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The relationship between brain size and digestive tract length do not support expensive-tissue hypothesis in *Hylarana guentheri*

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Abstract. The brain is among the most energetically costly organs in the vertebrate body. The expensive-tissue hypothesis (ETH) predicts that increasing the size of another costly organ, such as the gut, should compensate for the cost of a small brain. To date, this hypothesis has mainly been tested in homoeothermic animals and in some ectothermic animals (e.g., fishes and anurans). Here, we undertake a test of the ETH by analyzing the relationship between brain size variation and length of the digestive tract in *Hylarana guentheri*. After controlling for geographical situation and body size, we did not find a correlation between brain mass and the length of the digestive tract in *H. guentheri*. Our findings suggest that the variation of brain size did not follow general patterns in this species and that the effect of diet quality cannot play a role in the variation of brain.

Keywords. Brain size; energetic constraints; expensive-tissue hypothesis; *Hylarana guentheri.*

INTRODUCTION

As an important cognitive organ of animals, increased brains confirm to improve the cognitive ability (Striedter, 2005; Jiang et al. 2015; Luo et al., 2017; Wu et al., 2016; Yu et al., 2018). However, brains are one of the most metabolically costly tissues in the vertebrate body (Mink et al., 1981). The high amount of energy related to maintaining brain tissues should constrain on evolution of brain size (Striedter, 2005; Isler and van Schaik, 2006), although the brains can confer cognitive ability (Kotrschal et al., 2013; Liao et al., 2015a; Zeng et al., 2016; Kotrschal et al., 2017; Samuk et al., 2018). The expensive-tissue hypothesis (ETH) predicts that the enlarged brains will inevitably decrease the sizes of other metabolically costly tissues, such as gut (Aiello and Wheeler, 1995). There are evidences that the evolution of brain size follows the prediction of the ETH in animal kingdom (Isler and van Schaik, 2006; Navarrete et al., 2011; Jin et al., 2015; Tsuboi et al., 2015; Liao et al., 2016a; Sukhum et al., 2016).

Since the ETH was developed, primarily comparative studies have tested their predictions. While some studies have shown that negative associations between brain mass and gut size support the ETH (Kaufman et al., 2003; Tsuboi et al., 2015; Liao et al., 2016a), other studies do not find such patterns (Lemaître et al., 2009; Barrickman and Lin 2010; Navarrete et al., 2011). Most studies on the cost of brain size evolution have mainly focused on birds and mammals (e.g., Aiello and Wheeler, 1995; Jones and MacLarnon, 2004; Isler and van Schaik, 2006; Pitnick et al., 2006; Navarrete et al., 2011). In recent years, the cost of brain size evolution in ectothermic vertebrates has been investigated at inter-specific species level (Tsuboi et al., 2015; Liao et al., 2016a; Sukhum et al., 2016) and intra-specific species level (Kotrschal et al., 2013; Liu

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et al., 2014; Jin et al., 2015; Zhao et al., 2016; Gu et al., 2017; Yang et al., 2018). For instance, a negative correlation between brain mass and the length of the digestive tract have been found in *Rana omeimontis* (Jin et al., 2015) while Zhao et al. (2016) do not find any correlation between them in the dark-spotted frog (*Pelophylax nigromaculata*).

Here we provide the test of the relationship between the brain mass and length of the digestive tract among *Hylarana guentheri* populations. Based on the ETH, we predict that a larger brain should accompany a smaller digestive tract in the frog.

MATERIAL AND METHODS

The Guenther's frog, *Hylarana guentheri* is found in the subtropical forests in China at elevations ranging from 500 to 1100 m (Fei and Ye, 2001). The species exhibits a variable activity period. Males actively look for females and used advertisement calls to attract them (Liu et al., 2011). In this species, mating and egg-laying extends from March to May, as a prolonged breeder (Wells, 1977).

We collected a total of 135 frogs from six populations along a 420-km latitudinal and 341-m altitudinal transect across the Hunan, Hubei and Sichuan provinces in China between June and August in 2016 (Figure 1). All individuals were captured by hand in paddy fields and pools at night and confirmed at the adult stage by direct observations of secondary sexual characteristics (see details in Liao and Lu, 2010; Jin et al., 2015; Liao et al., 2015b). We recorded the altitude, latitude and longitude of each population because environmental factors will affect morphology and physiology of organism (Chen et al., 2016; Yang et al. 2017; Liao et al., 2016b; Tang et al., 2018; Zhong et al., 2017; Wang et al., 2017; Liao et al., 2018; Qin et al., 2018; Wu et al., 2018).

