**Down a rabbit hole: Burrowing behaviour and larger home ranges are related to larger brains in Leporids**

Orlin S. Todorov 1, Coen Hird 1, Brian Kraatz 2, Emma Sherratt 3, Narelle Hill, Jonathan Briganti, Alexandra A. de Sousa 4, Simone Blomberg 1, Vera Weisbecker 5

1 School of Biological Sciences, The University of Queensland, St. Lucia, Queensland

2 Department of Anatomy, Western University of Health Sciences, Pomona, CA, United States

3 School of Biological Sciences, The University of Adelaide, Adelaide, SA, Australia

4 Centre for Health and Cognition, Bath Spa University, Bath, BA2 9BN, UK

5 College of Science and Engineering, Flinders University

**Abstract**

Studies on the evolution of mammalian brain size variation usually focus on larger clades encompassing broader phylogenetic groups. This risks the introduction of ‘noise’ in the results, often obscuring effects that might be detected in less inclusive clades. In the current study we focus on a sample of 18 species of Leporids from 60 individuals, and test five different hypotheses related to endocranial volume (approximating brain size) evolution in mammals. This includes the evolution of the whole brain, as well as the olfactory bulb. We also address a pervasive issue in comparative phylogenetic studies by dealing with missing data via multiple phylogenetic imputation as to conserve the full sample size for all subsequent analyses.

Our analyses show that home range and burrowing behaviour are the only predictors of brain size variation in this clade. Additionally, the evolutionary increase in litter size (often found as a constraint in brain size evolution) is related to increased seasonality in temperature therefore tentatively masking the constraining effect of litter size on brain size in leporids. Unreasonable estimations of phylogenetic signal (Pagel’s lamba) additionally warrant caution when using small sample sizes in comparative studies.

**Introduction**

There are several non-mutually exclusive hypotheses that attempt to explain the evolution of brain size variation within mammals. Some of these relate to selection pressures that might be imposed on the individual [1-3] or social level [4]. Others emphasize the metabolic and developmental constrains on brain size [5, 6]. Lastly, some emphasise the effects of intelligence and culture as capable of mitigating both selection and constraints [7-10]. Among them, the cognitive buffer hypothesis (CBH) is probably the most general: it posits that larger brains have an adaptive function of buffering individuals against environmental challenges and might facilitate mitigating selection pressures and constraints by construction of novel behavioural responses [9].

The study of brain size variation can be impacted by high levels of ‘noise’ when studying patterns across all mammals as one taxon [11-13]. This is particularly the case when the inquiries are focusing on whole brain size, instead of certain brain partitions [14]. Therefore, testing different hypotheses might be best done by focussing on smaller clades and on different brain areas. Lagomorpha (rabbits, hares, and pikas) are particularly suitable in this respect. They are a mammalian order part of the superorder Euarchontoglires (rodents, lagomorphs, treeshrews, colugos, and primates) and are comprised of two extant families, the Ochotonidae (pikas) and the Leporidae (rabbits and hares). There are 109 extant species of lagomorphs, including 34 species of pikas, 42 species of rabbits, and 33 species of hares [15]. Lagomorphs appear in the fossil record around 52 Ma in present day China and Mongolia [16, 17] but currently the order is globally distributed, inhabiting every continent except Antarctica. They have a uniform body-plan that has been conserved through evolution and they inhabit forests, open scrub, or savannah in Eurasia, Africa, North, Central, and northern South America, and have also been introduced as an invasive species in Australia. Leporids exhibit different locomotor modes (saltatorial, generalist) but have evolved increased cursoriality [18]. They can reach very high maximal speeds of up to 72 km/h in *Lepus europaeus* and *Lepus alleni* [19].

A unique trait that sets leporids apart from other terrestrial mammals is cranial kinesis - the ethmoid-orbital and otic-occipital parts of their braincase are partially separated laterally by a piriform fenestra that passes subvertically along the posterior border of the os alisphenoideum and os squamosum [20] allowing substantial movement of skull bones relative to each other. Typical for snakes or water and suction feeding organisms, this adaptation in hares has been shown to be related to the demands of leporid’s locomotor behaviour, dissipating kinetic energy between the loading cycles of cursorial locomotion [21]. This adaptation might be related to different factors affecting brain evolution in the lineage. Leporids have been domesticated by humans in the 5th century in Europe by French monks, and used as a food source so the domesticated lineages have been mostly selected for body size [22]. All extant leporids are exclusively herbivorous and minimally sexually dimorphic. Also, as an important confounder of brain size variation, leporids have short gestation periods between 24–55 days, and there are both altricial and precocial species. Rabbits and hares are often under very high predation pressure which is related to both their high litter sizes and abundance cycles [23]. This results in around a 50% mortality rate within litters [24] which can also be attributed to infectious diseases and environmental variability (mainly annual rainfall) [25].

