



Published by the British
Herpetological Society

Evidence for the expensive-tissue hypothesis in the Omei Wood Frog (*Rana omeimontis*)

Long Jin*, Li Zhao^{1,*}, Wen Chao Liu¹, Yu Zeng^{1,2} & Wen Bo Liao¹

¹Key Laboratory of Southwest China Wildlife Resources Conservation (Ministry of Education), China West Normal University, Nanchong, 637009, P.R. China

²China Three Gorges Corporation, Beijing 100038, P.R. China

*Long Jin and Li Zhao contributed equally to the manuscript

Brain size variation across the animal kingdom can be interpreted as a trade-off between selective advantages of higher cognitive ability and the prohibitively high energy demands of a large brain. The Expensive-Tissue Hypothesis (ETH) predicts that brains are costly, and increases in brain size will decrease the size of other metabolically costly tissues. Here, we tested this prediction using the anuran *Rana omeimontis*. Brain size was negatively correlated with gut length, supporting the ETH. We did not find associations between brain size and the size of other organs (heart, liver, lungs, kidneys), but found positive correlations between brain mass and testes as well as limb muscle mass when correcting for the effects of body condition. The negative correlation between gut length and brain mass suggests that diet quality may play a role in the evolution of brain size in *R. omeimontis*.

Key words: brain size, expensive-tissue hypothesis, organs, *Rana omeimontis*

Brain performance is important for fitness associated with social acuity or the ability to manipulate others within a group (Allman, 2000). Brain size is often used as a proxy of the brain's evolutionary state of development in response to cognitive demands, and varies considerably at inter- and intra-specific levels (Striedter, 2005; Gonda et al., 2013). There is strong evidence that ecological, social and sexually selected pressures affect variation in brain size within species (Pitnick et al., 2006; Dunbar & Shultz, 2007; Barton & Capellini, 2011). Brains are however energetically expensive (Mink et al., 1981), and the expensive-tissue hypothesis (ETH) predicts that increases in brain size will inevitably decrease the size of other metabolically costly tissues (such as the gut, Aiello & Wheeler, 1995). Over the past 20 years, most studies of the ETH have focused on interspecific comparisons across vertebrates (Aiello & Wheeler, 1995; Kaufman et al., 2003; Jones & MacLarnon, 2004; Schillaci, 2006; Isler & van Schaik, 2006; Pitnick et al., 2006; Lemaître et al., 2009; Barrickman & Lin, 2010; Navarrete et al., 2011). Some studies support the ETH by highlighting negative size correlations between the brain and other

metabolically expensive issues such as gut, pectoral muscles and testes (Aiello & Wheeler, 1995; Kaufman et al., 2003; Isler & van Schaik, 2006; Pitnick et al., 2006; Barrickman & Lin, 2010; Kotrschal et al., 2013). However, other studies also found that correlations between brain size and other metabolically costly issues were either not significant or even positive (Isler & van Schaik, 2006; Lemaître et al., 2009; Barrickman & Lin, 2010; Navarrete et al., 2011).

One critical caveat when inferring causal mechanisms from comparative studies is that correlations between metabolically costly tissues across species may be based on mechanisms operating at different scales (Agrawal et al., 2010). Intraspecific studies of relative tissue investment in a single species can circumvent this problem (see discussion in Warren & Iglesias, 2012). The Omei Wood Frog (*Rana omeimontis*) is an anuran which devotes a large amount of effort to territory maintenance and defence (Liu et al., 2012a, b), which should make it a good model organism to test the ETH at an interspecific level. Brain, heart, lungs, kidneys, liver, testes and gut tissues have generally been recognised as being metabolically costly (Aiello & Wheeler, 1995; Isler & van Schaik, 2006; Barrickman & Lin, 2010; Navarrete et al., 2011; Warren & Iglesias, 2012). Although muscle tissue is not as expensive in energy consumption per unit mass as visceral organs (Caton et al., 2000), limb muscles in anurans makes up a large proportion of body mass and consume a large proportion of the available energy during locomotion (Duellman & Trueb, 1986). The aim of this study is to test the predictions of the ETH by investigating whether brain mass correlates negatively with other costly organs in *R. omeimontis*.

