

Mating patterns of *Bufo raddei* from three high altitude populations in southwestern China

Tong Lei Yu¹ & Xin Lu²

¹Department of Biology, College of Life Science, Xinyang Normal University, SD 464000, China

²Department of Zoology, College of Life Science, Wuhan University, SD 430072, China

This study investigated the mating system of *Bufo raddei* during two breeding seasons at three different elevations in southwestern China. The degree of sexual size dimorphism (SSD) differed significantly among populations. However, we did not find that amplexant males were larger in SVL than non-amplexant males within each population. Similarly, forelimb length of males found amplexed with females was not significantly different to non-amplexant males among sites. Moreover, we did not find a significant correlation between the body size of males and females from amplexant pairs within any of the sites or in any of the years, showing a random mating pattern with respect to size. Thus, we suggested that the large male aggregations and short pre-spawning period may limit opportunities for large males to have advantages in mating contests and for either sex to choose a large mate at a given site. A further possible explanation is that female toads compromise on mate quality to ensure conspecific matings.

Key words: body size, *Bufo raddei*, mate choice, mating system

INTRODUCTION

Traditionally, behavioural ecologists have studied mating patterns from the individual's point of view and have asked how mate choice and competition affect the fitness of females and males (reviewed by Andersson, 1994). Competition among males for females and selection of a male by a female may lead to a size-dependent or size-assortative mating pattern (Robertson, 1986; Tsuji & Matsui, 2002). In addition, factors other than sexual selection, such as the temporal or spatial distribution of breeding individuals, environmental conditions, the presence of predators and sex ratios may influence mating decisions (Olson, 1989; Höglund, 1989; Fawcett & Johnstone, 2003; Vaira, 2005; Lee & Park, 2009).

Although substantial evidence for non-random mating with respect to body size exists for anurans (Reading & Clarke, 1983; Howard, 1988; Woodward, 1982; Gatz, 1981; Briggs, 2008; Yu & Lu, 2010; Liao & Lu, 2011; Liao & Lu 2012), such patterns were not observed in all cases (Kruse, 1981; Okuno, 1986; Höglund & Robertson, 1987; Elmberg, 1987; Crump & Townsend, 1990; Friedl & Klump, 2005). Explanations for random mating include a short breeding season, high male densities and a lack of female choice (Höglund & Robertson, 1987; Okuno, 1986). Among anurans, different populations of the same species may also exhibit different mating tactics, because a wide range of traits (e.g., male vocalization, morphology, population density, sexual selection and climate) can vary spatially (Emlen & Oring, 1977, Wittenberger, 1979, Clutton-Brock, 1991, Olson et al., 1986, Davies, 1991).

Apart from a description of the morphology and vocalizations of *Bufo raddei* (Stöeck et al., 2001) there are few data concerning reproduction beyond anecdotal reports that document breeding behaviour and mating

patterns (Jia, 2002). To investigate reproduction further, we studied mating patterns across two breeding seasons in three distinct populations of *B. raddei*. We used field observations to address the following questions: (i) Do amplexant males constitute a random sample of all males? (ii) Is there a correlation between the size of males and females in amplexant pairs? (iii) What behavioural mechanisms are involved in shaping mating patterns?

MATERIALS AND METHODS

Study species

Bufo raddei is widely distributed in southwestern China and along the northern Yellow River (Fei & Ye, 2001). It is a typical "explosively" breeding species with a relatively short breeding season (6–14 days). In addition to *B. raddei*, *Rana kukunoris* and *Bufo minshanicus* regularly breed at our three study sites, and the breeding season of the three species usually overlaps. *Bufo raddei* activity is correlated with ambient temperature, with males calling between 0900 and 2300 hours (Smits, 1984; Zhang et al., 2007). Due to low temperatures, chorusing behaviour is absent at night, and the species is a strictly diurnal breeder. During explosive breeding events, males actively search for females on the water surface, clasping other anurans regardless of sex or species.

Study sites

In 2008 and 2009, the field study was carried out at three sites that differ in elevation: (1) Site A, Lanzhou (42°00'N, 121°45'E, elevation 2003m), where toads breed from mid-April to late-April; (2) Site B, Haiyan county (36°54'N, 100°59'E, elevation 2989m), where toads breed from late-April to early-May; and (3) Site C, Haibei county (36°57'N, 100°53'E, elevation 3100m),

where toads breed from late-April to early-May. Grasses and bulrushes dominate the vegetation at all three study sites. There are permanent pools near the Yellow River at site A and four small reservoirs at sites B and C, all of which serve as breeding sites.

