

# No evidence for the ‘expensive-tissue hypothesis’ in the dark-spotted frog, *Pelophylax nigromaculatus*

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**Abstract.** Increased brain size significantly contributes to the performance and fitness of organisms. The expensive-tissue hypothesis (ETH) based on studies of the correlation between brain size and size of the other energetically costly organs in mammals predicts that energy investment increased in one energetically costly tissue necessitates a decrease of investments in other costly tissues. Here, we test this hypothesis in an ectothermic species, the dark-spotted frog, *Pelophylax nigromaculatus*. We found that relative brain size was not correlated with relative sizes of testes, heart, liver, spleen, kidneys or limb muscles within each sex. Moreover, we also failed to find significantly negative correlation among the expensive organs (i.e., testes, heart, liver, spleen, kidneys or limb muscles) in this frog. However, we observed a significantly positive correlation between liver residuals and kidney residuals. Our finding suggests that energetic costs of one expensive tissue do not directly affect the investment in another expensive tissue, but rather may scatter its effect on all other expensive tissues.

**Keywords.** *Pelophylax nigromaculatus*, brain size, expensive-tissue hypothesis.

Brain size is an important characteristic that affects the performance and fitness of organisms (Allman, 2000; Liao et al., 2015a). Brain size is often used as an indicator of the brain's evolutionary development state in response to cognitive benefits (see review, Striedter, 2005). Previous studies have indicated that variations in brain size can be explained by the social brain hypothesis, the expensive-tissue hypothesis (ETH) and the sexual selection hypothesis (Aiello and Wheeler, 1995; Pitnick et al., 2006; Dunbar and Shultz, 2007; Barton and Capellini, 2011). However, the brain needs more energy per unit weight than the other somatic tissues, making it metabolically expensive (Mink et al., 1981). According to the ETH, investment in one metabolically costly tissue requires a decrease of investments in other tissues (Aiello and Wheeler, 1995). In the past two decades, the ETH has received mixed support in vertebrates (Aiello and Wheeler, 1995; Jones and Maclarnon, 2004; Isler

and van Schaik, 2006; Pitnick et al., 2006; Barrickman and Lin, 2010; Navarrete et al., 2011). Some studies find that investment in a metabolically expensive tissue is negatively significantly correlated with the other metabolically expensive tissues, thus supporting the ETH (Aiello and Wheeler, 1995; Kaufman et al., 2003; Isler and van Schaik, 2006; Barrickman and Lin, 2010; Jin et al., 2015; Tsuboi et al., 2015). However, other studies found that there were either non-significant or even positive correlations between metabolically costly tissues (Isler and van Schaik, 2006; Lemaitre et al., 2009; Barrickman and Lin, 2010; Navarrete et al., 2011; Liu et al., 2014).

Most studies of the ETH have been performed among species, though some intraspecific studies have been conducted (fish, Warren and Iglesias, 2012; Liu et al., 2014; frogs, Jin et al., 2015). However, no consistent results have confirmed the patterns of intraspecific variation in brain size, and the ETH in anurans need further study. In this

study, we tested the ETH in the dark-spotted frog *Pelophylax nigromaculatus*, a species widely distributed in plains, hilly paddy fields, ponds, lakes, rivers and mountains at an altitude below 2200m (Fei and Ye, 2001). Previous studies of the ETH have recognized brain, heart, kidneys, liver, testes and gut tissues as being metabolically costly (Aiello and Wheeler, 1995; Pitnick et al., 2006; Isler and van Schaik, 2006; Barrickman and Lin, 2010; Navarrete et al., 2011; Warren and Iglesias, 2012). Our aim was to explore whether brain size was negatively correlated with any other organs (i.e., heart, liver, spleen, kidneys, testes, and limb muscles mass) in *P. nigromaculatus*.

We captured specimens by hand in ponds in Yingxi Town of Nanchong city (30°50'N, 106°07'E, 338 m a.s.l.) in Sichuan, China (Mao et al., 2014). All individuals were caught at night during the breeding season from April 11 to 19 in 2010. We collected a total of 84 individuals (45 males and 39 females), and then brought them to the laboratory. The sex was determined by observing the differences in secondary sex characteristics. Before processing, the frogs were kept in rectangular tanks (1.0\*0.5\*0.4 m; L\*W\*H) with a water depth of 5 cm at room temperature. Animals were sacrificed by using double-pithing. We measured body size (snout to vent length, SVL) to the nearest 0.01 mm by a caliper, and body mass to the

