Animal Cells and Systems 12: 93-96, 2008

Animal Cells
and
Systems
http://www.AnimalCells.or.kr

Inability of Mate and Species Recognition by Male Asian Toads, *Bufo gargarizans*

Seokwan Cheong, Hacheol Sung and Shiryong Park*

Department of Biology Education, Korea National University of Education, Chungbuk 363-791, Korea

Abstract: In recent years, we frequently observed miss-matched pairs between male Asian toads, *Bufo gargarizans*, and bullfrogs, *Rana catesbeiana*, at the toad breeding ponds, where scramble competition for mating occurred among the male toads. Thus, we performed two-choice experiments to investigate recognition ability of mates and species in male toads. The test males did not discriminate sexes, but the clasped stimulus males immediately produced release calls and stopped it while the clasped stimulus female did not. In addition, the test male toads did not discriminate reproductive state of females and even species. However, male toads chose larger individuals. The present results indicate that the main reason of miss-matched amplexus by the male toads is due to 1) the lack of recognition cues of conspecifics, 2) the lack of communication tools like release calls, and 3) the larger size of bullfrogs than male toads themselves.

Key words: Asian Toad, *Bufo gargarizans*, two-choice experiment, bullfrog, *Rana catesbeiana*, recognition cue, release call

Communication occurs when cues given by senders influence the behavior of receivers (Endler, 1993). Receivers should benefit from recognizing between desirable and undesirable cues. When the recognition cues are overlapped, selection may favor an optimal balance between accepting undesirable cues (acceptance errors) and rejecting desirable cues (rejection errors), where the balance could be altered by changing decision rules or recognition templates of receivers (Sherman et al., 1997).

The recognition of species and mates may be important for successful reproduction in anuran amphibians. Species of prolonged breeders that have multiple mating opportunities mainly use vocalizations in relation to both interspecific and intraspecific mate selection (Sullivan, 1983; Wells, 1988; Schwartz, 1989; Ryan, 1990; Gerhardt, 1991): for example, the vocal characteristics of males, such as louder calls or lower frequency calls, play a role in female choice and male-male competition (Halliday, 1983). In addition, such calls possess species-specific characteristics functioning as a pre-mating isolating mechanism (Duellman and Pyles, 1983; Schneider et al., 1984). In contrast with prolonged breeders, very intense scramble competitions occur in explosive breeders because of synchronous arrival at a breeding site with limited mating opportunities of a short breeding period. Males may actively search for females rather than attract females with vocalizations, where males approach and attempt to clasp any moving objects nearby (Arak, 1983; Olson, 1989). The species may use other recognition cues, such as chemical cues and tactile cues, for sex and mate recognition (Davies and Halliday, 1978; Duellman and Trueb, 1994).

The Asian toad, *Bufo gargarizans*, is widely distributed over the large part of East Asia and is common in Korea (Yang et al., 2001). The species was once classified into *Bufo bufo gargarizans*, a subspecies of *Bufo bufo*, the European toad or the common toad (Kang and Yoon, 1975). They are typical 'explosive breeders', yearly visiting a breeding pond in early spring, where they are staying up to 1-3 weeks before returning to terrestrial areas for non-breeding season. The male toads usually experience intense scramble competition for possession of females without producing advertisement calls. In recent years, unusual mismatched pairing between bullfrogs (*Rana catesbeiana*) and male toads has been observed frequently in some breeding ponds (Fig. 1). The bullfrog is a large, an average length of 10 cm with weight of 142 g, and exotic invasive species rapidly spreading in the western and southern Korean Peninsula since being introduced from Japan at the end of 1970 (Kang and Youn, 1994). To date, no certain explanation has been given how it does happen.

In this paper, we hypothesized that male Asian toads lack

*To whom correspondence should be addressed.
Tel: +82-43-230-3719; Fax: +82-43-233-6263
E-mail: srpark@knue.ac.kr

Fig. 1. Miss-matched amplexus between a bullfrog (bottom) and several male Asian toads (top). The male toads strongly clasped the bullfrog to death.

an ability of mate recognition. Using choice experiments in the lab, we tested the hypothesis: whether male Asian toads discriminate 1) between the sexes of conspecifics, 2) between reproductive states of female toads, and 3) even between female toads and bullfrogs as a potential mate. Furthermore, we tested whether the males show size-assortative mating preference using different sizes of bullfrogs. This is the first study on the effects of alien species to breeding biology of native species occupying similar ecological status.

