Testis asymmetry in the dark-spotted frog

Rana nigromaculata

Cai Quan Zhou*, Min Mao*, Wen Bo Liao, Zhi Ping Mi & Yan Hong Liu

Key Laboratory of Southwest China Wildlife Resources Conservation, China West Normal University, Nanchong, China
Institute of Rare Animals and Plants, China West Normal University, Nanchong, China

INTRODUCTION

Testis asymmetry is common in a range of animal taxa (birds: Möller, 1989; Birkhead et al., 1997; anurans: Hettuy 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 (Möller, 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 (Möller, 1994; Hettuy 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 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).

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.
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) +1.618 ($F_{1, 46}=44.089$, $r^2=0.485$, $P<0.001$). 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 ($F_{1, 46}=2.866$, $P=0.011$) 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; $r=0.928$, $n=47$, $P<0.001$).

![Fig. 1. Age structure in male *Rana nigromaculata* in Yingxi Town, Nanchong city, northeastern Sichuan, western China.](image)

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: $r=–0.232$, $n=42$, $P=0.140$). 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 ($F_{1, 46}=44.089$, $r^2=0.485$, $P=0.001$).](image)
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.

ACKNOWLEDGEMENTS

We thank Long Jin, Lin Cai Yao and Ya Mei Li for assistance with lab work. We appreciate the improvements in English usage made by Robert Jehle. Financial support was provided by the Foundation of Key Laboratory of Southwest China Wildlife Resources Conservation (Ministry of Education), China West Normal University, P. R. China (XNYB01-3), New Century Training Programme Foundation for the Talents by the State Education Commission (NCET08-0906), the Key Scientific Research Foundation of China West Normal University (10A004), the Scientific Research Foundation of Sichuan Provincial Education Department (092ZC010) and the Scientific Research Foundation of China West Normal University (09B001). We declare that all animals used in the study were treated humanely and ethically following all applicable institutional animal care guidelines in China.

REFERENCES


Accepted: 10 May 2011