Male mating success in the Omei treefrog (*Rhacophorus omeimontis*): the influence of body size and age

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ABSTRACT. In anuran mate choice, advantaged males are generally known to be larger or older individuals. To test whether male mating success in the foam-nesting treefrog *Rhacophorus omeimontis*, a species distributed in western China, correlated with body size and age, we analysed differences in body size and age among three types of males. Males were classified as mated, joining or unmated at the time of sampling with joining males being additional males joining pairs in amplexus. Our results showed that there were no significant differences in body size among the three types of males. However, age was an important factor, with mated males being significantly older than joining and unmated males, which indicated that older individuals tended to have greater mating success than younger frogs.

KEY WORDS: Sexual selection, age, body size, *Rhacophorus omeimontis*

INTRODUCTION

Sexual selection has interested biologists for more than a century because it works differently to natural selection, leading to the evolution of sexual traits that are usually costly in fitness (Darwin, 1871; Zahavi & Zahavi, 1997; Andersson & Simmons, 2006). Intra-sexual competition and mate choice have stimulated the majority of recent investigations (West-Eberhard, 1979; Hunt et al., 2009; Rafael et al., 2010, Liao & Lu, 2011c). A majority of studies on sexual selection have shown that it usually results from a female choice for a particular sexual display, rarely from male mate choice. Under natural conditions, this sexual display (presence or value) is not only correlated to mating success but also to reproductive success (reviewed by Andersson, 1994; Hosken & Stockley, 2004; Plath et al., 2006; Hunt et al., 2009).

Studies of sexual selection on amphibians have shown that males with relatively high values of traits such as body size, forearm length, thumb-pad width, and advertisement calls will be preferred by females. Individuals of the sex undergoing selection usually intimidate their competitors and thus obtain mating advantages (Andersson, 1994; Poschadel et al., 2007; Bell, 2010). Body size represents the ability of an individual to have many resources and is therefore related to competition ability. Individuals with large body size use it in sexual display (Halliday, 1983; Andersson, 1994; Liao & Lu, 2009a; b). In some anurans male mating success is also correlated with age, which results from the indirect consequence that age is positively correlated to body size (Trivers, 1972; Höglund & Säterberg, 1989; Eggert & Guyétant, 2003; Okko & Jennions, 2008; Harrison et al., 2009; Bell, 2010; Somashekar & Krishna, 2011). In some species (Andersson, 1994; Byrne & Whiting, 2008), larger and older individuals, which have survived well and have better sexual displays, have more successful egg fertilization than do smaller and younger ones.

The Omei treefrog, *Rhacophorus omeimontis,* an arboreal breeder, is endemic to mountain ranges in the subtropical forests of Sichuan province in western China, where it occurs ranging from 750 to 2,000m a.s.l. (LIU & HU, 1961). During the prolonged breeding season, males gather in a pond and produce irregular advertisement calls to attract females. The males do not compete aggressively for call sites when they aggregate in groups in the pond (LIAO & LU, 2010d). Moreover, this species has a mating system where females will mate with more than one male (LIAO & LU, 2010d). Except for the knowledge presented above, there is no detailed information about the mating behaviour of this species. Here, we tested the hypothesis that body size and age in *R. omeimontis* affected male mating success in a subtropical high-elevation region in western China.

MATERIALS AND METHODS

Study area

The field study was conducted in Fengtongzhai National Nature Reserve in western China (102º48′-103º 00′E, 30º19′-30º 47′N, and total area about 39,039ha). The reserve ranges from 1,000-4,200m above sea level, has an annual average temperature of 5.9-7.2°C and an annual average precipitation of 700-1,300mm (over 60% of which falls during May–August, LIAO, 2009). During the breeding season, from 15 April to 20 May in 2008, we collected treefrogs in a permanent pond at Dengchigou Protection Station at an altitude of 1,700m. The pond was filled to a depth of 25cm with fresh water and was mainly covered by replanted broadleaf forest, which may provide hiding places for *R. omeimontis*.

