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## Female-biased sexual size dimorphism is driven by phenotypic selection on females in the Omei treefrog

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Sexual size dimorphism (SSD) is a widespread phenomenon commonly attributed to sex-specific patterns of selection on body size. Using data from Dengchigou population of the Omei treefrog (*Rhacophorus omeimontis*) in Fengtongzhai National Nature Reserve, southwestern China, we evaluated the hypothesis that direct phenotypic selection on both males and females can produce SSD. Female biased-SSD was generally driven by selection on females due to fecundity advantage of large body size rather than sexual selection favouring large male body size. Furthermore, differences in life-history traits (i.e. growth rate and/or time available for growth) between the sexes also resulted in female biased-SSD. Our findings suggest that the relationship between female size and fecundity supports the prediction that selection for fecundity drives female biased-SSD.

*Key words:* Omei treefrog, sexual size dimorphism, standardised selection gradients

Sexual size dimorphism (SSD) is a widespread phenomenon among animal species (Fairbairn, 1997). For example, in insects and anurans females are commonly larger than males and insects (e.g., Fairbairn, 1997; Liao et al., 2013), while the opposite is the case in many mammals and lizards (e.g., Cox et al., 2003; Lindenfors et al., 2007). The most prevalent explanation is that sexual selection drives SSD through inter-sexual mate choice or intra-sexual competition favouring large or small body size in one sex (Andersson, 1994). Alternatively, fecundity selection results in large females for increased reproductive output, thus driving SSD (Herczeg et al., 2010). In addition to the two evolutionary hypotheses, SSD can arise as consequence of sexual differences in age-specific mortality and growth rate, or differential age at maturity (Monnet & Cherry, 2002; Zhang & Lu, 2013).

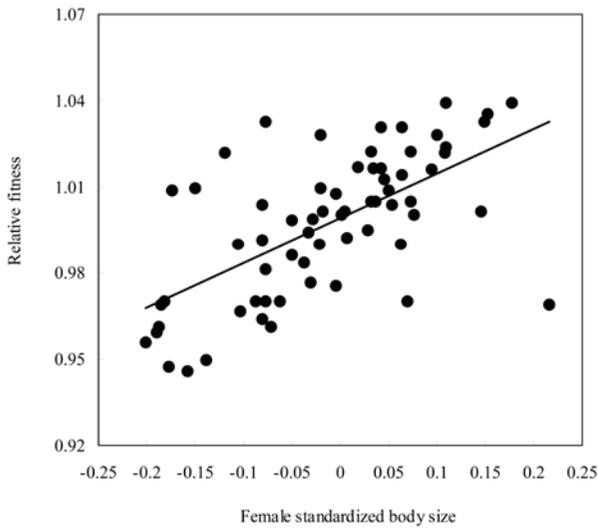
In amphibians, sexual size dimorphism is widespread (e.g., Wells, 2007), and females are larger than males in 90% of anuran species (Shine, 1979). For most species, a larger female would have a larger clutch size, thus a stronger fecundity. Fecundity selection predicts that

larger clutch size/fecundity favouring larger female size is thought to result in female-biased SSD in amphibians (Monnet & Cherry, 2002; Han & Fu, 2013). However, a phenotypic selection on male and female size by comparing the standardised selection gradients in amphibians is, as yet, rare (Liao et al., 2015). In this study, we tested the sexual and fecundity selection hypotheses that the phenotypic selection on size in both males and females by comparing the strength of selection acting on the sexes on male mating success and female fecundity in a treefrog (*Rhacophorus omeimontis*). We also determined the age of all individuals to control for age-related differences in SSD.

The Omei tree frog (*Rhacophorus omeimontis*) is a medium-sized anuran (body size: 65–78 mm) with female-biased SSD, widely distributed in subtropical forests in western China at elevations ranging from 760 to 2000 m (Fei & Ye, 2001). Breeding activity begins in mid-April, and lasts until late July. It is a lekking species in which males gather at pools in search for females which only visit pools for mating. Amplectant pairs release foam in leaves above water, with other males joining to form amplecting groups of 2 to 7 males. No physical competition between males has previously been observed (Liao & Lu, 2010). For this study, we classified males from amplecting groups as mated and all other males as unmated; mated males do not have a larger body size than unmated males (Liao & Lu, 2011a).

Field observations were conducted in two ponds (500 m apart) at Dengchigou (1700 m altitude, Fengtongzhai National Nature Reserve) during the breeding seasons of 2007 and 2008. After egg laying we captured all males and females in the breeding ponds, and recorded the clutch size at each nest. We collected a total of 270 frogs (203 males and 67 females). All individuals were confirmed to be adults by direct observation of secondary sexual traits and breeding behaviour. Body size (snout-vent length, SVL, in mm) of each individual was measured using calipers. The second phalange of the longest hind finger was surgically removed and stored in 10% neutral buffered formalin for age determination. All treefrogs were then released at their capture site. We used an improved method of paraffin sectioning and Harris's

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**Fig. 1.** The line relationship between relative fitness and female standardised body size in *Rhacophorus omeimontis*

haematoxylin staining to produce histological sections for aging adult females and males (Li et al., 2013; Huang et al., 2013). Cross-sections (13  $\mu\text{m}$  thick) of the phalanx with the smallest medullar cavity were selected to be mounted on glass slides. The number of lines of arrested growth (LAG), taken from mid-diaphyseal sections, were recorded using a Motic BA300 digital camera mounted on a Moticam2006 light microscope at  $\times 400$  magnification. LAG endosteal resorption and double lines was confirmed following Liao & Lu (2011b). Of the 270 adult specimens, 171 (143 males and 28 females) exhibited clear LAGs in their bone sections.

