The Evolution of Relative Brain Size in Marsupials Is Energetically Constrained but Not Driven by Behavioral Complexity

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Key Words

Brain size · Marsupials · Maternal investment · Seasonality · Life history · Constraint

Abstract

Evolutionary increases in mammalian brain size relative to body size are energetically costly but are also thought to confer selective advantages by permitting the evolution of cognitively complex behaviors. However, many suggested associations between brain size and specific behaviors – particularly related to social complexity – are possibly confounded by the reproductive diversity of placental mammals, whose brain size evolution is the most frequently studied. Based on a phylogenetic generalized least squares analysis of a data set on the reproductively homogenous clade of marsupials, we provide the first quantitative comparison of two hypotheses based on energetic constraints (maternal investment and seasonality) with two hypotheses that posit behavioral selection on relative brain size (social complexity and environmental interactions). We show that the two behavioral hypotheses have far less support than the constraint hypotheses. The only unambiguous associates of brain size are the constraint variables of litter size and seasonality. We also found no association between brain size and specific behavioral complexity categories within kanga-

roos, dasyurids, and possums. The largest-brained marsupials after phylogenetic correction are from low-seasonality New Guinea, supporting the notion that low seasonality represents greater nutrition safety for brain maintenance. Alternatively, low seasonality might improve the maternal support of offspring brain growth. The lack of behavioral brain size associates, found here and elsewhere, supports the general ‘cognitive buffer hypothesis’ as the best explanatory framework of mammalian brain size evolution. However, it is possible that brain size alone simply does not provide sufficient resolution on the question of how brain morphology and cognitive capacities coevolve.

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Introduction

One of the most impressive mammalian traits is an extremely large brain relative to body size, which reflects the superior cognitive abilities of mammals compared to most other vertebrates. It is presumed that some aspect of improved cognition selects for the evolution of large brains relative to body size (here referred to as ‘large brains’) in mammals [Healy and Rowe, 2007; Dunbar, 2009a; van Woerden et al., 2012]. Selection-focused research into cognitive correlates of brain size therefore

asks which behaviors are so cognitively complex that they require a larger brain [Dunbar, 1998; Weisbecker and Goswami, 2011]. A frustrating variety of selection factors [Healy and Rowe, 2007] have been proposed, which can be classified into two types [Dunbar, 1998]. The first, very popular, type is based on the ‘social brain hypothesis’ (SBH) [Dunbar, 1998]. This suggests that complex social bonds, particularly pair bonding, select for larger brain sizes [Marino, 2004; Shultz and Dunbar, 2006, 2010b; Walker et al., 2006; Dunbar and Shultz, 2007a]. The second type, whose constituent parameters could be called ‘environmental interaction traits’, includes interactions with the environment such as locomotor modes or circadian rhythms [e.g. Eisenberg and Wilson, 1981]. However, the practice of attaching specific behaviors to brain size evolution has been criticized. In particular, a third hypothesis not tied to specific behaviors – the ‘cognitive buffer hypothesis’ – simply states that large brains are advantageous because they allow for behavioral flexibility in novel or complex environments, for example during species invasions [Reader and Laland, 2002; Sol et al., 2007; Sol, 2009; Lefebvre et al., 2013].

The degree to which selection for large brains can act is limited by the considerable cost of large brains. Contrary to the relative inconclusiveness of selection-based research on mammals [Healy and Rowe, 2007], a large body of evidence shows that brain size is limited by maternal investment (the ability of females to provide sufficient nutrition for offspring brain growth) [Martin, 1981; Jones and MacLarnon, 2004; Martin et al., 2005; Finarelli, 2009a; Isler and Van Schaik, 2009; Shultz and Dunbar, 2010b; Weisbecker and Goswami, 2010, 2011; Barton and Capellini, 2011]. In addition, recent work on primates suggests that seasonality represents another energetic constraint on brain size [van Woerden et al., 2010, 2012] possibly because the brain depends on reliable nutrition.

