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No evidence for the expensive-tissue hypothesis in the Asiatic toad (*Bufo gargarizans*)

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Abstract. Conferring cognitive ability on animals, the brain is one of the most metabolically costly organs in vertebrates, and thus a large amount of energy associated with brain tissue maintenance should constrain brain size evolution. The expensive-tissue hypothesis (ETH) states that decrease in the sizes of other metabolically costly tissues compensates for increase in brain. In this paper, we tested ETH in a single species, *Bufo gargarizans*. We found no negative correlation between brain weight and intestinal length and no negative correlation between brain weight and the weight of other organs (heart, lungs, stomach, liver, kidneys, spleen, and testes). Therefore, our results do not support the ETH. We also found that intestinal length, stomach weight, liver weight and kidneys weight were positively correlated with each other, which may reflect the close functional relationships among these organs. However, a negative correlation was found between intestinal length and testes weight, indicating a trade-off between the testes and gut. Our findings suggest that energetic cost of a large brain in this species cannot be compensated by decreasing weight of other expensive organs.

Key words: brain size, expensive tissue hypothesis, evolution, trade-off, *Bufo gargarizans*.

Introduction

Relative brain size commonly varies among vertebrate species or organisms of a single species (Kotrschal et al. 1998). Variation in brain size underlies differences in individual cognitive performance (Isler & van Schaik 2006). The mechanism of brain size evolution remains a central endeavor for the evolutionary biologist (Mai & Liao 2019). In vertebrates, the brain is one of the metabolically high-cost organs (Mink et al. 1981). If relative brain size increases, the additional energy required to maintain brain tissue is bound to constrain brain size evolution (Striedter 2005). Therefore, brain size evolution may be limited by an organism's total energy budget (Liao et al. 2016). The expensive tissue hypothesis (ETH) states that animals can meet the metabolic cost of a large brain by reducing the amount of energy for other organs with high metabolic expenses (Aiello & Wheeler 1995). Aiello & Wheeler (1995) found a significant negative correlation between relative brain weight and relative intestinal weight in anthropoid primates. Since then, substantial evidence supporting the ETH was obtained in other mammalian groups (Jones & MacLarnon 2004), birds (Isler & van Schaik 2006), amphibians (Liao et al. 2016, Huang et al. 2018), and fishes (Kotrschal et al. 2013, Tsuboi et al. 2016). However, some studies do not support the ETH (Navarrete et al. 2011, Warren & Iglesias 2012, Sukhum et al. 2016, Zhao et al. 2016). Building on the ETH, Isler & van Schaik (2006) proposed the energy trade-off hypothesis (ETOH), which states that the metabolic cost for the evolution of a large brain must be compensated by decrease in the metabolic cost of body maintenance, locomotion, or reproduction and the total energy budget of the individual should not change in the process (Isler & van Schaik 2009, Jin et al. 2015).

Thus far, most comparative studies for testing the ETH or ETOH have been carried out on mammals and birds (Isler & van Schaik 2006, Navarrete et al. 2011, Pontzer et al. 2016), but studies of ectothermic vertebrates are few (Liao et al. 2015, Tsuboi et al. 2015, Mai & Liao 2019). However, ectothermic vertebrates should be especially suitable for investigating energetic constraints on brain size evolution (Liao et al. 2015, Tsuboi et al. 2015, Liao et al. 2016, Mai & Liao 2019). The brains of amphibians can differ in size and morphology between species and within a species (Jiang et al. 2015, Liao et al. 2015, Luo et al. 2017, Zhao et al. 2018, Huang et al. 2020, Mai et al. 2020). The lengths of the digestive tracts of some anurans considerably change with altitude (Naya et al. 2009, Lou et al. 2013, Wang et al. 2017, Mai et al. 2019), diet (Cai et al. 2020), temperature, and rainfall (Ma et al. 2016). The large plasticity of digestive tract length in anurans is suited to evolutionary reduction.

The Asiatic Toad (*Bufo gargarizans* Cantor, 1842) is endemic to East Asia and widely distributed in China, as well as in Russia and the Korean Peninsula. Information on its morphological anatomy (Feng 1990), mating behavior (Yu & Lu 2010), age structure (Mi 2015), and sexual dimorphism of limb muscles (Mi 2012, Mi 2013) is available. The aim of this study was to test the predictions of the ETH on the basis of the correlation between brain weight and the sizes of other energetically expensive organs in *Bufo gargarizans*.