Fieldwork was conducted in Caiba town of Yibin city (28°47' N, 104°33' E, 281 m a.s.l.), Sichuan, China. A total of 63 males were caught from artificial ponds by hand at night during the breeding season in 2011. The collection of frogs in the study was permitted by the Forestry Bureau of Yibin city, following all the applicable instructions of the Animal Care Guidelines in China. Frogs were kept individually in a rectangular tank (0.5×0.4×0.4 m) before being anaesthetised with benzocaine and then

Correspondence: Wen Bo Liao (liaobo_0_0@126.com)

Table 1. Regressions of brain mass residuals and body condition on other organ size residuals in *Rana omeimontis*. Coefficient estimates from regressions are given with 95% CI in brackets, and Beta, adjusted r^2 and p values associated with each regression are also provided.

Organ size	Brain mass			Body condition		
	Estimates [$\pm 95\%$ CI]	β	p	Estimates [$\pm 95\%$ CI]	β	p
Heart	0.227[0.069,0.386]	0.347	0.006	-0.070[-0.256,0.117]	-0.096	0.453
Lungs	0.157[0.042,0.273]	0.329	0.008	-0.062[-0.195,0.072]	-0.117	0.359
Liver	-0.072[-0.344,0.200]	-0.068	0.598	0.116[-0.182,0.413]	0.099	0.440
Kidneys	0.080[-0.066,0.225]	0.138	0.279	-0.012[-0.174,0.149]	-0.019	0.881
Gut	-0.483[-0.748,-0.219]	-0.424	0.001	0.386[0.082, 0.691]	0.309	0.014
Testes	0.122[0.067,0.178]	0.495	<0.001	-0.119[-0.182,-0.057]	-0.443	<0.001
Limb muscles	0.441[0.177,0.704]	0.394	0.001	-0.339[-0.641,-0.036]	-0.276	0.029

killed by double-pithing and preserved in 10% formalin in a phosphate buffer for tissue fixation and preservation. After one month, we measured body size (snout-vent length, SVL) to the nearest 0.01 mm with a caliper, and body mass to the nearest 0.1 mg with an electronic balance. Each frog was dissected and all major organs (brain, heart, lungs, kidneys, liver, testes and gut tissues) and limb muscles were removed. We then weighed and measured the brain, heart, lungs, kidneys, liver, testes and limb muscles (to the nearest 0.1 mg) as above.

All variables were log-transformed for parametric statistical tests (Type III sums of squares tests conducted with SPSS v.17.0). Correlations between organs were analysed using residuals from log-log regressions of brain, heart, lungs, kidneys, liver, limb muscles, testes mass and gut length on body mass. We used linear regression to estimate the correlations and 95% confidence intervals. Individual condition was estimated using the residuals from a log-log regression of body mass on SVL. We used least-squares linear regressions to test whether investment in any of the focal tissues was affected by individual condition. We then used partial correlation analysis to test for correlations between residual brain mass and each of the focal tissue residuals by treating body condition as covariate; this test can control for the possibility that energetic constraints are more pronounced in individuals with poor condition (Warren & Iglesias, 2012).

Brain mass residuals were negatively correlated with gut length residuals, and positively correlated with residuals of heart, lungs, testes and limb-muscle mass (Table 1). We did not find significant correlations between brain mass residuals and residuals of liver and kidney mass (Table 1). Correlations between other organs were not significant except for negative correlations between gut length residuals and residuals of testes, heart and muscle mass (Fig. 1).

