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

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# Significance box

This is 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 we make 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.

# Abstract (150 words)

# Introduction

Compared to other vertebrates, mammals have impressively large brains relative to body size (“relative brain size” herein), which confers advanced cognitive abilities to the class compared to the ancestral vertebrate condition (Jerison, 1973). It is assumed that this brain size increase is mostly the result of selection for the increase in relative brain size (Healy & Rowe, 2007). Similarly, evolutionary increases of relative brain sizes within mammals are widely considered to be due to selection for relative brain size (Reader & Laland, 2002; Sol, Bacher, Reader, & Lefebvre, 2008). However, the source of this selection pressure is unclear: several selection-focused hypotheses have been put forward in an attempt to explain the increase in relative brain size and its subsequent fitness benefits in a variety of cognitively demanding tasks. Among the more widely debated hypotheses, the ‘social-brain’ hypothesis suggests that increases in social complexity (such as social or foraging group size and mating system) can select for larger brain sizes, and particularly larger neocortex size (Dunbar, 1998). On the other hand, the ‘ecological brain’ hypothesis proposes that an increase in cognitive demands related to ecological factors (diet, home range, predation pressure) can select for an increase in relative brain size (DeCasien, Williams, & Higham, 2017; Milton, 1981) . There exists also a more holistic hypothesis regarding the evolution of brain variation which do not associate relative size increase with particular behavioural parameters. This ‘cognitive-buffer’ hypothesis claims that larger brains generally improve fitness and survival, due to advantages related to tackling novel and unpredictable environments and situations (Daniel Sol, 2009; van Woerden, Willems, van Schaik, & Isler, 2012). 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 (D. Sol, 2009).

It is widely recognized that processes of selection for relatively larger brains are antagonized by the high expense of brain growth and maintenance, as larger brains are known to be metabolically costly. Studies of brain size constraints under this ‘expensive tissue’ hypothesis (or more specifically the ‘expensive brain’ hypothesis’) have revealed indications of metabolic, maternal investment and general energetic constrains on the evolution of large brain size (Aiello & Wheeler, 1995; Isler & van Schaik, 2009).

Even though the two approaches to studying brain variation have 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 (Logan et al., 2018; V. Weisbecker, Blomberg, Goldizen, Brown, & Fisher, 2015). At the same time, many previous studies focus solely on rejecting or confirming only one of the many hypotheses. The same applies to studies focusing only on constraints or selection, as such processes may interact with each other. For example, home range is related to mating systems (Lukas & Clutton-Brock, 2014), social group sizes are related to predation pressure (Hintz & Lonzarich, 2018), maternal investment is dependent on mating systems (Shuster, 2009) and energy availability is dependent on diet.

A drawback of most studies on brain size evolution is their almost exclusive focus on placental mammals. Placentals include the largest-brained mammals (such as cetaceans and canid carnivores), but they also have highly varied life histories and reproductive modes. These may increase the risk of confounding constraints of reproduction with selection on traits such as behaviour (Logan et al., 2018). This makes the sister radiation of placentals – the Marsupial mammals – an attractive alternative to study. Marsupial mammals combine strikingly homogenous life histories and developmental regimes with a similarly diverse range of behavioural complexities to placentals (Carlisle et al., 2017; V. Weisbecker et al., 2015; V. Weisbecker & Goswami, 2010). Moreover, marsupials are a discrete, phylogenetically well-resolved, monophyletic radiation diverging from placentals around 130-160 mya (Luo, Yuan, Meng, & Ji, 2011). Their altricial neonates are born after very a short gestation period (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 and cognitive abilities (Todorov, 2019). 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 hypotheses about brain size evolution (Carlisle et al., 2017).

Previous work on marsupial brain size evolution, focusing on the Australasian radiation of marsupials, has yielded surprisingly little support for any of the main hypotheses of behavioural complexity, with a reproductive constraints model, and specifically litter size, representing the best model to explain brain size variation (V. Weisbecker et al., 2015). In addition, there was an unexpected highly significant effect of geological location, with New Guinean marsupials shown to be significantly larger-brained than their Australian relatives. However, the species in the dataset were nearly exclusively Australasian. In addition, like most other studies of brain size evolution, many cases in the dataset had multiple missing values, so that model comparisons could only be done one at a time due to constraints of sample size. With a very limited number of species and different species representation for each model the results of that study have been inconclusive. The statistical approach – namely PGLS – is also very sensitive to errors in phylogeny, hard to use on multiple imputed datasets, and assumes a single mode of evolution throughout the whole tree (Brownian motion) (Mundry, 2014).

