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

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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; Isler 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 anasthetised with benzocaine and then
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Regressions of brain mass residuals and body condition on other organ size residuals in Table 1. 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.

<table>
<thead>
<tr>
<th>Organ size</th>
<th>Brain mass</th>
<th>Body condition</th>
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<tbody>
<tr>
<td></td>
<td>Estimates [±95% CI]</td>
<td>$\beta$</td>
</tr>
<tr>
<td>Heart</td>
<td>0.227[0.069,0.386]</td>
<td>0.347</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.157[0.042,0.273]</td>
<td>0.329</td>
</tr>
<tr>
<td>Liver</td>
<td>-0.072[-0.344,0.200]</td>
<td>-0.068</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.080[-0.066,0.225]</td>
<td>0.138</td>
</tr>
<tr>
<td>Gut</td>
<td>-0.483[-0.748,-0.219]</td>
<td>-0.424</td>
</tr>
<tr>
<td>Testes</td>
<td>0.122[0.067,0.178]</td>
<td>0.495</td>
</tr>
<tr>
<td>Limb muscles</td>
<td>0.441[0.177,0.704]</td>
<td>0.394</td>
</tr>
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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; Pfefferle 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; Lemaitre et al., 2009), whereas no correlations are observed in other mammals (Schillaci, 2006; Lemaitre et al., 2009; Bordes et al., 2011) and fish (Liu et al., 2014). However, contrary to
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


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