Body condition was negatively correlated with the residuals of limb muscles and testes mass (Table 1). Conversely, there was a positive correlation between body condition and gut length residuals. Moreover, body condition was not correlated with residuals of liver, kidney and lung mass (Table 1), as well as brain mass residuals ($\beta = -0.244$, $p = 0.054$). However, when controlling for the effect of body condition by using partial correlation

analysis, brain mass residuals were negatively correlated with residuals of gut length ($r = -0.574$, $df = 59$, $p < 0.001$) and positively correlated with residuals of limb muscle and testes mass (Fig. 2; limb muscles, $r = 0.576$, $df = 59$, $p < 0.001$; testes, $r = 0.445$, $df = 59$, $p < 0.001$).

That physiological constraints force organisms to balance investments between metabolically costly tissues is a fundamental hypothesis in comparative biology (Isler & van Schaik, 2006). Consistent with the ETH, we found that brain mass and gut length were negatively correlated in *R. omeimontis*. Such a relationship was previously reported across a range of taxa (primates: Aiello & Wheeler, 1995; Aiello et al., 2001; Pfeifferle et al., 2011; Barrickman & Lin, 2010; fish: Kaufman et al., 2003; birds: Isler & van Schaik, 2006; cattle: Mau et al., 2009), but other studies also failed to reveal this relationship (bats: Jones & MacLarnon, 2004, mammals: Navarrete et al., 2011). High diet quality is associated with small guts (MacLarnon et al., 1986), and in birds is linked to large brains (Isler & van Schaik, 2006). Consistent with the ETH, our study suggests that individuals with larger brains might make use of better-quality food and therefore develop smaller guts.

Comparative analyses of the trade-offs between metabolically costly tissues demonstrate negative correlations between investment in testes and brains in bats (Pitnick et al., 2006; Lemaître et al., 2009), whereas no correlations are observed in other mammals (Schillaci, 2006; Lemaître et al., 2009; Bordes et al., 2011) and fish (Liu et al., 2014). However, contrary to

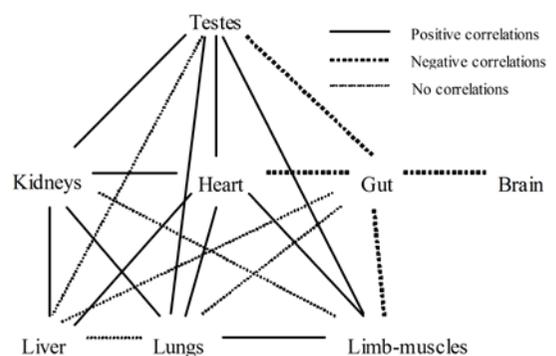


Fig. 1. Correlations between the sizes of other expensive organs in *Rana omeimontis*.

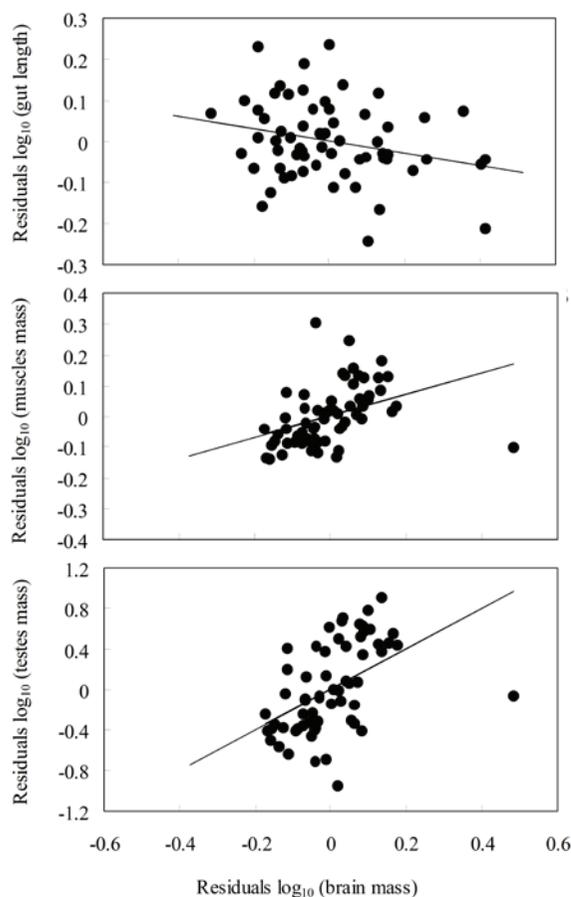


Fig. 2. Correlations between residuals brain mass and residuals of gut length, limb-muscles and testes mass in *Rana omeimontis*, controlling for body condition.