Within lagomorphs, the only species that has been studied extensively in terms of brain structure and function is the domesticated European rabbit (*Oryctolagus cuniculus*) and thus little is known about the evolution of the lagomorph brain [18]. Breeds of domesticated rabbits have been used in studies of timing of brain development [26], development of the visual cortex and neuronal morphology [27], brain anatomy [28] and the effects of domestication on the brain [29]. Despite the focus on the domesticated rabbit, larger scale studies on brain evolution in lagomorphs have not been performed. Leporids present a good system for studying brain evolution [18] as they are a homogeneous family with a similar diet, locomotion mode, comparable gestation periods and at the same time are distributed across many different habitats on each continent. They are also under high predation pressure and are subject to environmental variability which allows for the testing of hypotheses about seasonality. Given that olfaction is one of their main senses [30, 31] and the olfactory bulb (OB) is easily visible on endocasts, we measured the volume of the OB and the rest of the brain (ROB) in a sample of leporids from Kraatz et al. (2015)

The first virtual endocast of a fossil lagomorph, *Megalagus turgidus*, was described by López-Torres et al. [32]. It had larger OBs relative to its endocranial volume compared to extant leporids. The frontal lobes of *Megalagus* are wider than those observed in modern lagomorphs and not as expanded rostrally. Similar to extant leporids, the endocast of *Megalagus* exhibits only a lateral sulcus on the neocortex on an otherwise lissencephalic cerebrum, meaning brain anatomy in the lineage is consistent throughout evolution and has been stable since the emergence of stem lagomorphs around 42 Ma.

The primary neurological system processing chemical molecules in mammals is the OB comprised of the main and accessory olfactory systems [33]. The OB is one of the earliest structures to evolve in complex central nervous systems [34]. It is heavily involved during sexual and social behaviours, as well as in spatial orientation [35] and it has a different neuronal scaling relationship from the rest of the brain [36, 37]. Moreover, it is extremely plastic through ontogeny [38] and highly variable in size and structure across vertebrates. Previous research has shown that in different mammalian lineages it has undergone both reduction and expansion, independent of the rest of the brain [37, 39]. Additionally, the evolution of the OB has been shown to be related to the evolution of thermoregulatory function in some mammals [40]. Moreover, olfactory behaviour, mediated by the neural circuit devoted to olfaction has been shown to be important for processing spatial and social information from the environment in mammals and birds [41, 42]. This suggests the hypothesis that OB size and structure is related to social and spatial information processing in lagomorphs.

Leporids have a highly developed and conserved double path vomeronasal structure and its cellular structure is highly variable among species and between sexes [43]. Previous studies have shown that in mammals, rabbits represent one of the best models for studying chemocommunication (González-Mariscal, Caba, Martínez-Gómez, Bautista, & Hudson, 2016) and they remain the only mammal species in which a mammary pheromone (2-methylbut-2-enal) has been comprehensively characterised [44] . This pheromone is released by lactating females to awaken rabbit neonates and initiate the nipple-sucking reflex. Pheromones are a key means of communication in rabbits and are important in submissive and dominant behaviours [45]. Due to rabbits often being prey animals, olfaction is an essential sense for the detection of danger, predators, and mates [46].

We set out to test five models of brain evolution using a sample of 18 species of leporids derived from 60 individual virtual endocasts provided by Kraatz et al. (2015) [47]. Our first hypothesis is related to the CBH and we expect seasonality (as measured by seasonal variation in precipitation and temperature) to have a negative relationship with both the olfactory bulb (OB) and the rest of the brain (ROB) in leporids because of the challenges imposed by unpredictable food sources and sheltering opportunities and predator behaviour. Alternatively, if seasonality does not affect brain size in this family, this might be due to alternative behavioural strategies like burrowing, expansion of home or geographic range. Another set of hypotheses are related to maternal investment, where we expect litter size and gestation length to be negatively related to brain size [12, 48, 49], and dietary/ecological pressures as measured by diet niche breadth [1]. Previous studies have provided conflicting findings regarding the relationship between brain size variation and diet in other mammals [3, 50]. We focus on the OBs due to their prominence and the ease of measurement from brain endocasts, in addition to their important functional involvement in odour communication and spatial orientation [35, 51-53]. Moreover, it has been shown that the OBs in mammals do not follow the same allometric scaling relationship to body size as the ROB [37] thus making it a good candidate for brain evolution studies.

We expect that the OB and the ROB in leporids will be under different selective pressures, and while the ROB will be more constrained by developmental factors, the OB will be more sensitive to selection pressures, namely seasonality. Seasonality (especially in precipitation) has been shown to affect the perception of chemical cues, whereas environments with higher precipitation would not be conducive for olfactory orientation due to the reduced transmission of volatile chemical cues. On the other hand, it might be that leporids can buffer the effects of seasonality through behavioural and cognitive adaptations, like burrowing or activity cycle adjustment (diurnal vs nocturnal).

**Material and Methods**

All data and code are available online on https://github.com/orlinst/Leporid-brain-evo

**Study animals**

Morphometric and ecological data were collected from 60 leporid skulls spanning 18 species within all extant genera of Leporidae. The specimens were collected mostly from the American Museum of Natural History (AMNH) and The Los Angeles County Museum of Natural History (LACM). The samples reflect the phylogenetic coverage in the Kraatz and Sherratt [54] study, based off the Matthee et al. leporid phylogeny [55]. All specimens used were adults, characterised by fully fused occipital sutures [56]. For full detail on the origin of the skull specimens, refer to [54].

**Ecological data**

Ten ecological and life history variables relevant to our hypotheses were collated from existing literature including: activity cycle (diurnal, nocturnal, crepuscular), locomotor mode (generalised, saltatorial), diet breadth (three categories as defined by the number of host plants used, categorised by PanTHERIA [57]), gestation length, home range, geographic range, litter size, burrowing behaviour and seasonality in precipitation and temperature [47, 58]. PanTHERIA was used to source the following variables: adult body mass (grams); diet niche breadth; gestation length (days); home range (km2); litter size, 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 the coefficient of variation:

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.

