Relationship between brain size and digestive tract length support the expensive-tissue hypothesis in *Feirana quadranus*

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The brain is among the most energetically costly organs in the vertebrate body, while the size of the brain varies within species. 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. Here, the ETH was tested by analyzing the relationship between brain size variation and digestive tract length in a Swelled-vented frog (*Feirana quadranus*). A total of 125 individuals across 10 populations ranging from 586 to 1,702 m a.s.l. from the Qinling-Daba Mountains were sampled. With the increase in altitude, the brain size decreases and the digestive tract length increases. Different brain regions do not change their relative size in a consistent manner. The sizes of telencephalon and cerebellum decrease with the increase in altitude, while the olfactory nerve increases its size at high altitudes. However, the olfactory bulb and optic tectum have no significant relationship with altitude. After controlling for snout-vent length (SVL), a significant negative correlation could be found between brain size and digestive tract length in *F. quadranus*. Therefore, the intraspecific variation of brain size follows the general patterns of ETH in this species. The results suggest that annual mean temperature and annual precipitation are environmental factors influencing the adaptive evolution of brain size and digestive tract length. This study also suggests that food composition, activity times, and habitat complexity are the potential reasons driving the adaptive evolution of brain size and digestive tract length.

KEYWORDS

brain size, energetic constraints, expensive-tissue hypothesis, environmental factors, *Feirana quadranus*

Introduction

The brain has always been of interest to almost every field of biology and ecology dealing with animals as it is the central system of learning, memory, cognition, and information processing (Barton and Harvey, 2000; Gonda et al., 2013). Although the direct relationship between brain size and its functional capacity remains controversial, brain size has generally been taken as an important trait that reflects the number of neurons and cognition (Marhounová et al., 2019). The evolution of brain size could have profound effects on important ecological and evolutionary processes, such as selection pressures in the environment, thereby contributing to an enhanced understanding of the ecological fitness of species (Sayol et al., 2016a). The brains of higher vertebrates show great variation in size at interspecific and intraspecific levels (Striedter, 2006; Gonda et al., 2013), in absolute and relative items of the overall size of the brain or even the size of the main brain parts. Understanding the ultimate cause of brain size evolution has become a challenging task for evolutionary biologists (Minias and Podlaszczuk, 2017).

Considerable evidence showed that ecological, social, and sexual selection pressures influence brain size changes in species (Pitnick et al., 2006; Dunbar and Shultz, 2007; Barton and Capellini, 2011). Moreover, the brain is a metabolically costly organ (Mink et al., 1981), and the large amount of energy required for its metabolism limits the evolution of brain size (Striedter, 2006). Therefore, the brain size of an organism is predominantly determined by the tradeoff between the "selective advantage of increasing cognition" and the "energy expenditure of maintaining a larger brain" (Kotrschal et al., 2013a,b; Liu et al., 2022).

The expensive-tissue hypothesis (ETH) predicts that increasing brain size could inevitably reduce the size of other metabolically costly tissues, such as the gut (Aiello and Wheeler, 1995). The ETH was first used to explain the negative correlation between brain size and digestive tract length in primates and human evolution (Aiello and Wheeler, 1995). Later on, it was used to explain the negative correlation between the bat brain and testicular weight (Pitnick et al., 2006). However, the applicability and accuracy of this hypothesis have always been controversial. Currently, studies have verified the evolutionary relationship between brain size and digestive tract length in some animals. Some studies have shown a significant negative correlation between brain size and digestive tract length, thus supporting the ETH (Kaufman, 2003; Jin et al., 2015; Tsuboi et al., 2015; Liao et al., 2016). On the contrary, other studies have found no such relationship or even the opposite (Lemaître et al., 2009; Barrickman and Lin, 2010; Navarrete et al., 2011; Liu et al., 2018). The study of *Opsanus tau* shows that increased investment in one structure does not necessarily drive a loss of mass in one or more organs (Dornburg et al., 2018). Studies in mice have shown that evolutionary increase in cognitive abilities was initially associated with brain plasticity and fueled by an enlarged gut, which was not traded off for brain size, as the ETH posits (Konarzewski et al., 2020).

