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Evidence for directional testes asymmetry in *Hyla gongshanensis jindongensis*

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Abstract. The compensation hypothesis predicts that one testis may grow more for compensating for a reduced function in the other testis, thus exhibiting a directional asymmetry in testis size. In this study, we tested the prediction of the compensation hypothesis in the Chinese endemic Tree Frog *Hyla gongshanensis jingdongensis* in a population in Kegong Reserve site of Yunan Province in western China. For fifty-three male samplings, we found that the left testis size was significantly bigger than the right testis, which exhibited a significantly directional testis asymmetry, consistent with the evidence that mainly the left testis is functional with the right testis having a compensatory role, i.e. the left testis would increase in size if the right testis became non-functional. However, the relative testes size and the degree of testes asymmetry were not correlated with body condition in this species, suggesting that the testes asymmetry can not reflect male quality: high-quality individuals would not have more asymmetric testes.

Keywords. Jingdong tree frog, Hylidae, body condition, compensatory function, directional testes asymmetry.

The compensation hypothesis predicts that one testis may grow more for compensating for a reduced function in the other testis (Møller, 1994). As a result, testis size exhibits a directional asymmetry where the testis on one side, often the left, is bigger than the other side, the right one in bird because the right testis increases in size to compensate for any reduction in function of the left (Møller, 1994; Birkhead et al., 1997). Hence, the directional testes asymmetry (DTA) is assumed where the left testis size increases if the right testis serves a compensatory role in frogs due to developmental stress, such as growth season length (Hettyey et al., 2005). In recent years, the testis asymmetry has been confirmed in some anurans species (Hettyey et al., 2005; Zhou et al., 2011; Liu et al., 2011; Mi et al., 2012).

Testis size asymmetry is widely used as a measure of male body condition (Møller, 1994; Hettyey et al., 2005). As a result, the degree of directional asymmetry in testes size reflects male quality: high-quality individuals have more asymmetric testes. Because possibilities for energy acquisition were correlated with male quality, the energy acquisition affected the degree of directional testes asymmetry (Møller, 1994). For instance, male individual with good condition develops higher degree of directional testes asymmetry than that with poor condition in the common frog, *Rana temporaria* (Hettyey et al., 2005). By contrast, the degree of testes size asymmetry is not correlated with body condition in the Guenther's frog, *Hylarana guentheri* (Liu et al., 2011).

Our primary aim in this study was to test the compensation hypothesis in a frog. A prerequisite for testing the hypothesis is good evidence that a particular high-quality male is attractive to females (Jin et al., 2016a). We studied the Jingdong Tree Frog (*Hyla gongshanensis jingdongensis*), a species endemic to Sichuan and Yunnan province in China. The species is restricted to a nar-

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row range of altitudes, ranging from 1500 to 2470 m (Fei and Ye, 2001). Males have well-developed vocal sacs and attract their mates by their calls during the breeding period (Fei and Ye, 2001). Besides, little information on testes size asymmetry in *H. g. jingdongensis* is available. Here, we tested the prediction of the compensation hypothesis that the difference in size of the left and right testis arose in *H. g. jingdongensis*. Specially, we examined whether the directional testes asymmetry is occurred in all samplings, and whether there is a positive correlation between body condition and the degree of the directional testes asymmetry.

The population is located at Kegong Reserve site in Bama Snow Mountain Nature Reserve of Yuanan province, western China (27°33'N, 99°19'E; a.s.l. 2422 m). The Reserve is characterized by a subtropical climate with a strong seasonality due to the high altitude which has an annual average temperature of 14-18°C (Liao et al., 2015; Liao et al., 2016a). All individuals were caught by hand at night from March to May in 2011. We collected a total of 53 males and brought them to the laboratory. Until processing, males were kept individually in a rectangular tank (1 × 0.5 × 0 .4 m; L × W × H) with a water depth of 25 cm at room temperature.

Two day after collection, body size (snout–vent length, SVL) of each frog was measured to the nearest 0.1 mm using a caliper and body mass was weighted to the nearest 0.1 g using an electronic balance. We used the single-pithing to sacrifice all animals and then anatomized them (Liao et al., 2016b; Mai et al., 2017; Lüpold et al., 2017). Left and right testes were removed, and weighed to the nearest 0.1 mg using an electronic balance. Following the suggestion of Møller and Swaddle (1997), we calculated the degree of the directional testis size asymmetry as DTA = (left-testis mass – right-testis mass)/0.5(left-testis mass + right-testis mass). Relative testes size was calculated as the ratio of observed testes mass to that predicted by the allometric regression equation between testes mass and body size. Body condition was measured as the ratio of observed body mass to that predicted by linear regression by entering male body mass as a dependent variable and body size as an independent variable.

