Testis asymmetry in the dark-spotted frog *Rana nigromaculata*

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The left and right testes often differ in size, and testis asymmetry is particularly well studied in birds. The compensation hypothesis states that asymmetry in testes mass covaries with male quality. We tested this idea in the dark-spotted frog *Rana nigromaculata*, a species where large males have a mating advantage over small males. The left testes were significantly larger than the right testes. Larger and older males tended to have relatively larger testes, but did not show a higher degree of testis asymmetry than younger males. A negative correlation between male body size and testis asymmetry is in line with the hypothesis that the right testis has a compensatory role when the left testis is malfunctioning.

Key words: compensation hypothesis, directional testis asymmetry, male quality

INTRODUCTION

Testis asymmetry is common in a range of animal taxa (birds: Møller, 1989; Birkhead et al., 1997; anurans: Hettyey et al., 2005). It has been explained by the compensation hypothesis, which states that one testis may grow more in order to compensate for a reduced function in the other testis (Møller, 1989; Schärer & Vizoso, 2007). Often the left testis develops to be larger than the right testis (Lake, 1981; Jamieson et al., 2007), and it is therefore assumed that the right testis increases in size if the left testis is malfunctioning, e.g. due to developmental stress (Lake, 1981; Birkhead et al., 1997; Graves, 2004. The degree of testis asymmetry does indeed vary within a species. Males with poor body condition can have more symmetric testes because they are more sensitive to developmental stress (Møller, 1994; Hettyey et al., 2005), and the extent of asymmetry is higher in older individuals (Birkhead et al., 1997; Graves, 2004).

The dark-spotted frog *Rana nigromaculata* is a species living in meadows, forests, bushlands, deserts and rice fields (Zhao & Zhao, 1994). The frog is regarded as an explosive breeder with a short breeding season from April to mid-May (Khonsue et al., 2001). Body size is positively correlated with age in males (Liao et al., 2010), and larger males have better chances of obtaining mating partners (personal observation). Here, we analyse testis asymmetry in relation to body size and age. Our aims were to test whether individual frogs possessed significant (directional) differences in left and right testes size, to determine whether correlations exist between directional testis asymmetry (DTA) and body size, and to test whether age is correlated with DTA.

MATERIALS AND METHODS

From 11 to 19 April 2010, male *R. nigromaculata* were captured at a pond in Yingxi Town, Nanchong city (30°50'N, 106°07'E, 338 m a.s.l.) in northeastern Sichuan, western China. All individuals were caught by hand at night. A total of 47 males were collected and brought 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.

One day after collection, we measured body size (snout–vent length, SVL) of each frog to the nearest 0.1 mm using a caliper, and body mass to the nearest 0.1 mg using an electronic balance. We killed animals by double-pithing and anatomized all individuals. We removed both testes and weighed them to the nearest 0.1 mg. Following the suggestion of Möller & Swaddle (1997), relative directional testis asymmetry was defined as DTA = (left-testis mass – right-testis mass)/0.5(left testis mass + right-testis mass).

For determining the age of individual frogs, skeletochronology was used (for details see Liao et al., 2010). Paraffin section and Harris's haematoxylin stain was employed to produce histological sections of the phalanges. The 13 µm sections at the mid-shaft diaphysis of the phalanx were mounted on glass slides, and ages were determined under a light microscope by counting the number of lines of arrested growth (LAGs). Endosteal resorption of each individual was confirmed by noting the resorption line (RL, the division line between the endosteal and periosteal zones; Hemelaar & Van Gelder, 1980; Liao & Lu, 2010a,b,c, 2011).