Although the ETH is based on primates (Aiello and Wheeler, 1995), the best evidence supporting it comes from poikilotherms (Kaufman, 2003; Kotrschal et al., 2013a; Tsuboi et al., 2015). As ectotherms, amphibians are good models or subjects for brain research. The relative brain size of *Rana omeimontis* was significantly negatively correlated with the relative digestive tract length, and energy restriction could explain the change in brain size, thus supporting the ETH (Jin et al., 2015). However, some studies suggested that the relative brain size was not significantly correlated with the relative digestive tract length, and the change in brain size could not be explained by energy restriction, which did not support the ETH (Liao et al., 2016; Liu et al., 2018). Besides, the study found no negative correlation between brain mass and intestinal length, and even no negative correlation was discovered between brain mass and the mass of other organs in *Bufo gargarizans* (Mi and Liao, 2021).

Phenotypic plasticity in morphological and physiological traits is a universal phenomenon in animal groups (Balciauskas et al., 2020; Huang et al., 2021; Liang et al., 2021; Zamora–camacho, 2021; Zedda et al., 2021; Giacomini et al., 2022; Hinds et al., 2022). The phenotypic plasticity theory states that organisms change their morphology and physiological functions as an adaptive response to environmental conditions (Clifton et al., 2020). Therefore, changes in brain size are an evolutionary adaptation to environmental changes. Environmental conditions are crucial factors affecting the evolution of brain size adaptation. Nearly half a century ago, evolutionary biologists have carried out considerable research on the adaptive evolution of the brain size of warm-blooded animals. The results showed that in different environments, the types of food, habitat types, seasonality, activity time, breeding investment, and the difference in life history and the brain the size of the organism could change due to the differences in altitude gradient (Aiello and Wheeler, 1995; Barton and Harvey, 2000; Reader and Laland, 2002; Sol et al., 2005; van Woerden et al., 2011; Sayol et al., 2016b; Minias and Podlaszczuk, 2017; Baldwin et al., 2022). Changes in altitude, latitude, and longitude act on organisms primarily by affecting the temperature and rainfall of their habitat. In general, biodiversity and environmental complexity are higher where temperature and rainfall are higher (Carnaval et al., 2014). Therefore, the intrinsic mechanism of ETH could be explored by studying the relationship between the average annual temperature and rainfall and the brain and intestine.

In recent years, the adaptive evolution of brain size in amphibians and other poikilotherms has also received more attention (Liao et al., 2015, 2016, 2018; Luo et al., 2017; Yu et al., 2018; Huang et al., 2020). Research uncovered that amphibians' brain size evolution is closely related to environmental factors;

for instance, the seasonal variation in temperature could explain the variabilities of the relative brain size and the size of the optic tectum (TEC) in 30 amphibian species (Luo et al., 2017), and the toad's relative brain size decreased with the increase in altitude (Yao et al., 2021).

As the "backbone of China," the Qinling Mountains have unique topographic characteristics, and the regional response to climate change is more typical and representative. The Qinling Mountains stretch across the central part of China, which is a transitional region of north–south climate change in this country. Obvious spatial differences could be observed in temperature and annual precipitation changes (Xia et al., 2019). These advantaged environmental conditions provide convenience for studying the influence of environmental conditions on brain size. The Chinese endemic frog species swelled-vented frog (*Feirana quadranus*) is widely distributed in the Qinling-Daba Mountains. *F. quadranus* generally lives in mountain streams and near areas with an altitude range of 335–1,830 m a.s.l. in the Qinling-Daba Mountains. This species mainly preys on various insects and sometimes some other small animals. It is a common species in the Qinling-Daba Mountains with a high population density, thus especially suitable for studying intraspecific variation (Wang et al., 2019). The current declining population of *F. quadranus* requires urgent conservation-related research, and the present study could provide scientific advice that could better predict the species' endangerment and guide local biodiversity conservation measures (Gonzalez-Voyer et al., 2016).

