Commentary

Marsupials indeed confirm an ancestral mammalian pattern: A reply to Isler

Vera Weisbecker[1)2)3)*] *and Anjali Goswami*[3)4)]

In a recent publication [1] (see also our recent comment in BioEssays [2]), we demonstrated that marsupials are not, as frequently thought, systematically smaller-brained than placentals. We also showed that partial correlations of gestation length, weaning age, litter size, basal metabolic rate (BMR) and brain size – all adjusted for body size – differ in marsupials and placentals. The difference between these two clades consists of the existence of a partial correlation between BMR and brain size in placentals, which we did not find in marsupials. We suggested that placentals differ from what could be called an ancestral mammalian pattern (we prefer the term ancestral rather than general [3] for reasons of information content and accuracy) by having a placenta, through which increases in maternal BMR could benefit offspring brain sizes.

We agree with Isler's title assessment in her recent review of our work [3] that marsupials confirm an ancestral mammalian pattern – we hypothesise that it is the placentals that 'added' an additional avenue of energetically provisioning the growth of a large brain in their offspring. Also, as advocated by Isler, we took an energetic approach to our work (asking the question as to how the costs of increased brain size are met, by simultaneously testing multiple metabolic and reproductive variables). Isler's suggestion that allo-maternal care correlates with increases in marsupial brain size also fits our predictions very well. However, we disagree with several other aspects of Isler's characterisation of our study and her conclusions.

'Misleading' evidence on marsupial brain size

We never claimed (as Isler states) that marsupials are not, on average, less encephalised than placentals, as they certainly are. However, comparing average brain sizes obscures the full range of marsupial encephalisation, which extensively overlaps with that of placentals. Only the largest-brained placentals actually exceed marsupials in relative brain size. We also pointed out that marsupial brain size/body size scaling differs from that of placentals, resulting in exceptionally large brains in small marsupials compared to placentals (the regression slopes of both clades intersect at 43 g; thus, our comparison of relative brain sizes of species weighing $\leq$43 g was not arbitrary, as Isler claims). The different scaling slopes are clearly visible in our Fig. 1A [1] and Isler's Fig. 3A [3]. Despite this clear difference, Isler advocates the use of median slopes of the orders, partly because they better approximate a theoretical slope of 0.65 [4]. We contend that no intrinsic characteristic exists that makes the artificial taxonomic rank of 'Order' more suitable for deriving a true slope, than, for instance, between species of a genus or intra-specifically (in both cases, the slopes are shallower [5]). Furthermore, slopes within placentals are highly variable, ranging from 0.34 in Odontocetes to over 0.75 in Bats, Afrotheria and Primates, casting doubt on the value of the information content of the median of slopes (Table 1). Lastly, without further methodological details, we cannot replicate Isler's derivation of a median slope of 0.65 for marsupials. We obtain a much shallower median slope of 0.56 (Table 1; mirroring, perhaps, an overall shallower cross-species regression slope of marsupials also at ordinal level).

Although we disagree with Isler's approach of obtaining relative brain sizes from hypothetical or ordinal median slopes, the choice of slope does not affect our conclusions (Table 2): small marsupials are larger-brained than small placentals, even using Isler's 0.56 slope, showing that marsupials are not systematically constrained in brain size. Additionally, as Isler's Fig. 1B shows, most placentals have

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[1)] Department of Earth Sciences, University of Cambridge, Cambridge, UK
[2)] Institut für Spezielle Zoologie und Evolutionsbiologie, Friedrich-Schiller Universität Jena, Jena, Germany
[3)] Department of Genetics, Evolution and Environment, University College London, London, UK
[4)] Department of Earth Sciences, University College London, London, UK

***Corresponding author:**
Vera Weisbecker
E-mail: vw248@cam.ac.uk

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Table 1. Slopes and intercepts of a brain size/body weight regression for major mammalian clades

Clade	*n*	Slope	Error	Intercept	Error	*p*-Value
Placentals						
Afrotheria	19	0.78	0.17	−3.29	0.02	0.000
Xenarthra	6	0.47	0.17	−1.11	1.34	0.050
Primates	53	0.77	0.02	−2.34	0.24	0.000
Rodentia	78	0.64	0.016	−2.48	0.1	0.000
Artiodactyla	41	0.59	0.03	−1.38	0.31	0.000
Carnivora	47	0.64	0.02	−1.74	0.18	0.000
Odontocetes	9	0.34	0.03	3.03	0.52	0.000
Chiroptera	166	0.8	0.02	−3.14	0.07	0.000
Eulipotyphla	21	0.67	0.03	−3.06	0.13	0.000
Marsupials						
Dasyuridae	51	0.63	0.01	−2.59	0.06	0.000
Diprotodontia	116	0.62	0.01	−2.32	0.08	0.000
Didelphidae	10	0.49	0.03	−1.95	0.2	0.000
Peramelemorpha	14	0.51	0.08	−1.95	0.5	0.000