Body size, body mass and testes mass were $\log_{10}$-transformed to achieve normality. We compared the sizes of the left and right testes using a paired *t*-test. We performed line regressions to test the relationships between both body mass and body size and testes mass. We also analyzed the relationships between male body condition and both relative testis mass and the degree of the testis asymmetry using Pearson correlation analysis. All analyses were conducted using SPSS 21.0 (Statistical Product and Service Solutions Company, Chicago, USA).

Testis mass was positively correlated with body mass (Fig. 1A; $F_{1, 52}$ = 28.679, r^2 = 0.360, $P < 0.001$, log (testis mass (mg)) = 1.283 log (body mass (g)) +0.451). An increase in testes mass with body mass was larger than predicted by the power law ($\beta > 1$), providing an evidence for an allometric relationship. Testis mass was also positively correlated with SVL (Fig. 1B; $F_{1, 52}$ = 9.414, r^2 = 0.156, P = 0.003, log (testis mass (mg)) = 2.824 log (SVL (mm)) -3.470). The relative testes size was not correlated with body condition (Fig. 2; Pearson's correlation coefficient: r = -0.082, n = 53, P = 0.558).

The Jingdong Tree Frog exhibited a directional testes asymmetry: the left testis (4.5 mg ± SD 2.7) was significantly bigger than the right one (3.5 mg ± SD 1.9) in 71.69% of all individuals (Paired t-test: t = 3.67, df = 53, P = 0.001). Left testis mass was positively correlated with right testis mass (r = 0.723, n = 53, P < 0.001). We found a non-significant correlation between the degree of the directional testes asymmetry and body condition (Fig. 3; Pearson's correlation coefficient: r = -0.144, n = 53, P = 0.304).

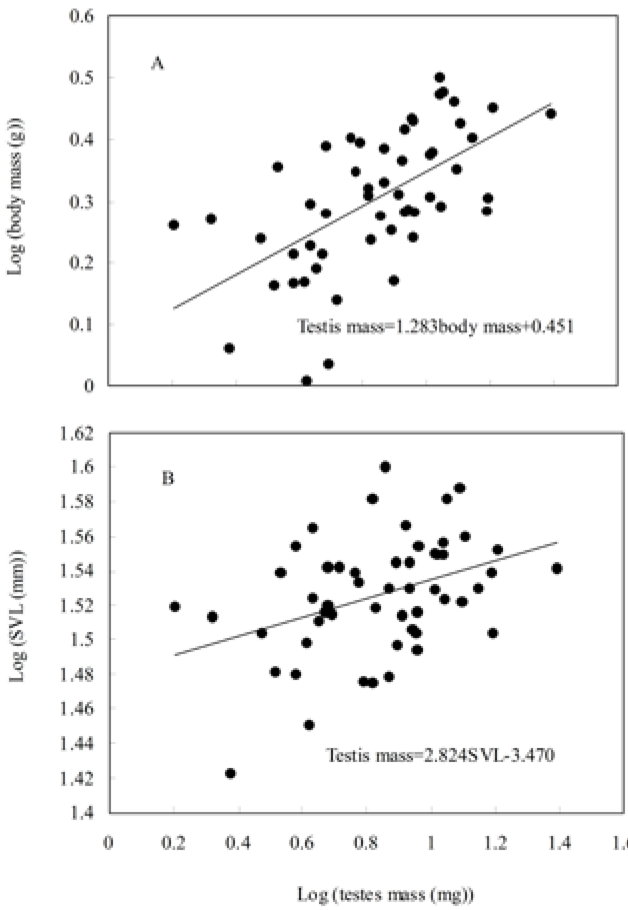

Fig. 1. Relationships between (A) body mass and testes mass, (B) SVL and testes mass. Displayed values are log-transformed data.

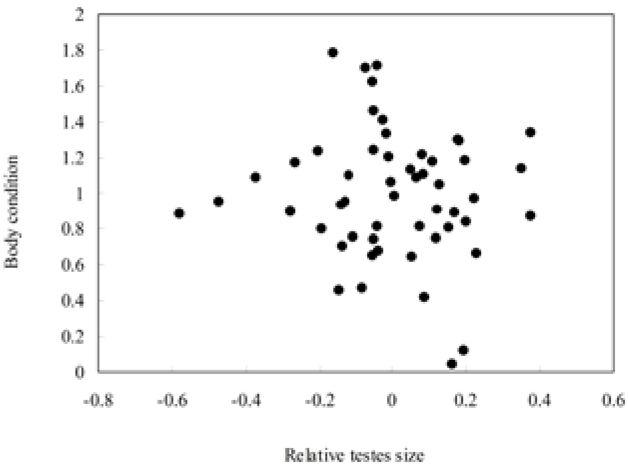

Fig. 2. Relationship between body condition and relative testes size in a *H. g. jingdongensis* population.

