

Short notes

Sex recognition and mate choice in male *Rana kukunoris*

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Mate recognition is important for successful reproduction, and consequently species have evolved various ways to recognize potential mates. Little is known about mate recognition in male *Rana kukunoris*, a temperate frog endemic to China. In the present study, we investigated experimentally whether male *R. kukunoris* can discriminate between conspecifics of different sexes, between gravid and non-gravid females of similar size, and between gravid females of different sizes. When offered the choice between a gravid female and a similar-sized male, the focal male attempted to clasp both sexes with equal frequency. Test males only released the stimulus frog when a release call was emitted by the stimulus male. Test males did not differentiate between gravid and non-gravid females of equal size either. However, male frogs preferred large females over smaller females. The results suggest that male *R. kukunoris* discriminate between the sexes based on the release calls of males, and between females of different sizes using visual cues.

Key words: mate recognition, mating pattern, visual cues

Mate recognition, including the ability to distinguish between species, sexes, kin and potential mates of different quality (Krebs & Davies, 1997), is crucial for successful reproduction. Choosing the wrong mating partner (such as a different species, sex, or a partner not ready for reproduction) wastes time and energy, and may result in lost opportunities for successful reproduction (Marco et al., 1998). Sexual selection has allowed species to evolve efficient signals (visual, acoustic, tactile, olfactory and chemical) and responses to allow individuals to locate potential mates (Halliday, 1983; Paterson, 1985).

For sexually dimorphic species, visual cues play important roles in both sex identification and mate attraction (Andersson, 1994; Plath & Tobler, 2007). Some sexually dimorphic amphibian species choose their mates primarily based on visual cues (Buchanan, 1993; Duellman & Trueb, 1994; Katsikaros & Shine, 1997). However, some male anurans base mate discrimination on the body size of potential mates (Berven, 1981; Marco et al., 1998; Liao & Lu, 2009), with males preferentially mating with larger females to maximize their reproductive success (Halli-

day, 1983; Andersson, 1994). Such studies have mainly focused on bufonids, whereas knowledge about mate choice in ranids is less complete.

Rana kukunoris is endemic to the eastern Tibetan plateau (29–41°N, 93–104°E) and is distributed at high elevations (Zhang, 1999; Xie et al., 2000). *Rana kukunoris* is an explosive breeder (Wells, 1977) with a short breeding period (one week; pers. obs.). Female fecundity is positively related to body size (Lu et al., 2008). Males lack vocal sacs and do not produce advertisement calls during the short breeding season. Therefore, males actively search for and approach females for mating during daytime. Amplexus takes place in water and lasts three or four days. Preliminary information on the distribution and habitat of *R. kukunoris* exists (Xie et al., 2000; Fei & Ye, 2001), but little is known about the mating behaviour of this Chinese endemic. In this study, we investigated mating patterns and used three choice experiments to test whether male frogs have the ability to discriminate between 1) females and males, 2) gravid and non-gravid females of similar body size, and 3) gravid females of different body sizes.

In April 2009 and 2010, we studied *R. kukunoris* at Gahai Lake (102°56'E, 30°33'N; elevation 3450 m) by hand-capturing frogs that were in amplexus or searching for mates in the water during the daytime. We sexed all individuals (Lu et al., 2008) and measured their snout-vent lengths (SVL) to the nearest 0.1 mm with a vernier caliper.

Mate choice experiments were conducted in Gahai Reserve Station (500 m from Gahai Lake) on 7–10 April 2009 and 8–10 April 2010. Experiments began immediately after collection of frogs. For each trial, we separated amplexed pairs and subsequently tested these individuals against frogs with different mating partners. Prior to trials, test animals were individually housed in separate containers containing water.