Note the variability of slopes within both marsupials and placentals, where many slopes are nowhere near the value of 0.65.

brain sizes similar to those of marsupials. This shows that marsupials are well within the middle of the mammalian range and can reach comparatively large brain sizes. This has been noted before [6, 7], but the importance of reiterating it is highlighted by Isler's version of the 'all-too-frequent' prejudice that perhaps marsupials cannot evolve big brains due to physiological adaptations to unfavourable conditions such as aridity. Aside from the fact that this hypothesis is countered by geological evidence (Australian marsupials encountered aridity quite recently, around 2.5 million years ago or later [8]), Isler herself points out that marsupials *can* have large brains, they just do not have them very often. This suggests that the lack of a large number of large-brained marsupials is due to circumstances rather than constraints. It is also worth remembering that marsupials are less ecologically and taxonomically diverse than placentals (approximately 6% of placental species diversity). For a small and relatively low-diversity clade, nearly a quarter of which belong to the relatively small-brained radiation of kangaroos, marsupials sport a sizeable number of large-brained representatives.

We do have one concession to make regarding our calculation of the brain size/body size ratios of small marsupials versus placentals. We only used ratios in a single instance (rather than across mammalian clades, as Isler suggests), as a shorthand way of checking whether marsupials in the ≤43 g category were significantly larger-brained than placentals, which we acknowledge was not an appropriate procedure. However, using the more correct residuals (from a common brain size/body size regression of marsupials and placentals) does not change the highly significant result. Isler's suggestion of removing the particularly small-brained bats and using shallower slopes of 0.65 or even 0.56 for the determination of residuals does not change the result (Table 2).

Table 2. Mann–Whitney U-tests of Marsupial encephalisation quotients (EQs, n = 41) compared to placental EQs (excluding the very small-brained bats; n = 33), including species weighing ≤ 43 g, and assuming brain size/body size relationships from our joint regression slope of 0.74, as well as Isler's suggested slopes of 0.65 and 0.56

Slope	*U*-Test	*p*-Value
0.74	360	0.001
0.65	1.12	0.002
0.56	435	0.008

Small marsupials have significantly larger-brains than placentals in all tests.

Correlations of BMR with brain size

The presence and nature of a correlation of brain size with BMR is one of the most contentious issues in studies of brain size evolution. After much debate as to whether this link exists at all, Isler and van Schaik [9, 10] have shown that a correlation indeed exists, at least across large placental datasets. However, the biological meaning of this link remains controversial, having been interpreted as reflecting the need to maintain a larger-brain (direct metabolic constraints hypothesis), an avenue of maternal investment (maternal investment hypothesis), or as both (Isler and van Schaik's [10] expensive tissue hypothesis). As Isler [3] herself points out, statistical distinction of the direct constraints versus the maternal investment hypothesis with regards to BMR is currently impossible, but we believe that there is much circumstantial evidence against the direct constraints link. In particular, the lack of a correlation between BMR and brain size in marsupials (as well as in carnivores [11], rodents [9], bats [12], 'Insectivores' [9] and birds [13]; primates are the only clade where a robust correlation exists [9]) strongly argues against the direct constraints hypothesis, since a relationship between BMR and brain size would be expected in *all* mammals, and not just some. We believe that a facultative reproductive link explains better the rarity and tenuousness of brain size/BMR correlations.

It should be added that the main premise of the direct constraints hypothesis – namely, that a large brain is metabolically extremely expensive – is by no means widely accepted. It appears that non-primates require only

a modest part of their energy budget to maintain the brain [14, 15]. Furthermore, the extra energy required to bridge the differential between a brain of 'predicted'-size and a comparatively large one appears to translate into only minute increases in calorie intake or decreases in activity [16].

