Sex recognition and mate choice by male western toads, *Bufo boreas*

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Abstract. In field-based choice experiments, we examined sex recognition and mate choice in male western toads, *Bufo boreas*. When given a simultaneous choice between a male and a female of equal size, males did not discriminate between the sexes and attempted to amplex a male or a female with equal frequency. When a test male clasped a stimulus male, the stimulus male uttered a release call that caused the test male to release the stimulus male. Male–male amplexus never lasted more than 3 s, but male–female amplexus was tenacious and prolonged. Furthermore, males discriminated between gravid females that differed in body size, choosing larger gravid females over smaller ones, but they did not discriminate between gravid females or non-gravid females of equal size. In choice tests that excluded chemical cues, males jumped more frequently towards large females than small ones. Given that females are significantly larger than males, selecting larger individuals as potential mates increases the probability that males amplex with a female.

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An efficient system of signals and responses is important for successful reproduction (Paterson 1985). Individuals should not mate indiscriminately, but instead should choose their mates carefully (Halliday 1983). This may be especially important in species that have a single opportunity to mate, such as occurs for many explosive breeders. Sexual selection theory indicates that scramble competition is predicted to favour enhanced sensory adaptations in the competing sex to improve sex recognition and discrimination of the quality of potential mates (Andersson 1994). We speculate, however, that under some conditions scramble competition may not favour effective sex and mate recognition. In some situations, the cost of incorrect discrimination can be low; thus, discriminatory mechanisms may not be favoured.

Male anuran amphibians usually produce vocalizations that function in sex recognition and mate choice (Halliday & Tejedo 1995; Stebbins & Cohen 1995). Frog and toad advertisement calls may be considered a form of long-range courtship (Rand 1988) and function as an important premating isolating mechanism (Duellman & Pyles 1983). Advertisement calls are the main form of male display in most anuran species. The vocal characteristics of males can play an important function in mate selection by attracting females or in establishing territories (Wells 1988). However, explosive breeders that show scramble competition in high-density populations usually lack chorusing behaviour (Hoglund & Robertson 1988). In certain species with non-calling mating systems, chemical cues, visual displays and tactile cues are used for sex recognition and mate choice (Duellman & Trueb 1994). Some explosive-breeding anurans apparently do not discriminate between sexes, however. In these species, males show active searching for females and typically approach and attempt to clasp any small objects moving nearby (Arak 1983). This is also true for the western toad, *Bufo boreas* (Brown 1977; Olson 1989).

Western toads may move long distances to breeding sites and use the same reliable sites year after year (Nussbaum et al. 1983). Chemical cues are important to localize their 'home' areas (Tracy & Dole 1969). Furthermore, western toads are explosive breeders and males usually undergo scramble competition for mates (Olson et al. 1986). They concentrate synchronously in small breeding sites often containing several hundred

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individuals. Up to 5000 western toads have been recorded in a single population (Nussbaum et al. 1983). During the short mating period (4–6 nights), males do not produce advertisement calls. They rest quietly at the water surface and swim vigorously towards other moving toads. There is male choice with a strong clasping response. Amplexus with females is usually tenacious and prolonged. However, male toads give bird-like twittering calls (release calls) in response to amplexus attempts by other males (Nussbaum et al. 1983).

Using choice experiments in the field, we tested the ability of male western toads to discriminate between the sexes of conspecifics. Furthermore, we tested whether males choose gravid females over non-gravid females of equal size and large gravid females over small ones. We also examined the existence of size-assortative mating in a natural breeding population.

MATERIALS AND METHODS

We conducted four experiments to examine sex recognition and mate choice in a population of western toads at Lost Lake (Cascade Mountains, Linn County, Oregon, U.S.A.; elevation 1220 m). We also observed their mating behaviour and recorded, to the nearest 0.1 mm, the snout–vent length of 208 toads (104 pairs) involved in amplexus. We used choice tests to determine whether males (1) recognized the sex of conspecifics (experiment 1, 20 trials), (2) discriminated between gravid versus non-gravid females of the same size (experiment 2, 20 trials) and (3) discriminated between large versus small gravid females (experiment 3, 24 trials). Furthermore, we tested whether males discriminated between large and small gravid females in the absence of chemical cues (experiment 4, 22 trials).

All experiments were conducted in the field during the mating period (10–16 May 1996). All subjects were captured in the lake when they were in amplexus or presumably searching for mates in the water. For each trial, we used a rectangular tank ($120 \times 50 \times 30$ cm) filled with approximately 10 cm of fresh lake water as a testing chamber. Water was replaced after each trial. In every trial, we put one stimulus toad on each end of the tank in a position selected at random. In experiments 1, 2 and 3, the stimulus toads were tied to the tank

ends with string 15 cm long. The string was attached to the toad's leg, permitting movement over a short distance. In experiment 4, the stimulus toads were housed in transparent chambers that prevented the diffusion of chemical cues. The test animals could not make physical contact with the stimulus animals, but they could see them and detect mechanical/acoustic vibrations through the chambers.

