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Bufo gargarizans minshanicus males exhibit size-dependent mate choice but lack sex recognition: an experimental approach

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Bufo gargarizans minshanicus is an explosively breeding species with intense male-male competition, leading to a large-male mating advantage and little opportunities for mate choice by males. In the present study, we experimentally show that *B. g. minshanicus* discriminates between the sizes of potential mating partners, however without discriminating between the sexes. We also show that their choice is limited or overruled by strong male-male competition in a natural population.

Key words: Bufo gargarizans minshanicus, male density, mate choice, sex recognition

Many anuran species have non-random mating patterns with regard to body size (Howard, 1988; Briggs, 2008; Lu et al., 2009, 2010; Yu & Lu, 2010a), which may result from competition for mates or mate choice, the two proximate components of sexual selection (Andersson, 1994). Males should mate preferentially with larger females in species for which female fecundity increases with body size, because male fitness increases with the number of mating and offspring sired (Halliday, 1983; Andersson, 1994). However, the effects of male density on mating system in anurans remain unclear. Many studies showed that sexual selection is promoted when male densities are high (e.g., Höglund, 1989; Telford & Van Sickle, 1989; Bourne, 1992). However, high male densities coinciding with a male-biased operational sex ratio (OSR) increases the intensity of male–male competition (Emlen, 1976; Kvarnemo & Ahnesjö, 1996; Byrne & Roberts, 2004), thus reducing the opportunity for mate choice (Petersson et al., 1999; Shuster & Wade, 2003).

In a previous study on *Bufo gargarizans minshanicus*, an explosively breeding toad endemic to the Tibetan Plateau, we showed that the majority of pairs in this species formed non-randomly with respect to body size (Yu & Lu, 2010a). In this study, we used three choice experiments in which male density were set at lower levels than in nature and toads were allowed to interact for a sufficiently long time to provide evidence for sexual selection. We tested whether male *B. g. minshanicus* have the ability to discriminate between i) females versus males of similar body size, ii) large female versus small males, iii) large versus small females.

The field study was carried out at Zecha Conservation Station in Gahai-Zecha National Nature Reserve of Luqu County, Gansu, China (102°41’E, 34°29’N, total area 132,969 ha, elevation 3028 m). Between 31 March to 9 April 2013, 174 unpaired males and 96 mated pairs were caught by hand. The OSR was 1.81:1 in favour of males (significantly different from equality; Chi-square test: χ^2=22.53, df=1, p<0.001), at high male densities (6–9 toads/m^2). Snout–vent length (SVL) of each individual was recorded to the nearest 0.5 mm using a ruler. Prior to trials, animals were individually housed in separate containers containing water. For each trial with moderate male density (2–4 toads/m^2), amplexed pairs were separated and subsequently tested against toads with different mating partners.

Each trial was conducted in a rectangular tank (110×45×25 cm) filled with water to a depth of 15 cm. Tank water was changed between trials and the tanks were flushed twice to minimise olfactory contamination. At the start of each trial, a transparent plastic container (15 cm in diameter, 40 cm high) was placed in the centre of the tank and the test toad was given two minutes to acclimatise. Two stimulus toads were then introduced into the tank (at opposite ends) tied to the tank ends with a 15 cm long string, permitting movement over a short distance. Trials were observed for a maximum of 15 minutes from the time of introduction of the test male. Trials were considered complete if the test male was in amplexus with another individual. If there was not in amplexus after 15 minutes then the trial was excluded from further analyses. The direction of the initial movement of the test male was recorded along with the time spent on each side of the tank; the position of the head was used to assign a location. After each trial, test males were released. We used parametric tests or non-

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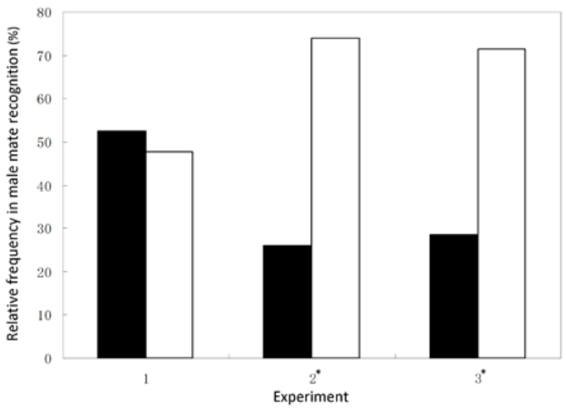