Material and Methods

We collected specimens by hand in fields in Jintai Town, Nanchong City (30° 93′ N, 106° 12′ E, 319 m a. s. l.), Sichuan, China. A total of 43 male toads were collected on the evening of March 25, 2016. Collection was permitted by the Forestry Bureau of Nanchong city, following all applicable instructions of the Animal Care Guidelines in China. After the toads were brought to the laboratory, they were temporally kept in an artificial tank (0.6×0.5×0.8m; L*W*H) before processing. Animals were anesthetized with ether and then killed by the single-pithing method (i.e., the spinal cord was destroyed with a needle, and the brain was preserved). The snout-to-vent length (SVL) was measured with an electronic caliper to the nearest 0.01 mm, and body weight was determined with an electronic balance to the nearest 10 mg. The toads were immediately dissected, the intestines were extracted, and intestine length was measured. The heart, lungs, liver, stomach, kidneys, spleen, and testes of each toad were removed and placed on filter paper to dry the organ surfaces, and then weighed to the nearest 0.1 mg. After the visceral organs were removed, the toads were fixed and preserved in 10% formaldehyde solution. After 1 month, the brains were weighed.

First, the values of all variables were log_{10}-transformed, and normal distribution was detected using the Kolmogorov–Smirnov test. The residual value obtained through the linear regression analysis of body weight on SVL was used as the body condition index (BCI). For controlling the influence of body weight, residual values from the linear regression of brain, heart, lungs, liver, stomach, kidneys, spleen, testes weight, and intestinal length on body weight, were used as proxies of relative organ size. We performed least-squares linear regression to estimate the correlations and 95% confidence intervals between brain and other organs and between BCI and other organs. Pearson correlation analysis was used in estimating the correlations among the organs except the brain, the significance level was set to 0.05. When BCI was significantly related to an organ, a partial correlation analysis was conducted, and BCI was used as the covariate in testing the relationship between the brain and other organs. Statistical analyses were performed using SPSS 23.0.

Table 1. Means and standard deviation of body weight, SVL and size of several organs (e.g. brain, gut, stomach, liver, spleen, kidneys, heart, lungs and testes) in *Bufo gargarizans*.

Variable	Mean (1SD)	Range	N
Body weight (mg)	141997.21(1SD: 22575.73)	103380 ~ 222130	43
SVL (mm)	110.38 (1SD: 5.77)	98.75 ~ 124.09	43
Brain (mg)	155.45 (1SD: 26.39)	111.80 ~ 218.80	43
Gut length (mm)	126.22 (1SD: 27.22)	69.48 ~ 191.32	43
Stomach (mg)	1771.03 (1SD: 599.78)	939.10 ~ 3445.60	43
Liver (mg)	3557.80 (1SD: 1359.36)	1675.60 ~ 7836.60	43
Spleen (mg)	94.81 (1SD: 48.03)	38.30 ~ 341.40	43
Kidneys (mg)	490.79 (1SD: 107.01)	310.90 ~ 833.80	43
Heart (mg)	467.74 (1SD: 92.02)	305.40 ~ 774.20	43
Lungs (mg)	1717.87 (1SD: 352.76)	1130.90 ~ 2531.70	43
Testes (mg)	298.01 (1SD: 109.32)	130.60 ~ 583.50	43

Results

The means and ranges of body weight, SVL, and size of other organs were summarized in Table 1. The results showed no correlation between brain weight residual and heart, lungs, liver, stomach, kidneys, spleen, testes weight residual, or intestinal length residual (Table 2). Linear regression of body weight on SVL was highly significant (Fig. 1; R^2 = 0.568, n = 43, $P < 0.001$). The BCI showed no correlation with the residuals of heart, lungs, liver, stomach, kidneys, spleen, testes, or intestinal length (Table 2). Given the absence of correlation between the BCI and the above-mentioned organs, a partial correlation analysis was not conducted between the brain and other organs. In other organs, we found that intestinal length residuals were positively correlated with liver, stomach and kidneys weight residuals; stomach weight residuals were positively correlated with kidneys and liver weight residuals; kidneys weight residuals were positively correlated with liver weight residuals; and liver weight residuals were positively correlated with spleen weight residuals. However, intestinal length residuals were negatively correlated with testes weight residuals (Table 3, Fig. 2).

