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A Comparison of Somatic Condition and Testis Mass in Black-Spectacled Toad (*Duttaphrynus melanostictus*) between Two Populations at Different Altitudes

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ABSTRACT

Altitude, served as one common geographical gradient has been considered to have a major influence on the evolution of life-history traits, which leads to variation in temperature, food availability and duration of breeding season in amphibians. Here, we examined differences in somatic condition and testis mass of male *Duttaphrynus melanostictus* distributed in two altitudes (120 m and 1673 m above sea level) in Hunan and Yunnan Province, southwest China. We found that *D. melanostictus* displayed significant differences in body mass and relative testis mass between low- and high-altitude populations. Body mass and relative testis mass from the high-altitude population were larger than that of the low-altitude population in the species. We also found that testis mass increased with body mass and somatic condition. Furthermore, the somatic condition exhibited a significantly positive correlation with testis mass, indicating the condition-dependent testis mass in *D. melanostictus*.

INTRODUCTION

The same species in different geographical locations tend to have different morphology, physiology, and life-history traits (e.g., egg size, clutch size, sperm length and number) due to variations in climate, resource availability, competition or predation risk (Bergmann, 1847; Gould and Johnston, 1972; Atkinson and Sibly, 1997; Blanckenhorn and Demont, 2004; Zhang and Lu, 2012; Zhang *et al*., 2012; Liao *et al*., 2015a; Jin *et al*., 2016a; Lüpold *et al*., 2017; Kenthao and Jearranaiprepame, 2018; Samani, 2018; Yu *et al*., 2018; Liu *et al*., 2018; Yang *et al*., 2018; Zhong *et al*., 2018; Mai and Liao, 2019). Since poikilotherms are dependent on the relatively systematic climatic gradients along altitude and/or latitude to create chances for clinal variation in trait expression through local adaptation, they are often used as one of the major indicators of climatic variations (Morrison and Hero, 2003; Liao *et al*., 2014). For example, activity periods and breeding activity are often affected by seasonality and environmental temperature (Endler, 1977; Hjernquist *et al*., 2012; Alton *et al*., 2017).A relatively short activity period at high altitude and/or latitude is often associated with larger eggs but relatively smaller clutches (Dziminski and Alford, 2005; Liao and Lu, 2011, 2012). Differences in egg size and clutch size have been studied intensively across a wide range of taxa in females (Roff, 2002; Morrison and Hero, 2003; Liao *et al*., 2016a). However, the information on variation in testis mass remains poorly known in males.

Relative testis mass is widely used as a measure of male reproductive investment, and is considered under strong sexual selection (Gomendio and Roldan, 1991; Byrne *et al*., 2002; Zhou *et al*., 2011; Mi *et al*., 2012; Chen *et al*., 2016; Zeng *et al*., 2014; Tang *et al*., 2018; Cai *et al*., 2018). Therefore, when sperm competition is intense, males increase the investments in testes to enhance sperm production at inter- or intra-specific species level (Dziminski *et al*., 2010; Liao *et al*., 2011). Recent works have focused on between-population variation in testis mass linked with environmental factors (Hettyey *et al*., 2005; Hettyey and Robert, 2006; Liao *et al*., 2013a; Jin *et al*., 2016a). Individuals inhabiting at high altitude/latitude have smaller relative testes mass because the total energy collection is limited due to the decreased environmental temperature and shortened breeding season, possibly

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allowing relatively less energy to be allocated to reproduction (Hettyey *et al*., 2005; Hettyey and Robert, 2006; Chen *et al*., 2014; Zhang *et al*., 2018).

Duttaphrynus melanostictus is a species widely distributed in South Asia, southeast Asia and southern China where they are found in ponds near grass clumps, kaleyard and stream near farmhouse with altitude ranging from 10 to 1700 m. The period of egg laying extends from March to July (Fei *et al*., 2010). So far, little information on difference in somatic condition and testis mass of this species across altitudinal populations is available. Here, we explored differences in somatic condition and testis mass in *D. melanostictus* between two populations at different altitudes. Previous studies have suggested that, at high altitude/latitude, lower environmental temperature and shorter breeding season decreased energy acquisition (Morrison and Hero, 2003), thereby limiting somatic condition and investments in sperm production (Chen *et al*., 2014; Jin *et al*., 2016b). Hence, we tested the hypotheses (1) that somatic condition and testis mass decrease with increasing altitude; (2) that testis mass increases with somatic condition.