Fig. 1. Male mate choice in *Bufo gargarizans minshanicus*. Experiment 1: choice between males (black column) and gravid females (open column) with similar size (*p*=0.83); experiment 2: choice between small males (black column) and large females (open column, *p*=0.02); experiment 3: choice between small (black column) females and large females (open column, *p*=0.05).

parametric tests to analyse data. All statistical tests were two-tailed, values are presented as mean±standard error (SE), and analyses were run with SPSS v.13.0 (SPSS Inc., 2004).

Female toads averaged 7.33±0.47 cm (range 6.27–9.14 cm) in SVL, and were significantly larger than males (6.55±0.28 cm, range 5.68–7.31 cm; Student's *t*-test: t_{268}=16.97, p<0.001). Amplectant males were significantly larger than non-amplectant males (SVL: 6.62±0.28 cm vs. 6.47±0.26 cm, t_{172}=3.53, p=0.001). A correlation between SVL of female size and clutch size revealed that larger females were more fecund (Pearson correlation: *r*=0.77, *n*=64, *p*<0.001). However, a comparison of SVL of mated pairs did not reveal size-assortative mating (*r*=-0.04, *n*=96, *p*=0.68).

When given a simultaneous choice between a male and a female of identical size, males did not discriminate between the sexes, and attempted to clasp a male or a female with equal frequency (Fig. 1, Table 1). When two males with distinct size differences were provided with a male, the male chose the larger individual. Furthermore, males preferred large gravid females over smaller ones.

Our experiments suggest that *B. g. minshanicus* males show an apparent lack of sexual discrimination even at low male density. This is consistent with patterns previously reported for other species, and might be linked to low costs when making a wrong choice (Marco et al., 1998; see also Marco & Lizana, 2002; Liao & Lu, 2009; Yu & Lu, 2010b; Chen & Lu, 2011; Yu & Sharma, 2012; Yu & Lu, 2013). A male-male amplexus in *B. g. minshanicus* only lasts a few seconds before males continue their search for females, and large males can displace small males from their mating partners (Yu, personal observation). In another *Bufo* species, large males can outcompete small males at more even OSR, and size-assortative pairing occurs at highly skewed OSRs (Lee & Park, 2009; see also Höglund, 1989; Arntzen, 1999).

Indiscriminate mating in association with male-biased OSR and high male density should arise if females have a high probability to be amplexed by non-vocalising or searching males (Arak, 1983; Lee & Salzburg, 1989). Such a situation appears to be typical for the mating system of *B. g. minshanicus* (Yu & Lu, 2010a). Since larger females may provide direct or indirect benefits through generally more fecund and higher-quality offspring (Halliday, 1983; Yu & Sharma, 2012), males attempt to mate with larger females, thereby potentially increasing their fitness (Andersson, 1994). A further possible reason for the size-dependent mate choice of *B. g. minshanicus* males is their local co-occurrence with the smaller species *Rana kukunoris*, which might lead *B. g. minshanicus* males attempting to avoid interspecific matings (see also Pfennig, 2000).

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Table 1. Body size of *Bufo gargarizans minshanicus* used in the three experiments. M: male, F: female, LF: large female, SM: small male, SF: small female, NC: number of choices, TBA: time before amplexus. For more details see text.

		Tested males	Candidates			Results			
		SVL (cm)		SVL (cm)		NC		TBA (s)	
Experiment	*n*	Mean, SE		Mean, SE	*t*-test (*p*)		Chi square (*p*)	Mean, SE	*t*-test (*p*)
1	31	6.63, 0.21	M	6.92, 0.15	0.81	11	0.83	53.05, 21.28	0.22
			F	6.92, 0.16		10		23.81, 9.77	
2	28	6.54, 0.32	SM	6.37, 0.33	< 0.001	6	0.02	15.14, 16.11	0.04
			LF	7.79, 0.46		17		45.91, 15.14	
3	24	6.59, 0.32	SF	6.78, 0.18	< 0.001	6	0.05	37.81, 12.76	0.07
			LF	7.78, 0.35		15		11.95, 5.02	

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