**3D reconstruction of endocasts**

Virtual reconstructions of endocasts from Micro-CT scans obtained by [54] (voxel size between 125–160μm) from skulls of the 60 leporid specimens were prepared in Mimics version 18 (Materialise NV, Leuven, Belgium, 1992–2020). Brain endocasts were prepared through “flood-filling” the cranial cavities of scanned specimens in Mimics and 3Matic.

**Endocranial volume measurements**

After measuring total brain volume the endocasts were divided into olfactory bulb volume (OB); and total minus olfactory bulb volume (Rest of Brain or ROB). To compartmentalise the brain into OB and ROB, brain endocasts were virtually segmented using the software Mimics. First, the brain stem was digitally removed by placing a line along the axial plane from the dorsal cerebellum to the point which removes most of the brain stem without cutting any other brain compartments. This was done to standardise brain stem size between endocasts, which may otherwise confound volumetric analyses. OBs were digitally separated by the placement of a coronal plane surface plane immediately after the proximal point of the olfactory bulb. Partition volume for the OB and ROB endocast were calculated using Mimics.

**Statistical analyses**

All analyses were conducted in the R 4.1.1 statistical environment [59] using the following packages for the analysis: MCMCglmm [60], MulTree [61], ape [62], phytools [63], mice [64], phylomice (Drhlic P, Blomberg S.P, unpubl. Available from SPB), and naniar [65], ggplot2 [66], bayesplot [67], hdrcde [68] and performance [69] for plotting.

**Phylogeny**

We used a tree derived by Matthee et al. [55] including the 18 species studied here (Figure 1). The tree had two polytomies that needed to be resolved due to the requirements of some of the analyses. We did so by adding 0.01% of the median branch length. Subsequently the tree was ultrametricized using the function extension, from the package phytools.

**Imputation**

Testing hypotheses related to brain evolution within small clades inadvertently suffers from issues related to sample size, constraining the scope of such inquiries. Issues related to limited sample sizes include low statistical power, faulty estimation of various model parameters, and limited scope of the analytical conclusions. Because of the scarcity of data, both of anatomical traits and traits related to life-history and ecology, many studies omit whole cases due to one or a few missing traits, as most statistical comparative methods cannot deal with missing data. One way to solve this pervasive issue is through multiple imputation [70], and in this study, we have used phylogenetic multiple imputation, so we can conserve the complete sample (n=18) for all analyses.

Our 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 as shown by our analysis using the sensiPhy package. The detailed description of the missingness pattern is included in the supplementary material.

We imputed 4 datasets using the phylogenetic predictive mean 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 [12, 70-73]. Analysis of the pattern of missingness are included in the supplementary material and have been conducted using the R package naniar.

**Modelling**

We tested five different models as described in Table 1.

*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.*

|  |  |
| --- | --- |
| 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 |

All models were analysed using MCMCglmm (imputed datasets) and pGLS (complete cases with case-wise deletion included in the supplement). A full description of the models tested is included in the supplementary material. Each model was assessed using the performance R package and all met the assumptions for linear regression (see the supplementary material).

For the MCMCglmm models we used improper, uniform, uninformative priors, which assume that all values of the parameters are equally likely. We ran the MCMCglmm for 1 000 042 iterations with a burn-in of the first 100 000 iterations and a 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 [74].

The results from models based on the multiply-imputed datasets (two parallel chains of MCMC on 4 imputed data-sets) were pooled using Rubin's rules [70]. The fit of all models was compared using the deviance information criterion (DIC) [75]. This is an estimator of prediction error, similar to AIC, where the estimate is based on the posterior mean. Only model parameteres with substantial posterior distribution away from 0, defined as at least 95% above or below 0, were selected as being significant.

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

Chart, bar chart

Description automatically generated

Figure 1. Phylogenetic tree of the 18 species used in the study. Black circles indicate burrowing species, red bars – log (Brain size) and blue bars log (Home range). For species with no data for home range only brain size is displayed.

**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 an effect (see Table 2). We tested 4 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: leporids with larger home ranges that exhibit burrowing behaviour have larger brains than non-burrowers (Figure 2) or species with smaller home ranges. Our pGLS analysis (see Supplement) included Pagel’s 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).

Table 2, Results from the MCMCglmm analysis on the full MI dataset: ROB – Rest of brain, OB – Olfactory bulb, β – regression coefficient, SE – standard error, PD > 0 – posterior distribution above 0 in percentage, DIC – deviance information criterion.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **MCMCglmm** | | | |
| **Model** | **β** | **SE** | **PD > 0** | **DIC** |
| Seasonality  **ROB**  Temperature  Precipitation  **OB**  Temperature  Precipitation | 0.0005  -0.0007  0.0336  0.0032 | 0.052  0.005  0.0791  0.0075 | 51  44  67  68 | -25.8  -22 |
| Maternal investment  **ROB**  Litter Size  Gestation Length  **OB**  Litter Size  Gestation Length | 0.0242  0.003  0.0324  -0.0024 | 0.0345  0.007  0.054  0.012 | 77  67  74  41 | -28.3  -22.3 |
| Spatial  **ROB**  Home Range  Geographic Area  **OB**  Home Range  Geographic Area | 0.0534  -0.006  0.0628  -0.0195 | 0.0179  0.012  0.0285  0.0206 | **100**  30  **98**  16 | -42.8  -38.3 |
| Diet  ***ROB***  Diet Breadth  ***OB***  Diet Breadth | 0.0858  0.0661 | 0.0939  0.1189 | 88  73 | -26.8  -23.9 |
| Behavioural (activity)  ***ROB***  Locomotor mode Generalised  Locomotor mode Saltatorial  Diurnal  Nocturnal  Burrow  ***OB***  Locomotor mode Generalised  Locomotor mode Saltatorial  Diurnal  Nocturnal  Burrow | -0.0902  0.0106  0.1243  -0.0502  0.1775  -0.2017  0.0079  0.1948  -0.0221  0.1728 | 0.1135  0.0814  0.0994  0.1037  0.0540  0.2262  0.1560  0.2111  0.2094  0.1094 | 20  55  90  30  **100**  17  52  84  45  94 | -35.1  -24.8 |