MATERIALS AND METHOD

We collected the Asian toads at a pond (36°17'43"N, 127°30'30"E) in Okcheon Gun, Chungbuk Province, Korea, in March 1999. We measured snout-vent lengths (SVL) of the toads to the nearest 0.01 mm with vernier calipers and isolated them by sex. The sex of each toad was determined by the presence of nuptial pads of males. We performed five experiments to investigate the recognition ability of male Asian toads for species and mate choice: we used choice experiments to determine whether the males discriminate 1) between the sexes of conspecifics, 2) between gravid versus non-gravid females of the similar size, 3) even between female toads and bullfrogs as a potential mate, 4) between small (7~9 cm of SVL) versus large (12~15 cm) stimulus, and 5) between medium (9~12 cm) and large stimulus. As a stimulus, we used bullfrogs because we are interested in revealing whether they tend to have size-assortative selection in mate choice even if the bullfrogs are not the same species. We captured the bullfrogs in Youngjong island (37°29'12"N, 126°30'E), Incheon City, in November 1998. We kept the bullfrogs provided loaches for food in an aquarium till the next spring of the toad breeding season.

We designed the five experiments considering the methods used by Marco et al. (1998). We prepared for a rectangular tank (120×50×30 cm) filled with about 5 cm of pond's water for all experiments. We replaced the water after each trial in order not to contaminate the tank with chemicals. We put stimulus individuals on each end of the tank at random position. The stimulus individuals were tied with string 15 cm long to the tank ends so that they were allowed to move for a short distance. We placed a single test male in a small container (20×20×20 cm) constructed of fiberglass screen into the center of the tank. The test male was acclimatized for two minutes. All experiments were started with removing the container, and we counted the number of attempts to amplex one of the stimulus individuals. We decided the male selected a stimulus subject if there was an amplexus more than 30 seconds. We stopped the trial if any amplexus attempt was not observed for 10 minutes.

We used student's t-test to examine the variation of SVL between stimulus individuals and used Binomial test to determine whether there is a significant difference between the observed and the expected frequencies in mate choice experiments. Numerical data in the text are presented as mean±SD. Data were analyzed using SPSS statistical software (v.11.5; SPSS 2002).

RESULTS

We captured 133 males and 46 females of the Asian toads. Mean SVL of males was 8.08 cm (±0.67) and female was 10.77 cm (±0.78). Females were significantly larger than males (student's t-test, t=20.75, $p \leq 0.001$). The mean SVL of used stimulus toads and bullfrogs in each trial are shown in Table 1: there were no significant differences between two groups of stimulus individuals in Experiment 1, 2, 3. However, the large size of bullfrogs was significantly larger than small or medium size of bullfrogs as expected.

In experiment 1, when similar sizes of a female toad and a bullfrog were presented to male toads, 7 of 20 males (35%) attempted to amplex female toad while 13 males to bullfrogs, which was not significantly different from random (z=1.34, p=0.13, Fig. 2). The test males tightly clasped not only female toad but also bullfrog. The bullfrog more struggled to escape from being amplexed than female toad, but failed till we stopped the trial at 30 seconds.

In experiment 2, when test males received a choice between a female and a male toad in similar sizes, 9 of 20 males (45%) chose female over male, which did not significantly differ from random (z=0.41, p=0.45, Fig. 2). The stimulus male produced release calls in amplexus while the stimulus female did not. Male-male amplexus never lasted more than 5 seconds in response to the call,

Table 1. Comparisons of body sizes between stimulus 1 and 2 in the five experiments. Mean (±SD) SVL (snout-vent length) of stimulus individuals, where N means number of trials

Experiment (N)	Stimulus 1		Stimulus 2	
	Individual	SVL (cm)	Individual	SVL (cm)
1 (20)	Female toad	10.79±0.73	Bullfrog	11.78±0.17
2 (20)	Female toad	9.72±0.28	Male toad	9.39±0.21
3 (20)	Non-gravid female	10.75±0.98	Gravid female	10.76±0.95
4 (21)	Small bullfrog	7.93	Large bullfrog	13.17
5 (20)	Medium bullfrog	11.35	Large bullfrog	14.62