We took all individuals to the laboratory and kept individually in rectangular tanks (0.5 × 0.4 × 0.4 m) before being anesthetized with benzocaine. Each individual was killed by single-pithing and preserve in 4% buffered formalin in a phosphate buffer (Liao et al., 2016a; Lüpold et al., 2017; Mai et al. 2017a, b). We measured body size of all individuals (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. All frogs were dissected, and their brains were removed (see detail in Liao et al., 2015a; Jin et al., 2016). We then weighed and measured all brains to the nearest 0.1 mg with an electronic balance.

We used a Canon SX530 HS digital camera to take digital images of digestive tract with a caliper. We then measured length of digestive tract using a Motic Images 3.1 digital camera mounted on a Moticam 2006 light microscope at a 400× magnification.

All statistical analyses were performed using SPSS 22.0 (Statistical Product and Service Solutions Company, Chicago, USA). We ran a Linear Mixed Models (LMMs) with $\log_{10}$-

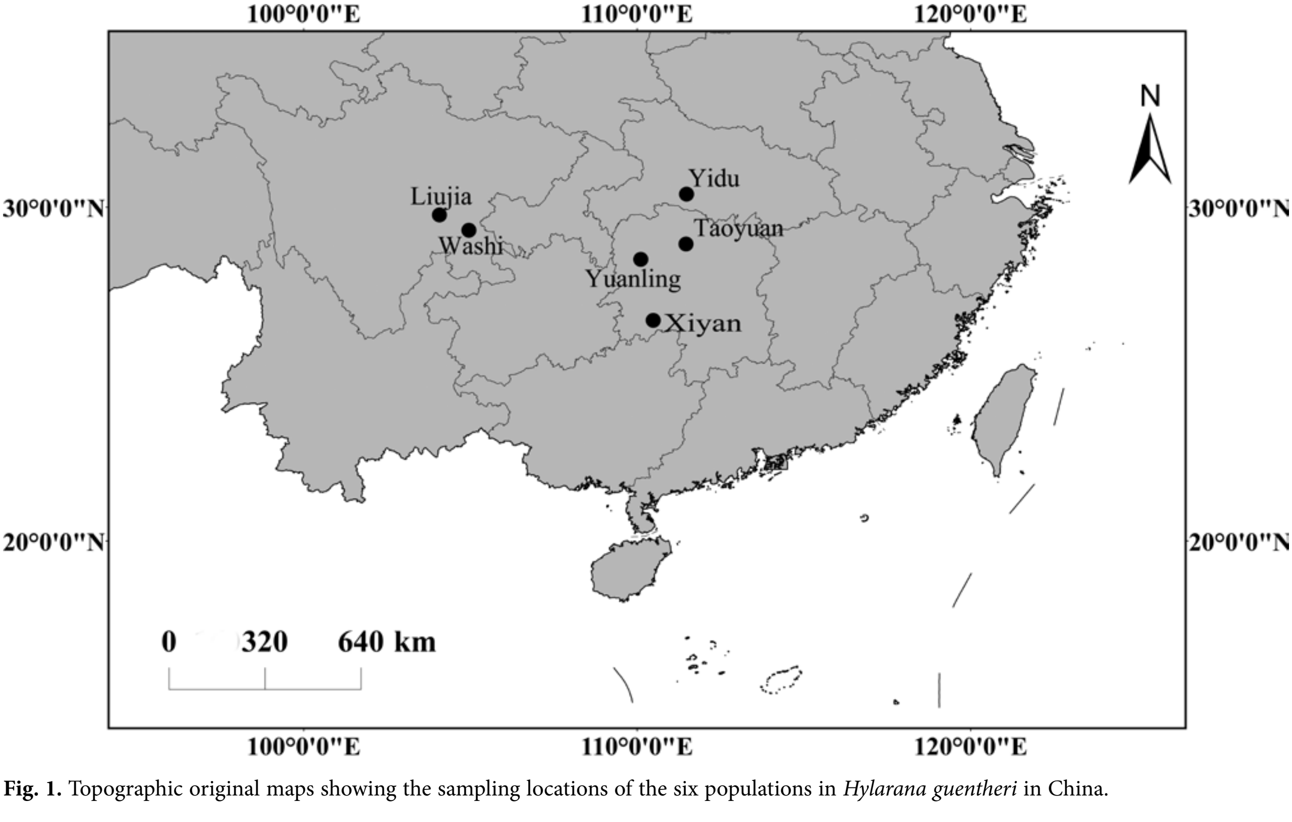

Fig. 1. Topographic original maps showing the sampling locations of the six populations in *Hylarana guentheri* in China.

transformed brain mass as a dependent variable, population as a random factor, and $\log_{10}$-transformed digestive tract length, latitude, longitude, sex and altitude as fixed effects, $\log_{10}$-transformed body size as covariate to test the original ETH. Within each sex, we also used LMMs to test the correlation between brain size and digestive tract length.

