Bayesian comparative framework for testing hypotheses of brain size variation: the case of marsupials

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# Abstract

# 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

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>

1. Show ANC on residuals (vs absolute?) (Figure 1)
2. Show convergent clades and parameters of similar adaptive regimes (Figure 2)
3. Report all MCMCglmm models on the multiple imputation with graphs (Multiple figures)

## MCMCglmm models:

### Developmental model

The developmental model included litter size and weaning age as predictors. Both were shown to not have a pronounced effect on brain size, but litter size had marginal negative effect (94.12% of the posterior distribution below zero, β = -0.034, SE=0.022).

### 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, but when the interaction with body size is taken into account, the relationship reverses (95.31% of the posterior distribution above zero, β = 0.032, SE=0.019).

### Origin model

Species from New Guinea were shown to have larger brains (99.5% of the posterior distribution above zero, β = 0.042, SE=0.016), but the effect reversed when the interaction with body size was taken into account (94.07% of the posterior distribution below zero, β = -0.044, SE= 0.028), despite the latter effect being only marginally present.

### Evolutionary mode variation and regime changes

To investigate if evolutionary mode or evolutionary regime show up as related to any of our models, we looked at where differences in evolutionary mode occur within all species 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 did BM vs OU vs EB.

# 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, caper, MCMglmm, mulTree, mice, phylomice, geiger, SURFACE. For plotting we use ggplot2 and hdrcde.

## 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 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 (1) 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 *(Jackson, S. (2010). Koala: Origins of an Icon (2nd ed.). Allen & Unwin. ISBN 978-1-74237-323-2).* 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 (2).

## Statistical methods

We use a combination of Bayesian statistical methods combined in a framework for phylogenetically informed comparative analyses. 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 our analyses and conclusions. (See framework scheme)

### 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 (3–5) which has previously been shown to be a better solution to the problem, than omitting missing cases (6).

For imputation of missing data we used the R package phylomice (Blomberg and Drhlik). It is an extension for the package mice (7), 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 (8, 9), 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, we had 25% missing values, which we used as reference for the number of multiple imputations. Following an established rule of thumb (10), 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 and min-max standardised continuous variables, and raw values of categorical variables (see strip plot). 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.

### SURFACE

We used the SURFACE method for detecting convergence over similar selective regimes (11). It fits Ornstein-Uhlenbeck stabilizing selection model to first identify regime shifts on branches of the tree, where a proposed regime shift would improve the AIC score of the model.  Employing such information criterion allows for balancing the trade-off between improving the log‐likelihood versus increasing the complexity. During the forward phase, a nonlinear optimization is used to find the maximum likelihood estimate for α in the OU model (α represents the rate of adaptive evolution towards a hypothetic optima – θ) from which the maximum likelihood estimates for σ2 (Brownian rate parameter) and θ (optimal trait value) are obtained. During a second, ‘backward phase’, it collapses regimes at different branches and evaluates the AIC again. This time AIC can improve when reducing the number of the parameters outweighs the potential decrease in the log-likelihood. After collapsing all pairs of identified regime shifts it accepts the set of collapses that reaches optimal AIC, indicating that collapsed regimes are convergent.

The analysis was conducted on natural logged and min-max standardised body size and the natural logged residuals from the phylogenetic regression of brain and body size (residual brain size) due to high collinearity between brain size and body size.

### < Model selection

We performed model selection using the dredge function in the MuMIn package on a set especially imputed for that purposes, using the procedure explained above (see Imputation section). The initial models were based on expectations from previous studies, but included as many interactions as possible, which were subsequently reduced. We ran each full model twice for 250 000 iterations, with burn in of the first 10000 iterations and sampling rate of 101. Convergence was verified visually and tested with Gelman-Rubin criterion (< 1.1) and effective sample size was always above 2000.  
Subsequently, one of the chains was ‘dredged’ and the preferred candidate model was chosen based on the requirement to be within Δ <= 3 of the best candidate model ranked by DIC, and containing the most exhaustive set of variables, and least interactions. >

### MCMCglmm

Due to its convenient wrapper functions we used the package mulTree (12) to conduct MCMCglmm (13) 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 |  | (1) + |
| Body | grams |  | (14–18) |
| Origin | 1 – Australia, 2 – New Guinea, 3 - Americas | Different origins predispose different influence of seasonality, predation pressure, food abundance. | (15, 16, 18) |
| Status | 1 - Common, abundant, 2 - Vulnerable, endangered, rare, declining, limited  3 - Extinct | Highly threatened mammals are known to have larger relative brain sizes (19) | (15, 17) |
| 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. | (1, 15, 16) |
| Arboreality | 1 - Arboreal or scansorial, 2 - Terrestrial | Arboreal environment is considered more cognitively demanding. | (1, 15, 16) |
| 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. (20) | (1, 15, 16) |
| 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 | (1, 15, 16) |
| Group living | 1 – No, 2 - Yes | Measure of social complexity, which imposes greater interaction and recognition demands | (1, 15, 16, 18) |
| Parental care | 1 – No, 2 - Yes | Parental investment is known to positively influence brain size (21) | (1, 15, 16, 18) |
| Mating system | 1 – Promiscuous, 2 - Complex (polygamous/monogamous) | Complex mating systems require more cognitive complexity and usually result in higher parental investment (22) | (1, 15, 16) |
| Litter size | Average litter per reproductive episode | Constraint on maternal investment. | (1, 15) |
| Weaning age | Months | Constraint on maternal investment. | (1) |
| Home range | Hectares | Larger home ranges usually imply increased cognitive complexity related to orientation (23) | (1, 15, 18) |
| Population density | Individuals per hectare | Increased population density is a proxy of increased interaction and social tolerance. | (1, 15) |
| FMR | Field metabolic rate | Measure of metabolic turnover in the wild. | (24) |
| Torpor | 0 – No, 1 – Yes | Torporing has been shown to be costly to the maintenance of large brains (25) | (26–28) |
| Play | 1 – No, 2 – Rudimentary, 3 - Complex | Proxy for cognitive ability. Play has been shown to correlate with larger brains in birds and mammals (29) | (29, 30) |