In this study, we incorporate a new dataset of 62 South American marsupial species into existing data, thus increasing the amount of marsupial brain size data by one third. Most of the body weight data has been updated with the latest information from (van Dyck, Gynther, & Baker, 2013), and many other trait data had been updated. We then address the common limitation of incomplete data coverage by using phylogenetically adjusted multiple data imputation technique on a comparative dataset (Nakagawa & Freckleton, 2008; Rubin, 1987).

We then use a phylogenetically corrected Bayesian generalised linear mixed-effects model – MCMCglmm – which has fewer assumptions about the distribution of the source data and can test multiple hypothesis on multiple datasets (Hadfield, 2010).

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).

We then firstly ask whether this more comprehensive approach improves on previously tested models of behavioural complexity in marsupials; we then go on to test three additional important hypotheses of brain size evolution, namely whether play behaviour, hibernation and conservation status are associated with brain size increases. To better understand the evolutionary patterns leading to relative brain size variation in marsupials, we compare the evolutionary modes of relative brain size increase in the three landmasses (Australia, New Guinea and the Americas) and test whether evolutionary rate shifts had occurred as a result of invasion in a novel landmass. We test whether New Guinean marsupials had an increased rate of evolution compared to Australian or American species, which might explain why this latest marsupial radiation has the largest relative brain size.

# Results

|  |  |  |  |
| --- | --- | --- | --- |
| Model | β | SE | Posterior distribution above zero |
| Developmental  Weaning age  Litter size | 0.03  -0.09 | 0.03  0.05 | 77.5%  **95.88%** |
| Environmental  Diurnal  Crepuscular  Shelter Safety - intermediate  Shelter Safety - open  Terrestrial  Diet - 2  Diet - 3  Diet - 4  Home Range | 0.03  -0.05  0.03  0.05  -0.05  0.05  -0.07  -0.03  0.01 | 0.08  0.04  0.04  0.07  0.04  0.06  0.07  0.08  0.01 | 67.4%  9.99%  80.89%  76.06%  13.96%  79.89%  14.41%  33.62%  81.21% |
| Social  Group living  Parental care  Mating system  Populations density | 0  -0.02  0.03  0 | 0.05  0.07  0.05  0.01 | 47.68%  34.07%  77.07%  54.85% |
| Metabolic  FMR | 0.04 | 0.08 | 68.95 |
| Hibernation  Torpor | -0.13 | 0.15 | 19.22% |
| Play  Play – 2  Play - 3 | 0.1  0.08 | 0.18  0.17 | 70.37%  69.36% |
| Vulnerability  Status – 2\*  Status – 3\* | 0.02  0.06 | 0.01  0.06 | **96.94%**  84.72% |
| Origin  Origin – 2  Origin - 3 | -0.03  -0.05 | 0.02  0.04 | **4.74%**  12.15% |

## 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 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 larger brains within larger bodied marsupials, but smaller brains within small bodied ones (96.94% of the posterior distribution above zero, β = 0.023, SE=0.012 for the interaction between vulnerability and body size).

(need to produce graphs of the interaction)

### Origin model

Species from New Guinea were shown to have larger brains within small and average body sized marsupials, compared to Australian or American (4.74% of the posterior distribution above zero, β = -0.031, SE= 0.019).

(need to produce graphs of the interaction)

## Evolutionary models

We show that marsupials in Australia have undergone early burst (EB) of both brain and body size evolution. In contrast, in New Guinea marsupials, we detected EB of evolution of the brain only but Brownian motion (BM) for body size evolution. In America we determined that BM is the best fit for both brain and body size evolution.

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

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.

(Need to produce the ANCOVA graph)

# Discussion

In this comprehensive study of brain size evolution, we find an intriguing lack of selection-related brain size correlates across the radiation of marsupial mammals. 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 (Smaers, Dechmann, Goswami, Soligo, & Safi, 2012).

Our models included the most comprehensive dataset of marsupials to date, including species from all geographic regions, along with updated body mass and trait data. Nevertheless, these new and more rigorous analyses confirmed previous findings, namely, that the only major determinant of brain size evolution in marsupials is their developmental mode, and more specifically litter size is inversely related to relative brain size (V. Weisbecker et al., 2015). Additionally, we were able to confirm that marsupials from New Guinea do have the largest brains among marsupials, and this relationship is dependent upon body size – the largest bodied species from both localities do not differ in brain size. Similar to previous studies (Byers, 1999; Iwaniuk, Nelson, & Pellis, 2001), we did not find any clear-cut evidence that play behaviour and its complexity is related to brain size.