Data collection

We captured calling unmated male toads and amplexant pairs by hand about once every two days throughout the breeding season during daylight hours. Wading in the ponds allowed us to find more toads than by searching for toads along the edges of ponds, thus allowing better determination of population structure and sex ratio. We conservatively characterized non-amplexant males as unsuccessful, although we acknowledge that these males may have been successful in securing a mate prior to or after capture. Because we collected non-amplexant males after we had already located amplexant pairs, any differences between amplexant and non-amplexant males are likely to reflect true differences in the sample population (Lee & Crump, 1981; Howard et al., 1994; Lee, 2001; Briggs, 2008). Body length (measured as snout-vent length, SVL) was measured to the nearest 1 mm using a plastic ruler with the toads placed belly-down on a flat surface. Forelimb length was measured from the axilla to the tip of the longest toe on the left foreleg. All captured toads were marked individually by a toe-clip for later identification (Lüddecke & Amézquita, 1999) and released at their original site of capture. Individuals were not measured more than once or counted as amplexant at one time and non-amplexant at another. Some individuals from site B were transported back to the laboratory for experiments on amplexus displacement. We also noted whether amplexant males had an abnormal grip, which we defined as a behaviour in which a male clasped a female from her centre or around her waist with his head opposite to hers. Finally, we noted whether males continually clasped objects or animals other than gravid chorusing females, and these males were not included in analyses as amplexant males.

Statistical analysis

All data met normal distribution according to One-Sample Kolmogorov-Smirnov Tests, so we used parametric tests. To test whether mating patterns and the magnitude of SSD differed across the three populations, we first compared SVL with ANCOVAs, comparing sexes, populations and years as factors, with date (number of days from the capture date, 1 April) as a covariate. Then, for males only, we used ANCOVAs (with date as the covariate) to examine the effects of mating status (amplexant or non-amplexant), year (2008 or 2009) and population (A, B or C) on SVL. Since we were interested in interactive effects of some variables, we first examined interactions between mating status \times year \times population, mating status \times year, mating status \times population, and year \times population. We conducted the same two ANCOVAs for forelimb length, but also included SVL as a covariate. To test for size-assortative mating, we calculated for each site and year separately, Pearson's correlations or Kendall's correlation between SVLs of males and females that were found as

pairs. Statistical analyses were performed with SPSS v.13 (SPSS institute, Inc. 2002–2003), and all *P*-values given are two-tailed, with values presented as means \pm standard error.

RESULTS

A total of 924 toads (638 males, 286 females) were marked and included in our study. The sex ratios of breeding adults were strongly male-biased at all three sites (male/female = 3.41 ± 0.25 , range: 2.33–3.97). When examining SVL, there was a significant sex \times population interaction (Table 1), suggesting that dimorphism in SVL differed among populations. There was also a significant year \times population interaction (Table 1), suggesting that yearly variation in SVL differed among populations. When examining only male morphology, we found a significant year \times population interaction, but no other interactions or main effects were significant (Table 2). This result indicates that amplexant males did not differ significantly from non-amplexant males in SVL. The results for forelimb length (with SVL as a covariate) were similar, with a significant year \times population interaction (Table 2). Larger females did not tend to be paired with larger males: there was not any significant correlation between the SVL of males and females found in amplexus within any of the sites or in any of the years (all *P* > 0.05, Table 3).

DISCUSSION

One trait commonly implicated in sexual selection is male body size. Being a large male may indicate survival ability or faster juvenile growth, both of which are thought to indicate high genetic quality (Wilbur et al., 1978; Cooper & Vitt, 1993; Miyazaki & Waas, 2003; Chandler & Zamudio, 2008). According to this assumption, females that mate with large males would

Table 1. The generalized linear model for toads' SVL (with dates as a covariate) among the three populations in different years. Significant interaction effects have *P*-values in *italics*.

Factor	SVL			
	df	MS	<i>F</i>	<i>P</i> -value
Dates	1	0.04	0.37	0.54
Sex	1	1.31	13.69	<0.001
Year	1	3.85	40.37	<0.001
Population	2	6.79	71.14	<0.001
Sex \times year	1	0.11	1.14	0.28
Sex \times population	2	1.69	17.70	<0.001
Year \times population	2	1.58	16.51	<0.001
Sex \times year \times population	2	0.05	0.56	0.57
Error	893	0.10		

Table 2. The generalized linear model for males' SVL (with dates as a covariate) and forelimb length (with male's and dates as covariate) among the three populations in different years. Significant interactions have *P*-values in *italics*.