For all experiments, we introduced a single test male in a small container constructed of fibreglass screen ($20 \times 15 \times 12$ cm). The container was placed into the centre of the tank and the test toad was given 3 min to acclimatize. We then removed the container and recorded the first side of the tank chosen by the male. We also recorded the time that the test toad spent on each side of the tank. If the toad was in the middle of the enclosure, we used the position of the head to assign location. In experiments 1, 2 and 3, we recorded the time it took the test animal to attempt to amplex one of the stimulus animals and which stimulus animal was selected. We recorded the duration of amplexus (up to 30 s) and the behaviour of the animals involved in the amplexus. If there was no amplexus after 10 min, we stopped the trial and recorded the animal as making no choice. In experiment 4, all trials lasted 10 min. In experiment 4, in addition to the above criteria, we also recorded the number of jumps that the test male made towards each stimulus female.

RESULTS

Breeding took place from 10 to 16 May 1996. Many toads (>200) arrived synchronously at the breeding site. Lost Lake is approximately 34 ha in area, but toads bred in only a 0.3-ha area of the lake. The Pacific tree frog, *Hyla regilla*, was the only other amphibian observed during this time and we did not observe toad amplexus with *H. regilla*.

We captured 208 toads (104 pairs) in amplexus. Females were, on average, significantly larger than males (Student's *t*-test: $t=30.467$, $P<0.001$). Average ± SD body size of females was 108.4 ± 5.5 mm (range=93–118 mm). Average male body size was 85.9 ± 5.2 mm (range= 72–96 mm). Comparison of snout–vent length of mated pairs revealed size-assortative mating

Table I. Body size of western toads used in the four experiments

Experiment (N)		Test		Stimulus 1			Stimulus 2			P
1 (20)	♂	87.65	(3.88)	♂	95.75	(0.44)	G♀	95.50	(0.51)	NS
2 (20)	♂	86.55	(3.36)	G♀	110.75	(4.94)	N♀	110.25	(4.55)	NS
3 (24)	♂	86.65	(4.30)	L♀	117.42	(1.80)	S♀	94.33	(1.09)	***
4 (22)	♂	88.00	(4.11)	L♀	116.05	(1.36)	S♀	94.48	(0.93)	***

Average ($\pm$ SD) snout–vent length of test and stimulus western toads used in the four experiments. N=number of trials. Test: adult males in every experiment; Stimulus 1: adult male in experiment 1, gravid female (G) in experiment 2 and large gravid female (L) in experiment 3 and 4; Stimulus 2: adult gravid female in experiment 1, non-gravid female (N) in experiment 2 and small gravid female (S) in experiments 3 and 4. P: significance level of differences between snout–vent length of Stimulus 1 and 2 using the Wilcoxon signed-ranks test. ***$P<0.001$.
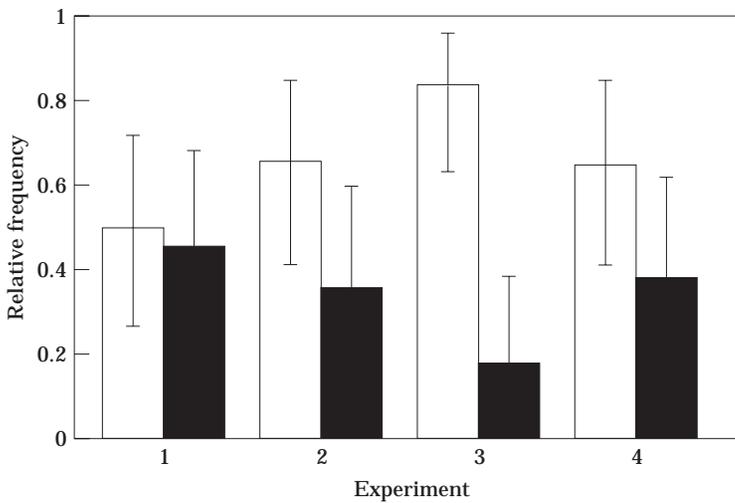
Figure 1. Male mate choice in *B. boreas*. Experiment 1: choice between female (□) and male (■) matched for size (NS); experiment 2: choice between gravid (□) and non-gravid (■) female matched for size (NS); experiment 3: choice between large (□) and small (■ females ($P<0.05$); experiment 4: choice between large (□) and small (■) females when the access to the females was restricted (NS). Error bars indicate the 95% confidence interval for the proportion (Fisher's exact test).

(Pearson's product moment correlation: $r=0.512$, $F_{104}=36.6$, $P<0.001$).