The life history of amphibian anurans requires experiencing both aquatic and terrestrial environments, and the habitat is more complex compared to other taxa. Environmental factors may have different effects on different tissues and organs, and the difference in energy consumption of different tissues and organs may be more obvious. Therefore, we studied intraspecific size variation in brain size, the size of the brain region, and digestive tract length of *F. quadranus* along an altitudinal gradient. In particular, the ETH related to brain size evolution was tested using this system. To gain insight into the physiological adaptability of this species in response to environmental changes, we also tested whether brain size and digestive tract length were correlated with annual mean temperature and annual precipitation.

Materials and methods

Field sampling

A total of 125 individuals, including 50 males and 75 females, were collected from 10 sample sites during the breeding season in 2021 (Figure 1 and Table 1). The main locations were in the Qinling-Daba Mountain area, with an elevation range of 586–1,702 m. The specimen for this study was approved

by the Animal Ethics Committee at China West Normal University.

Brain and digestive tract measurements

All specimens were taken to the laboratory, kept individually in rectangular tanks (0.5 m × 0.4 m × 0.4 m) before being anesthetized with benzocaine, and preserved in 4 % buffered formalin in a phosphate buffer. After 2 months of preservation, snout-vent SVL was measured to the nearest 0.01 mm with calipers. Brains were dissected out and weighed (to the nearest 0.1 mg) with an electronic balance (Jiang et al., 2015). The complete brain regions taken out in this study mainly included the olfactory nerve (OLF-N), olfactory bulb (OLF-B), telencephalon (TEL), optic tectum (OPT), and cerebellum (CER), a total of five parts (Jiang et al., 2015).

A digital camera was used to take photographs of these brains from dorsal, lateral, and ventral views (Figure 2). The coronal and sagittal planes were parallel to the camera sensor, and a caliper with scale was placed in all photographs as a reference. Finally, the brain size was measured three times with the tpsDig software (Huber et al., 1997). All the measurements were conducted by the same investigator (YF) to eliminate interobserver variability (Burns et al., 2009). The length (L), width (W), and height (H) of each brain and the total volume of fluid displacements were used to obtain the ellipsoid model brain volume (V) as follows: $V = (L*W*H)*\pi/(6*1.43)$. This formula was adopted to calculate the total brain volume and the volume of different brain parts for each individual OLF-N, OLF-B, TEL, OPT, and CER. For paired structures, only the right hemisphere was measured and the volume was doubled (Jiang et al., 2015).

Similarly, the digestive tract was dissected out, and the digestive tract was placed on a foam board and secured with a pin to avoid overlap and bumps affecting its length measurement. Then, a digital camera was used to take images of the digestive tract with a caliper. Finally, the tpsDig software was used to measure the length of the digestive tract.