Figure 2. ANCOVA of total brain volume plotted against body weight of all specimens in the sample (n=61). Different species are indicated in different colours (listed in the legend in increasing order related to body size and the n per species in brackets). The red line indicates burrowing species, and the green line non-burrowing species. Red X-s mark the mean value per species used in the pGLS and MCMCglmm regressions.

**Discussion**

We set out to test 5 different hypotheses related to brain evolution in leporids: we expected seasonality (as measured by seasonal variation in precipitation and temperature) to have negative effects on both the OB and the ROB or alternatively, if seasonality does not affect brain size, alternative behavioural strategies like burrowing, expansion of home or geographic range were expected to relate to an increase in brain size. Another set of hypotheses we tested were related to maternal investment, where we expected litter size and gestation length to be negatively related to brain size, so as dietary/ecological pressures as measured by diet breadth

We did not find support for any effect of seasonality on the evolution of brain size variation in leporids. On the other hand, our analysis indicated that the only correlates of brain size in this family are burrowing behaviour and home range. Seasonality in temperature or precipitation has previously been shown to have both positive [8] and negative [76, 77] effects on the evolution of brain size in vertebrates, but we were unable to detect any effect in our sample. One reason for the lack of such an effect in leporids might be due to the fact that burrowing behaviour compensates for climatic variation, and as such, could be a selection force responsible for an increase in brain size. Another possible mechanism for overcoming seasonality pressures due to unpredictability of food sources and predatory pressure might be through extending home ranges which is also supported by our findings. This is in line with the cognitive buffering hypothesis [9] where individuals can overcome some environmental challenges by behavioural and cognitive adaptations.

Our analyses do not support the notion that species with larger geographic ranges are better at avoiding seasonality selective pressures related to brain size. The only variable that is positively related to brain size from out spatial model is the species’ home range. Despite leporid home ranges being relatively small (from 0.004 to 0.86 km2) relatively larger home ranges might provide better an opportunity for niche construction through burrowing, predator avoidance and other opportunities related to seasonality buffering [78].

The fact that the size of the olfactory bulb was shown to be related to the size of the home range in leporids is consistent with the finding in mice that processing of olfactory information is related to processing of spatial information [79]. It has also been previously shown that the size of the olfactory bulb is related to home range in carnivores [51] and this confirms that larger olfactory bulbs are beneficial in maintaining extended home ranges and mitigation of predation and seasonality pressures. However, the exact mechanism behind this relationship in not yet clear.

We did not find support for any maternal investment effect (as measured by litter size and weaning age) limiting brain size evolution in leporids. This is unexpected because many previous studies have found that litter size correlates negatively with brain size. This might be due to the fact that our sample is relatively homogeneous in terms of these two reproductive variables, and while such an effect has been shown in larger clades (marsupials [12] and mammals in general [48]), focussing on the family level, such an effect might not be detectable in out small sample. Additionally, the limiting effect of maternal investment on brain size variation might be ubiquitous and uniform in most leporids, as in the lineage, larger litter sizes are maintained to buffer environmental variability, predation and infectious disease [78]. It has previously been shown that an increase in seasonality is related to an increase in litter size, at least in rodents [80]. We confirmed this in our sample of leporids (pGLS Litter Size ~ Seasonality in temperature p=0.0336, beta=0.66, t=2.32, λ=1.07, df=16; but no relationship to Seasonality in precipitation (p=0.64, t=-0.48, df=16). This might indicate that this is one of the mechanisms that has evolved to buffer seasonality in rodents and leporids - instead of selecting for cognition, the reproductive rate has increased.

Additionally, our full activity model was also not supported (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, and thus differences in activity mode are not related to brain variation.

Surprisingly, in most of our pGLS models (included in the Supplement) including ROB and one including OB, the lambda estimates were unreasonable. This is a common situation in many phylogenetic comparative studies [81] but it is often ignored. Pagel’s lambda [82], as a measure of phylogenetic signal in the residuals, can only take values between 0 (indicating lack of phylogenetic signal), and 1 (indicating fit with Browninan motion), so any value larger than 1 or lower than 0 is a result of a flawed restricted maximum likelihood estimation and therefore unrealistic. However, in all pGLS models including ROB the estimated maximum likelihood lambda values always fell outside the 0-1 interval (see Supplement graphs). This is most probably due to the small sample size, which renders all our lambda estimates unreliable. This and other cases of the same issue [81] warrant caution when using small sample sizes in phylogenetic comparative studies, as then, maximum likelihood estimations become unreliable. Unfortunately, the limitations of sample size are common in many comparative studies, and while such analyses lay a foundation for further inquiries after more extensive data collection, they should be taken with substantial caveats. Authors of such studies should pay more attention to the statistical artefacts arising from small sample size limitation and analyse them in more detail before reporting their results [83]. One potential solution to this problem is doing the analysis assuming Brownian motion (λ = 1) and verifying whether the outcome of the regression with the flawed estimate corresponds to the one with the fixed lambda value.