nearest 0.1 mg by an electronic balance. Frogs were dissected and all tissues (i.e., brains, livers, hearts, spleen, kidneys, testes, and limb muscles) were removed and weighed. We analyzed the male and female data separately. All data were log-transformed prior to analysis. In order to control the effect of body mass on energetically expensive tissues, we used relative size of expensive tissues to analyze correlations between brain size and the other organs. The relative size of the expensive tissue was the residual of observed expensive tissues size to that predicted on the basis of the regressions of brain, heart, spleen, kidneys, liver, testes mass and limb muscles on body mass. We estimated body condition using the residuals from a regression of body mass on SVL. Some tissues were affected by body condition. If the effect of body condition on each of the expensive tissues was significant, we then used a partial analysis and body condition as a covariate to test relationships between brain mass and each of the expensive tissues. Statistical tests were parametric, using Type III sums of squares tests with the SPSS (22.0) statistical package.

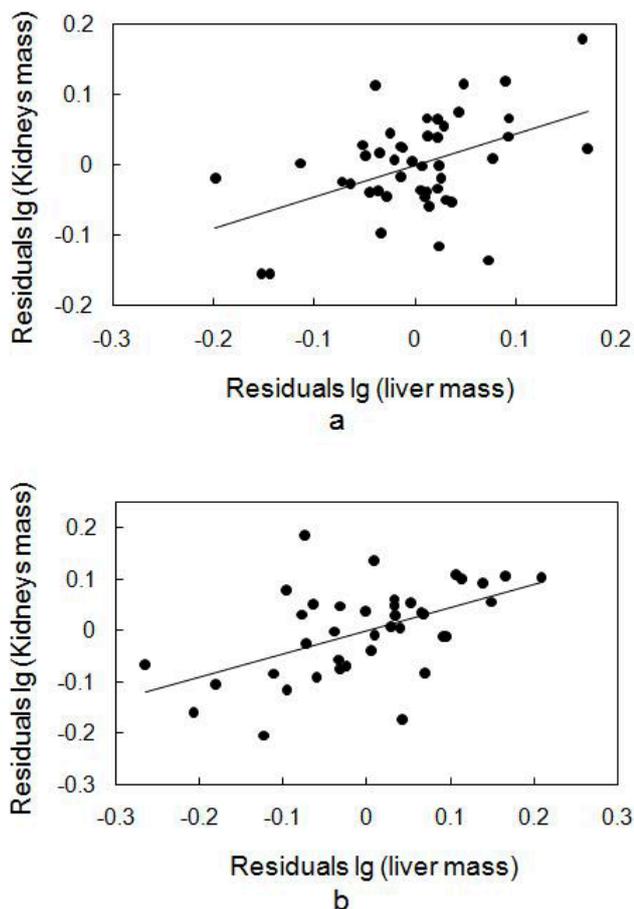
In the male dark-spotted frog, *P. nigromaculatus*, the results showed that brain residuals did not negatively correlate with residuals of testes, heart, liver, spleen, kidneys or limb muscles (Table 1). For other tissues (testes,

**Table 1.** Regressions of brain mass residuals and body condition on other organ size residuals in male *Pelophylax nigromaculatus*. Coefficient estimates from regressions are given with 95% CI in brackets, and  $\beta$  and P-values associated with each regression are also provided.

Organ size	Brain mass			Body condition		
	Estimates [ $\pm$ 95% CI]	$\beta$	P-value	Estimates [ $\pm$ 95% CI]	$\beta$	P-value
Testes	0.010[-0.205,0.224]	0.015	0.927	0.017[-0.082,0.115]	0.056	0.731
Heart	-0.051[-0.388,0.286]	-0.047	0.761	0.111[-0.265, 0.043]	-0.217	0.152
Liver	0.086[-0.223,0.394]	0.085	0.578	0.104[-0.038,0.055]	0.220	0.147
Spleen	-0.027[-0.144,0.090]	-0.071	0.644	-0.009[-0.064,0.046]	-0.052	0.733
Kidneys	0.158[-0.158,0.473]	0.152	0.320	-0.003[-0.153,0.146]	-0.007	0.965
Limb muscles	0.428[-0.324,1.179]	0.172	0.257	0.048[-0.309,0.405]	0.041	0.788

**Table 2.** Regressions of brain mass residuals and body condition on other organ size residuals in female *Pelophylax nigromaculatus*. Coefficient estimates from regressions are given with 95% CI in brackets, and  $\beta$ , P-values associated with each regression are also provided.