MATERIAL AND METHODS

Study sites

We visited two *D. melanostictus* populations located at different altitudes in Hunan and Yunnan Province in Southwest China, from July to August, in 2018. The low-altitude population was located at 120 m in Yuanling (110°24.41′E, 28°26.18′N) in which toads were collected in a stream at the distance of 1 km to farmhouse. The high-altitude population was located at 1673 m in Midu (100°28.51′E, 25°20.24′N), where toads are captured in a stream at the distance of 0.5 km to farmhouse.

Samplings collection

A total of 45 adult males were collected. We captured all individuals by hand at night through using a 12-V flashlight in streams. Before processing, the toads were kept in individual rectangular tanks (1.0×0.5×0.5 m; L×W×H) with a water depth of 2 cm (Jin *et al*., 2015). All the individuals were killed by using single-pithing (Mai *et al*., 2017a; Liao *et al*., 2015b). Body size (snout-vent length: SVL) was measured to the nearest 0.1 mm with a vernier caliper and body mass to the nearest 0.1 mg using an electronic balance (Zhong *et al*., 2016). Toads were dissected, and testes were removed and weighed to the nearest 0.1 mg using an electronic balance (Liu *et al*., 2011; Wu and Liao, 2017).

Statistical analyses

Body mass, soma mass (i.e., body mass–testis mass), testis mass, and body size were log-transformed to achieve normality and improve homogeneity of the variances. We ran a linear regression treating log10 (body mass) as a dependent variable and log10 (body size) as an independent variable, and residual mass was used as an index to measure body condition (Schulte-Hostedde *et al*., 2001, 2005; Jin *et al*., 2016a). Differences in body mass, testes mass and somatic condition (i.e., $\log_{10}$(soma mass) / $\log_{10}$(SVL)) between populations were tested using One-way ANOVA. To test statistical differences in relative testis mass (i.e., $\log_{10}$(testis mass) / $\log_{10}$(soma mass)) between the low- and high-altitude populations, we ran a generalized linear models (GLMs) treating testis mass as a dependent variable, population as fixed factors, and soma mass or somatic condition as covariate. All statistical tests were two-tailed, and the nominal significance level was set at $P = 0.05$. All analyses were performed by SPSS 22.0.

RESULTS

Body mass significantly differed between the populations, being larger for the high- altitude population than the low-altitude population (One-way ANOVA: $F_{1,43} = 217.182$, $P < 0.001$; Table I, Fig. 1). Testis mass at the high-altitude population was significantly larger than that at the low-altitude population (One-way ANOVA: $F_{1,43} = 172.640$, $P < 0.001$; Table I, Fig. 2). The difference in somatic condition between high- and low-altitude population was significant (One-way ANOVA: $F_{1,43} = 214.527$, $P < 0.001$). Relative testis mass from the high-altitude was still larger than the low-altitude population when removing the influence of soma mass (GLMs: $F_{1,43} = 19.203$, $P < 0.001$) or somatic condition (GLMs: $F_{1,43} = 20.375$, $P < 0.001$). Moreover, testis mass was positively correlated with soma mass (GLMs: $F_{1,63} = 8.417$, $P = 0.006$) and somatic condition (GLMs: $F_{1,43} = 9.151$, $P = 0.004$).

DISCUSSION

The results demonstrate that the black-spectacled toad exhibits striking differences in somatic condition between different populations, and this somatic condition displays an increase with altitude. In addition, testis mass significantly differed between the populations, and this testis mass displayed an increase with increased altitude. Moreover, testis mass was positively correlated with soma mass and somatic condition, suggesting that males invest more in their gonads in good somatic condition.

Testis mass relative to soma mass is widely used as a measure of reproductive investment in males

Table I. The body mass and testis mass for two populations *viz.* low altitude and high altitude of *Duttaphrynus melanostictus* males.

Characters	Low-altitude population		High-altitude population	
	Mean ± SD	Range	Mean ± SD	Range
Body mass (g)	12.83 ± 2.44	9.43 - 19.16	31.77 ± 6.57	15.50 - 40.64
Testis mass (mg)	16.60 ± 7.43	5.40 - 37.80	82.34 ± 26.08	40.70 - 133.40