Data analysis

All continuous variables were $\log_{10}$ transformed to meet the assumption of normality and enhance the homogeneity of variances (type III sums of squares tests conducted with SPSS v.22.0). To analyze the differences in brain volume and digestive tract length among populations, we used one-way analyses of variance (ANOVA) for brain volume and digestive tract length separately, controlling for SVL as a covariate. Next, we ran a general linear model (GLM) with brain volume as a dependent variable, digestive tract length as fixed effects, and SVL as a
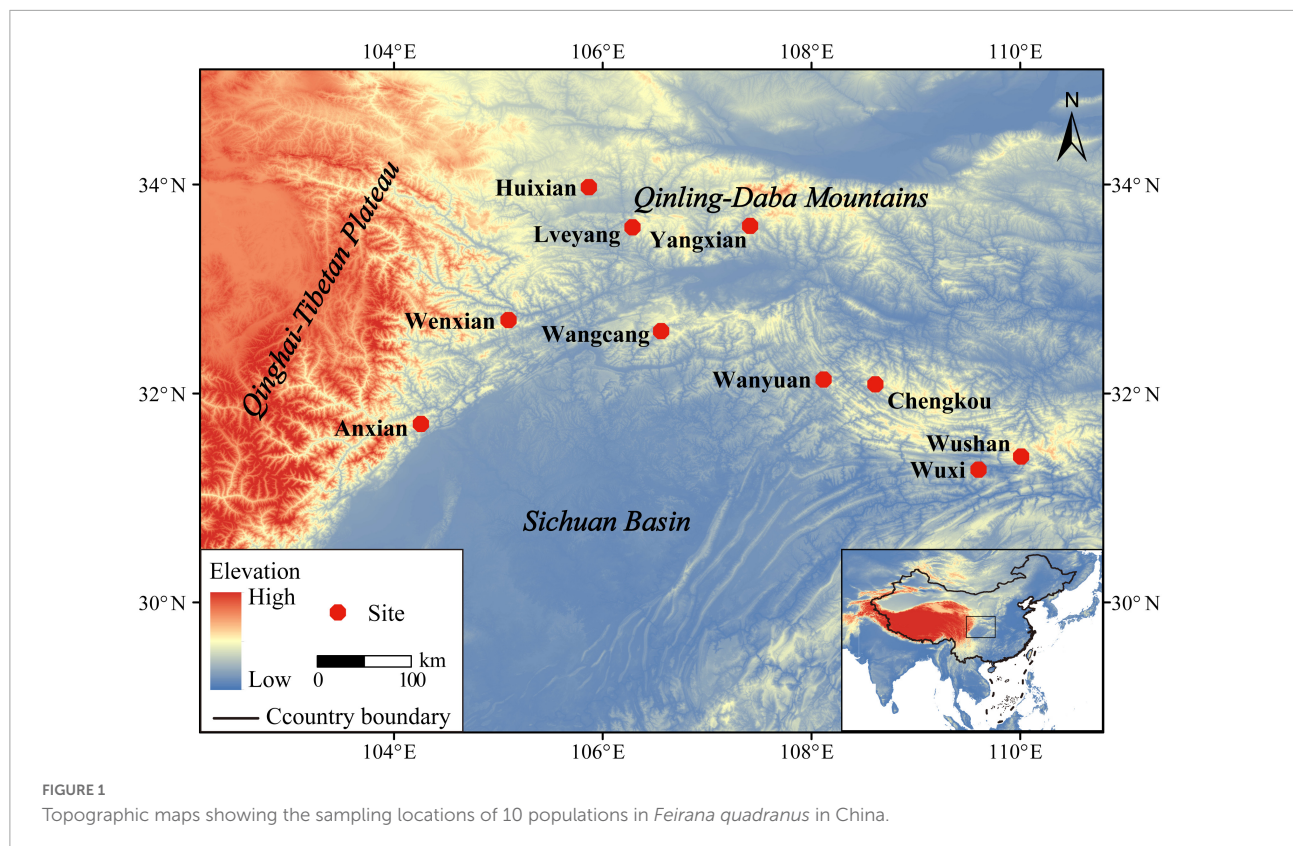
FIGURE 1

Topographic maps showing the sampling locations of 10 populations in *Feirana quadranus* in China.

TABLE 1 Sampling sites and collecting information.

Objected	Population	Longitude	Latitude	Altitude	♂/♀
1	Wuxi	109.60069	31.27111	586	2/6
2	Wangcang	106.55908	32.59732	756	10/4
3	Wenxian	105.09679	32.70333	860	4/3
4	Wanyuan	108.11598	32.13377	1,066	4/9
5	Yangxian	107.41203	33.60417	1,148	5/19
6	Wushan	110.01034	31.39667	1,249	4/3
7	Anxian	104.25767	31.70826	1,326	13/4
8	Huixian	105.86727	33.97528	1,488	5/15
9	Lveyang	106.28531	33.59056	1,625	0/4
10	Chengkou	108.61244	32.08716	1,702	3/8