Apart from confirming previous findings, we were able to analyse the relationship between species’ vulnerability and brain size, showing for the first time that larger brained marsupials are more vulnerable to extinction. This effect, again, was dependent on body size (Abelson, 2016; Gonzalez-Voyer, Gonzalez-Suarez, Vila, & Revilla, 2016), where vulnerable, endangered, rare, declining or species with very limited habitats were shown to have larger brains among species with larger body sizes, but smaller brains within species with smaller body sizes. This observation may be due to the fact that large bodied marsupials with larger brains may fall easier pray to introduced predators in areas with human activity (such as cats and dogs) due to their increase in prosociallity. On the other hand, smaller bodies marsupials with larger brains might be more adaptable to human modified environment due to increase in intelligence and behavioural plasticity, where their small sizes facilitate the ability to avoid predation risks related to human activities and introduced predators(Snell-Rood & Wick, 2013). The interaction we see might also not in fact relate fully to brain size, but rather to the different reproductive mode of big and small brained mammals. E.g. small mammals tend to be k strategists whose survival is aided by their large reproductive output, which combined with an increase in brain size and the concomittant increase in intelligence and behavioural plasticity, might put the in a more favourable position to hedge around exctinction related to human activity.

(Discuss the NG effect in more detail?)

(Discuss the imputations and the MCMCglmm in more detail?)

(finish the discussion with smth like) Despite the hugely improved sophistication of data completeness through imputation and modelling, the only model that explains brain size variation best is the maternal constraint one and specifically litter size. Marsupials with their low level of reproductive confoundment may therefore indicate that a majority of correlations found in placentals have their ultimate cause in reproductive traits.

# Materials and Methods

All analyses were conducted in R. The code to replicate all analyses, including all data, can be found on LINK TO GITHUB. 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 use body mass as an estimate for body size, while endocranial volume (ECV) was 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 (Ashwell, 2008; V. Weisbecker et al., 2015). Most ECV volumes were obtained from Ashwell (2008) which included: 472 skulls from 52 species of Dasyuromorph (carnivorous/insectivorous) marsupials and the marsupial mole, 146 skulls from 14 species of Peramelemorphia (bilbies and bandicoots) and 639 skulls from 116 species of Diprotodontia (koala, wombats, gliders, possums, kangaroos, wallabies, from the collection of the Australian Museum in Sydney. 29 skulls from 16 species of Ameridelphian marsupials from the Museums of Victoria and Queensland. We had added 62 new species of American marsupials provided by (REF). Body weight data were taken from (Vera Weisbecker, Ashwell, & Fisher, 2013) but thoroughly updated using latest data from (van Dyck et al., 2013). As a result, we collated the largest and most comprehensive dataset on marsupial brain size and body weight to date comprising 176 species.

While endocranial volumes are a reliable proxy for brain size (Jerison, 1973) they do suffer from certain drawbacks. For example, in marsupials, the koala’s (*Phascolarctos cinereus*) 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. To our knowledge, no other species in our dataset has such stark discrepancy between ECV and actual brain size.

Moreover, the dataset includes 16 traits chosen so to allow for testing most of the hypothesis about brain variation (See table for traits and sources). 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. The full dataset used can be found both as Supplement herein and on Github.

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).

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 exception of the extinct Thylacine) obtained from Time Tree. This was deemed appropriate because the tree provided full coverage of all species investigated, using for most taxa the recent marsupial phylogeny of Mitchell et al.

The tree had 12 branches with length of 0 (used as means for resolving polytomies), 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 (cite the Tail paper).

## Statistical methods

### Multiple imputations

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 are imputed based on several 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 using Rubin’s rule (REF).

### Evolutionary mode variation and regime changes

To assess the suggestion of Weisbecker et al. (2015) that switches to different land masses may change patterns of marsupial brain evolution, we assessed if differences in evolutionary mode on brain/body mass evolution regimes occurred in Australian, New Guinean, and American marsupials. To investigate if changes in evolutionary mode (i.e. whether Brownian motion or a specific optima-driven mode of evolution occurs) and particularly 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 the function fastBM from the geiger package,

### Model assessment

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 assumes 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). Subsequently, the results from all 50 model runs (25 datasets on 2 chains) were pooled using the Rubin’s rule (REFERENCE). Finally, the fit of all models to explain brain size was compared using heritability and BIC.

# 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 et al., 2001) | (Ashwell, 2008; Iwaniuk et al., 2001) |

Dataset

Imputed datasets

R Code

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