Despite our sample size being small (n=18) it comprises 25% of all extant leporid species (n=75) and the data have been collated based on measurements from 60 specimens (see Fig 2 per number of specimens per species). Measuring brain partitions from endocasts can be challenging and given the quality of our sample, we were only able to measure the size of the OB along the whole endocranial cavity (but see [84]). Future data collection and inquiries related to brain evolution will benefit from higher resolution imaging techniques (such as CT scans and MRIs) which might facilitate measurements of other brain partitions which will in turn allow for testing hypotheses related to the mosaic evolution of the brain.

Besides the scarcity of anatomical data, there is very limited behavioural, ecological and life history data on leporids. Further effort should be directed into collecting data on various aspects of the life history and ecology of this family, and the whole order Lagomorpha as to facilitate further comparative studies.

Acknowledgements:

Supplementary material:

1. Table with the MCMCglmm (imputed) and pGLS (list-wise deletion) analyses
2. Graphs of the likelihood estimations of all the flawed lambda estimates
3. Analysis of the missingness pattern
4. Graphs of meeting requirements for linear regression of the pGLS models
5. Density distributions and model evaluations of each MCMCglmm model
6. Data, R code, imputed datasets and tree used in the study

1. Milton K. 1981 Distribution Patterns of Tropical Plant Foods as an Evolutionary Stimulus to Primate Mental Development. *American Anthropologist* **83**(3), 534-548. (doi:10.1525/aa.1981.83.3.02a00020).

2. Rosati A.G. 2017 Foraging Cognition: Reviving the Ecological Intelligence Hypothesis. *Trends Cogn Sci* **21**(9), 691-702. (doi:10.1016/j.tics.2017.05.011).

3. DeCasien A.R., Williams S.A., Higham J.P. 2017 Primate brain size is predicted by diet but not sociality. *Nat Ecol Evol* **1**(5), 112. (doi:10.1038/s41559-017-0112).

4. Dunbar R.I.M. 1998 The social brain hypothesis. *Evolutionary Anthropology: Issues, News, and Reviews* **6**(5), 178-190. (doi:10.1002/(sici)1520-6505(1998)6:5<178::Aid-evan5>3.0.Co;2-8).

5. Isler K., van Schaik C.P. 2009 The Expensive Brain: a framework for explaining evolutionary changes in brain size. *J Hum Evol* **57**(4), 392-400. (doi:10.1016/j.jhevol.2009.04.009).

6. Aiello L.C., Wheeler P. 1995 The Expensive-Tissue Hypothesis: The Brain and the Digestive System in Human and Primate Evolution. *Current Anthropology* **36**(2), 199-221. (doi:10.1086/204350).

7. Holekamp K.E., Benson-Amram S. 2017 The evolution of intelligence in mammalian carnivores. *Interface Focus* **7**(3), 20160108. (doi:10.1098/rsfs.2016.0108).

8. van Woerden J.T., Willems E.P., van Schaik C.P., Isler K. 2012 Large brains buffer energetic effects of seasonal habitats in catarrhine primates. *Evolution* **66**(1), 191-199. (doi:10.1111/j.1558-5646.2011.01434.x).

9. Sol D. 2009 Revisiting the cognitive buffer hypothesis for the evolution of large brains. *Biol Lett* **5**(1), 130-133. (doi:10.1098/rsbl.2008.0621).

10. Muthukrishna M., Doebeli M., Chudek M., Henrich J. 2018 The Cultural Brain Hypothesis: How culture drives brain expansion, sociality, and life history. *PLoS Comput Biol* **14**(11), e1006504-e1006504. (doi:10.1371/journal.pcbi.1006504).

11. Smaers J.B., Rothman R.S., Hudson D.R., Balanoff A.M., Beatty B., Dechmann D.K., de Vries D., Dunn J.C., Fleagle J.G., Gilbert C.C. 2021 The evolution of mammalian brain size. *Science Advances* **7**(18), eabe2101.

12. Todorov O.S., Blomberg S.P., Goswami A., Sears K., Drhlík P., Peters J., Weisbecker V. 2021 Testing hypotheses of marsupial brain size variation using phylogenetic multiple imputations and a Bayesian comparative framework. *Proceedings of the Royal Society B: Biological Sciences* **288**(1947), 20210394. (doi:doi:10.1098/rspb.2021.0394).

13. Tsuboi M., van der Bijl W., Kopperud B.T., Erritzoe J., Voje K.L., Kotrschal A., Yopak K.E., Collin S.P., Iwaniuk A.N., Kolm N. 2018 Breakdown of brain-body allometry and the encephalization of birds and mammals. *Nat Ecol Evol* **2**(9), 1492-1500. (doi:10.1038/s41559-018-0632-1).

14. Barton R.A. 2010 Mosaic evolution of brain structure in mammals. *Evolution of Nervous Systems* **3**(6790), 97-102. (doi:10.1016/B0-12-370878-8/00052-5).

15. Murphy W.J., Eizirik E., O'Brien S.J., Madsen O., Scally M., Douady C.J., Teeling E., Ryder O.A., Stanhope M.J., de Jong W.W. 2001 Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* **294**(5550), 2348-2351.