covariate to test the original ETH. Correlations between organs were analyzed using the residuals from the log–log regressions of the brain and digestive tract length on SVL as relative values to offset the effect of SVL on organs. Then, we used multiple linear regression model (MLRM) to assess the effect of geographical gradients (e.g., latitude, longitude, and altitude) on relative brain volume, the relative size of brain regions, and relative digestive tract length. Finally, we analyzed the relationship between relative brain volume and relative digestive tract length and environmental parameters (e.g., annual mean temperature and annual precipitation) of each site among all populations. We also analyzed the relationship between the relative size

of brain regions and environmental parameters. The annual mean temperature and annual precipitation in this study were obtained from the World Climate Database.[1]

Results

One-way ANOVA revealed a significant difference in brain volume ($F_{9,115} = 4.583$, $P < 0.001$) and digestive tract length
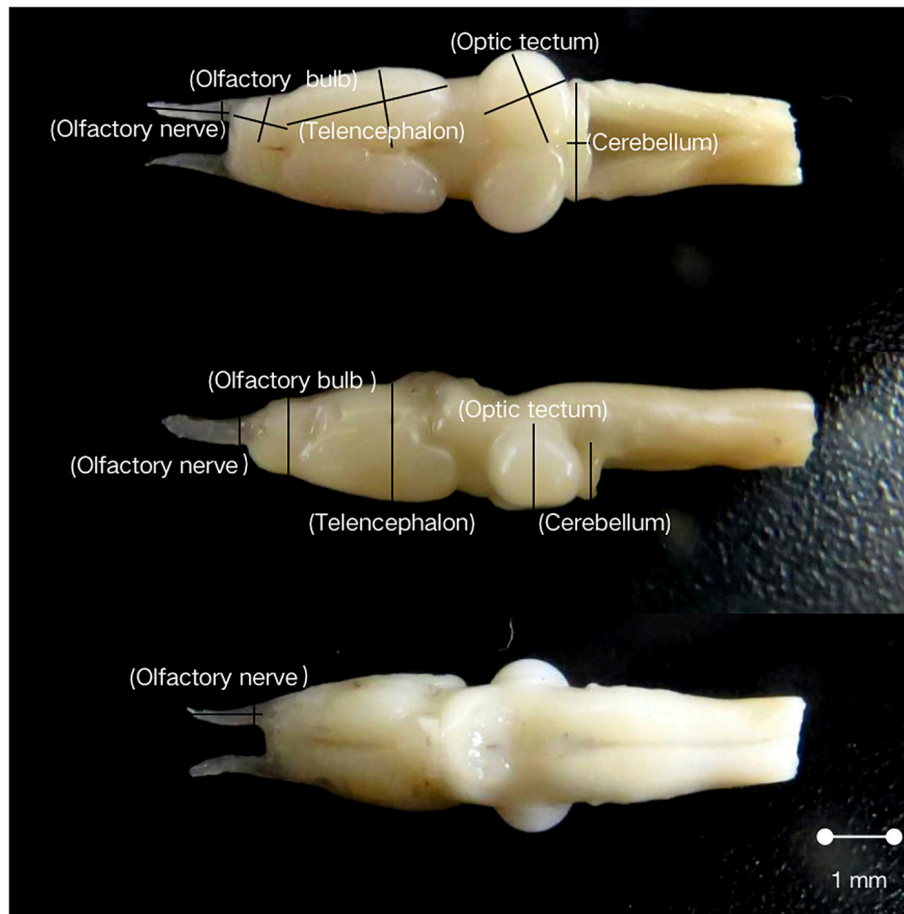
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1 https://worldclim.org/

FIGURE 2
Dorsal, lateral, and ventral views of the brain in *Feirana quadranus*. Length, width, and height measures for each of the five brain structures (olfactory nerve, olfactory bulb, telencephalon, optic tectum, and cerebellum) are shown. Further details are found in the "materials and methods" section.