the predictions of the ETH, we find that the increase of brain size is accompanied by an increase in testes size. Muscle tissue can consume a considerable proportion of the organisms' energy even when at rest (Aiello & Wheeler, 1995), and we also found a positive correlation between brain mass and limb muscles mass. For anurans, locomotory capability is linked with the ability to search for mates and high-quality diet, as well as predator avoidance (Duellman & Trueb, 1986). Hence, a positive correlation between brain mass and limb muscle mass in *R. omeimontis* might reflect the ability of perception alongside higher capabilities for locomotion. This contrasts the trade-off between muscles mass and brain mass in birds which are characterised by weight-limited, energy-demanding flight styles (Isler & van Schaik, 2006).

Organs that are of critical importance to the functioning of the organism may vary in size and metabolic activity during different life-cycle periods (Piersma, 2002). Similar to the results of Navarrete et al. (2011), we fail to find correlations between brain mass and heart, liver, kidney or lung mass. This may be explained by the fact that the energetic costs of one tissue may be reflected in investment in all other costly tissues combined rather than a specific tissue (Lemaître et al., 2009).

Acknowledgements: Financial support was provided by Sichuan Province Outstanding Youth Academic Technology Leaders Program (2013JQ0016) and the

Innovative Team Foundation of China West Normal University. The reported experiments comply with the current laws of China concerning animal experimentation, and permission to collect frogs was received from the Ethical Committee for Animal Experiments in China and Canadian Council on Animal Care (CCAC) guidelines.

REFERENCES

- Agrawal, A.A., Conner, J.K. & Rasmann, S. (2010). Trade-offs and adaptive negative correlations in evolutionary ecology. In Bell, M., Eanes, W., Futuyma, D. & Levinton, J. (Eds). *Evolution after Darwin: the First 150 Years*, pp. 243–268. Sinauer Associates, MA, Sunderland.
- Aiello, L.C. & Wheeler, P. (1995). The expensive-tissue hypothesis – the brain and the digestive system in human and primate evolution. *Current Anthropology* 36, 199–221.
- Aiello, L.C., Bates, N. & Joffe, T. (2001). In defence of the expensive tissue hypothesis. In Falk, D. & Gibson, K.R. (Eds). *Evolutionary Anatomy of the Primate Cerebral Cortex*. pp. 57–78, Cambridge University Press, Cambridge.
- Allman, J. (2000). *Evolving brains*. Scientific American Library, New York.
- Barrickman, N.L. & Lin, M.J. (2010). Encephalization, expensive tissues, and energetics: an examination of the relative costs of brain size in Strepsirrhines. *American Journal of Physical Anthropology* 143, 579–590.
- Barton, R.A. & Capellini, I. (2011). Maternal investment, life histories and the costs of brain growth in mammals. *Proceedings of the National Academy of Sciences of the United States of America* 108, 6169–6174.
- Bordes, F., Morand, S. & Krasnov, B.R. (2011). Does investment into “expensive” tissue compromise anti-parasitic defence? Testes size, brain size and parasite diversity in rodent hosts. *Oecologia* 165, 7–16.
- Caton, J.S., Bauer, M.L. & Hidari, H. (2000). Metabolic components of energy expenditure in growing beef cattle—Review. *Asian-Australasian Journal of Animal Sciences* 13, 702–710.
- Duellman, W.E. & Trueb, D.L. (1986). *Biology of Amphibians*. McGraw-Hill Inc, New York.
- Dunbar, R. & Shultz, S. (2007). Evolution in the social brain. *Science* 317, 1344–1347.
- Gonda, A., Herczeg, G. & Merilä, J. (2013). Evolutionary ecology of intraspecific brain size variation: a review. *Ecology and Evolution* 3, 2751–2764.
- Isler, K. & van Schaik, C. (2006). Costs of encephalization: the energy trade-off hypothesis tested on birds. *Journal of Human Evolution* 51, 228–243.
- Jones, K.E. & MacLarnon, A.M. (2004). Affording larger brains: testing hypotheses of mammalian brain evolution on bats. *American Naturalist* 164, E20–E31.
- Kaufman, J.A., Hladik, C.M. & Pasquet, P. (2003). On the expensive-tissue hypothesis: independent support from highly encephalized fish. *Current Anthropology* 44, 705–707.
- Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., et al. (2013). Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Current Biology* 23, 168–171.
- Lemaître, J.F., Ramm, S.A., Barton, R.A. & Stockley, P. (2009). Sperm competition and brain size evolution in mammals. *Journal of Evolutionary Biology* 22, 2215–2221.
- Liu, W.C., Huang, Y., Liao, Y.M. & Li, C. (2012a). Testes asymmetry of Chinese endemic frog (*Rana omeimontis*) in relation to body condition and age. *North-Western Journal of Zoology*