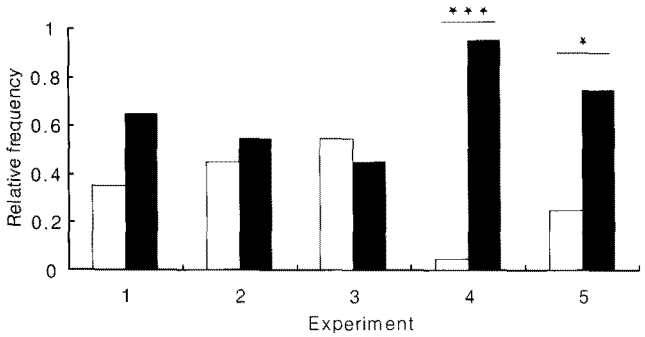

Fig. 2. Mate choice by male Asian toads. Experiment 1: female toad (□) vs. bullfrog (■); Experiment 2: female toad (□) vs. male toad (■); Experiment 3: Non-gravid female toad (□) vs. gravid female toad (■); Experiment 4: small bullfrog (□) vs. large bullfrog (■); Experiment 5: medium bullfrog (□) vs. large bullfrog (■). Significant level of binomial test: *p<0.05, ***p<0.001.

while male-female amplexus lasted till we separated paired couples.

In experiment 3, when test males received a choice between a gravid female and a non-gravid female in similar sizes, 9 of 20 males (45%) chose gravid female over non-gravid female, which did not significantly differ from random (z=0.41, p=0.45, Fig. 2).

In experiment 4 and 5, when test males received a choice between small or medium vs. large stimulus, the males significantly preferred large ones: 20 of 21 males (95%) in small vs. large stimulus experiment (z=4.15, p<0.001, Fig. 2) and 15 of 20 males (75%) in medium vs. large stimulus experiment (z=2.24, p=0.02, Fig. 2).

DISCUSSION

The present results suggest that male Asian toads do not recognize sex, reproductive state of females, and even species. The males randomly chose similar sizes of male toads over female toads, bullfrogs over female toads, non-gravid over gravid females. However, the males preferred large individual to small or middle one.

As a typical explosive breeder, the Asian toads aggregate synchronously into breeding sites forming high-density populations, where they have a short breeding period with a male-biased sex ratio (Sung et al., 2007). This allows male to have few opportunities to amplex a female. This may lead to sexual selection favoring males that actively search for females, but not vice versa. The active males may attempt to vigorously clasp close individuals without discriminating sex, gravid female, and even conspecifics. Due to such a behavioral strategy for male reproductive success, the toads may not use an advertisement call to attract a potential mate. The advertisement call has been extensively used in one of recognition cues indicating male quality (i.e. large males) by female choice (Davies and Halliday, 1978; Arak, 1983; Wells, 1988). The western toad (*Bufo boreas*) is the similar species that lacks this kind of advertisement calls (Marco et al., 1998). Like the Asian toad, the western toads use a serious of release calls as only recognition cue whether the amplexed individual is male or female.

Males chose larger individuals given a choice, which may be another reproductive strategy to increase the probability of selecting correct females rather than males. Females are usually about 1.3 times larger than males (Sung et al., 2007). The sexual dimorphism in anurans has been described as resulting from the interaction between natural and sexual selection (Lande and Arnold, 1983; Ryan, 1985; Arak, 1988). Males tended to favor larger females as a mate because the larger females lay more eggs so that males in amplexus increase the number of their fertilizing eggs (Davies and Halliday, 1977; Arntzen, 1999). It may be true in an excess of females, where males may be able to evaluate female size to choose the larger ones. However, in case female choice by male is temporally and spatially limited, the male may attempt to claps a potential female quickly using size cues as well as release calls.

In recent years, we frequently observed miss-matched amplexus of male toads and the bullfrogs in the fields, which, in turn, a toad kept clasping a bullfrog to death. The bullfrogs do not have ribs protect internal organs from external pressure by toad clasp. Thus, the main reason of miss-matched amplexus by the male toads is due to 1) the lack of recognition cues of conspecifics, 2) the lack of communication tools like release calls, and 3) the larger

size of bullfrogs than male toads themselves. As an exotic invasive species, the bullfrogs are evolutionary absent in this country so that it is an unusual phenomenon in nature in these days.