($F_{9,115} = 2.803$, $P = 0.005$) among populations. We found that brain volume was significantly negative correlated with digestive tract length when controlling for SVL (**Figure 3**; digestive tract length, $F_{44,79} = 1.923$, $P = 0.006$; SVL, $F_{1,112} = 12.559$, $P = 0.001$).

We further examined the variation in the geographic gradients of organ size at the population level based. The relative brain volume was negatively correlated with altitude and latitude, but did not vary significantly with longitude (**Table 2**). The relative digestive tract length was positively correlated with altitude and longitude, but did not vary significantly with latitude (**Table 2**). The relative volume of OLF-N enlarged with the increase in altitudes ($F_{3,118} = 2.145$, $t = 2.304$, $P = 0.023$). Meanwhile, the relative volume of TEL and CER reduced with the increase in altitudes (TEL, $F_{3,118} = 2.445$, $t = -2.334$, $P = 0.021$; CER, $F_{3,118} = 7.009$, $t = -2.403$, $P = 0.018$) and the relative volume of OPT reduced with the increase in latitude ($F_{3,118} = 8.837$, $t = -3.977$, $P < 0.001$). However, the relative sizes of OLF-B had no significant relationship with

geographical gradients (all $P > 0.05$). The results of MLRM show that the relative brain size was positive correlated with annual mean temperature ($F_{2,121} = 20.641$, $t = 5.611$, $P < 0.001$), while relative digestive tract length was negative correlated with annual mean temperature ($F_{2,121} = 5.107$, $t = -3.118$, $P = 0.002$). The MLRM showed that the relative TEL volume and relative OPT volume was positively correlated with annual mean temperature, while relative OLF-N volume was negatively correlated with annual mean temperature, and relative OPT volume size and relative CER volume size were positively correlated with annual precipitation (**Table 3**). No significant correlation was found between the other relative brain regions' size and environmental factors (**Table 3**).

Discussion

The results uncovered a negative correlation between brain size and digestive tract length after controlling for
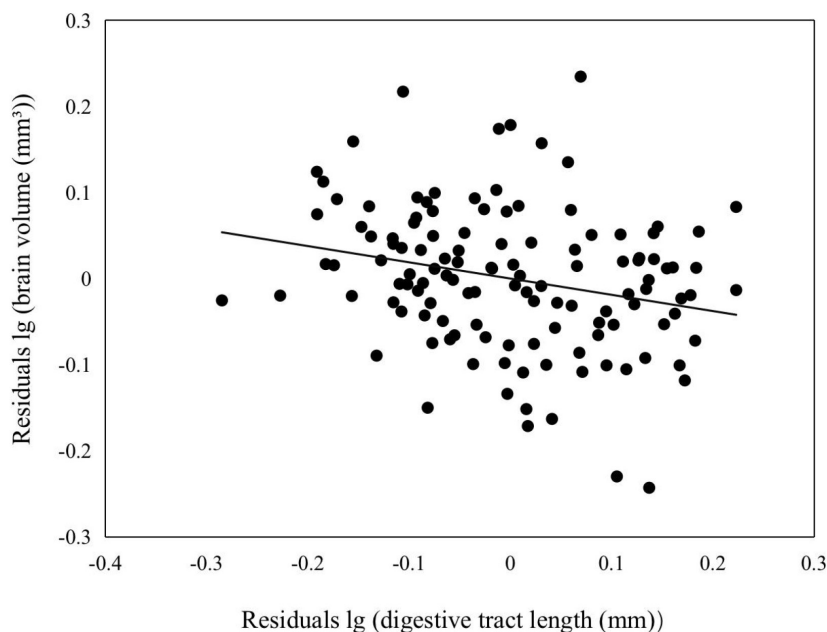
FIGURE 3

Correlations between relative brain volume and relative digestive tract length in *Feirana quadranus*.

SVL in *F. quadranus*, supporting the original "brain versus gut" prediction arising from the ETH. The brain size of *F. quadranus* increased and the digestive tract length decreased with the increase in annual mean temperature. Thus, this study supports the existence of energetic constraints as important factors influencing the patterns of brain size diversification among frogs.

