Testing hypotheses of brain size variation using Bayesian comparative framework: the case of marsupials

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# Abstract (150 words)

# Introduction

1. A word about comparative studies on evolution of brain size variation in general outlining the major hypothesis that will be tested
2. Zone in into marsupials and previous findings
3. A few words about convergence and mode of evolution
4. Present the new approach (framework)

# Significance box

# Results

## Ancestral state estimations

Show ANC on residuals (vs absolute?) (Figure 1)

## MCMCglmm models:

### Developmental model

The developmental model included litter size and weaning age as predictors. Weaning age did not show a pronounced effect on brain size, but litter size had a negative effect (95.88% of the posterior distribution below zero, β = -0.086, SE=0.052).

### Environmental model

Predictors in this model included activity period, shelter safety, arboreality, diet and home range. We did not find any effect of any of the predictors on brain size.

### Social model

Predictors in this model were group living, parental care, mating system and populations size. None of them had any clear effect on brain size.

### Metabolic model

The model revealed no effect of field metabolic rate on brain size, including no interaction between body size and metabolic rate.

### Hibernation model

Torpor had no effect on brain size, including no interaction between body size and torpor.

### Play model

Species with larger brain sizes did not exhibit more or more complex play behaviour compared to smaller-brained species. The interaction between body size and play behaviour also did not reveal any effect of brain size.

### Vulnerability model

Vulnerable, endangered, rare, declining or species with very limited habitats were shown to have slightly smaller brains (96.08 of the posterior distribution below zero, β = -0.14, SE=0.081), but when the interaction with body size is taken into account the relationship becomes positive (96.94% of the posterior distribution above zero, β = 0.023, SE=0.012).

### Origin model

Species from New Guinea were shown to have larger brains (99.42% of the posterior distribution above zero, β = 0.31, SE=0.12), but the interaction with body size was positive (95.26% of the posterior distribution below zero, β = -0.031, SE= 0.019).

## Evolutionary models

In Australia marsupials EB is the best fitting model of evolution for both body and brain. In Ng EB best fits as a model of evolution of the brain but BM is a better fit for body size evolution. In America we determined that BM was the best fit for both brain and body size.

|  |  |  |
| --- | --- | --- |
| Origin | Brain | Body |
| Australia | EB | EB |
| New Guinea | EB | BM |
| Americas | BM | BM |

1. **Evolutionary models of BM, OU, EB**

**Prediction:** Later invasions into new ecospaces have involved bursts of variation as the clade adapts.

**Rationale:** We would expect this for Australia because of the invasion from Gondwana and for NG because of the invasion from Australia; We would not expect this because crown marsupials have been in S. Am. Since the isthmus of panama formed.

**Result:**

In Australia we have EB for body and brain

In Ng we have EB for brain but BM for body

In America we have BM for both brain and body

**Conclusion:** Prediction supported – VW: in Ng we have significantly greater relative brain size and there seems to have been a jump in brain size that body mass for some reason has not participated in. The polarity of this is interesting – it really is the brain that jumps, not body mass. Why??? Seasonality? Human hunting pressure? Competition with placentals? Cognitive buffer?

An additional pANCOVA showed that a model including ‘Origin’ as an interaction term was significantly better than a model including maruspials from all origins (F=5.07, P=0.0072 on 4, 2 degrees of freedom), while variance inflation factor (VIF) was <2.   
  
<figure 1 around here – report slopes and intercepts for the 3 origins>

## Rate shifts

RRphylo report here (Figures)

# Discussion

1. Discuss the framework and elaborate on advantages and some drawbacks ( i.e. the case of a lot of missing data)
   1. Imputation as a useful tool and extending the phylo-part of MICE
   2. MCMC as better (more flexible) compared to pgls
   3. Pooling
2. ANC and further explorations after incorporating fossil data
3. Discuss convergence and the further directions using this method in brain evolution studies (maybe shape too?)
4. Red line about ECV vs brain
5. Whinge about more data in B(F)MR and cog ability (play, etc)
6. Discuss all models and stress on the new ones. Discuss differences and similarities with previous attempts in the field and propose further work (maybe suggest neuronal morphology, numbers, and density gradients?)

# Materials and Methods

Packages that we use for the analysis are phytools (Revell, 2012), caper (Orme, 2012), MCMglmm (Hadfield, 2010), mulTree (Guillerme & Healy, 2014), mice (Buuren & Groothuis-Oudshoorn, 2011), phylomice (Blomberg and Drhlik), geiger (Harmon, Weir, Brock, Glor, & Challenger, 2007), RRphylo (Raia et al., 2019). For plotting we use ggplot2 (Wickham, 2016) and hdrcde (Hyndman, Einbeck, Wand, & Hyndman, 2018).