Constraint-focused and selection-focused research on brain size are traditionally conducted separately, an approach that has sometimes been explicitly advocated [Dunbar and Shultz, 2007b; Dunbar, 2009b]. However, this separation is problematic because of the well-known connections between hypothesized behavioral selectors and constraining traits on brain size [Shultz and Dunbar, 2010b; van Woerden et al., 2010, 2012; Weisbecker and Goswami, 2011]. Social traits are particularly susceptible to such confounding factors because they often coevolve with particular life histories and developmental regimes [Temrin and Tullberg, 1995; Geffen et al., 1996; Bhumstein and Armitage, 1997; Hatchwell and Komdeur, 2000; Olson et al., 2008; González-Lagos et al., 2010; Shultz and

Dunbar, 2010b]. For example, pair bonding requires complex interactions and is proposed to be a key selector for larger brains [Dunbar and Shultz, 2007b], but it has also been argued to improve offspring provisioning by allowing females to invest more energy into their offspring’s brain development [Isler and Van Schaik, 2009, 2012]. This issue is particularly relevant because of the near-exclusive research focus on placental mammals [exceptions are Eisenberg and Wilson, 1981; Legendre et al., 1994; Iwaniuk et al., 2000]. Placentals are interesting because they contain several extremely large-brained clades, such as primates (including humans), canid carnivores, and cetaceans. However, placentals vary greatly in life history and reproduction [Capellini et al., 2010; Barton and Capellini, 2011; Weisbecker and Goswami, 2011], which increases the risk that behavioral associates of placental brain size are confounded with life-history factors that exert maternal investment constraints.

Marsupial mammals are much more homogenous in their life history than placentals, thus presenting an opportunity to test hypotheses of mammalian brain size evolution in a framework that is less confounded by reproductive traits. For at least 60 million years [when the ancestors of modern-day marsupials diverged; Horowitz et al., 2009], marsupials have been born after an extremely short gestation (12–30 days) requiring little maternal energy [Tyndale-Biscoe, 2005]. Marsupial neonates are extremely altricial. Milk composition and lactation duration are more homogenous across marsupials than they are across placentals [Green and Merchant, 1988; Tyndale-Biscoe, 2005]. Marsupials display nearly all ecotypes and social behaviors considered relevant by selection-based studies of brain size evolution (including pair bonding and allomaternal care, considered key constraining [Isler and Van Schaik, 2012] as well as selecting [Dunbar, 2009a] traits on brain size). In addition, marsupials allow the evaluation of a constraint of seasonality on brain size [van Woerden et al., 2010, 2012] because they are distributed from seasonal temperate to aseasonal equatorial biogeographical zones. In particular, representatives of all orders are also found in New Guinea (NG), which experiences little environmental seasonality [Feng et al., 2013], no seasonally predictable fluctuation in insect activity, and continuous rather than seasonal breeding of dasyurids, unlike most of Australia [Fisher et al., 2013]. This allows for testing of the hypothesis that seasonality associates with increased brain sizes, presumably due to a more consistent availability of food which allows the nutritional requirements of large brains to be met [van Woerden et al., 2010, 2012].

Table 1. Description of the traits used in the pgls models

Trait	Units/categories	Reasons proposed for the impact on brain size evolution	References
Population density	Individuals per hectare	A dense population increases cognitively intense social interactions	Walker et al., 2006; Bailey and Geary, 2009
Group living	Solitary (1), living in a group (2)	Group living requires greater recognition and interaction skills	Shultz and Dunbar, 2006; Walker et al., 2006; Dunbar and Shultz, 2007a
Parental care	No parental care (1), paternal/family care (2)	Highly complex behavior due to the requirement of interactions	Shultz and Dunbar, 2007, 2010a
Mating system	Promiscuous (1), polygamous/monogamous/complex mating systems (2)	A greater mating bond complexity requires more cognitively complex social interaction	Pitnick et al., 2006; Schillaci, 2006; Shultz and Dunbar, 2006
Home range	Hectares	The spatial complexity of an increased home range requires improved cognition	Clutton-Brock and Harvey, 1980; Walker et al., 2006; Dunbar and Shultz, 2007a
Diet	1 = >50% grass/browse; 2 = seeds, grass, roots, leaves, fruit, invertebrates; 3 = nectar, fruit, invertebrates; 4 = >50% invertebrates/vertebrates	A constraint due to either gut size or energy content or selecting for large brain size via foraging complexity	Clutton-Brock and Harvey, 1980; Eisenberg and Wilson, 1981; Mace et al., 1981; Barton, 2006; Hartwig et al., 2011
Arboreality	1 = Arboreal or scansorial; 2 = terrestrial	A complex arboreal environment is cognitively demanding	Eisenberg and Wilson, 1981; Mace et al., 1981; Meier, 1983
Diurnality	0 = Strictly nocturnal; 1 = not strictly nocturnal	Nocturnal birds, primates, marsupials are considered larger-brained	Clutton-Brock and Harvey, 1980; Eisenberg and Wilson, 1981; Mace et al., 1981; Garamszegi et al., 2002
Shelter safety	1 = Protected (burrow/constructed nest in a tree hollow); 2 = intermediate (tree canopy/hollow log/under a rock/nest on the ground or in a soil crack); 3 = open (under shrubs/in grass/tree shade)	Used as proxy for predation particularly on juveniles [Fisher et al., 2001]; predation pressure is considered a selection factor for larger brains	Palombo et al., 2008; Dunbar, 2009b; Kondoh, 2010
Litter size	Average litter size/species	Maternal investment constraint	Isler and Van Schaik, 2009, 2012; Weisbecker, 2010
Weaning age	Age at weaning	Maternal investment constraint	Martin, 1981; Weisbecker, 2010; Isler and Van Schaik, 2012