After controlling for SVL, a negative correlation between brain size and digestive tract length was observed in this species. Although the applicability and accuracy of the ETH have always been controversial, the brain–gut relationship in this species typically conforms to ETH, consistent with some previous studies. For example, the relationship between brain size and other energy-consuming organs in 30 anuran species

was consistent with the ETH (Liao et al., 2016). The ETH was originally proposed to explain why the brain of several anthropoid primates and humans accounts for 1–2% of body weight (Aiello and Wheeler, 1995; Striedter, 2006). Ectotherms were believed to have small brains and be less likely to produce energy tradeoffs due to energy constraints in the evolutionary process, but this study and other studies demonstrated that the brain and digestive tract were negatively correlated in anuran species (Jin et al., 2015; Liao et al., 2016). These studies indicated that energy constraints and tradeoffs are prevalent in the evolution of the brain and digestive tract in vertebrates, especially in ectotherms.

Populations live in different environments with different altitudes, latitudes, and longitudes, and climate conditions. These environmental differences could be expressed in temperature, rainfall, ultraviolet light, oxygen pressure, and even indirectly related factors, such as food (Naya et al., 2009; Lou et al., 2013). When altitude was included in the statistical analysis, brain size was found to be decreased with the increase in altitudes, whereas a trend of digestive tract length increase with increased altitudes was observed. The altitudinal gradient is one of the most commonly used ecological differences to reveal biodiversity and its evolutionary mechanisms (Hodkinson, 2005; Keller et al., 2013). High-altitude environments are characterized by hypoxia, low temperature, high ultraviolet radiation, high climatic diurnal, and seasonal variability (Storz et al., 2010). Therefore, high altitude represents strong directional selection (Yao et al., 2021). Altitude, temperature, and rainfall are closely related to environmental factors.

TABLE 2 The effects of geographical gradients on variation in relative brain volume and relative digestive tract length of the *Feirana quadranus* using multiple linear regression model (MLRM).

Source	d.f.	Predictor	t	P
Relative brain volume				
	3,120	Altitude	−3.996	<0.001
	3,120	Longitude	−1.390	0.167
	3,120	Latitude	−3.099	0.002
Relative digestive tract length				
	3,120	Altitude	2.736	0.007
	3,120	Longitude	2.713	0.008
	3,120	Latitude	0.389	0.698

TABLE 3 The effects of annual mean temperature and annual precipitation on variation in the relative volume of brain region of the *Feirana quadranus* using multiple linear regression model (MLRM).

Brain region	Environmental factor	Df	t	P
Relative olfactory nerve volume	Annual mean temperature	2, 119	−2.231	0.028
	Annual precipitation	2, 119	1.634	0.105
Relative olfactory bulb volume	Annual mean temperature	2, 119	−0.662	0.509
	Annual precipitation	2, 119	−0.438	0.662
Relative telencephalon volume	Annual mean temperature	2, 119	2.904	0.004
	Annual precipitation	2, 119	0.339	0.735
Relative optic tectum volume	Annual mean temperature	2, 119	3.195	0.002
	Annual precipitation	2, 119	3.178	0.002
Relative cerebellum volume	Annual mean temperature	2, 119	1.795	0.075
	Annual precipitation	2, 119	3.053	0.003

The combination of dehydration and cold temperatures in high-altitude areas leads to shorter periods of activity in the diurnal and seasonal cycles, thus also limiting energy supplies. Organisms at high altitudes must allocate the limited energy for all their functional organs to sustain basic life activities. With altitude increase, the energy investment in the brain decreases, whereas in the digestive tract increases. This could be an important reason for causing the negative correlation between brain size and digestive tract length.

Normally, the temperature is extremely important in shaping the characteristics of an organism. Temperature and rainfall could affect activity time, food abundance, natural enemy pressure, and habitat complexity. Therefore, this study not only focused on the relationship between brains and guts; it could also help gain fresh and deeper insights into how these environmental factors drive the evolution of the brain and high energy-consuming tissues. Three possible explanations could help understand the underlying mechanism of the evolution of brain size and digestive tract length in *F. quadranus*.