RESULTS

We at first analyzed the association between relative brain size and relative digestive tract length to evaluate the original ETH. We found that relative brain size was not correlated with relative size of digestive tract when controlling for the effect of the body size (Table 1). There was a non-significant variation in brain size among populations ($Z = 0.656$, $P = 0.512$). Moreover, sex, latitude, longitude and altitude did not affect the relative brain size (Table 1).

Table 1. Regression models of brain size on digestive tract in relation to various predictor variables in species when controlling for body size.

Source	d.f.	Predictor	F	P
Brain size				
	1, 133.961	Digestive tract	1.182	0.279
	1, 134.993	Sex	1.104	0.295
	1, 2.002	Altitude	3.517	0.201
	1, 1.999	longitude	2.152	0.280
	1, 1.981	Latitude	0.028	0.883
	1, 110.113	Body size	150.942	<0.001

The LMMs revealed that for males relative brain size was not correlated with relative length of digestive tract (Fig. 2; $F_{1,\, 80.413} = 0.573$, $P = 0.451$) and the environmental factors (e.g., latitude: $F_{1,\, 1.97} = 1.069$, $P = 0.411$; longitude: $F_{1,\, 2.117} = 0.01$, $P = 0.93$; altitude: $F_{1,\, 2.1} = 0.674$, $P = 0.494$). However, there was a significant correlation between brain size and body size ($F_{1,\, 79.354} = 96.174$; $P < 0.001$).

For females, the relative brain size was not correlated with digestive tract length (Fig. 3; $F_{1,\, 49} = 2.034$, $P = 0.16$) and the environmental factors (e.g., latitude: $F_{1,\, 49} = 0.002$, $P = 0.969$; longitude: $F_{1,\, 49} = 0.050$; $P = 0.943$). However, we also found that brain size was positively correlated with the body size ($F_{1,\, 49} = 57.732$; $P < 0.001$).

DISCUSSION

Our results uncover a non-significant association between brain size and digestive tract size after controlling for several environmental factors and body size in *H. guentheri*, which do not support the original "brain versus gut" prediction arising from the ETH. Thus, our study does not support the existence of energetic constraints as important factors influencing patterns of brain size diversification among individuals in frogs. Below, we discuss our results in more detail considering the main hypothesis addressed.

Although the data set on which the ETH is based refers to primates (Aiello and Wheeler, 1995), most con-

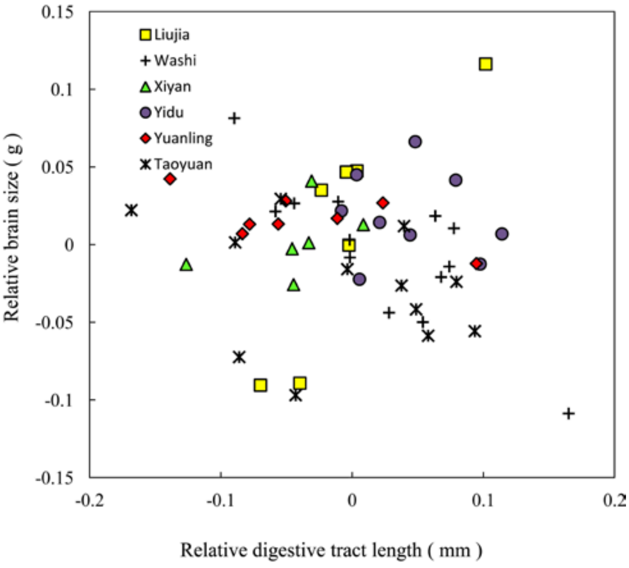

Fig. 2. Plot of relative brain size and relative digestive tract length of the sampled male individuals of *Hylarana guentheri.*