Dataset

Imputed datasets

R Code

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# References

1. Weisbecker V, Blomberg S, Goldizen AW, 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):125–35.

2. Revell LJ (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* 3(2):217–223.

3. Nakagawa S, Freckleton RP (2008) Missing inaction: the dangers of ignoring missing data. *Trends Ecol Evol* 23(11):592–596.

4. Rubin DB (1987) *Multiple imputation for nonresponse in surveys Donald B. Rubin.* doi:10.1002/9780470316696.

5. Resche-Rigon M, White IR (2018) Multiple imputation by chained equations for systematically and sporadically missing multilevel data. *Stat Methods Med Res* 27(6):1634–1649.

6. Fisher DO, Blomberg SP, Owens IPF (2003) Extrinsic versus intrinsic factors in the decline and extinction of Australian marsupials. *Proc R Soc London Ser B Biol Sci* 270(1526):1801–1808.

7. Buuren S van, Groothuis-Oudshoorn K (2011) mice: Multivariate Imputation by Chained Equations in R. *J Stat Softw* 45(3):1–67.

8. Little RJA (1988) Missing-Data Adjustments in Large Surveys. *J Bus Econ Stat* 6(3):287–296.

9. Buuren S van (2018) *Flexible imputation of missing data* (CRC Press).

10. White IR, Royston P, Wood AM (2011) Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med* 30(4):377–399.

11. Ingram T, Mahler DL (2013) SURFACE: detecting convergent evolution from comparative data by fitting Ornstein-Uhlenbeck models with stepwise Akaike Information Criterion. *Methods Ecol Evol* 4(5):416–425.

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

13. Hadfield JD (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *J Stat Softw* 33(2):1–22.

14. Weisbecker V, Ashwell K, Fisher D (2013) An improved body mass dataset for the study of marsupial brain size evolution. *Brain Behav Evol* 82(2):81–82.

15. Gynther I, Baker A (2013) *Field companion to the mammals of Australia* Available at: http://eprints.qut.edu.au/69784 [Accessed September 22, 2018].

16. Flannery T (1995) *Mammals of New Guinea* (Cornell University Press, Ithaca, United States).

17. IUCN (2018) The IUCN Red List of Threatened Species. *Version 2018-2*. Available at: http://www.iucnredlist.org [Accessed August 15, 2018].

18. Myers P, et al. (2006) The animal diversity web. *Accessed June 2018* 12(2006):2.

19. Abelson ES (2016) Brain size is correlated with endangerment status in mammals. *Proceedings Biol Sci* 283(1825):20152772.

20. Reddon AR, Chouinard‐Thuly L, Leris I, Reader SM (2018) Wild and laboratory exposure to cues of predation risk increases relative brain mass in male guppies. *Funct Ecol* 32(7):1847–1856.

21. Isler K, van Schaik CP (2012) Allomaternal care, life history and brain size evolution in mammals. *J Hum Evol* 63(1):52–63.

22. Schillaci MA (2006) Sexual selection and the evolution of brain size in primates. *PLoS One* 1(1):e62.

23. Seyfarth RM, Cheney DL (2002) What are big brains for? *Proc Natl Acad Sci U S A* 99(7):4141–2.

24. Riek A, Bruggeman J (2013) Estimating field metabolic rates for Australian marsupials using phylogeny. *Comp Biochem Physiol - A Mol Integr Physiol* 164(4):598–604.

25. Heldstab SA, Isler K, van Schaik CP (2018) Hibernation constrains brain size evolution in mammals. *J Evol Biol*. doi:10.1111/jeb.13353.

26. Ruf T, Geiser F (2015) Daily torpor and hibernation in birds and mammals. *Biol Rev Camb Philos Soc* 90(3):891–926.

27. McNab BK (2008) An analysis of the factors that influence the level and scaling of mammalian BMR. *Comp Biochem Physiol - A Mol Integr Physiol* 151(1):5–28.

28. Geiser F, Körtner G (2010) *Hibernation and daily torpor in Australian mammals* doi:10.7882/AZ.2010.009.

29. Iwaniuk AN, Nelson JE, Pellis SM (2001) Do big-brained animals play more? Comparative analyses of play and relative brain size in mammals. *J Comp Psychol* 115(1):29–41.

30. Ashwell KWS (2008) Encephalization of Australian and New Guinean marsupials. *Brain Behav Evol* 71(3):181–199.