## Dataset

We collated the largest and most comprehensive dataset on marsupial brain sizes to date (See table for sources). It includes 18 traits intdrcluding brain and body size. The final dataset comprises 176 species of marsupials from all three continents inhabited by the infra-class. Those comprise around 53% of all marsupial species, approximated to be around 330 in total. In particular, we provide an extensive dataset on X ameridelphian species, which have to date been underrepresented in marsupial brain size datasets

Brain size, body size, origin and activity cycle had no missing values, while the rest had around 25% missing values on average (see Multiple Imputations section and Supplementary Information for the pattern of the missing data). We use body mass as an estimate for body size, while brain volume is used as an estimate for brain size. Data on brain volumes were derived from measurements of endocranial volumes (ECV) and were obtained from several different sources (Weisbecker, Blomberg, Goldizen, Brown, & Fisher, 2015) WHO ELSE. While endocranial volumes are a reliable proxy for brain size, they do suffer from certain drawbacks. In marsupials, the koala (*Phascolarctos cinereus*) is a remarkable example for the pitfalls of using it as a direct proxy. Koala’s endocranial cavity is exceptionally large compared to the brain contained in it, comprising only around 60% of the total ECV (Taylor, Rühli, Brown, De Miguel, & Henneberg, 2006)*.* Therefore, using ECV without correction in such species might lead to the misleading observation that they have very large brains. (HAVE WE CORRECTED FOR THAT???!). To our knowledge, no other species in our dataset has such stark discrepancy between ECV and actual brain size.

For detailed description on rationale for inclusion and sources of the data, see the table with data sources.

## Phylogeny

We included information on phylogenetic non-independence in all our analyses using an ultrametric phylogenetic tree of 176 extant marsupial species obtained from Time Tree (with the one exception of the Thylacine which is extinct). The tree had 12 branches with length of 0 (used as means for resolving politomies), which due to the requirements of some of the approaches had to be resolved. We did that by adding 0.01% of the median branch length, and then ultrametricized the tree again, using extension, with the package phytools.

## Statistical methods

We use a combination of Bayesian statistical methods combined in a framework for phylogenetically informed comparative analyses (see Figure). We start off with multiple imputations of missing data resulting in a number of biased estimations based on chained equations (check Multiple Imputations section). We run MCMCglmm on all the imputed datasets running on 2 chains. Subsequently, we pool all the solutions from both chains into an ‘average’ model, on which we base all analyses and conclusions.

### Multiple imputations

Dealing with missing data has been a pervasive issue in comparative studies. The most common solution to the problem has been to omit cases with missing values, which often results in losing whole cases only because of one or two missing values. A proposed and tested approach is multiple data imputation (Nakagawa & Freckleton, 2008; Resche-Rigon & White, 2018; Rubin, 1987) which has previously been shown to be a better solution to the problem, than omitting missing cases(Fisher, Blomberg, & Owens, 2003).

For imputation of missing data, we used the R package phylomice. It is an extension for the package mice, which allows for multiple imputations with the addition of taking the phylogenetic non-independence of the data into account. We use the method of predictive means matching(Demirtas, 2018; Little, 1988), a semi-parametric stochastic regression method in which a small set of candidate values (‘donors’) is found for each missing data point based on multiple regression model, whose predicted regression score is closest to the missing value. The choice of donor is then biased by the phylogenetically closer cases. Because the beta coefficients values in the regression models are chosen at random from the joint posterior distribution, such model introduces considerable stochastic variation, simulated by a Markov chain Monte Carlo procedure.

This imputation method has the advantage that missing data is imputed based on values observed elsewhere in the set, so they are usually realistic. The pattern of missing values in our dataset is reported in the supplementary material. We have variables with 0 missing values - brain size, body size, origin, diurnality; and such with more than half of the values missing, i.e play (68% or 120 missing), torpor (53% or 94 missing). On average, the dataset contained 25% missing values, which we used as reference for the number of multiple imputations. Following an established rule of thumb (White, Royston, & Wood, 2011), the number of datasets we imputed was equal to the percentage of missing data – twenty-five.

We ran the imputations for 500 iterations each, on natural log transformed continuous variables, and raw values of categorical variables (see strip plot of imputations). As predictors, only values with less than 35% missing values were used, which rendered 13 predictors in total. Convergence of the chained equations was assessed visually on the diagnostic plots of mice, using both strip plots and density plots.

All subsequent analysis conducted on variables containing missing values were done on all twenty-five imputed datasets, and final results were pooled from all twenty-five imputations.

### Ancestral state estimation

For estimation of ancestral states, we used the package phytools and the function fast anc. We estimated ancestral traits on absolute brain size, and on the phylogenetically corrected residuals from the regression with body size.