Factor	<i>Males' SVL</i>				Factor	<i>Males' forelimb</i>			
	df	MS	<i>F</i>	<i>P</i> -value		df	MS	<i>F</i>	<i>P</i> -value
Dates	1	0.18	2.03	0.16	Dates	1	12.24	298.75	< 0.001
Mating status	1	0.07	0.82	0.37	Males' SVL	1	0.42	10.34	< 0.001
Year	1	1.60	18.43	< 0.001	Mating status	1	< 0.001	< 0.001	0.98
Population	1	2.50	28.67	< 0.001	Year	1	0.87	21.26	< 0.001
Mating status × year	2	0.09	1.06	0.30	Population	2	0.08	1.97	0.14
Mating status × population	1	0.19	2.20	0.11	Mating status × year	1	0.10	2.56	0.11
Year × population	2	0.63	7.27	<i>0.001</i>	Mating status × population	2	0.02	0.49	0.61
Mating status × year × population	2	0.01	0.12	0.73	Year × population	2	0.78	18.94	<i>0.001</i>
					Mating status × year × population	1	0.07	1.72	0.19
Error	617	0.09			Error	581	0.04		

gain indirect genetic benefits in terms of high offspring fitness. However, a large male mating advantage was not found in *B. raddei* with its explosive breeding season and female mate choice, and seems to be absent in many other anuran populations (Sullivan, 1983; Cherry, 1993; Jacobson, 1985; Passmore & Telford, 1983; Dyson et al., 1992). Moreover, the data did not show that female SVL is significantly correlated with male SVL among sites and years, respectively. Therefore, by investigating natural mate pairings in *B. raddei* across two breeding seasons at three sites, the results showed a random mating pattern with respect to body size.

Based on natural mating behaviour of *B. raddei*, four conditions might explain the presence of random mating patterns with respect to size: (1) although male densities were high, *B. raddei* has a short pre-spawning period, without sufficient time for male-displacement. Accordingly, we recorded no instances of male combat or takeovers in the field; (2) At higher densities where

there is a strong male-biased operational sex ratio, the intensity of male-male competition is increased (Byrne & Roberts, 2004) and female choice may be obscured (Shuster & Wade, 2003). Similarly, 19 mismatings were observed in the field, 52.6% involved one male clasping heterospecific animals, 26.3% involved females that had already released eggs and 21.1% involved dead conspecific females. However, the strong male-male competition may limit opportunities for large males to have advantages in mating contests and for either sex to choose a large mate at a given site (Lu et al., 2009); (3) High male densities or male advertisement calls are masked by considerable background noise, including calls of other anuran species, and background sounds may prevent females from accurately choosing a large male (Ehret & Gerhardt, 1980; Narins & Zelick, 1988; Schwartz & Gerhardt, 1989; Telford et al., 1989; Narins, 1992). Owing to these limitations on mate assessment, female anurans would have to move around the chorus to sample multiple males.

Table 3. Results of Pearson's or Kendall's product moment correlations of mean body size traits within males and females found as amplexant pairs of *Bufo raddei*.

<i>Study sites</i>	<i>Size trait</i>	<i>Paired females</i>	<i>Paired males</i>	<i>n</i>	<i>r/τ</i>	<i>p</i>
Site A	2008	5.84±0.19	5.79±0.09	8	-0.07	0.81
	2009	5.00±0.08	5.03±0.08	34	0.21	0.24
Site B	2008	6.25±0.11	5.78±0.09	15	0.25	0.20
	2009	5.99±0.04	5.77±0.03	60	0.07	0.58
Site C	2008	5.72±0.06	5.66±0.06	27	-0.09	0.52
	2009	5.62±0.03	5.67±0.02	114	0.11	0.27

However, extended mate-searching behaviour of females may increase their risk of predation (Gerhardt et al., 1996). Therefore, indiscriminate mating may be adaptive for females in a high male-male competition environment, as has been suggested by others (Berven, 1981; Elmberg, 1987; Crump & Townsend, 1990; Friedl & Klump, 2005). A fourth possible explanation is that females compromise on mate quality to ensure conspecific matings. Pfennig (2000) found that female spadefoot toads preferred an extreme call characteristic resembling the call of heterospecifics, and that more eggs were fertilized in the absence of other species. However, in populations that overlapped with other species, females preferred call characteristics close to the norm for their population without the benefit of enhanced fertilization success. In this study, *B. raddei* co-occurs with *R. kukunoris* and *B. minshanicus*, and females may make use of a similar strategy to enhance the probability of conspecific matings.

Although the mating pattern in *B. raddei* can probably be best described as a random mating system with respect to body size, females might select mates based on other morphological (e.g., nuptial pad size and colouration), acoustic or multiple traits (Friedl & Klump, 2005). Overall, in addition to expanding the spatial and temporal scope of future studies, an investigation of interactions among ecological factors, female selection based on male chorus attendance and breeding demography is needed to improve our understanding of the dynamics of the anuran mating process.

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