This study exploits the suitability of marsupials for studies of brain size evolution to jointly evaluate four hypotheses: the constraint-based ‘maternal investment hypothesis’ and ‘seasonality hypothesis’ and the selection-based SBH and ‘environmental interaction hypothesis’. Using phylogenetically corrected general least squares modeling and detailed within-clade analyses, we search for significant associations of the model variables with brain size and evaluate the relative explanatory power of the models.

Materials and Methods

Data Collection

Data on marsupial endocranial volumes (proxies for brain size) and body mass were taken from Ashwell [2008] and Weisbecker et al. [2013], respectively. Based on a literature review, a set of potential brain size correlates representing the maternal investment, seasonality, social brain, and environmental interaction hypotheses were chosen (table 1) to form part of our data set (online suppl. material 1; see www.karger.com/doi/10.1159/000377666 for all online suppl. material). Note that basal metabolic rate, which is often included in constraint-based explanatory frameworks of mamma-

lian brain evolution, is unlikely to associate with marsupial brain size [Weisbecker and Goswami, 2010, 2011, 2014] and was therefore excluded from the analysis. Data on maternal investment, social behaviors, and environmental interaction traits were mostly from Fisher et al. [2001, 2014]. Additional references for data on South American and NG marsupials and macropodoid (kangaroos, wallabies, and rat kangaroos) group complexity are listed in online supplementary material 2. Latitudinal data for Australian and NG species are from Fisher et al. [2014], with the addition of latitudinal estimates for some NG species based on averages of the maximum and minimum latitude [International Union for Conservation of Nature, 2014]. To avoid atomization and for sufficient sample sizes in each variable bin, broad categories were employed for the modeling analyses. For finer-grained assessment specifically of the SBH, relative brain sizes were separately investigated in the following categories of group complexity: (1) macropodoids (kangaroos; $n = 25$), with (a) solitary, (b) fission-fusion groups, or (c) polygynous social systems; (2) dasyurids (marsupial predators; $n = 43$), with (a) communal (genus *Antechinus*, which nests in family groups) or (b) solitary social systems, and (3) petaurids (ringtail possums and gliders; $n = 25$), with (a) no paternal/family care, (b) extended family care, or (c) paternal care. If varying behaviors were reported for different populations of a species, we scored the one considered more complex (e.g. monogamy over polygyny in brush-tailed possums [Brown et al., 2007; Martin and Martin, 2007]).

Model Selection and Fitting

Four models, all with logarithm-transformed brain volume as the dependent variable, were compiled from the data and compared. The predictor variables of the SBH model ($n = 63$) included group size, parental care, mating system, and population density. The 'environmental interaction model' ($n = 97$) included home range size, diurnality, shelter safety, diet, and locomotor type. The 'maternal investment constraint model' ($n = 92$) included litter sizes and weaning age. The 'seasonality constraint model' ($n = 178$) compared marsupials from NG to marsupials from Australia. The assembly of the four models according to established hypotheses allowed us to distribute the variables into biologically meaningful sets that have previously been associated with brain size, while allowing a good balance between the sample sizes and the variables tested. However, the models are not mutually exclusive as constituent variables of several models could conceivably associate with brain size.