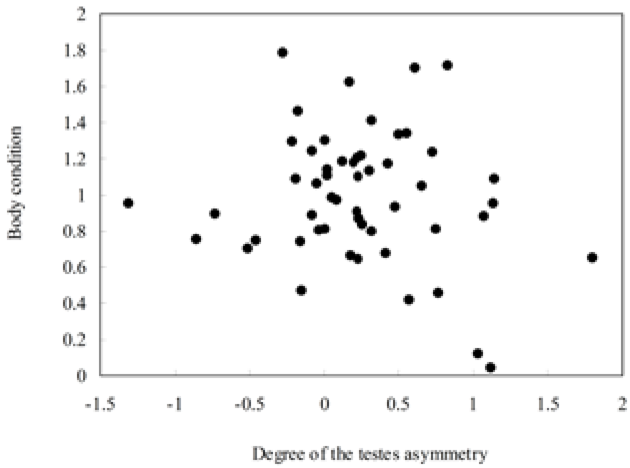

Fig. 3. Relationship between body condition and the degree of testes asymmetry in a *H. g. jingdongensis* population.

Our results uncovered positive correlations between testis mass and both body mass and SVL in *H. g. jingdongensis* in a population. Furthermore, the left testis was significantly bigger than the right one, which exhibited a directional testes size asymmetry. However, body condition was non-significantly correlated with both the relative testes size and the degree of the testes asymmetry.

Previous studies have shown that the directional testes asymmetry is common in animal taxa (anurans: Hettyey et al., 2005; Liao and Lu, 2010; Zhou et al., 2011; Liu et al., 2011; Liu et al., 2012; Jin et al., 2016b; Chen et al., 2016; birds: Wright and Wright, 1944; Møller, 1994; Birkhead et al., 1997; mammals: Yu, 1998). For *H. g. jingdongensis* left testis was bigger than right testis in most individuals, demonstrating a directional testes asymmetry. Meanwhile, most individuals had at least one testis smaller than the median for the population, which was considered as natural selection for compensation, consistent with the compensation hypothesis (Møller, 1994). Furthermore, a larger left testis and a smaller right testis might be advantageous because constraints during embryonic development results in the higher efficiency in sperm production of the left testis (Kempenaers et al., 2002; Liao and Lu, 2012). However, if a bigger sperm production on left testicle influences for the decrease on sperm production on right testicle, that otherwise would maintain the same rate of production, this condition does not represent an advantage on testes asymmetry.

There is a heritability evidence of male body condition that sexual selection supports females making use of the genetic correlation between body condition and sperm traits in frogs (Byrne et al., 2003). As a result, male individuals with good body condition have larger testis size than that with poor body condition to obtain mates. In the study, we found that the relative testis size was not correlated with male body condition, suggesting that males in good condition did not exhibit the higher ability of sperm competition. Hettyey and Roberts (2007) found that sperm-depletion after mating led to a non-significant correlation between body condition and testis mass. In our study, a non-significant correlation between body condition and relative testes size in *H. g. jingdongensis* might result from sperm depletion. Hence, we showed a consistent hypothesis for the lack of body condition and asymmetrical directional testicles.

Previous studies have shown that the degree of the testes asymmetry may not be a good measure of body condition in birds (Birkhead et al., 1997; Birkhead et al., 1998; Kimball et al., 1997; Kempenaers et al., 2002). By contrast, the house sparrow and the barn swallow exhibit a positive correlation between testes asymmetry and body condition (Møller, 1994). For anurans, some species exhibit a positive correlation between body condition and testes asymmetry while other species do not exhibit correlation between them (Hettyey *et al.,* 2005; Zhou et al., 2011; Liu et al., 2011). For instance, the degree of the testes asymmetry is significantly correlated with body mass in *R. temporaria* (Hettyey *et al.,* 2005). However, Zhou et al. (2011) found that the degree of the testes asymmetry is negatively correlated with body size in *Pelophylax nigromaculata*. In this study, the degree of the testes asymmetry did not co-vary with male body condition, suggesting that the degree of the directional testes asymmetry may not be a good indicator of male quality. Similar results have been observed in two species of anurans (*Hylarana guentheri*, Liu et al., 2011; *Rana omeimontis*, Liu et al., 2012). Although the degree of the testis asymmetry in *H. g. jingdongensis* did not reflect good body condition, males developed larger left testes and smaller right testes for supporting the compensation hypothesis.

In conclusion, consistent with the prediction of the compensation hypothesis, our analyses of difference in left and right testes in *H. g. jingdongensis* showed a directional testes asymmetry, suggesting that the left testis exhibited functional and the right testis had a compensatory role. However, body condition was not significantly correlated with the degree of the testes asymmetry, suggesting that directional asymmetry in testes size may not be a good measure of male quality.

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