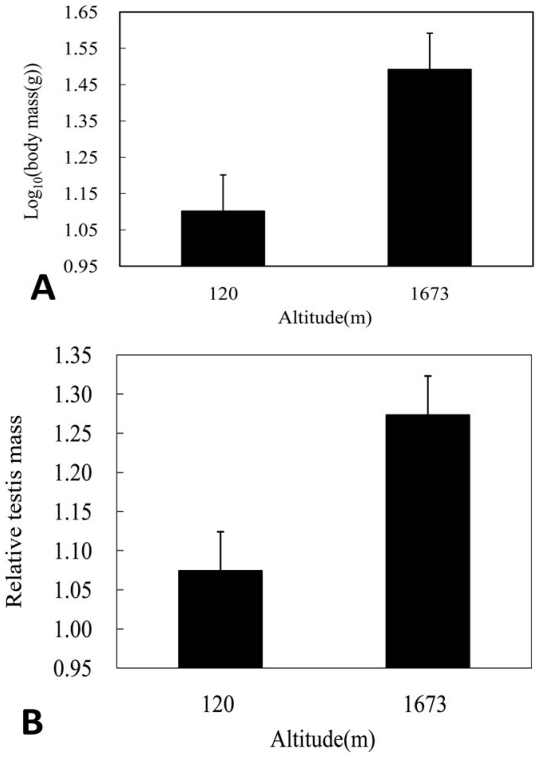

Fig. 1. The difference in body mass (A), relative testis mass between low- and high-altitude population.

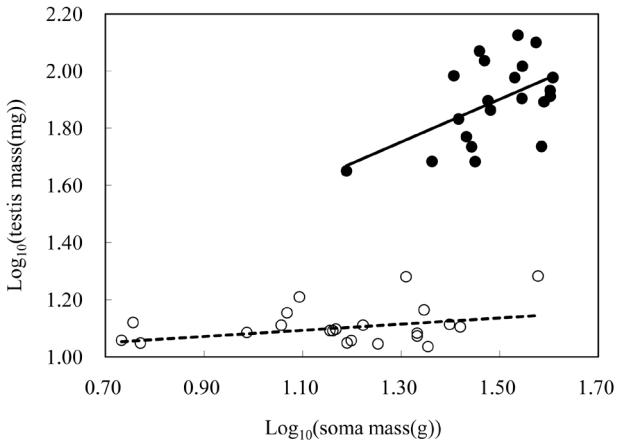

Fig. 2. Relationships between testis mass and soma mass of *Duttaphrynus melanostictus*. Open circle/dashed line and close circle/real line represent the low- and high-altitude populations, respectively.

(Jin *et al*., 2016b; Tang *et al*., 2018). It has been suggested by previous studies that there is a condition-dependent testis mass across a wide range of taxa (Byrne *et al*., 2002; Schulte-Hostedde and Millar, 2004; Burness *et al*., 2008; Jin *et al*., 2016b; Tang *et al*., 2018). Males with good somatic condition have relatively large relative testis mass than males in poor somatic condition (Simmons and Kotiaho, 2002; Jin *et al*., 2016b). In the study, testis mass was significantly positive associated with soma mass and somatic condition, suggesting that males in good body condition may have larger reproductive investment than males with poor body condition. Similar results have been reported previously in other studies (Simmons and Kotiaho, 2002; Mai *et al*., 2017b; Zhou *et al*., 2011; Chen *et al*., 2014; Lüpold *et al*., 2017; Tang *et al*., 2018).

Life-history strategy with respect to resource allocation for reproduction reflects a trade-off between current reproductive effort and future reproductive success and/or survival (Lack, 1966; Roff, 2002). In high-altitude or latitude, individuals experience less time for reproduction and the extended re-acquisition of resources necessary for survival, as a result, there was a negative relationship between testis mass and altitude or latitude in male frogs (Hettyey *et al*., 2005; Chen *et al*., 2014). However, we found a positive correlation between relative testis mass and altitude in this species, although there was a lower environmental temperature and shorter time available for reproduction in high-altitude population. Our evidence suggests that the increased resources required for survival in high-altitude cannot decline the energetic investment allocated to testis mass in *D. melanostictus*. Similar result has been reported in *Dianrana pleuraden* (Mai *et al*., 2017b) and *Feirana quadranus* (Tang *et al*., 2018). In addition, somatic condition displays an increase with altitude in this study suggesting that males with good somatic condition in high-altitude population have more resources available to invest in testes.

In general, we observed significant variation in

somatic condition and testis mass in *D. melanostictus* between different populations. In addition, we found that somatic condition and testis mass displayed an increase with altitude and testis mass was positively correlated with somatic condition, suggesting that males with good somatic condition have more energy available to invest in testes.

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Ethical approval

All experiments involving the sacrifice of live animals were approved by the Animal Ethics Committee at China West Normal University.

Statement of conflict of interest

The authors declare that there is no conflict of interests.

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