The experimental arena consisted of a rectangular tank (150 cm × 100 cm × 50 cm; L × W × H) filled with water to a depth of 5 cm and maintained at 6–8 °C. Following each trial, we flushed the tank twice with fresh water to minimize any interference of olfactory cues in subsequent trials. At the start of each trial the test male was placed in the centre of the experimental tank and covered with a transparent plastic cylinder (20 cm in diameter × 40 cm in height). We then simultaneously introduced the two stimulus frogs (one at each end of the tank), whose legs were tied to the tank ends with a 40 cm long string, permitting movement over a short distance. After all frogs were allowed to acclimate for 10 minutes, we lifted the cylinder and began the trial. During each trial, the test males had full access to the stimulus animals. We recorded the first individual clasped by the test male, the time it took for this to occur, and the duration of amplexus (up to 30 s) with a stopwatch. If amplexus did not occur within 20 min, we terminated the trial and recorded the test male as not making a choice. Each animal was used once in experiments and then released after the trials were completed.

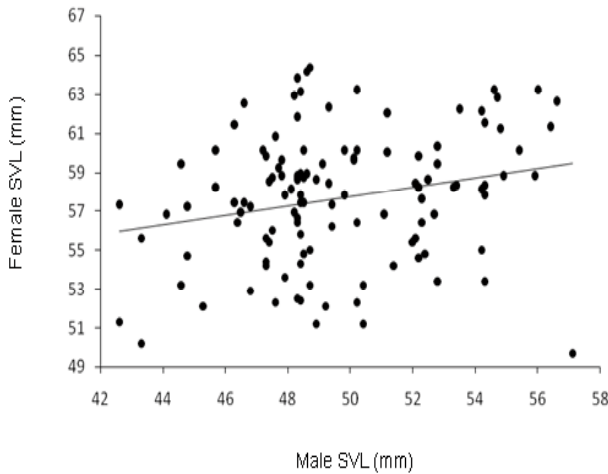


Fig. 1. Correlation between the body size (snout-vent length, SVL) of male and female *Rana kukunoris* found in amplexus in Gansu province, northwestern China.

We used Pearson's correlation coefficients to assess the relationship between the body sizes of amplexed pairs. Paired student's *t*-tests were used to test whether the body sizes of the two stimulus frogs were significantly different. Mann-Whitney tests were used to compare the time it took for stimulus animals to choose a partner and the duration of amplexus. Binomial tests were used to determine whether there was a significant difference in male choice. All statistical tests were two-tailed, values are presented as mean \pm standard deviation (SD), and analyses were run with SPSS 13.0 (SPSS Inc., 2004).

We recorded a total of 620 frogs (432 males and 188 females) at our site, which resulted in a male-biased sex ratio of 2.3:1 that differed significantly from 1:1 ($\chi^2=96.03$,

$df=1$, $P<0.001$). We collected 120 amplexant pairs for our experimental trials. Female frogs were significantly larger than males (females: 57.64 ± 3.31 mm, range 52.8–67.1 mm; males: 49.58 ± 3.21 mm, range 42.2–61.2 mm, paired *t*-test: $t=21.92$, $P<0.001$). Correlation between SVL of the mated pairs revealed that larger males were more likely to mate with larger females (Pearson correlation: $r=0.238$, $n=120$, $P=0.009$; Fig. 1).

Males were unable to recognize the sex of potential mates, or whether females were gravid (Table 1). However, males could discriminate between mates of different size (Table 1). Test males released male stimulus frogs only when the stimulus male uttered a specific release call, which occurred immediately after initiating amplexus (i.e. <2 s). When test males attempted amplexus with a stimulus female, release calls were never emitted. Amplexus with females was usually tenacious and always lasted until we stopped the trials at 30 s.

Size-assortative mating has been reported in several amphibian species (Marco et al., 1998; Marco & Lizana, 2002; Liao & Lu, 2009; Yu & Lu, 2010) and is adaptive in terms of increased fertilization success (Arak, 1983). In this study, the positive correlation between the body size of females and males in amplexant pairs may have evolved from strong male-male competition and mate choice for large size by one or both sexes (Crespi, 1989; Harari et al., 1999).