16. Lopatin A., Averianov A. 2008 The earliest lagomorph (Lagomorpha, Mammalia) from the basal Eocene of Mongolia. In *Doklady Biological Sciences* (p. 131, Springer Nature BV.

17. Wang Y., Meng J., Beard C.K., Li Q., Ni X., Gebo D.L., Bai B., Jin X., Li P. 2010 Early Paleogene stratigraphic sequences, mammalian evolution and its response to environmental changes in Erlian Basin, Inner Mongolia, China. *Science China Earth Sciences* **53**(12), 1918-1926. (doi:10.1007/s11430-010-4095-8).

18. Kraatz B., Belabbas R., Fostowicz-Frelik Ł., Ge D.-Y., Kuznetsov A.N., Lang M.M., López-Torres S., Mohammadi Z., Racicot R.A., Ravosa M.J., et al. 2021 Lagomorpha as a Model Morphological System. *Frontiers in Ecology and Evolution* **9**(366). (doi:10.3389/fevo.2021.636402).

19. Garland T. 1983 The relation between maximal running speed and body mass in terrestrial mammals. *Journal of Zoology* **199**(2), 157-170. (doi:<https://doi.org/10.1111/j.1469-7998.1983.tb02087.x>).

20. Wible J.R. 2007 On the cranial osteology of the Lagomorpha. *Bulletin of Carnegie Museum of Natural History* **2007**(39), 213-234.

21. Bramble D.M. 1989 Cranial Specialization and Locomotor Habit in the Lagomorpha. *American Zoologist* **29**(1), 303-317. (doi:10.1093/icb/29.1.303).

22. Clutton-Brock J. 1989 Five thousand years of livestock in Britain. *Biological Journal of the Linnean Society* **38**(1), 31-37. (doi:10.1111/j.1095-8312.1989.tb01560.x).

23. Krebs C.J., Boonstra R., Boutin S., Sinclair A.R.E. 2001 What Drives the 10-year Cycle of Snowshoe Hares? *BioScience* **51**(1), 25. (doi:10.1641/0006-3568(2001)051[0025:wdtyco]2.0.co;2).

24. Rödel H.G., Starkloff A., Seltmann M.W., Prager G., von Holst D. 2009 Causes and predictors of nest mortality in a European rabbit population. *Mammalian Biology* **74**(3), 198-209. (doi:<https://doi.org/10.1016/j.mambio.2008.04.003>).

25. Tablado Z., Revilla E., Palomares F. 2012 Dying like rabbits: general determinants of spatio-temporal variability in survival. *Journal of Animal Ecology* **81**(1), 150-161. (doi:10.1111/j.1365-2656.2011.01884.x).

26. Lim S.Y., Tyan Y.-S., Chao Y.-P., Nien F.-Y., Weng J.-C. 2015 New Insights into the Developing Rabbit Brain Using Diffusion Tensor Tractography and Generalized q-Sampling MRI. *PLOS ONE* **10**(3), e0119932. (doi:10.1371/journal.pone.0119932).

27. Murphy E.H., Magness R. 1984 Development of the rabbit visual cortex: A quantitative Golgi analysis. *Experimental Brain Research* **53**(2), 304-314. (doi:10.1007/BF00238159).

28. Schneider N.Y., Datiche F., Coureaud G. 2018 Brain anatomy of the 4-day-old European rabbit. *Journal of Anatomy* **232**(5), 747-767. (doi:<https://doi.org/10.1111/joa.12789>).

29. Brusini I., Carneiro M., Wang C., Rubin C.-J., Ring H., Afonso S., Blanco-Aguiar J.A., Ferrand N., Rafati N., Villafuerte R., et al. 2018 Changes in brain architecture are consistent with altered fear processing in domestic rabbits. *Proceedings of the National Academy of Sciences* **115**(28), 7380-7385. (doi:10.1073/pnas.1801024115).

30. González-Mariscal G., Caba M., Martínez-Gómez M., Bautista A., Hudson R. 2016 Mothers and offspring: the rabbit as a model system in the study of mammalian maternal behavior and sibling interactions. *Hormones and Behavior* **77**, 30-41.

31. Xi J., Si X.A., Kim J., Zhang Y., Jacob R.E., Kabilan S., Corley R.A. 2016 Anatomical Details of the Rabbit Nasal Passages and Their Implications in Breathing, Air Conditioning, and Olfaction. *The Anatomical Record* **299**(7), 853-868. (doi:<https://doi.org/10.1002/ar.23367>).

32. López-Torres S., Bertrand O.C., Lang M.M., Silcox M.T., Fostowicz-Frelik Ł. 2020 Cranial endocast of the stem lagomorph Megalagus and brain structure of basal Euarchontoglires. *Proceedings of the Royal Society B* **287**(1929), 20200665.

33. Swaney W.T., Keverne E.B. 2009 The evolution of pheromonal communication. *Behavioural brain research* **200**(2), 239-247.

34. Eisthen H.L. 1997 Evolution of Vertebrate Olfactory Systems. *Brain, Behavior and Evolution* **50**(4), 222-233. (doi:10.1159/000113336).

35. Ihara S., Yoshikawa K., Touhara K. 2013 Chemosensory signals and their receptors in the olfactory neural system. *Neuroscience* **254**, 45-60.