- 8, 390–393.
- Liu, W.C., Liu, Y.H., Huang, Y., Mi, Z.P. & Li, C. (2012b). Skeletochronological study on age structure of a Chinese endemic frog (*Rana omeimontis*). *Asian Herpetological Research* 3, 252–257.
- Liu J., Zhou, C.Q. & Liao, W.B. (2014). Evidences for neither the compensation hypothesis nor the expensive-tissue hypothesis in *Carassius auratus*. *Animal Biology* 64, 177–187.
- MacLarnon, A.M., Chivers, D.J. & Martin, R.D. (1986). Gastro-intestinal allometry in primates and other mammals including new species. In Else, J.G. & Lee, P. (Eds). pp. 75–85, *Primate Ecology and Conservation*. Cambridge University Press, Cambridge.
- Mau, M., Sudekum, K.H. & Kaiser, T.M. (2009). Why cattle feed much and humans think much—new approach to confirm the expensive tissue hypothesis by molecular data. *Bioscience Hypotheses* 2, 205–208.
- Mink, J. W., Blumenschine, R. J. & Adams, D.B. (1981). Ratio of central nervous system to body metabolism in vertebrates—its constancy and functional basis. *American Journal of Physiology* 241, R203–R212.
- Navarrete, A., van Schaik, C.P. & Isler, K. (2011). Energetics and the evolution of human brain size. *Nature* 480, 91–93.
- Pfefferle, A.D., Warner, R.L., Wang, W.C., Nielsen, J.W., et al. (2011). Comparative expression analysis of the phosphocreatine circuit in extant primates: implications for human brain evolution. *Journal of Human Evolution* 60, 205–212.
- Piersma, T. (2002). Energetic bottlenecks and other design constraints in avian annual cycles. *Integrative and Comparative Biology* 42, 51–67.
- Pitnick, S., Jones, K.E. & Wilkinson, G.S. (2006). Mating system and brain size in bats. *Proceedings of the Royal Society B: Biological Sciences* 273, 719–724.
- Schillaci, M.A. (2006). Sexual selection and the evolution of brain size in primates. *PLoS ONE* 1, e62.
- Striedter, G.F. (2005). *Principles of brain evolution*. Sinauer Associates, Sunderland.
- Warren, L.D. & Iglesias, L.T. (2012). No evidence for the ‘expensive-tissue hypothesis’ from an intraspecific study in a highly variable species. [Journal of Evolutionary Biology](#) 25, 1226–1231.

Accepted: 8 August 2014