We further collected a total of 66 amplexant treefrogs in 2007 and 2008 and transported them to laboratories close to the breeding sites. Pairs were kept separately in tanks (40 $\times$ 50 $\times$ 60 cm) filled with pond water. Once oviposition was completed, we counted the total number of eggs in a clutch. After the experiments, all individuals and egg were returned to the places from which they were collected.

We estimated standardised selection gradients (Lande & Arnold, 1983) with data on male mating status and female fecundity to compare the strengths of selection on male and female size. Body size was standardised within each sex by subtracting the population mean from individual values, divided by the sample standard deviations (following Lande & Arnold, 1983). The standardised linear selection gradients were calculated from a regression of relative fitness on body size. The nonlinear selection gradients were calculated from a

multiple regression of relative fitness on standardised body size and its square (i.e. quadratic selection gradient, Fairbairn & Preziosi, 1994). Relative fitness was calculated by dividing the individuals' fitness (clutch size [females] or mating status [males]) with the mean fitness (i.e. mean clutch size or mating status) in the given population. Univariate linear selection gradients ( $\beta_1$ ) were estimated based on the following equation: relative fitness ( $w'$ )= $c+\beta_1$  (standardised body size), and univariate nonlinear selection gradients ( $\gamma_1$ ) were estimated based on relative fitness ( $w'$ )= $c+\beta_1$  (standardised body size) +  $\beta_2$  (standardised body size)<sup>2</sup>, where  $\gamma_1=2\beta_2$ .

All analyses were performed using SPSS v.17.0. Body size, age and clutch size were  $\log_{10}$ -transformed. We used a one-way ANOVA to test for differences in body size and age between males and females. We also ran a GLM treating body size as dependent variable, sex as fixed factor and age as covariate to test whether difference in body size between the sexes remained significant after correcting for age.

Average body size and age differed significantly between the sexes (body size,  $F_{1,269}=1078.107$ ,  $p<0.001$ ; age,  $F_{1,170}=58.718$ ,  $p<0.001$ ), also when removing the effect of age ( $F_{1,171}=275.981$ ,  $p<0.001$ ), and a significant effect of age on SSD was observed in the population ( $F_{1,171}=25.158$ ,  $p<0.001$ ). Furthermore, the significant sex\*age interaction on body size (growth rate) revealed differences in growth rates between the sexes ( $F_{1,171}=3.274$ ,  $p=0.040$ ).

Selection gradients between males and females from both the linear and nonlinear regression were different (Table 1). The standardised selection gradients revealed that selection did not favour large males in the population. The linear gradients were positive and non-significant, and the non-linear gradients were negative and non-significant. The standardised estimates of strengths of selection on females revealed positive selection on large females (Fig. 1).

The Omei tree frog exhibits striking sex differences in body size, age and growth rate in the population studied. Females have longer average age and longevity than males, contributing to a female-biased SSD. Age and growth (an indicator of the sex\*age interaction on body size) affect body size within each sex. As a result, differences in age and growth rate between the sexes can explain SSD. Selection gradients on males show that sexual selection on larger males cannot explain the female-biased SSD, whereas fecundity selection acting on large female size can explain the female-biased SSD based on a positive relationship between fitness and female body size.

**Table 1.** Standardised linear selection gradients and their standard errors (SE) for selection on body size in both sexes. Note:  $\beta_1$ : linear standardised selection gradients;  $\gamma_1$ : univariate nonlinear selection gradients. \* $p<0.001$ .

Sexes	Linear gradients		Nonlinear gradients	
	$\beta_1$	SE	$\gamma_1$	SE
Males	0.035	0.020	0.034	0.012
Females	0.157*	0.024	0.674*	0.264

Life-history traits for indeterminate growing ectothermic organisms, such as anurans, usually operate through ontogeny in sex-specific ways to affect the size of individuals (Morrison & Hero, 2003). Differences in growth rate and/or time available for growth can result in SSD (Shine, 1979). When females experience a longer duration of growth than males, SSD is increasingly biased towards females (Zhang & Lu, 2013). For *R. omeimontis*, females attain maturity later and grow more slowly toward the asymptotic body size than males (Liao & Lu, 2011b). Consequently, later maturation and slower growth in females result in more time and energy devoted to somatic growth to achieve larger body size, resulting in a female-biased SSD. We found that SSD was significantly correlated with age and growth, suggesting that phenotypic traits promoted difference in body size between the sexes.

Female-biased SSD is common for anuran species (Shine, 1979). Selection gradients on both males and females can produce SSD. Usually, male-biased SSD is driven by selection on large males as a consequence of sexual selection (large male-advantage in male-male competitions, Fairbairn & Preziosi, 1994). For *R. omeimontis*, selection gradients on males were non-significant, and sexual selection did not favour large male body size (as previously confirmed in Liao & Lu, 2011a). Conversely, sexual selection may also favour small male size in taxa with female-biased SSD due to increasing mobility or agility in small individuals (Székely et al., 2004). However, male *R. omeimontis* is not characterised by marked mobility or agility (Liao & Lu, 2010).

It is worthwhile to note that the alternative hypotheses - fecundity advantage - can also explain female-biased SSD due to the increased reproductive output of large females (Herczeg et al., 2010). In this study, the standardised estimates of strengths of selection suggested that fecundity selection acting on larger female size resulted in female-biased SSD in *R. omeimontis*. This pattern coincides with previous evidence that fecundity is positive correlated with female body size (Liao & Lu, 2011c). Taken together, female-biased SSD in *R. omeimontis* is driven by fecundity advantage, in addition to sex-specific differences in the life-history traits.

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