36. Ribeiro P.F.M., Manger P.R., Catania K.C., Kaas J.H., Herculano-Houzel S. 2014 Greater addition of neurons to the olfactory bulb than to the cerebral cortex of eulipotyphlans but not rodents, afrotherians or primates. *Frontiers in Neuroanatomy* **8**(23). (doi:10.3389/fnana.2014.00023).

37. Finlay B.L., Darlington R.B., Nicastro N. 2001 Developmental structure in brain evolution. *Behav Brain Sci* **24**(2), 263-278; discussion 278-308. (doi:10.1017/S0140525X01003958).

38. Wu A., Yu B., Komiyama T. 2020 Plasticity in olfactory bulb circuits. *Current Opinion in Neurobiology* **64**, 17-23. (doi:<https://doi.org/10.1016/j.conb.2020.01.007>).

39. Meisami E., Bhatnagar K.P. 1998 Structure and diversity in mammalian accessory olfactory bulb. *Microscopy Research and Technique* **43**(6), 476-499. (doi:<https://doi.org/10.1002/(SICI)1097-0029(19981215)43:6><476::AID-JEMT2>3.0.CO;2-V).

40. Martinez Q., Clavel J., Esselstyn J.A., Achmadi A.S., Grohé C., Pirot N., Fabre P.-H. 2020 Convergent evolution of olfactory and thermoregulatory capacities in small amphibious mammals. *Proceedings of the National Academy of Sciences* **117**(16), 8958-8965. (doi:10.1073/pnas.1917836117).

41. Corfield J.R., Price K., Iwaniuk A.N., Gutierrez-Ibañez C., Birkhead T., Wylie D.R. 2015 Diversity in olfactory bulb size in birds reflects allometry, ecology, and phylogeny. *Frontiers in Neuroanatomy* **9**(102). (doi:10.3389/fnana.2015.00102).

42. Benhamou S. 1989 An olfactory orientation model for mammals' movements in their home ranges. *Journal of Theoretical Biology* **139**(3), 379-388. (doi:<https://doi.org/10.1016/S0022-5193(89)80216-4>).

43. Villamayor P.R., Cifuentes J.M., Quintela L., Barcia R., Sanchez-Quinteiro P. 2020 Structural, morphometric and immunohistochemical study of the rabbit accessory olfactory bulb. *Brain Structure and Function* **225**(1), 203-226. (doi:10.1007/s00429-019-01997-4).

44. Schaal B., Coureaud G., Langlois D., Ginies C., Sémon E., Perrier G. 2003 Chemical and behavioural characterization of the rabbit mammary pheromone. *Nature* **424**(6944), 68-72.

45. Melo A.I., González-Mariscal G. 2010 Communication by olfactory signals in rabbits: its role in reproduction. *Vitamins & Hormones* **83**, 351-371.

46. Apfelbach R., Blanchard C.D., Blanchard R.J., Hayes R.A., McGregor I.S. 2005 The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neuroscience & Biobehavioral Reviews* **29**(8), 1123-1144.

47. Kraatz B.P., Sherratt E., Bumacod N., Wedel M.J. 2015 Ecological correlates to cranial morphology in Leporids (Mammalia, Lagomorpha). *PeerJ* **3**, e844-e844. (doi:10.7717/peerj.844).

48. Isler K., van Schaik C.P. 2012 Allomaternal care, life history and brain size evolution in mammals. *J Hum Evol* **63**(1), 52-63. (doi:10.1016/j.jhevol.2012.03.009).

49. Weisbecker V., Blomberg S., Goldizen A.W., Brown M., Fisher D. 2015 The evolution of relative brain size in marsupials is energetically constrained but not driven by behavioral complexity. *Brain Behav Evol* **85**(2), 125-135. (doi:10.1159/000377666).

50. Todorov O.S., Weisbecker V., Gilissen E., Zilles K., Sousa A.A.d. 2019 Primate hippocampus size and organization are predicted by sociality but not diet. *Proceedings of the Royal Society B: Biological Sciences* **286**(1914), 20191712. (doi:doi:10.1098/rspb.2019.1712).

51. Gittleman J.L. 1991 Carnivore olfactory bulb size: allometry, phylogeny and ecology. *Journal of Zoology* **225**(2), 253-272. (doi:<https://doi.org/10.1111/j.1469-7998.1991.tb03815.x>).

52. Pager J. 1986 Neural correlates of odor-guided behaviors. *Experientia* **42**(3), 250-256. (doi:10.1007/bf01942505).

53. Yi S., Wang M., Ju M., Yi X. 2021 Olfaction alters spatial memory strategy of scatter-hoarding animals. *Integrative Zoology* **16**(1), 128-135. (doi:10.1111/1749-4877.12498).

54. Kraatz B., Sherratt E. 2016 Evolutionary morphology of the rabbit skull. *PeerJ* **4**, e2453. (doi:10.7717/peerj.2453).

55. Matthee C.A., Van Vuuren B.J., Bell D., Robinson T.J. 2004 A molecular supermatrix of the rabbits and hares (Leporidae) allows for the identification of five intercontinental exchanges during the Miocene. *Systematic biology* **53**(3), 433-447.

56. Hoffmeister D.F., Zimmerman E.G. 1967 Growth of the skull in the cottontail (Sylvilagus floridanus) and its application to age-determination. *American Midland Naturalist*, 198-206.

57. Jones K.E., Bielby J., Cardillo M., Fritz S.A., O'Dell J., Orme C.D.L., Safi K., Sechrest W., Boakes E.H., Carbone C. 2009 PanTHERIA: a species‐level database of life history, ecology, and geography of extant and recently extinct mammals: Ecological Archives E090‐184. *Ecology* **90**(9), 2648-2648.