We do not know how the male sizes influence male mating success. However, from the study of the common toad, when breeding period is short, mating in relation to body size is random (Davies and Halliday, 1977; Hoglund and Robertson, 1987). Although small clasped males is often displaced by large ones at the time of spawning, small males, considered to be disadvantageous in male-male competition, still have some opportunities for mating (Davies and Halliday, 1977). Thus, the male mating success in this species is almost density and time dependent. We more need to investigate the male size effects of mate selection processes in the Asian toad.

REFERENCES

ARAK A (1983) Male-male competition and mate choice in anuran amphibians. In: P. Bateson (ed), Mate Choice, Cambridge, Cambridge University Press, pp 181-210.

Arak A (1988) Sexual size dimorphism in body size: a model and a test. *Evolution* 42: 820-825.

Arntzen JW (1999) Sexual selection and male mate choice in the common toad, *Bufo bufo*. *Ethol Ecol Evol* 11: 407-414.

Davies NM and Halliday TR (1977) Optimal mate selection in the toad *Bufo bufo*. *Nature* 269: 56-58.

Davies NB and Halliday TR (1978) Deep croaks and fighting assessment in toads *Bufo bufo*. *Nature* 274: 683-685.

Duellman WE and Pyles RA (1983) Acoustic resource partitioning in anuran communities. *Copeia* 1983: 639-649.

Duellman WE and Trueb L (1994) Biology of Amphibians. Baltimore: The Johns Hopkins University Press.

Endler JA (1993) Some general comments on the evolution and design of animal communication systems. *Phil Tran R Soc London B* 340: 215-225.

Gerhardt HC (1991) Female mate choice in treefrogs: static and dynamic acoustic criteria. *Anim Behav* 42: 615-636.

Halliday TR (1983) The study of mate choice. In: P. Bateson (ed), Mate choice, Cambridge Univ. Press, Cambridge, pp 3-52.

Hoglund J and Robertson JG M (1987) Random mating by size in a population of common toads (*Bufo bufo*). *Amphibia-Reptilia* 8: 321-330.

Kang EJ and Youn CH (1994). The settlement and distribution of the introduced bullfrog, *Raja catesbeiana*, in Korea. *nature conservation report* 13: 231-250.

Kang YS and Yoon IB (1975) Illustrated encyclopedia of fauna and flora of Korea Vol 17 Amphibia'Reptila. The Korean ministry of education. Seoul.

Lande R and Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37: 1210-1226.

Marco A, Kesecker JM, Chivers DP, and Blaustein AR (1998) Sex recognition and mate choice by male western toads, *Bufo boreas*. *Anim Behav* 55: 1631-1635.

Olson DH (1989) The ecological and behavioral dynamics of breeding in three sympatric anuran amphibians. Ph.D. thesis, Oregon State University.

Ryan MJ (1985) The Tungara Frogs: A Study in Sexual Selection and Communication. Univ Chicago Press, Chicago and London.

Ryan MJ (1990) Sensory systems, sexual selection, and sensory exploitation. *Oxford Surv Evol Biol* 7: 157-195.

Schneider H, Sofianidou TS, and Kyriakopoulou-Sklavounou (1984) Bioacoustic and morphometric studies of water frogs (genus *Rana*) of Lake Ioannina in Greece, and description of a new species (Anura, Amphibia). *J Zool Syst Evolut-forsch.* 22: 349-366.

Schwartz JJ (1989). Graded aggressive calls of the spring peeper, *Pseudacris crucifer*. *Herpetologica* 45: 172-181.

Sherman PW, Reeve HK, and Pfennig DW (1997) Recognition systems. In: Krebs JR and Davis NB (eds.), Behavioural Ecology: An Evolutionary Approach, Oxford, Blackwell, pp 69-96.

Sung HC, Park OH, Kim SK, Park DS, and Park SR (2007) Abundance and breeding migration of the Asian Toad (*Bufo gargarizans*). *J Ecol Field Biol* 30: 287-292.

Sullivan BK (1983) Sexual selection in Wood house's toad (Bufo woodhousei). II. Female choice. *Anim Behav* 31: 1011-1017.

Wells KD (1988) The effect of social interactions on anuran vocal behavior. In: Fritzsch B, Ryan MJ, Wilczynski W, Hetherington TE, and Walkowiak W (eds), The Evolution of the Amphibian Ear, New York, John Wiley, pp 433-454.

Yang SY, Kim JB, Min MS, Suh JH, and Kang YJ (2001) Korean amphibians. Academic Press.

[Received May 9, 2008[accepted June 13, 2008]