At first, changes in food composition in different environments may lead to a tradeoff between the brain size and the digestive tract length. The mean temperature of the environment often determines food availability. In general, areas with low average annual temperature and rainfall have low biodiversity and environmental complexity (Connell and Orias, 1964). The adult *F. quadranus* mainly feeds on insects; however, insect mortality is closely related to

annual average temperature (Fitt, 1989; Morecroft et al., 2002; Savage et al., 2004; Staley et al., 2007; Wen and Zhang, 2010; Shi et al., 2011). There is evidence supporting that, in such harsh environments with high altitudes, decreased temperature may result in decreasing animal-based foods and increasing plant-based foods in anuran species (Naya et al., 2009; Lou et al., 2013; Wang et al., 2017). To better digest the nutrients in the food and improve survival in high altitudes, an increase in indigestible materials may drive the increased relative size of the digestive tract in these individuals (Wang et al., 2017). As a result, the amount of energy allocated to the brain reduces and the size of the brain decreases.

Under different environmental conditions, the variation of individual activity time is also one of the main reasons affecting the evolution of brain size. Shorter activity times may result in smaller brain size and increased digestive tract length. For *Fejervarya limnocharis*, individuals foraging at higher altitudes with shorter activity times have increased digestive tract lengths (Wang et al., 2017). A study in *Bufo andrewsi* revealed that the brain became smaller due to the decrease in its activity time at a high latitude with low temperature. In addition, populations of *B. andrewsi* with longer activity times developed larger optic tectum than those with shorter activity times, which may be an adaptation to a life of high risk at lower latitudes (Naya et al., 2009; Lou et al., 2013; Wang et al., 2017).

The habitat complexity including the difference in habitat type and predation risk is also an important factor driving intraspecific brain size evolution in different environments (Striedter, 2006; Liao et al., 2022). Direct experimental evidence showed that brain size evolution is intimately linked to the evolution of neuron number and cognition (Marhounová et al., 2019). Recent studies suggested that environmental factors have a significant influence on the evolution of some brain regions in amphibians. For example, habitat type could explain the change in telencephalon size, and natural enemy pressure could explain the change in bulbus olfactorius and optic tecta size in 43 amphibious species (Liao et al., 2015). Studies have shown that the increase in brain size is closely related to the recognition of predators (Liao et al., 2015; van der Bijl et al., 2015). With the increase in altitude, the changes in annual mean temperature and annual precipitation can not only lead to fewer food resources but also fewer types and numbers of natural predators. In such a simpler environment, the reduced cognitive demands drive the evolution of decreased brain size correspondingly, providing excess energy to develop the digestive tract. Meanwhile, places with high annual average temperature and high rainfall have high biodiversity and complex environment. The factors mentioned above could encourage individuals living in these environments to increase their brain size and cognitive abilities to cope with more complex environments and predation stress, thereby increasing their chances of survival. Besides, although the overall brain size decreases with altitude, the olfactory nerve increases its size

along the altitudinal gradients. The function of the olfactory nerve is mainly to perceive odor information, which is essential for locating predators and finding and capturing prey. This finding suggested that *F. quadranus* at high altitudes, cold temperature, and dry conditions likely faces additional cognitive challenges, which demand an enlarged olfactory nerve to cope with it.

In conclusion, the findings suggested that the ETH is supported in *F. quadranus* because brain size and digestive tract length are inversely related. By influencing food availability, activity times, and habitat complexity, environmental factors such as annual mean temperature and annual precipitation are the potential mechanisms driving the adaptive evolution of brain size and digestive tract length.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by the Animal Ethics Committee at China West Normal University.

Author contributions

YF and YH conceived, wrote, and edited the manuscript. YH secured funding. YF, YS, CY, XL, and YL participated in laboratory work and collected the data. YF conducted data analysis and visual representation of the data. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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