Sampling

Females are quickly grasped by a male when they enter the breeding pond. The pairs then move to a neighbouring plant and release foam in leaves above the water surface of the pond. Immediately, other males, which we call joining males here, come to join the pairs. Treefrogs were caught by hand at night whilst they were in amplexus or searching for mates in the pond. Following the protocol of LEE & CRUMP (1981), we conservatively characterized unmated males as unsuccessful although these males may have been successful in securing a mate prior to or after capture. However, unmated males were collected after amplectant males and joining males had been located, thus any differences between mated, joining and unmated males are likely to reflect true differences at that moment. Each adult specimen was sexed by direct observation of the secondary sexual characteristics (the nuptial pads on the first finger for male, the eggs readily visible by the skin of the abdomen for female). We confirmed the three types of males by direct observation at the breeding sites. Body size (from snout to vent, SVL) was measured to the nearest 0.1mm using a calliper. All treefrogs were individually marked by toe clipping and then released at their capture site. Toe clipping can reduce frog survival and affect their behaviour (BYRNE & ROBERTS, 1999), but the technique did not affect or inhibit behaviour of individuals in *R. omeimontis* during two successive years (LIAO & LU, 2010d). The longest toe from the right limb of the hind leg was removed and stored in 10% neutral buffered formalin for skeletochronological analysis. Since there was no substantial regeneration of the toe pads, the individuals could be easily identified throughout the study period. We used the Lincoln-Petersen index to estimate population size (CAUGHLEY, 1977).

Age determination

Skeletochronology was applied to the phalanges of adults (141 males and 28 females). The selected digits were washed in water for 2h and then decalcified in 5% nitric acid for 24h. They were washed in running tap water overnight and then stained with Ehrlich's haematoxylin for 75min. Thin sections (13μm thickness) were selected at the mid-shaft diaphysis of the phalanx with the smallest medullar cavity and mounted on glass slides. The analysis of lines of arrested growth (LAG) was made under a light microscope by two observers, who had similar experience in the technique and who agreed on the identification criteria and the final age estimation. As recommended by ROZENBLUT & OGIELSKA (2005), we confirmed LAG endosteal resorption based on the occurrence of the Kastschenko Line (KL, the division line between endosteal and periosteal zones). In the samples, endosteal resorption was only found in four individuals, and we added one year to the age of these individuals. Of 169 adult specimens, 166 (138 males and 28 females) exhibited clear LAGs in their bone sections.

Statistical analyses

We also used parametric tests to perform homogeneous analyses for comparing differences in body size and age between the two sexes. We applied ANCOVA with age and sex as factors to test for differences in body size between the sexes. One-way ANOVA was used to test for differences in body size and age among mated, joining and unmated males. We ran an ANCOVA with age as covariate to test whether statistical significance of differences in body size of mated, joining and unmated males still remained after removing the effects of age. The total breeding sex ratio in the population was evaluated using a Chi-square test. All statistical tests were two-tailed and values given are shown as mean ± SD.

RESULTS

During the study period, a total of 169 adult individuals (141 males in amplexus, joining an amplexus or alone, and 28 females) were individually marked by toe-clipping. We recaptured 101 individuals during the subsequent 35-day period. The mark-recapture data produced an estimate of the population size of 284 individuals. Therefore, our sample containing 169 frogs (59.9% of 284) provided a representative part of the adult population. The total breeding sex ratio was significantly male-biased (χ^2=72.62, *df*=1, *P*<0.001). Females were significantly larger than males (Table 1; Students *t*-test: *t*=7.508, *df*=84, *P*<0.001). The average body size of females was 76.7±3.1mm, while males were 64.7±2.4mm. Female frogs were on average 4.3±1.2 yrs in age, and thus significantly older than males with 3.6±1.0 yrs (Students *t*-test: *t*=22.054, *df*=81, *P*<0.001). ANCOVAs