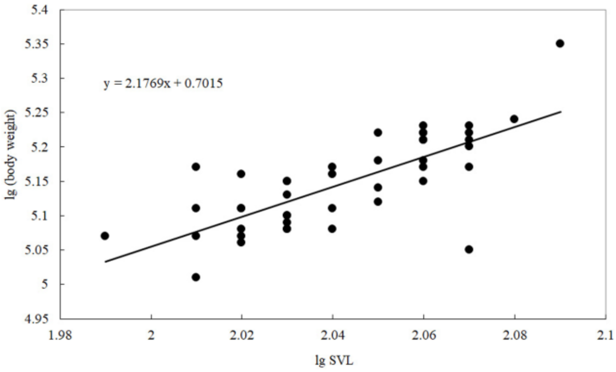

Figure 1. Linear regression of the body weight on SVL in *Bufo gargarizans*.

Discussion

The expensive tissue hypothesis states that a large brain size is maintained by reducing the size of other metabolically expensive tissues, and this mechanism ensures that the energy demand for maintaining a large brain is met without increase in total energy budget (Aiello & Wheeler 1995). Based on this hypothesis, comparative studies had been performed on many vertebrate groups (Isler & van Schaik 2006, Navarrete et al. 2011, Tsuboi et al. 2015, Liao et al. 2016, Yang et al. 2018). Previous studies can be divided into two different scales: interspecific and intraspecific brain size evolution. Intraspecific studies exclude interspecific difference in selection pressure on tissues and may be more suitable for testing the ETH (Warren & Iglesias 2012). However, the ETH has received mixed support in interspecific or intraspecific studies, that is, some comparative studies support the ETH (Isler & van Schaik 2006, Jin et al. 2015, Tsuboi et al. 2015, Liao et al. 2016), whereas other studies do not (Jones &

Table 2. Regressions of brain weight residuals and BCI on other organ size residuals in *Bufo gargarizans*. Coefficient estimates from regressions and corresponding 95% confidence intervals (CI) in brackets and β and *P* values are provided. The significance level is 0.05.

Organ	Brain weight			BCI		
	Coefficient [95% CI]	β	*P*	Coefficient [95% CI]	β	*P*
Gut	-0.018 [-0.219, 0.183]	-0.029	0.855	0.083 [-0.059, 0.226]	0.182	0.243
Stomach	-0.044 [-0.196, 0.107]	-0.092	0.558	0.019 [-0.091, 0.128]	0.053	0.733
Liver	0.041 [-0.116, 0.198]	0.082	0.599	-0.040 [-0.160, 0.065]	-0.133	0.395
Spleen	0.035 [-0.086, 0.156]	0.091	0.561	-0.013 [-0.100, 0.074]	-0.048	0.758
Kidneys	0.044 [-0.198, 0.285]	0.057	0.717	0.030 [-0.144, 0.204]	0.055	0.728
Heart	0.074 [-0.197, 0.346]	0.086	0.582	-0.077 [-0.272, 0.117]	-0.125	0.426
Lungs	0.222 [-0.042, 0.486]	0.256	0.097	-0.121 [-0.314, 0.072]	-0.195	0.211
Testes	0.125 [-0.008, 0.257]	0.284	0.065	-0.085 [-0.181, 0.011]	-0.269	0.081

Table 3. Pearson correlations analysis on organ size in *Bufo gargarizans*. A plus sign indicates positive correlation, minus sign indicates negative correlation, and n.s indicates no significant correlation, correlation coefficient r and *P* values (in r, *P* sequence) are also provided. The significance level is 0.05.

Organ	Gut	Stomach	Liver	Spleen	Kidneys	Heart	Lungs	Testes
Gut		**0.688, 0.000**	**0.341, 0.025**	-0.002, 0.989	**0.472, 0.000**	-0.006, 0.969	-0.119, 0.449	**-0.320, 0.036**
Stomach	+		**0.447, 0.003**	0.215, 0.167	**0.607, 0.000**	-0.021, 0.893	-0.185, 0.235	-0.186, 0.231
Liver	+	+		**0.315, 0.040**	**0.455, 0.002**	-0.021, 0.892	-0.069, 0.658	0.072, 0.648
Spleen	n.s	n.s	+		0.260, 0.092	0.122, 0.436	-0.090, 0.566	0.194, 0.213
Kidneys	+	+	+	n.s		0.290, 0.059	-0.115, 0.463	-0.118, 0.451
Heart	n.s	n.s	n.s	n.s	n.s		0.233, 0.133	0.201, 0.197
Lungs	n.s	n.s	n.s	n.s	n.s	n.s		0.068, 0.663
Testes	-	n.s	n.s	n.s	n.s	n.s	n.s	