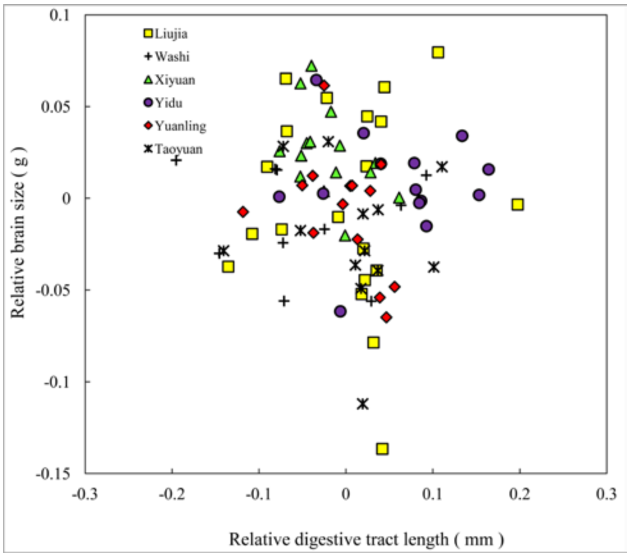

Fig. 3. Plot of relative brain size and relative digestive tract length of the sampled female individuals of *Hylarana guentheri.*

vincing evidences favoring the ETH come from ectothermic animals (e.g., *Gnathonemus petersii*: Kaufman et al., 2003; *Rana omeimontis*: Jin et al., 2015; *Poecilia reticulata*: Kotrschal et al., 2013; Tanganyika cichlids: Tsuboi et al., 2015; anurans: Liao et al., 2016a). The negative relationship between brains and guts is not observed in homothermic animals (Isler and van Schaik, 2006, Jones and MacLarnon, 2004; Barrickman and Lin, 2010; Navarrete et al., 2011). Hence, the ETH might explain cases of encephalization in specific lineages of homoeotherms but it is not valid in overall these groups (Aiello et al., 2001). In contrast to the homoeotherms where brain mass corresponds to 1-2% of total body mass (Striedter, 2005), frogs and toads have smaller brain mass that, on average, corresponds to 0.3% of body mass (Liao et al., 2016a). The robust demonstrations of negative associations between brain and gut across species suggest that energetic constraints play an important role in vertebrate, especially ectotherm brain evolution. For *H. guentheri*, the energetic constraints cannot explain brain size variation because of the lack of correlation between brain size and digestive tract length.

There is at first an inverse correlation between one very costly organ, the brain, and the very organ that procures energy, the gut. Potentially this correlation may materialize if an increased brain enables a potentially cognitively driven shift towards a more nutritious diet (Liao et al., 2016a). There is evidence that the smallest-brained species are primates, with the largest guts feed on plant matter, while the larger-brained species are more omnivorous and humans, with the largest brain and smallest gut, even outsourced part of its gut function to cooking pots (Aiello and Wheeler, 1995). Previous studies have argued to find support for the ETH in primates by using diet quality as a proxy of gut morphology (Fish and Lockwood, 2003). However, diet quality does not correlate with brain size in lemurs and lorises (Gonzalez-Voyer et al., 2009; Allen and Kay, 2011). Within the anurans, because most are insectivorous as adults, the potential for dietary divergence may be limited. However, anurans may feed on insects of different nutritive value, which may demand varying levels of cognitive ability to catch (Liao et al., 2016a). Hence, diet quality might affect the brain size variation in anuran species.

The energetic constraints play an important role in promoting brain size variation within single species because there are negative associations between brains and guts (Kotrschal et al., 2013; Jin et al., 2015). However, inconsistent with the ETH, the relative brain size did not exhibit a negative correlation with digestive tract length in *H. guentheri*. Similar pattern has been supported in the other two frogs (e.g., *P. nigromaculata*: Zhao et al., 2016; *Fejervarya limnocharis*: Yang et al., 2017). The discrepancy among above studies also may result from the diet quality (Isler and van Schaik, 2006). There is evidence that a high diet quality combined with a large brain and a low diet quality combined with small brain has been reported in a frog *R. omeimontis* (Jin et al., 2015), which suggests that more clever individuals (larger brains) make use of better food and therefore develop smaller guts. However, we did not find a correlation between brain and gut in *H. guentheri*, suggesting that diet quality is not combined with brain size variation.

In conclusion, our findings suggest that the expensive-tissue hypothesis is not supported in *H. guentheri* because brain size and gut length are not inversely related. Thus, the energetic constraints do not play an important role in promoting brain size variation for this species.

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