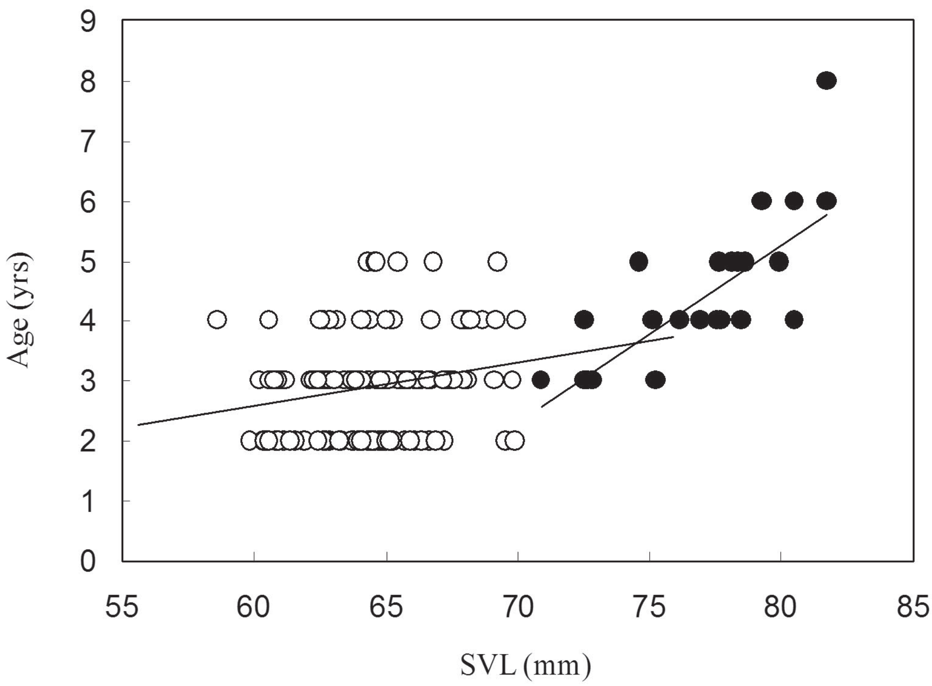

Fig. 1. – The interaction between sex and age of *Rhacophorus omeimontis* (female, black circles; male, white circles) in western China. SVL=Snout-vent length.