Previous studies have shown that some male anurans do not discriminate between the sexes due to high population densities and strong scramble competition among males in populations with male-skewed operational sex ratios (Marco et al., 1998; Marco & Lizana, 2002; Liao & Lu, 2009; Yu & Lu, 2010). We found that male *R. kukunoris* do not discriminate between females and males. This may be because the costs of discriminating behaviours are higher when the mating period is relatively short and females are a limited resource (Sullivan et al., 1995). In this context, males adopt the strategy of actively searching

Table 1. Results of mate recognition experiments offering a male *Rana kukunoris* the choice between two stimulus animals. Abbreviations: male (M), female (F), gravid female (GF), non-gravid female (NGF), larger-than-average female (LF), smaller-than-average female (SF), number of choices (NC), time before amplexus (TBA), time spent trying to grasp (TTA), time of amplexus (TA), binomial test (BT), paired student's *t*-test (PST), Mann-Whitney test (MWT). Exp. = experiment.

Exp.	Tested males		Alternatives				Result of choices					
	<i>n</i>	SVL (mm), mean/SD	SVL (mm)		NC	TBA (s)		TTA (s)		TA (s)		
			Mean/SD	PST <i>t</i> /P		Mean/SD	MWT Z/P	Mean/SD	MWT Z/P	Mean/SD	MWT Z/P	
1	46	48.6/3.2	M	56.4/2.5	20	0.65	11.4/4.2	0.04/0.97	4.2/1.7	0.09/0.93	1.6/0.50	6.3/<0.001
			F	56.5/2.5								
2	34	49.9/3.0	GF	60.9/1.9	18	0.73	10.9/4.9	0.35/0.73	5.2/2.1	0.09/0.92	30/0	0/1
			NGF	60.9/2.0								
3	40	47.3/2.3	LF	61.0/2.0	33	<0.001	12.2/2.8	0.39/0.68	3.4/0.6	1.25/0.21	30/0	0/1
			SF	54.3/2.0								

for females and attempting to clasp any nearby individual (Arak, 1983; Brown, 1977; Olson, 1989). Although this strategy can increase the costs of obtaining a mating partner, it results in a high chance of mating success.

Olfactory cues also can be used for mate recognition (Tracy & Dole, 1969; Verrell, 1985). During our experimental trials, *R. kukunoris* males were in contact with two stimulus frogs and therefore were exposed to potential chemical cues. However, because males did not discriminate between the sexes before attempting amplexus, this implies that sex recognition is not based on chemical cues from the stimulus animals. In fact, the test male was only able to discriminate between the sexes after the stimulus male uttered a release call. We speculate that this release call is the only mechanism by which *R. kukunoris* can discriminate between the sexes.

Male *R. kukunoris* were also unable to discriminate between gravid and non-gravid females, which has been reported in other amphibians (Marco et al., 1998; Liao & Lu, 2009; Yu & Lu, 2010). One possible reason is that the probability of males' amplexing non-gravid females in nature is relatively low, because formerly gravid females quickly leave the breeding site immediately after laying eggs.

In some amphibian species, males can discriminate between mating partners based on size, potentially using visual cues (Berven, 1981; Andersson, 1994; Marco et al., 1998; Liao & Lu, 2009). The evolution of visual cues by sexual selection may have given male amphibians the ability to distinguish the body size of females (Duellman & Trueb, 1994). Males benefit from selecting large females as mates because larger females are generally more fecund (Rhainds et al., 1995; Marco et al., 1998; Savalli & Fox, 1998). Our results are consistent with this pattern, with male *R. kukunoris* discriminating between gravid females based on body size. Large females have a higher fecundity than smaller females (Lu et al., 2008), and by mating with larger females male *R. kukunoris* can maximize their reproductive success (Marco et al., 1998; Liao & Lu, 2009).

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