The sizes of left and right testes were compared using a paired t-test. We analysed the allometric relationship between body mass and testis mass using a linear regression. We tested the hypothesis that the increase in testis size with body size is larger than predicted by the power law using a reduced major axis regression. Relative testes mass was recalculated as the ratio of observed testes mass to that predicted by the allometric regression equation. We investigated variation in testis mass with a general linear model (GLM) by entering male size as a fixed factor

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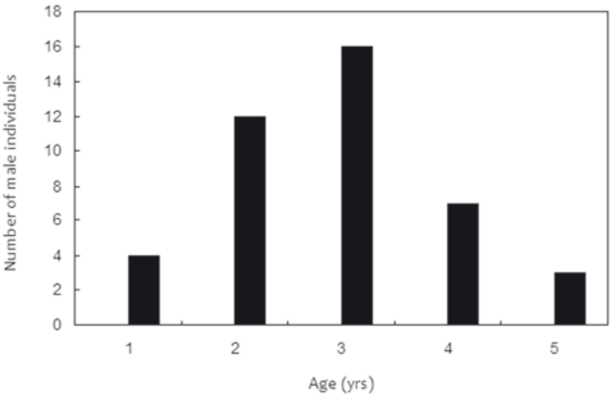

Fig. 1. Age structure in male *Rana nigromaculata* in Yingxi Town, Nanchong city, northeastern Sichuan, western China.

and soma mass (body mass – testes mass) as a covariate. To assess between-male variation in the degree of testis asymmetry, we used a GLM treating DTA as a dependent variable, together with male size as a fixed factor, and soma mass as a covariate. We performed Pearson correlations to test for relationships between male size and relative testis mass and the degree of testis asymmetry. The relationships between male age, relative testis mass and DTA were tested using Pearson correlations, which were also used to relate soma mass to relative testis mass and DTA. All analyses were conducted using SPSS version 15.0.

RESULTS

Testis mass of the dark-spotted frog demonstrated directional asymmetry: the left testes were significantly larger than the right testes (paired *t*-test: t=6.24, df=47, P<0.001). Of 47 males, 42 (89.36%) had at least one testis smaller than the median for the population, and therefore can be considered to be under selection for compensation.

The average age of male *R. nigromaculata* was 2.83±1.06 years, ranging from one to five years (Fig. 1); 66.67% of males were either two or three years old. Age was significantly correlated with body size (Pearson's correlation coefficient: r=0.412, n=42, P=0.007).

Testis mass was positively related to body mass (Fig. 2: $F_{1, 46}$=44.271, r^2=0.496, P<0.001, testis mass (mg) = 0.001 body mass (mg) +1.593). Body size was also positively correlated with relative testis mass (r=0.867, n=47, P<0.001); the increase in testes size with body size was larger than predicted by the power law (β>1), providing evidence for an allometric relationship**.** Accordingly, there was a positive correlation between age and relative testis mass (r=0.337, n=42, P=0.029). Average testis mass was significantly different among individuals ($F_{1.45}$=2.866, P=0.011) when the effect of male size was controlled for ($F_{1.46}$=1.171, P=0.370). Relative testis mass showed a positive relationship with soma mass (Fig. 3; r=0.928, n=47, P<0.001).

The degree of testis asymmetry was negatively correlated with body size (r= –0.296, n=47, P=0.007), although there was no correlation between male age and testis asymmetry (Fig. 3: r_s= –0.232, n=42, P=0.140). Variation in the degree of testis asymmetry among individuals was not significant (F=1.032, P=0.483). Male body size did not significantly affect the variation in testis asymmetry (Fig. 3; $F_{1.45}$=0.982, P=0.528). The degree of testis asymmetry was not correlated with soma mass (Pearson's correlation coefficient: r= –0.225, n=47, P=0.128).

DISCUSSION

Our study revealed a directional testis asymmetry in *R. nigromaculata*, as has been previously observed in *R. temporaria* (Hettyey et al., 2005). Consistent with the compensation hypothesis (Lake, 1981; Rising, 1987; Møller, 1994; Jamieson et al., 2007), we found that the mass of the left testis was significantly larger than that of the right testis. However, this asymmetry is different from previous studies describing on average larger right testes (Friedmann, 1927; Merilä & Sheldon, 1999; Hettyey et al., 2005).