The average snout–vent length of test and stimulus toads in every test are shown in Table I. In experiment 1, when males were offered a male and a female of similar snout–vent length, nine of 20 males (45%) attempted to amplex another male. Ten males (50%) amplexed a female, and one male (5%) made no choice (Fig. 1). The percentage of males that attempted to amplex another male was not significantly different from random (binomial test: $t<0.01$, NS). The first side of the tank selected by males apparently did not influence their final choice. Males in 38% of the tests chose the individual from the side opposite the one they moved towards first. In 16% of the trials, test males selected the side with the female and then moved towards the side with the male to attempt amplexus. There was no significant difference between the time that test males spent on either side of the tank (Wilcoxon signed-ranks test: $T=0.382$, NS). The average time that males took to clasp one of the stimulus animals was 37.5 s (range=6–197 s). There was no difference in the amount of time for the test male to amplex a male or female (Mann–Whitney test: $U=1.84$, NS). When test males clasped a stimulus male, the stimulus male produced a release call and the test male released him. Male–male amplexus never lasted more than 3 s. When test males attempted

amplexus with a stimulus female, there was never a release call, and amplexus always lasted until we stopped the trials at 30 s. The absence of sex recognition by male toads in our experiments is consistent with our observations of natural behaviour of unrestricted toads, where we often observed male toads attempting to amplex another male.

When males received a choice between gravid and non-gravid females matched for size, 13 of 20 males (65%) chose the gravid female over the non-gravid one (Fig. 1). This percentage was not significantly different from random (binomial test: $t=1.118$, NS). On only 11 of 20 trials, the first side of the chamber selected by the test male coincided with the side of final choice. The average time taken by males to attempt an amplexus was 42.35 s (range=4–111 s). There was no difference in the amount of time for the test male to amplex a male or female (Mann–Whitney U-test: $U=0.63$, NS). These results are consistent with our observations that male toads do attempt to amplex non-gravid females.

In tests where males could select between two gravid females that differed by approximately 20 mm, 20 of 24 males (83.3%) chose the larger female over the smaller one (Fig. 1). This percentage was significantly different from random (binomial test: $t=3.354$, $P<0.001$). The first side of the tank selected by test males was the same side of the final choice in the 70.8% of the trials.

When we introduced both stimulus animals into transparent isolated chambers without chemical cues, 14 of 22 males spent more time on the side with the large female (63.6%, binomial test: $t=1.066$, NS). There were no significant differences between the times that test males spent on either side of the tank (Wilcoxon signed-ranks test: $T=1.721$, NS). However, test males jumped more frequently towards large females than small ones (Wilcoxon signed-ranks test: $T=3.005$, $P<0.01$). The average ± SD number of jumps towards large females was 6.91 ± 3.45; for small females it was 3.41 ± 2.06.

DISCUSSION

Our results suggest that male *B. boreas* do not discriminate between sexes before attempting amplexus. The absence of a release call in female toads seems to be the only element of sex recognition in the western toad.

Male toads have nuptial pads and longer, more muscular forelimbs than do females (Olson 1989; Halliday & Tejedo 1995). However, males did not discriminate between sexes using these sexually dimorphic features. Western toads have a well-developed olfactory capacity that is used in homing behaviour (Tracy & Dole 1969). Moreover, evidence suggests that chemical cues can be important in male choice of some amphibians (e.g. Verrell 1985; Mathis et al. 1995). If male and female western toads have different chemical signals, however, males apparently did not use them to discriminate between the sexes. Consistent with this result, Tracy & Dole (1969) observed that anosmic toads can clasp females and attempt amplexus.

In explosive-breeding species with high-density populations, temporal constraints may increase the costs of highly discriminating behaviour by females (Sullivan et al. 1995). In the population we studied, females do not appear to search for mates. Males actively search for females and their attempts at amplexus are fast, strong and tenacious. In this context, there was probably weak selection pressure for the evolution of an advertisement call.

Perhaps explosive breeding in this species has led to little selection pressure for sex recognition by males. Thus, the reproductive strategy for male *B. boreas* may be to clasp quickly every conspecific and then determine whether it is a female. Once a female is encountered, the male and female mate and there is little competition from other males for the female. In fact, Olson et al. (1986) found only one of 271 paired female western toads clasped by two males and found no evidence of males being displaced by other males. In that study, only 16 of 286 gravid females were captured individually. In our study, males attempted to amplex other males, but male–male amplexus only lasted a few seconds, after which males can continue their searching behaviour. Consequently, the cost of the absence of sex recognition in terms of lost time and energy is small. The benefits of being first to encounter a female probably outweigh the costs of repeated clasping with other males (Wells 1977).

Females are on average larger than males. Because males can discriminate the size of a conspecific and select a larger animal to mate, the likelihood that males will choose a female is high. Furthermore, with this strategy males will mate

with larger and potentially more fecund females, perhaps increasing their reproductive success.

Males did not discriminate between gravid and non-gravid females, and amplexus with non-gravid females was tenacious under experimental conditions. Males that clasp non-gravid females could waste time and energy and miss opportunities to mate with gravid females. After egg laying, however, females quickly leave the breeding site. Thus, the probability that males encounter non-gravid females in nature is relatively low.

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