Organ size	Brain mass			Body condition		
	Estimates [ $\pm$ 95% CI]	$\beta$	P-value	Estimates [ $\pm$ 95% CI]	$\beta$	P-value
Heart	-0.080[-0.393,0.239]	-0.085	0.605	-0.292[-0.308,-0.277]	-0.252	0.121
Liver	0.050[-0.193,0.293]	0.069	0.678	-0.083[-0.238,0.073]	-0.175	0.287
Spleen	0.012[-0.111,0.135]	0.032	0.847	-0.063[-0.140,0.014]	-0.264	0.104
Kidneys	0.215[-0.058,0.489]	0.254	0.119	-0.023[-0.206,0.161]	-0.041	0.804
Limb muscles	0.063 [-0.591,0.716]	0.032	0.847	-0.370[-0.775,0.036]	-0.290	0.073



**Fig. 1.** Correlations between residuals liver mass and residuals of kidneys mass in *Pelophylax nigromaculatus*, controlling for body condition (a: male; b: female).

liver, spleen, kidneys, limb muscles), we found only one significant positive correlation between the liver residuals and the kidney residuals (Fig. 1a). We found the same relationships between metabolically expensive tissues in females (Table 2, Fig. 1b).

The results of our study did not find a significant correlation between body condition and other organs either in males or females (Table 1, Table 2). However, a significantly positive correlation between liver residuals and kidney residuals still existed when controlling for the effect of body condition (male:  $r = 0.442$ ,  $P = 0.005$ ; female:  $r = 0.527$ ,  $P < 0.001$ ).

The expensive tissue hypothesis states that organisms can reduce the size of other expensive tissues in their body to maintain a relatively larger brain size (Aiello and Wheeler, 1995). Previous most convincing studies in favor of the expensive tissue hypothesis result from ectothermic animals such as the elephant nose fish *Gnathopeternus petersii* (Kaufman et al., 2003), or the guppy *Poecilia reticulata* (Kotrschal et al., 2013), 73 species of Lake Tanganyika cichlids (Tsuboi et al., 2015), the Omei wood frog *Rana omeimontis* (Jin et al., 2015). In contrast to the ETH, we did not find clear evidence to support this hypothesis in *P. nigromaculatus*. There is a similar study investigated ETH in a fish which did not find support for ETH (Liu et al., 2014).

The energy trade-off hypothesis predicts that organisms, in order to maintain relatively larger brain, will reduce investment in reproduction (Isler and van Schaik, 2006). Pitnick et al. (2006) found significantly negative correlations between investment in testes and brains in bats, supporting this theory. However, we did not find a negative correlation between the size of the brain and testes in the dark-spotted frog. There are two possible evolutionary paths to fuel energetic requirements for brain enlargement in animals: (i) increase overall energy budget and (ii) re-allocate energy budget. The metabolic constraints hypothesis concerns the first possibility, and ETH and the energy trade-off hypothesis concern the second possibility. The lack of support for the ETH found in this study suggest that energetic constraints operates in frog brain size evolution, despite copious evidence that brain tissue is metabolically costly to develop and maintain in another frog (Jin et al., 2015). This difference in the ETH between the two frogs' species might relate to difference in their habitat use (Fei and Ye, 2001).

Muscle tissue can consume a considerable proportion of the organism's energy at rest and it must therefore be included in the ETH (Aiello and Wheeler, 1995). For amphibians, individuals with high energy costs of locomotive capability have strong ability to search for mates and to avoid predation (Duellman and Trueb, 1986; Liao et al., 2012; Jin et al., 2015; Liao et al., 2015b). As a result, individuals with greater reproductive fitness selected larger brains are associated with an elevated cognitive ability (i.e., the ability to process information; Striedter, 2005). However, we did not find an increase brain mass increasing with the mass of limb muscles, as proxy for the costs of locomotion. The kidneys are the primary organs for excreting metabolic waste and maintaining pH balance in organisms (Moore, 1995; Ganong, 2005). The liver is one of the most important energy storage organs in animals (Ji et al., 2002; Yang and Wu, 2006). The ETH predicts a significant negative correlation between the size of the brain and both the liver and the kidneys. Nonetheless, our findings demonstrated that there were no significant relationships among them in *P. nigromaculatus*.

The critically important organs (testes, liver, spleen, kidneys, and limb muscles) can change in size and metabolic activity in different life-cycle periods (Piersma,

2002). In this study, we did not find significant relationships between brain mass and organ mass of any of the major metabolically expensive organs. This lack of correlation supports the hypothesis that energetic costs of one expensive tissue do not necessarily directly affect the investment in another expensive tissue, but rather may scatter its effect on all other expensive tissues (Lemaître et al., 2009). Moreover, we did not find evidence that there were negative correlations among testes, spleen, limb muscles, liver or kidneys, suggesting that there was no trade-offs between metabolically expensive tissues due to differences in activity level or growth among individuals. However, we found a positive correlation between liver and kidneys in both sexes.

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