **0.02**  0.17 | -0.33\*  0.72 | 18 |

Table 2, Results from the MCMCglmm and pGLS analysis, ROB – Rest of brain, OB – Olfactory bulb, Asterisk indicates lambda values <0 and >1. SE – standard error, DIC – deviance information criterion.

**Discussion**

We did not find support for any effect of seasonality on the evolution of brain size variation in leporids. Seasonality in temperature or precipitation has previously been shown to have both positive (REFS) and negative (REFS) effects on the evolution of brain size in vertebrates. One reason for the lack of such effect in leporids might be due to the fact that burrowing behaviour compensates for such climatic variation, and as such appears to be a selection force behind increase in brain size. This is in line with the cognitive buffering hypothesis (REF) and is also additionally supported by our finding that species with larger home ranges have larger brains. (ELABORATE ON BURROWING IN LEPORIDS)

We did not find support for any maternal investment effect limiting brain size evolution in leporids, as measured by litter size and weaning age. This might be due to the fact that our sample is relatively homogenous in terms of these two reproductive variables, and whilst such effect has been shown in larger clades (marsupials, etc REF), focusing on a family level, such effect might not be detectable. (ELABORATE ON THIS WITH A FEW MORE SENTENCES)

Additionally, our activity model was not supported either (including locomotor mode and activity cycle) indicating that leporid species, being mainly terrestrial are unable to buffer any environmental effects relating to brain size, besides using burrowing behaviour.

The size of the olfactory bulb in leporids did not seem to be related to any variable in any of our models. (EXPLAIN)

One observation in our pGLS models was the fact that in all models including ROB the lambda estimates were unrealistic. This is a common situation in many phylogenetic comparative studies (lizard paper, other REFS) that does not receive enough attention from scholars in the field. Lambda, as a measure of phylogenetic signal in the residuals, can only take values between 0 (indicating lack of phylogenetic signal), and 1, so any value larger than 1 or lower than 0 is a result of a flawed maximum likelihood estimation and is unrealistic. As shown on graph XXX, in all pGLS models including ROB the estimated lambda values’ maximum likelihood always falls outside the 0-1 interval. This is most probably due to the small sample size, which renders all lambda estimates unreliable. This and other cases of the same issue (REFS) warrant caution when using small sample sizes in phylogenetic comparative studies, as then, maximum likelihood estimations become unreliable. Such suggestion is supported by the fact that none of our MCMCglmm models confirmed the results obtained by pGLS, as MCMCglmm uses similar maximum likelihood algorithm to obtain probability densities of the posterior distribution. Obviously, with a sample size of 18 species, the available data were not enough for conclusive analysis using Bayesian inference. Unfortunately, the limitations of sample size are common in many comparative studies (REFS), and while such analyses lay a foundation for further inquiries after more extensive data collection, they should be taken with a grain of salt. Authors of such studies should pay more attention to the statistical artifacts arising from sample size limitation and analyse them in more details before reporting their results. PROPOSE A METHODOLOGICAL STEP BY STEP SOLUTION.

Conclusion