Body size heavily influences many biological variables [Martin et al., 2005], often in a nonlinear and complex way [Sibly and Brown, 2007; Anderson-Teixeira et al., 2009], which might result in different associations between brain size and the predictor variables at different body sizes. To address this, aside from including body size as a covariate in all models, we initially computed ANOVA tables of models in which an interaction of each variable with log body size was included. Due to the resulting strong collinearity in the models, determined by assessment of variance inflation factors as computed in the car package [Fox and Weisberg, 2011] in R, we first computed type II SSQ ANOVA tables to assess whether any of the interaction terms with body mass were significant. Nonsignificant interaction terms were dropped from the final model ANCOVAs, resulting in substantially lower variance inflation factors.

Models were fit using phylogenetic generalized least squares (pgls), computed by the nlme [Pinheiro et al., 2014] and ape [Paradis et al., 2004] packages in R 2.15.2 [R Core Team, 2014]. This

avoids problems of phylogenetic nonindependence [Felsenstein, 1985] by incorporating an estimate of the sample's phylogenetic correlation structure and branch lengths. We collated a compound phylogeny based on the recent literature (online suppl. material 2, 3) and computed several branch length transformations (ultrametricized according to Grafen [1989], Nee [Purvis, 1995], and Pagel [1992] in Mesquite version 2.75 [Maddison and Maddison, 2011]) and evolutionary models (Brownian motion, Pagel [1999], and Grafen [1989]). We determined the best combination of branch length and evolutionary model for each of our models by determining the relative likelihoods of each combination based on Akaike scores [Burnham and Anderson, 2002; Grigione et al., 2002].

ANOVA tables of all models were computed using type II sums of squares (type II SSQ) and the most likely phylogenetic settings as described above. The use of type II SSQ is a conservative approach that calculates associations of each variable with the dependent variable after adding all other variables to the model. This also avoids the issue of needing to specify a variable input order, which in our case would have been arbitrary and artificial.

We also fit three of the models (social brain, environmental interaction, and maternal investment constraints) based on a smaller joint data set ($n = 59$; it is a prerequisite of Akaike comparisons that the models are run with the same data and the same correlation structure). This allowed us to use Akaike weight score comparisons to determine which of the models was most likely. To this comparison, we also added a model with body size as the only predictor variable, to assess whether body size is the only likely predictor of brain size. The scant information available on the life history and behaviors of NG marsupials would have diminished the number of species in a joint data set, so that the seasonality constraint model was not compared to the other three models. Mammals on islands such as NG have been suggested to both increase and decrease body size faster than brain size [Palombo et al., 2008; Weston and Lister, 2009]. To determine whether our results might be influenced by such an island effect, a pgls model comparing body size in NG marsupials and all other species was also computed.

pgls Residual and Within-Clade Analyses

From the within-clade data, we used ANOVA tables of pgls models as described above to assess whether behavioral categories within macropodoids, dasyurids, and petaurids were associated with brain size. A pgls ANOVA table to assess whether the overall more social radiation of petaurid possums (ringtail possums, gliders, and allies) was larger-brained than all other marsupials was also computed.

Results

Model Fitting

The only variables that unambiguously associated with brain size (aside from body size) were litter size, NG origins, and, with weak significance, latitude. For a visual representation of the relative brain sizes of species of NG origins, as well as mating complexity (one of the most important variables of the SBH), see figure 1.

Table 2. ANOVA tables of the pgl models, including model formulae and phylogeny settings

	F	p	AIC	λ or ρ
<i>Maternal investment model</i> ¹			0.57	0.89
Body mass	532.04	0.000		
Litter size	5.4	0.0223		
Weaning age	1.39	0.2408		
<i>Social brain model</i> ²			0.000	1
Body mass	367.08	0.000		
Group size	8.34	0.0052		
Parental care	0.03	0.86		
Mating system	0.00	0.96		
Population density	5.76	0.02		
Group size:body mass	8.36	0.0052		
Population density:body mass	6.2	0.01		
<i>Environmental interaction model</i> ³			0.000	1
Body mass	312.89	0.000		
Diurnality	2.03	0.16		
Shelter safety	0.68	0.52		
Arboreality	1.56	0.22		
Diet	0.83	0.49		
Home range	0.17	0.68		
<i>Body size only model</i> ⁴			0.43	0.29
Body mass	1,163.02	0.000		
<i>Geographical distribution model</i> ⁵			Not compared	0.84
Body mass	977.27	0.000		
NG origins	10.36	0.0016		
Latitude	4.12	0.044		

Significant associations between brain size and variables are shown in bold. Intercepts of all models were highly significant at $p = 0.000$. AIC = Akaike W score of a comparison of the first three models based on a joint data set ($n = 59$); λ or ρ = Pagel's lambda or Grafen's rho (measuring phylogenetic signal in the data set; Brownian motion is 1 for λ or ρ).

