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

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# Abstract (150 words)

# Introduction

Compared to other vertebrates, mammals have impressively large brains relative to body size (from now on, relative brain size). This is reflected by the advanced cognitive abilities within the class and it is assumed that this has, in part, selected for the increase in relative brain size. Several selection-focused hypotheses has been put forward in an attempt to explain the increase in relative brain size and its subsequent fitness benefits in variety of cognitively demanding tasks. The ‘social-brain’ hypothesis suggests that increase in social complexity (such as social or foraging group size and mating system) can select for larger brain sizes, and particularly larger neocortex size. On the other hand, the ‘ecological brain’ hypothesis proposes that increase in cognitive demands related to ecological factors (diet, home range, predation pressure) can select for increase in relative brain size. There exists also more general hypothesis regarding the evolution of brain variation which don’t associate relative size increase with particular behavioural parameters. The ‘cognitive-buffer’ hypothesis claims that larger brains generally improve fitness and survival, due to advantages related to tackling novel and unpredictable environments and situations. Additionally, it has been proposed that the buffer function of the brain could result in the generation of positive feedback processes which can even accelerate brain evolution.

Another approach to studying brain variation focuses on the constraints of maintaining a large brain, which has been shown to be extremely metabolically costly. Generally dubbed the ‘expensive tissue’ hypothesis (or more specifically the ‘expensive brain’ hypothesis’) has been able to point to metabolic, maternal investment and general energetic constrains on the evolution of large brain size.

Even though the two approaches to studying brain variation have many times been applied separately, a conclusive picture about the evolutionary processes shaping brain size can only be drawn by simultaneously investigating both the effects of selection pressures and the limitations imposed by developmental constraints. The same is valid for studies focusing only on particular constraint or selection, as many times such processes interact with each other – home range in related to mating systems, social group sizes are related to predation pressure, maternal investment is dependent on mating systems and energetic availability is dependent on diet.

A major drawback of most studies on brain size evolution is their almost exclusive focus on placental mammals. Irrespective of the fact that all of the extremely-large brained mammals are placentals, it is known that in that infra-class the variation of life history and reproductive modes is so varied that these can be a significant confound. At the same time, marsupial mammals present another radiation of mammals with strikingly homogenous life histories and developmental regimes. They have diverged from placental mammals around 60 mya and altricial neonates are born after very short gestation periods (12-30 days) and most receive little to no maternal investment. Moreover, marsupials exhibit a diverse array of social and mating systems, diet types, home ranges, cognitive abilities. They are distributed in various habitats with various levels of seasonality (New Guinea, Australia and the Americas). This unique combination of reproductive homogeneity and ecological, behavioural and social diversity, makes marsupials perfectly suited for testing hypothesis about brain size evolution.

The current study builds upon previous studies focused on marsupials by making use of the most comprehensive dataset of marsupial species (176 from all known habitats). Using novel advances in phylogenetic comparative methods, data imputation technique and analysis of rate of evolution, we test several hypothesis of brain evolution, including some that have not been tested in the infra-class before. To our knowledge, this is the most comprehensive study on marsupial brain evolution to date, and the only one applying phylogenetically informed imputation techniques and Bayesian statistical methods.

# Significance box

The most comprehensive study on marsupial brain evolution to date including data on 176 species from Australia, New Guinea and the Americas. We are applying phylogenetically informed imputation techniques and Bayesian statistical methods for the first time in such studies and are confirming previous findings (brain size is constrained by maternal investment; NG marsupials have larger relative brains) and contributing with several new findings (vulnerability is related to brain size and providing details on the rate of evolution in different marsupial radiations). Additionally, we provide a framework for Bayesian analysis of brain size evolution, incorporating data imputations for the first time.

# 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

In this comprehensive study of marsupial brain size evolution, we find an intriguing lack of selection-related brain size correlates across the radiation of marsupial mammals. However, our use of RRphylo/SURFACE suggests that this very common issue in brain size evolution studies may arise because there is no single correlate of brain size. Rather, increases in brain size seem to be associated with different factors in different radiations, which appears to make it all so noisy that an overarching signal is lost.

Our models were really comprehensive because XXX And awesome because YYY. Nevertheless… all the old finds were replicated. Intruiguingly, we found X in terms of conservation status. This contrasts with finds in birds, where …. . In addition, play behaviour came up …. Which is consistent with Y.

reveals solid support for the two hypotheses that posit an energetic constraint on mammalian brain size, while adding the subclass of Marsupialia to the considerable number of placental clades for which no connections between social complexity and relative brain size are apparent [Walker et al., 2006; Healy and Rowe, 2007; Finarelli, 2009b; Shultz and Dunbar, 2010b]. Specifically, the maternal investment constraint model has far more support than the environmental interaction and social brain models. In addition, the constraint-based variables of litter size and NG origins (as well as possibly latitudinal distribution, which was close to the significance cutoff) are the only unambiguous associates of marsupial brain size. The significantly larger brains of NG marsupials in particular provide good support for the notion that low seasonality represents ‘nutrition safety’ for the brain [van Woerden et al., 2010, 2012]. It is possible that this effect is amplified by the better soils of NG, which might contribute to better food availability overall [Flannery, 1994; Ashwell, 2008]. However, this needs further research, since many NG ecosystems – particularly rainforest communities – are on fairly poor soil.

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

1. ANC and further explorations after incorporating fossil data
2. Discuss convergence and the further directions using this method in brain evolution studies (maybe shape too?)
3. Red line about ECV vs brain
4. Whinge about more data in B(F)MR and cog ability (play, etc)
5. 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 were used for the analysis: 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 ggplot2 (Wickham, 2016) and hdrcde (Hyndman, Einbeck, Wand, & Hyndman, 2018) were used.

## Dataset

We collated the largest and most comprehensive dataset on marsupial brain sizes to date (See table for sources). It includes 18 traits including 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 other traits 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 175 extant marsupial species (with the one exception of the Thylacine which is extinct) obtained from Time Tree. 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 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 fastBM 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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