58. Myers P., Espinosa R., Parr C.S., Jones T., Hammond G.S., Dewey T.A. 2006 The animal diversity web. (pp. 2-2.

59. R Core Team. 2021 R: A language and environment for statistical computing. (Vienna, Austria: R Foundation for Statistical Computing.

60. Hadfield J.D. 2010 MCMC Methods for Multi-Response Generalized Linear Mixed Models: TheMCMCglmmRPackage. *Journal of Statistical Software* **33**(2), 1-22. (doi:10.18637/jss.v033.i02).

61. Guillerme T., Healy K. 2014 mulTree: a package for running MCMCglmm analysis on multiple trees. *Zonodo*. (doi:10.5281/zenodo. 12902).

62. Paradis E., Schliep K. 2019 ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**(3), 526-528. (doi:10.1093/bioinformatics/bty633).

63. Revell L.J. 2012 phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**(2), 217-223. (doi:10.1111/j.2041-210X.2011.00169.x).

64. Buuren S.v., Groothuis-Oudshoorn K. 2011 mice: Multivariate Imputation by Chained Equations inR. *Journal of Statistical Software* **45**(3), 1-67. (doi:10.18637/jss.v045.i03).

65. Tierney N., Cook D., McBain M., Fay C., O'Hara-Wild M., Hester J. 2019 Naniar: Data structures, summaries, and visualisations for missing data. *R Package*.

66. Wickham H. 2016 *ggplot2: elegant graphics for data analysis*, Springer.

67. Gabry J., Simpson D., Vehtari A., Betancourt M., Gelman A. 2019 Visualization in Bayesian workflow. *Journal of the Royal Statistical Society: Series A (Statistics in Society)* **182**(2), 389-402. (doi:10.1111/rssa.12378).

68. Hyndman R.J., Einbeck J., Wand M., Hyndman M.R. 2018 Package ‘hdrcde’.

69. Lüdecke D., Ben-Shachar M., Patil I., Waggoner P., Makowski D. 2021 performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *Journal of Open Source Software* **6**(60), 3139. (doi:10.21105/joss.03139).

70. Rubin D.B. 1987 *Multiple Imputation for Nonresponse in Surveys*.

71. White I.R., Royston P., Wood A.M. 2011 Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med* **30**(4), 377-399. (doi:10.1002/sim.4067).

72. Barnard J., Rubin D.B. 1999 Small-Sample Degrees of Freedom with Multiple Imputation. *Biometrika* **86**(4), 948-955.

73. Little R.J.A. 1988 Missing-Data Adjustments in Large Surveys. *Journal of Business & Economic Statistics* **6**(3), 287-296. (doi:10.1080/07350015.1988.10509663).

74. Brooks S.P., Gelman A. 1998 General Methods for Monitoring Convergence of Iterative Simulations. *Journal of Computational and Graphical Statistics* **7**(4), 434-455. (doi:10.1080/10618600.1998.10474787).

75. Spiegelhalter D.J., Best N.G., Carlin B.P., van der Linde A. 2014 The deviance information criterion: 12 years on. *Journal of the Royal Statistical Society Series B (Statistical Methodology)* **76**(3), 485-493.

76. Luo Y., Zhong M.J., Huang Y., Li F., Liao W.B., Kotrschal A. 2017 Seasonality and brain size are negatively associated in frogs: evidence for the expensive brain framework. *Sci Rep* **7**(1), 16629. (doi:10.1038/s41598-017-16921-1).

77. Heldstab S.A., Isler K., van Schaik C.P. 2018 Hibernation constrains brain size evolution in mammals. *J Evol Biol* **31**(10), 1582-1588. (doi:10.1111/jeb.13353).

78. Bond B.T., Burger Jr L.W., Leopold B.D., Godwin K.D. 2001 Survival of cottontail rabbits (Sylvilagus floridanus) in Mississippi and an examination of latitudinal variation. *The American Midland Naturalist* **145**(1), 127-136.

79. Wiedenmayer C.P., Myers M.M., Mayford M., Barr G.A. 2000 Olfactory based spatial learning in neonatal mice and its dependence on CaMKII. *Neuroreport* **11**(5), 1051-1055. (doi:10.1097/00001756-200004070-00030).

80. Stewart T.A., Yoo I., Upham N.S. 2020 The coevolution of mammae number and litter size. *bioRxiv*, 2020.2010.2008.331983. (doi:10.1101/2020.10.08.331983).

81. De Meester G., Huyghe K., Van Damme R. 2019 Brain size, ecology and sociality: a reptilian perspective. *Biological Journal of the Linnean Society* **126**(3), 381-391. (doi:10.1093/biolinnean/bly206).

82. Pagel M. 1999 Inferring the historical patterns of biological evolution. *Nature* **401**(6756), 877-884. (doi:10.1038/44766).

83. Freckleton R.P. 2009 The seven deadly sins of comparative analysis. *J Evol Biol* **22**(7), 1367-1375. (doi:<https://doi.org/10.1111/j.1420-9101.2009.01757.x>).

84. Carlisle A., Selwood L., Hinds L.A., Saunders N., Habgood M., Mardon K., Weisbecker V. 2017 Testing hypotheses of developmental constraints on mammalian brain partition evolution, using marsupials. *Sci Rep* **7**(1), 4241. (doi:10.1038/s41598-017-02726-9).