¹ log brain size~litter size + log weaning age + log body mass, d.f. = 90, assuming a Pagel-ultrametricized tree and Pagel's correlation structure.

² log brain size~group size~log body mass + parental care + mating system + log population density~log body mass + log body mass, d.f. = 56, assuming a Pagel-ultrametricized tree and Brownian motion.

³ log brain size~diurnality + shelter safety + arboreality + diet + log home range + log body mass, d.f. = 60, assuming a Pagel-ultrametricized tree and Brownian motion.

⁴ log brain size~log body mass, d.f. = 180, assuming a Grafen-ultrametricized tree with Grafen's correlation structure.

⁵ log brain size~NG origins + latitude + log body mass, d.f. = 178, assuming a Nee-ultrametricized tree and Pagel's correlation structure.

In all models except the SBH model, interaction terms with body size were nonsignificant so that the final models were run without interactions (table 2). However, the final SBH model included significant interactions of population density and group size variables with body size, as well as significant associations of these two variables with brain size. The significance of the interaction terms was weak (near $p = 0.01$), the model was highly collinear, and

the model's coefficients revealed interaction term parameter values an order of magnitude smaller than those of the main effects (online suppl. material 4). Nevertheless, we retained the interaction terms in the model because, although they are of a small magnitude, they are unlikely to be exactly zero (for the model without interactions and extended reports of model coefficients, see online suppl. material 4).

Fig. 1. Log brain volume plotted against log body mass, with a pgl's fit line, with NG species and species with nonpromiscuous mating systems highlighted.

Table 3. ANOVA tables of pgl's models for within-clade analysis

	F	p	λ or ρ
<i>Macropodoids</i> ¹			0.72
Intercept	7.6	0.012	
Body mass	87.8	0.000	
Group complexity	0.56	0.58	
<i>Dasyurids</i> ²			0.87
Intercept	70.59	0.000	
Body mass	174.65	0.000	
Communal nesting	0.00063	0.98	
<i>Within petauroids</i> ³			1
Intercept	48.49	0.012	
Body mass	108.92	0.000	
Parental care type	1.34	0.29	
<i>Petauroids vs. other marsupials</i> ⁴			0.27
Intercept	80.06	0.000	
Body mass	1,217.8	0.000	
Petaurid affinity	1.74	0.189	

¹ log brain size~group complexity + log body mass, n = 25, assuming a Grafen-ultrametricized tree and Pagel's correlation structure. ² log brain size~communal nesting + log body mass, n = 43, assuming a Grafen-ultrametricized tree and Pagel's correlation structure. ³ log brain size~parental care type + log body mass, n = 25, assuming a Pagel-ultrametricized tree and Brownian motion. ⁴ log brain size~petaurid clade affinity + log body mass, n = 180, assuming a Grafen-ultrametricized tree and Grafen's correlation structure.

p-values indicating significance ($p \leq 0.05$) are bold. λ or ρ = Pagel's lambda or Grafen's rho.

A possibly confounding impact of an island effect on body size, where a fast change in body size would result in either relatively smaller or larger brains, could be excluded as NG species did not have significantly different body sizes ($p = 0.450$; Pagel's correlation structure, Nee-transformed branch lengths).

Akaike weight comparisons (table 2) showed that the likelihood of the maternal investment model was far greater relative to that of the social complexity and environmental interaction models. The maternal investment constraint model was also more likely than the body mass only model.