Male relative testis mass is positively related to male condition (Simmons & Kotiaho, 2002). Evidence of heritability of body condition has suggested that females may use the genetic association between sperm traits and body condition for the purposes of sexual selection (Merilä et al., 2001; Simmons & Kotiaho, 2002; Schulte-Hostedde & Millar, 2004), although it is difficult to identify the traits of interest and their associated heritability (Wilson & Nussey, 2010; Lailvaux & Kasumovic, 2011; McGuigan et al., 2011). In our study, relative testis mass was associated with male body size, soma mass and age, suggesting that experienced males in good body condition may have the highest chances of reproductive success.

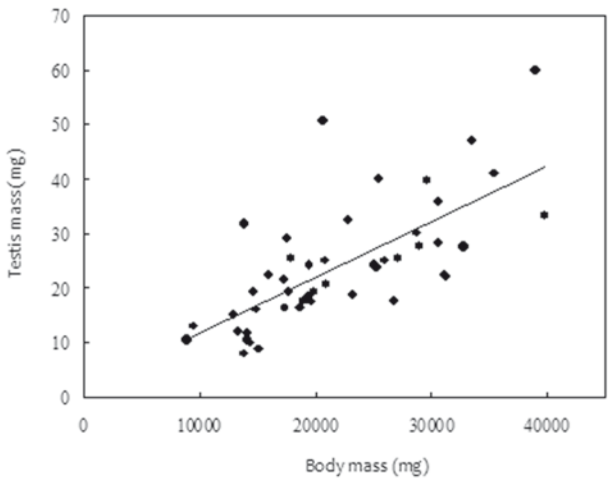

Fig. 2. Allometric relationship between body mass and testes mass in male *Rana nigromaculata*. The regression equation is as follows: testes mass (mg) = 0.001 body mass (mg) +1.618 ($F_{1,46}$=44.089, r^2=0.485, P<0.001).

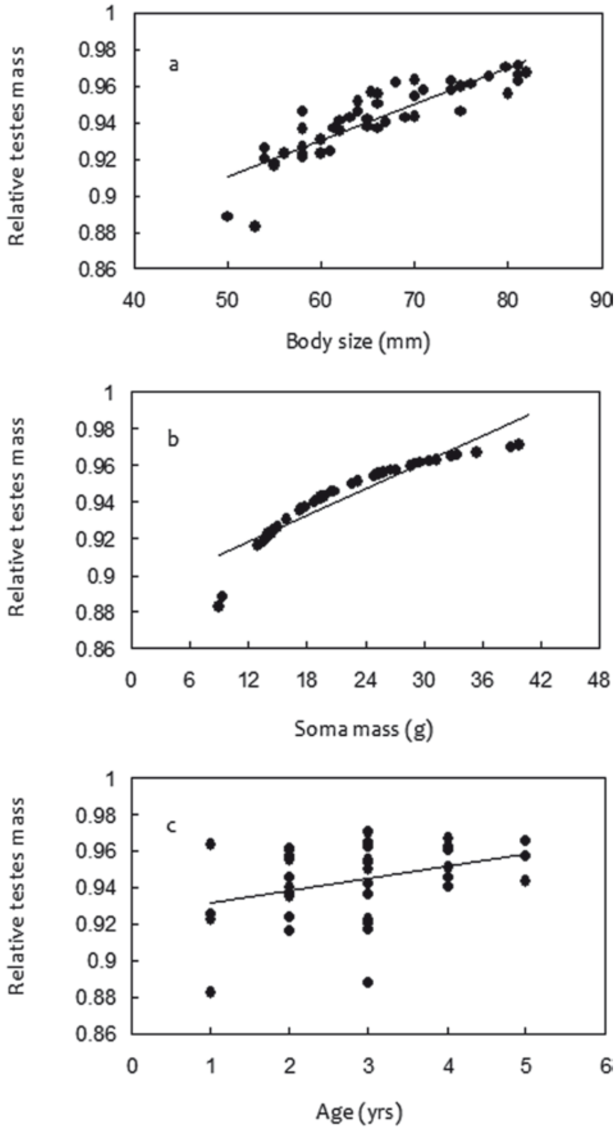

Fig. 3. Relationships between testis mass and a) body size, b) soma mass and c) age. Values displayed are untransformed data for ease of interpretation.