The difficulty of dealing statistically with BMR is explained in two ways by Isler. Firstly, the data are not good enough because BMR measurements are variable and problematic to measure; and secondly, the link is masked. With regard to the first point, as originally noted by McNab [17], the regression of BMR against body size leads to a tighter linear fit in marsupials than in placentals, suggesting that the marsupial data are at least equivalently robust to those of placentals. We agree with Isler on the masking issue, as BMR consistently explains no (see above) or very little variation in brain size, except for in primates (Isler and van Schaik's [9] own results suggest that, after phylogenetic correction, BMR accounts for 2.6% of brain size variation across mammalian species and a maximum of 13% in precocial placentals; whereas, in Primates, BMR explains 20%). Hence, any existing link with brain size may well be easily masked. The BMR/brain size relationship obtained by adjusting by r_{max} (a theoretical, multi-parameter proxy of maximum population growth [18]), as presented by Isler [3], is problematic because the results are marginally significant and may have been heavily influenced by a high-leverage outlier (Fig. 3 in [3]; perhaps *Tarsipes rostratus*, representing an unreliable value which the author of the BMR data set advises against using [19]). The distribution of data points in Isler's Fig. 3 [3] further emphasizes the (at best) weak, if not non-existent, relationship between BMR and brain size in marsupials. Furthermore, r_{max} is a reproduction-related parameter, so that the pattern suggested by Isler would confirm our view of BMR as a maternal investment, rather than maintenance-related parameter.

Methodological issues

Isler [3] aims a suite of accusations at comparative analyses such as ours, claiming that they 'spread confusion by creating contradictory results'.

(1) We are reminded to check published data for outliers and homogeneity issues before use. Aside from the usual checking of scatter plots and accuracy checks of our life history data, we were very aware that the BMR data were particularly controversial. We, therefore, used data from two independently compiled datasets [17, 20] with identical results, and also re-calculated published BMRs for more than 200 species to ensure accuracy.

(2) Although Isler implies otherwise, we ran analyses using phylogenetically independent contrasts in our study, and discussed the results explicitly. While Isler provides a protocol for using phylogenetic corrections, she does not rigorously adhere to this standard in her own work [9, 10, 13, 21]. We agree that phylogenetic correction is essential to demonstrate that comparative results are not due to phylogenetic influences. However, if a simple, well-assembled, phylogenetically-corrected analysis confirms the results from an uncorrected analysis, further investigation using a gamut of different parameters seems to us unnecessary.

(3) Isler advocates running a PCA to avoid collinearity issues. Collinearity is an issue if two variables in a dataset are highly correlated and, hence, provide redundant information, which is definitely not the case in our analyses. Collinearity in the marsupial dataset that we used for partial correlations is 4.1, as measured by the matrix condition number [calculated as (Maximum/Minimum PCA Eigenvalue)$^{0.5}$], which is far below the threshold of 30 for suspected collinearity.

(4) Isler cautions against exploring data 'until some significant relations are obtained', effectively accusing us of 'fishing'. Our question was whether the relationships between reproductive traits and BMR with brain size are the same in placentals as in marsupials. How this approach does not justify the use of comparative data or constitutes 'fishing' remains a mystery to us.

(5) In Box 1, Isler suggests that body mass should be included in a multivariate analysis rather than using other methods such as body-size adjusted residuals. This is a problem in the case of BMR, which is published with specimen-specific body sizes rather than species means. We, therefore, used separate body size adjustment for BMR and brain size in our partial correlations [1]. Isler and van Schaik [10] calculated a 'corrected' BMR in which they determined what the BMR for a specimen would be at mean species weight, using an empirical slope of BMR/body mass across mammals. However, given that the precise scaling relationship of BMR and body sizes is itself an issue of debate [20], this method of correction is at least as error-prone as the use of residuals.

(6) Isler mentions that parameters of maternal investment and offspring production proxies (specifically, r_{max}) 'have to be considered simultaneously in any model', although this is the first time Isler uses r_{max} in an analysis of the interaction between BMR, life history and brain size. The analysis she presents is also not a simultaneous treatment of maternal investment and offspring production (which would likely violate collinearity), as she provides no data from a multivariate analysis.

Conclusions

Large-scale comparative analyses, with phylogenetic correction, careful checking of data and specific questions, are an important tool in understanding macro-evolutionary patterns and influences on morphological evolution. Our original analysis followed these standards and demonstrated that long-standing hypotheses on the evolution of placental mammal brain size are not simply extendable to all mammals. Disagreements are inevitable, and often helpful, in contentious topics such as brain evolution, but, in this case, the points raised by Isler [3] do not alter the conclusions of our analysis.

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