None of the social behavior types within the separate models of dasyurids, macropodoids, within-petauroids and between petauroids versus marsupials were significantly associated with brain size (fig. 2; table 3). Notably, NG marsupials of all Australidelphian orders were well represented within the 5% of species with the relatively largest brains (6 out of a total of 9), as determined by the residuals from the regression shown in figure 1 (for largest- and smallest-brained species, see table 4). Of these, the only potentially social species was the petaurid *Dactylopsila palpator*, a little-studied close relative of the striped possum *Dactylopsila trivirgata*, which can nest in pairs or groups that interact frequently [Handasyde and Martin, 1996; McKenna, 2005]. In a zoo colony, *Dactylopsila* showed no signs of pair bonding or paternal care [McKenna, 2005]. The remaining species within the top 5% bracket include the solitary omnivorous bilby, the

Fig. 2. Log brain volume plotted against log body mass, with a pgl fit line, in dasyurids (a), macropodoids (b), and petauroids (c) with two different types of social organization.

probably solitary carnivorous NG dasyurids *Myoictis melas* and *Murexia longicauda*, the solitary omnivorous NG spiny bandicoot *Peroryctes raffrayana*, the solitary folivorous NG tree kangaroo *Dendrolagus dorianus*, and the solitary NG folivorous phalanger *Phalanger carmelitae*. By contrast, two petauroid possum species with strong pair bonds – the rock-haunting possum *Petroseudes dahli*, which displays obligate pair bonding and paternal care [Runcie, 2000], and the lemuroid ringtail possum *Hemibelideus lemuroides*, which is organized in family groups [Winter and Atherton, 1984] – are among species with the lowest 10% of pgl residuals; even without phylogenetic corrections, these species are very small-brained relative to similarly sized marsupials.

Discussion

Our study 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)

Table 4. Top and bottom 10% of residuals from a pgl's fit of brain size versus body size in all marsupials (n = 180; assuming Pagel-ultrametricized trees and Grafen's correlation structure)

Bottom 10%		Top 10%	
<i>Sminthopsis douglasi</i>	-3.24186794	<i>Dendrolagus goodfellowi</i>	1.253399
<u><i>Hemibelideus lemuroides</i></u>	-2.51278287	<i>Sminthopsis griseoventer</i>	1.31274
<i>Marmosa robinsoni</i>	-2.48021863	<i>Microperoryctes longicaudata</i>	1.339236
<i>Planigale gilesi</i>	-2.12058413	<i>Macropus giganteus</i>	1.381831
<i>Cercartetus concinnus</i>	-2.00173976	<i>Antechinus stuartii</i>	1.46412
<i>Phalanger orientalis</i>	-1.98449353	<i>Dorcopsis atrata</i>	1.513139
<i>Macropus greyi</i>	-1.98237692	<i>Thylacinus cynocephalus</i>	1.531801
<i>Echymipera rufescens</i>	-1.92536399	<i>Caluromys philander</i>	1.53887
<i>Sminthopsis virginiana</i>	-1.92057437	<i>Dendrolagus lumholtzi</i>	1.543503
<i>Phalanger lullulae</i>	-1.80482822	<i>Antechinus swainsonii</i>	1.564659
<i>Cercartetus nanus</i>	-1.63024674	<i>Phalanger carmelitae</i>	1.568368
<u><i>Petropseudes dahli</i></u>	-1.62178071	<i>Peroryctes raffrayana</i>	1.942001
<i>Pseudantechinus roryi</i>	-1.55530299	<i>Myoictis melas</i>	1.969024
<i>Monodelphis brevicaudata</i>	-1.38261595	<i>Dendrolagus dorianus</i>	2.093844
<i>Dasyurus maculatus</i>	-1.26887549	<i>Perameles bougainville</i>	2.184201
<i>Pseudocheirus canescens</i>	-1.26119522	<i>Murexia longicaudata</i>	2.29615
<i>Didelphis marsupialis</i>	-1.1925408	<u><i>Dactylopsax palpator</i></u>	2.368372
<i>Antechinus laniger</i>	-1.16566976	<i>Macrotis leucura</i>	2.450916

Dark grey covers 0–5%, and light grey covers 5–10%. NG species are bold, and social or potentially social species are bold and underlined.

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.