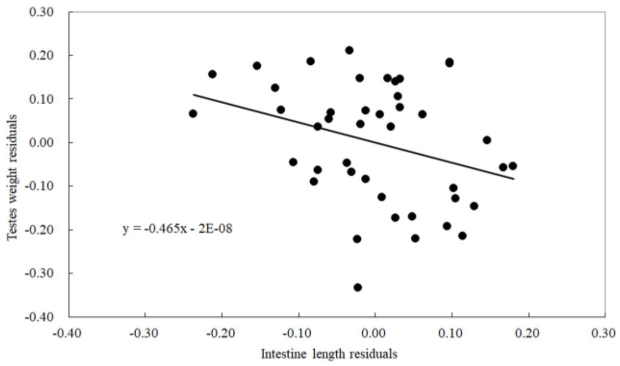

Figure 2. Linear regression of the residuals of testes weight on the residuals of intestine length in *Bufo gargarizans*.

MacLarnon 2004, Navarrete et al. 2011, Zhao et al. 2016, Liu et al. 2018, Yang et al. 2018). In this study, we did not find obvious evidence to support the ETH in *Bufo gargarizans*. However, this finding does not completely rule out the presence of a trade-off between the brain and other metabolically expensive tissues because only organ weight and length were used as macroscopic diagnostic indices in this study. An organism may not sacrifice the weight of any expensive organ, but trade-off relationships at the cellular and subcellular levels or material metabolism may still be present (Fedrigo et al. 2011, Warren & Iglesias 2012), which needs to be further explored in *Bufo gargarizans*.

The ETOH predicts a negative relationship between brain size and testes weight (Isler & van Schaik 2006, Pitnick et al. 2006), but we did not find any significant correlation between brain weight and testes weight in *Bufo gargarizans*. This finding is consistent with the findings of previous studies (Schillaci 2006, Bordes et al. 2011, Liu et al. 2014, Zhao et al. 2016) that do not support the ETOH. However, we found a significant negative relationship between intestine length and testes weight. Sperm competition success is usually decided by the relative testes sizes of rivals (Ball & Parker 2000). Male–male competition is intensive in *Bufo gargarizans* (Mi 2012), so to grow large testes and increase sperm production, toads may reduce intestine length. Consumption of high-quality food can potentially compensate the loss in intestinal length (Jin et al. 2015, Mai et al. 2019, Cai et al. 2020). This trade-off between the testes and gut needs further experimental verification.

At the interspecific level, Liao et al. (2016) studied the relationship between brain size and digestive tract length in 30 anuran species through phylogenetically controlled generalized least squares (PGLS) regression analyses and found a significant negative correlation between brain size and digestive tract length. Within a species, Jin et al. (2015) found a significant negative correlation between brain weight and intestinal length in *Rana omeimontis*. By contrast, no significant correlation was found between brain weight and intestinal length in *Pelophyllax nigromaculatus* (Zhao et al. 2016), *Hylarana guentheri* (Liu et al. 2018), *Fejervarya limnocharis* (Yang et al. 2018), and *Bufo gargarizans* (this study), let alone a negative relationship. As noted above, the ETH has gained mixed support in studies of anurans. The seemingly contradictory results may be attributed to two reasons: on the one hand, different research scales may lead to different results. From the perspective of interspecies macro-evolution, the evolution of brain size in anurans conforms to the ETH, but from the perspective of intraspecies micro-evolution, each anuran species is not necessarily consistent with the ETH; on the other hand, the difference in living environment or life history may cause difference in the driving force of brain size evolution among species. Habitat type, diet, predator risk, sexual selection, and life-history traits affect the evolution of brain size (Liao et al. 2015, Wu et al. 2016, Yu et al. 2018, Mai & Liao 2019, Mai et al. 2020).

In *Bufo gargarizans*, we found several positive correlations among metabolically expensive tissues except the brain, that is, intestinal length was positively correlated with liver, stomach or kidneys weight; stomach weight was positively correlated with liver and kidneys weight; liver weight was positively correlated with kidneys and spleen weight. According to the above results, obtaining a general pattern is difficult. The positive correlations among the stomach, intestine, liver, and kidneys may be due to the fact that the gastrointestinal tract digests food and absorbs nutrients and then transports them to the liver for metabolism and metabolic wastes are excreted by the kidneys to maintain the stability of the internal environment inside the body. The positive correlations among metabolically expensive tissues had been reported in previous studies (Chappell et al. 2007, Jin et al. 2015, Zhao et al. 2016). These results may indicate that change in energy demand may be greater than change in energy distribution within a single species (Glazier 1999).

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