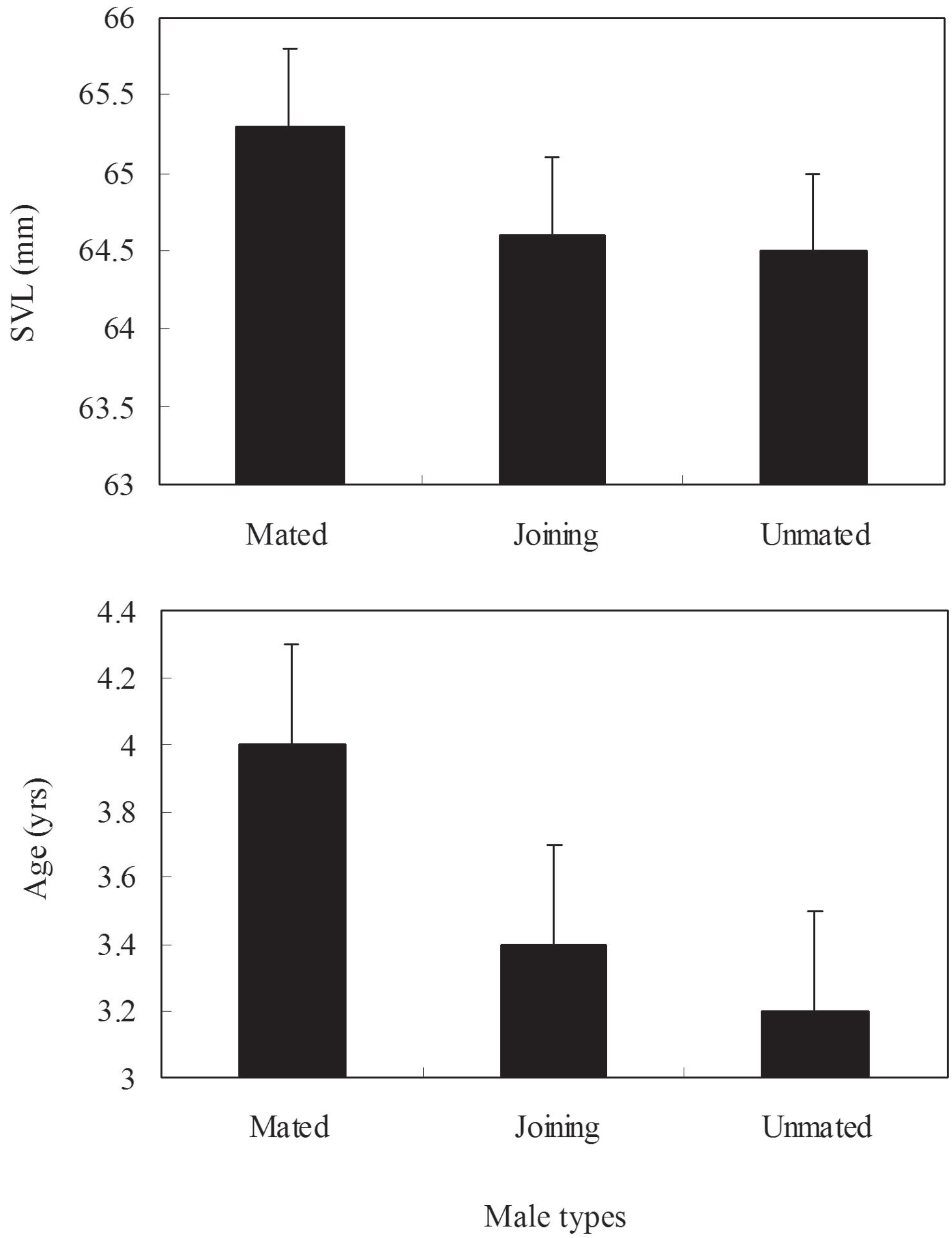

Fig. 2. – Differences in body size and age of *Rhacophorus omeimontis* among mated males, joining males and unmated males in western China. Vertical bars are standard deviations. SVL=Snout-vent length.

with age and sex as factors showed that their interaction was significant (Fig.1; $F_{2, 165}$=5.041, P=0.008), and age and sex resulted in significant difference in body size between the sexes (age, $F_{5, 157}$=7.182, P<0.001; sex, $F_{1, 165}$=304.056, P<0.001). The sexual difference in body size remained significant when the effect of age was controlled ($F_{1, 165}$=335.858, P<0.001).

Body sizes did not differ significantly among mated, joining and unmated males (one-way ANOVA: $F_{2, 138}$=1.12, P=0.33; Fig.2). On the other hand, average age did differ significantly among the three categories of males (one-way ANOVA: $F_{2, 136}$=3.86, P=0.01; Table 1), with mated males tending to be older than joining (P<0.03 for Tukey HSD post hoc test) or unmated males (P<0.04 for test) while there was no significant difference between the average age of joining and unmated males (P=0.90 for Tukey HSD post hoc test). ANCOVAs with age and male types as factors showed that their interaction was not significant ($F_{4, 108}$=0.972, *P*=0.322). There were no differences in SVL between mated, joining and unmated males even when controlling for the effect of age (Fig.3; ANCOVA: $F_{2, 136}$=0.55, *P*=0.47).

DISCUSSION

We found a positive correlation between age and body size in both sexes of *Rhacophorus omeimontis*, similar to what has been described from several other anurans (*R. perezi*, ESTEBAN et al., 1996; *Mantidactylus microtympanum*, GUARINO et al., 1998; *R. nigrovittata*, KHONSUE et al., 2000; *R. ridibunda*, KYRIAKOPOULOU-SKLAVOUNOUA et al., 2008; *Amolops mantzorum*, LIAO & LU, 2010a; b; *Hyla annectans chuanxiensis*, LIAO & LU, 2010c; *Rana nigromaculata*, LIAO et al., 2010; *R. omeimontis*, LIAO & LU, 2011a; *Bufo andrewsi*, LIAO & LU, 2011b). However, in our study, it was not possible to predict age from body size because there was considerable overlap of body size among the different age classes (Fig.1).

We found no differences in body size of *R. omeimontis* among mated, joining and

TABLE 1

Body size and age by *Rhacophorus omeimontis* among mated, joining and unmated males in western China.

		MATED MALES	JOINING MALES	UNMATED MALES
Samplings	n	28	29	81
Snout-vent-length	mm	65.3±2.2	64.6±2.2	64.5±2.6
Age	yrs	4.0±1.1	3.4±0.9	3.5±0.9