### Evolutionary mode variation and regime changes

To investigate if changes in evolutionary mode or regime changes are related to any of our models, we looked at where differences in evolutionary rate change occur within our phylogenetic tree and whether there was a coincidence of a model parameter (e.g. origin) and changes in mode. Similarly, to particularly investigate if the deepest split in the marsupial tree (Ameri-vs. Australidelphia) resulted in different evolutionary patterns, we investigated which mode of evolution best fitted our data - BM vs OU vs EB. Best fitting evolutionary models were assessed using fasBM from the geiger package, while rate shifts were evaluated using the package RRphylo.

### MCMCglmm

Due to its convenient wrapper functions we used the package mulTree (Guillerme & Healy, 2014) to conduct MCMCglmm (Hadfield, 2015) on each of the 25 imputed datasets. We ran the MCMC for 1 000 042 iterations, with burn in of the first 150 000 iterations, and sampling rate of 250. All priors were set to uniform and uninformative, which supposes that all values of the parameters are equally likely. Each model was run on 2 chains which produced an effective sample size of at least 3000 and all converged successfully (Gelman-Rubin criterion < 1.1).

# Supplementary material

Table with data sources

|  |  |  |  |
| --- | --- | --- | --- |
| Trait | Units | Rationale | Reference |
| Brain | mm3 |  | (Weisbecker et al., 2015) + |
| Body | grams |  | (Birdlife International, 2016; Flannery, 2013; Myers et al., 2006; van Dyck, Gynther, & Baker, 2013; Weisbecker, Ashwell, & Fisher, 2013) |
| Origin | 1 – Australia, 2 – New Guinea, 3 - Americas | Different origins predispose different influence of seasonality, predation pressure, food abundance. | (Flannery, 2013; Myers et al., 2006; van Dyck et al., 2013) |
| Status | 1 - Common, abundant, 2 - Vulnerable, endangered, rare, declining, limited  3 - Extinct | Highly threatened mammals are known to have larger relative brain sizes (Abelson, 2016) | (Birdlife International, 2016; van Dyck et al., 2013) |
| Diurnality | 1- Nocturnal, 2 – Diurnal, 3 - Crepuscular or not fully nocturnal | Nocturnal animals are considered larger brained, but daily activity is related to more complex predator avoidance techniques. | (Flannery, 2013; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Arboreality | 1 - Arboreal or scansorial, 2 - Terrestrial | Arboreal environment is considered more cognitively demanding. | (Flannery, 2013; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Shelter safety | 1 - Protected (burrow/nest in a tree hollow), 2 - Intermediate (tree canopy/hollow log/under rock/nest on the ground or in a soil crack), 3 - Open (under shrubs/in grass/tree shade) | Proxy for predation as selection pressure for larger brains. (Reddon, Chouinard-Thuly, Leris, & Reader, 2018) | (Flannery, 2013; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Diet | 1 - >50% grass/browse, 2 - Seeds, grass, roots, leaves, fruit, invertebrates, 3 - Nectar, fruit, invertebrates, 4 - >50% invertebrate/vertebrate | Foraging complexity and diet rich in nutrients have been shown to influence brain size | (Flannery, 2013; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Group living | 1 – No, 2 - Yes | Measure of social complexity, which imposes greater interaction and recognition demands | (Flannery, 2013; Myers et al., 2006; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Parental care | 1 – No, 2 - Yes | Parental investment is known to positively influence brain size (Isler & van Schaik, 2012) | (Flannery, 2013; Myers et al., 2006; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Mating system | 1 – Promiscuous, 2 - Complex (polygamous/monogamous) | Complex mating systems require more cognitive complexity and usually result in higher parental investment (Schillaci, 2006) | (Flannery, 2013; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Litter size | Average litter per reproductive episode | Constraint on maternal investment. | (van Dyck et al., 2013; Weisbecker et al., 2015) |
| Weaning age | Months | Constraint on maternal investment. | (Weisbecker et al., 2015) |
| Home range | Hectares | Larger home ranges usually imply increased cognitive complexity related to orientation (Clutton‐Brock & Harvey, 1980) | (Myers et al., 2006; van Dyck et al., 2013; Weisbecker et al., 2015) |
| Population density | Individuals per hectare | Increased population density is a proxy of increased interaction and social tolerance. | (van Dyck et al., 2013; Weisbecker et al., 2015) |
| FMR | Field metabolic rate | Measure of metabolic turnover in the wild. | (Riek & Bruggeman, 2013) |
| Torpor | 0 – No, 1 – Yes | Torporing has been shown to be costly to the maintenance of large brains (Heldstab, Isler, & van Schaik, 2018) | (Geiser & Körtner, 2010; McNab, 2008; Ruf & Geiser, 2015) |
| Play | 1 – No, 2 – Rudimentary, 3 - Complex | Proxy for cognitive ability. Play has been shown to correlate with larger brains in birds and mammals (Iwaniuk, Nelson, & Pellis, 2001) | (Ashwell, 2008; Iwaniuk et al., 2001) |

Dataset

Imputed datasets

R Code

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