The significant associations between low-seasonality NG origins and litter size with marsupial brain size raise the intriguing possibility that the effects of these two variables on brain size are mechanistically linked. It is possible that the improved food availability in NG allows females to provide their offspring with more consistent or better nutrition, thus allowing their young to grow larger brains. In this context, it is worth noting that lactational investment may possibly play a role in the raising of large-brained offspring, despite the lack of an association of weaning age with brain size in our analyses. This is because such an association may be obscured by the strong phylogenetic signal from peramelids (bandicoots) and didelphids (opossums), which are extremely fast-weaning and small-brained [Weisbecker and Goswami, 2014].

However, at present the notion of a link between low seasonality and improved maternal investment cannot be distinguished from the current view that low seasonality facilitates the consistent nutrition of adult brains [van Woerden et al., 2010, 2012].

Our results show that selection for social complexity – such as pair bonding and group living – is not a pervasive influence on the evolution of large mammalian brains [Dunbar, 1998, 2009b; Shultz and Dunbar, 2006, 2010b; Dunbar and Shultz, 2007b]. This result is consistent with our suspicion that many of the social complexity/brain size associations found in placentals arise from a confounding influence of reproductive life history traits on social complexity patterns. We found no indication that more socially complex dasyurids, macropodoids, and petauroids are larger-brained than other members of their respective clades; indeed, the anecdotal evidence from single species shows that several marsupials with high degrees of social complexity are particularly small-brained. The lack of an association between brain size and parental care is interesting because allomaternal care (where mothers are helped to raise their offspring) has been convincingly linked to larger brain sizes because it improves the ability of mothers to supply energy to their offspring. Canid carnivores in particular show this pat-

tern [Isler and Van Schaik, 2012], but it clearly is not a factor in marsupial brain size evolution.

A caveat remains that significant interactions between population density and group size with body mass preclude a decision as to whether these two parameters influence brain size. As discussed above, however, significant interactions within the model were of small magnitude while a noninteraction model returned no association of population density or group size with brain size. The model performed extremely poorly relative to other models in the Akaike information criterion comparisons, suggesting that the variables contained in the model do not explain much of brain size variation regardless of the details of their association.

The lack of reliable behavioral correlates of increased brain size, found here and in other studies [Walker et al., 2006; Healy and Rowe, 2007; Finarelli, 2009b; Shultz and Dunbar, 2010b], is in stark relief to the clearly high costs of raising large-brained offspring [Dunbar, 2009b]. Why was this selection gradient so spectacularly overcome first by the ancestors of mammals [Rowe et al., 2011] and again in several mammalian radiations? It is possible that searching for specific behaviors that might have caused such selection is the wrong approach, as proponents of the cognitive buffer hypothesis suggest. Instead, larger-brained mammals and birds seem to be more successful when faced with challenges that require a general ability to deal with cognitive challenges [Sol, 2009; González-Lagos et al., 2010; van Woerden et al., 2012]. This is supported by the fact that extremely large-brained mammals, such as odontocetes and primates, exhibit high social complexity but also excel in many other areas of cognition (including learning, spatial orientation ability, and sensorimotor skills [González-Lagos et al., 2010; Kondoh, 2010]).

Another issue is highlighted by an increasing body of evidence that relative brain size per se contains too much noise to be an accurate predictor of mammalian cognition, as it does not reflect the brain infrastructure very

well. Recent success in using brain components to understand the evolution of brain macromorphology [Sakai et al., 2011; Swanson et al., 2012; Gómez-Robles et al., 2014] and particular behaviors [de Winter and Oxnard, 2001], together with the realization that cell density and connectivity differ substantially among mammals [Herculano-Houzel et al., 2007, 2011; Sarko et al., 2009; Huang et al., 2014], also suggest that overall brain size may be too vague a predictor of cognitive ability. There is mounting evidence that relatively large brains often arise through selection for a smaller body size, rather than larger brain size [Fitzpatrick et al., 2012; Smaers et al., 2012; Swanson et al., 2012], so that in many cases no cognitive selectors of large brain size may exist.

In summary, our results suggest that marsupial brain size is not driven by selection for specific behaviors, as suggested by work on various placental clades. We suspect that much of the behavioral signal from placentals might arise from the confounding influence of their diverse reproduction. The only clear signal arising from our work and that of others is that marsupial brain size and mammalian brain size in general are constrained by nutritional requirements whose precise nature remains to be elucidated. Further understanding of this cost-benefit conundrum might be improved by linking work on brain macromorphology with the physiological and cellular properties of the evolving mammalian brain and consideration of the structure and physiology of brain tissue.

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