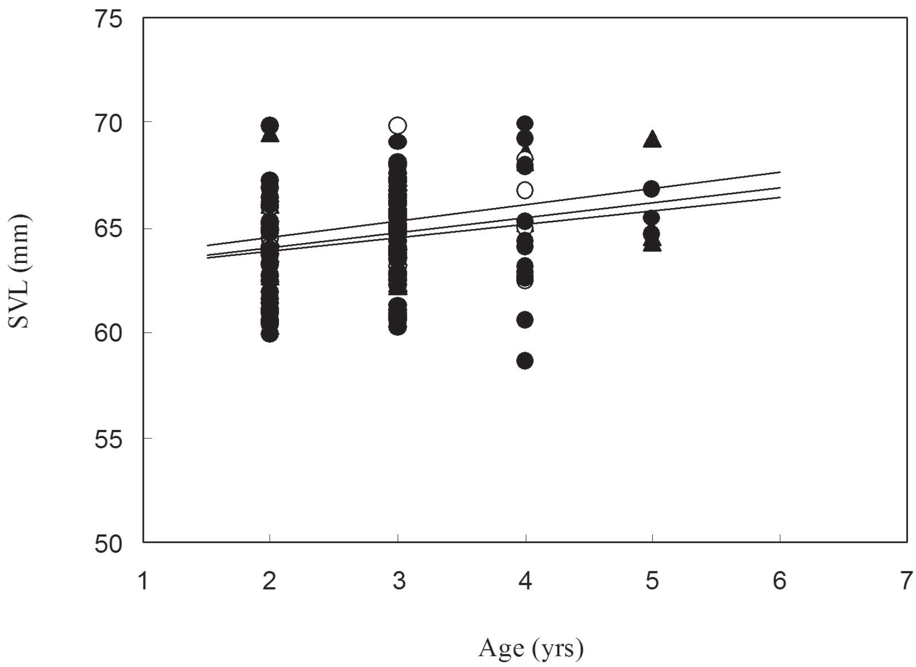

Fig. 3.– The interaction between sex and male type of *Rhacophorus omeimontis* (mated males, black triangles; joining males, white circles; unmated males, black circles) in western China. SVL=Snout-vent length.

unmated males even when controlling for the effect of age, suggesting that larger males did not have higher chances to obtain mates. This is in line with the prediction that sexual selection does not act on body size in the related foam-nest species because female choice depends on male calls (i.e. *R. schlegelii*, FUKUYAMA, 1991; *R. arboreus*, KUSANO et al., 1991; *Chiromantis xerampelina*, JENNIONS et al., 1992). Thus, body size may not be a good indicator of male competing advantages in *R. omeimontis*, suggesting that larger size does not reflect a male's ability to compete for a mate (LIAO, 2009). Similar results have been obtained for other frogs and toads (HALLIDAY & VERRELL, 1988). Moreover, we postulate that the absence of any relationship between body size and mating success could result from our experimental design. Our three defined male categories may not be good indicators for mating success. Future studies should include consideration of calling abilities as an indicator for mating success, as well as age or body size.

Although we did not find any obvious effect of body size on mating success, older males clearly had more success than younger males (see above). Male attributes that females may consider important include resources controlled and genetic quality (HOWARD, 1978). Males with strong competitive abilities are males with high genetic quality. Because these males have "good genes", they have higher mating success and their offspring benefit from these genes (TRIVERS, 1972). In our study on *R. omeimontis,* older males tended to have greater mating success than younger ones. This could be because older males possess genes that have enabled them to survive through a longer succession of different environments in the past (WILBUR et al., 1978; HOWARD, 1984; SOMASHEKAR & KRISHNA, 2011). According to this hypothesis, females that mate with older males would gain indirect genetic benefits in terms of increased offspring fitness.

FELTON et al. (2006) reported that age, independent of body size, was negatively correlated to call dominant frequency and female preference for low dominant frequency equates with choosing older males. In *R. omeimontis*, older males may have more attractive calling because they have larger SVL (WELCH et al., 1998). Previous studies have indicated that female anurans show clear preferences for older males' advertisement calls with a high rate or long duration, because females may gain indirect benefits in terms of increased offspring viability (GERHARDT, 1994; GERHARDT & HUBER, 2002; PROKOP et al., 2007). Indeed, WELCH et al. (1998) found for the grey treefrog, *Hyla versicolor*, that there may be indirect genetic benefits for females choosing males with long calls. Female *R. omeimontis* preferences seem to be (also) based on male advertisement calls (LIAO, 2009). However, data on the relationships between male call rates, female preferences and male age are still missing. Further investigation is warranted.

ACKNOWLEDGEMENTS

We thank Ben Qing Yang of the Fengtongzhai National Nature Reserve for assistance with the field work. We appreciate improvements to the English made by Emma Houston and Isa Schön. Financial support was provided by the National Natural Science Foundation of China(31101633) and the Scientific Research Foundation of China West Normal University (10A004). We declare that all animals used in the study were treated humanely and ethically following all applicable institutional animal care guidelines in China.

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Received: May 5th, 2009

Accepted: June 26th, 2011

Branch editors: Schön Isa/Blust Ronny