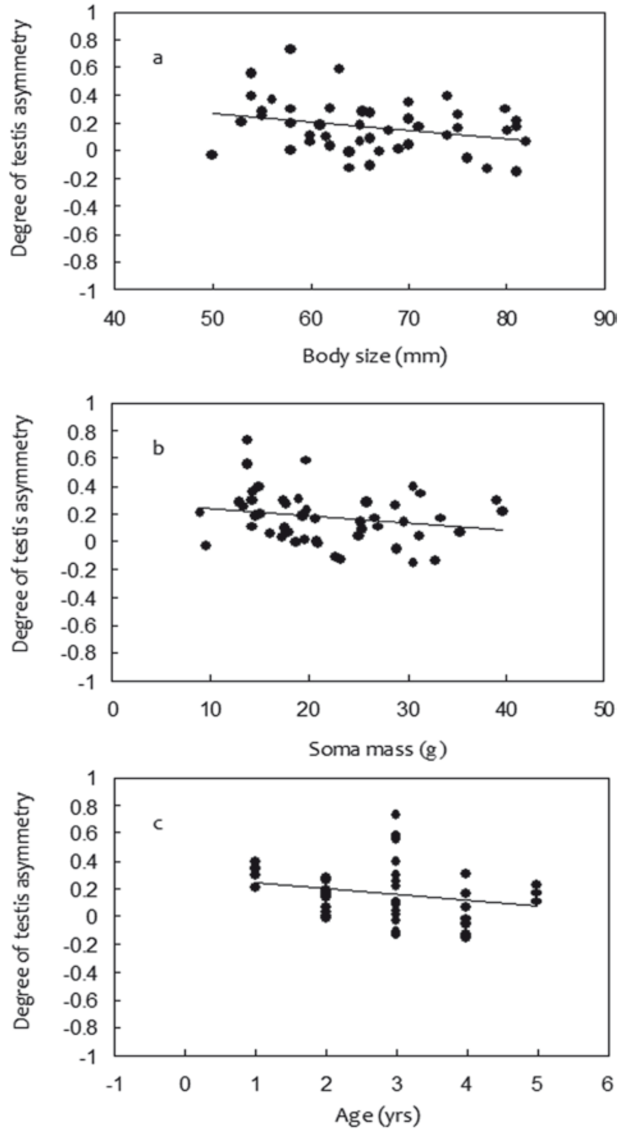

Fig. 4. Correlations between directional testis asymmetry and a) body size, b) soma mass and c) age. Values displayed are untransformed data for ease of interpretation.

The association between the degree of testis asymmetry and body condition may be understood as adaptive, by causing physiological or morphological handicaps that individuals in good condition can cope with (Møller, 1994). The degree of testis asymmetry was negatively correlated with body size and not correlated with soma mass; individuals with good body condition did not tend to have a higher degree of testis asymmetry. This result is contrary to the finding that testis asymmetry in the common frog (*R. temporaria*) is positively correlated with body mass (Hettyey et al., 2005) and that testis mass asymmetry represents the optimal phenotype and thus should be the norm (Møller, 1994). Numerous other studies have also shown that asymmetry in testis size may not be a good measure of male body condition (Birkhead et al., 1997, 1998; Kimball et al., 1997; Merilä & Sheldon, 1999; Calhim & Birkhead, 2009). Similarly, *R. nigromaculata* males develop larger left testes, but this is not indicative of good body condition as reflected in body size and soma mass.

Previous studies have shown that older males exhibited a higher degree of testis asymmetry than younger males (Sheldon, 1994; Birkhead et al., 1997; Graves, 2004), indicating that males with a higher DTA might have higher survival rates (Catchpole et al., 1984). However, we did not find such an effect. Future work will look at mechanisms behind variation in testis asymmetry in relation to age in other anurans.

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