

Influence of Amplexus on Phonotaxis in the Cricket Frog *Acris crepitans blanchardi*

KLAUDIA WITTE, KUEI-CHIU CHEN, WALTER WILCZYNSKI, AND MICHAEL J. RYAN

We investigated phonotactic responses of amplexed and unamplexed females to different male calls in the Cricket Frog *Acris crepitans blanchardi*. A significantly higher proportion of amplexed females responded to male calls than did unamplexed females. When unamplexed females responded to male calls their phonotactic preferences did not differ from that of amplexed females. Amplexed and unamplexed females did not differ in their latency to respond to different male calls. Thus, the use of unamplexed females in phonotaxis experiments does not seem to bias any interpretation of preferences and would increase the number of testable females available.

FEMALE phonotaxis experiments with anurans have proven to be a reliable bioassay for assessing female preferences for male advertisement calls. There is no consistent protocol, however, regarding the use of amplexed females. In some studies, only females clasped by males, that is, in amplexus, were used in phonotaxis experiments (e.g., Gerhardt, 1981; Ryan et al., 1992; Bodnar, 1996), whereas in other studies, females not in amplexus when collected were tested (e.g., Martof, 1961; Martof and Thompson, 1964; Lopez and Narins, 1991). Because there is some evidence that amplexus might change ovulatory state (Bragg, 1941) and that hormonal state might influence female phonotaxis (Schmidt, 1984, 1985), it is reasonable to suspect that amplexed and unamplexed females might differ in their responsiveness and/or preferences.

Comparisons among species and studies suggest that amplexed females are more responsive in choice tests than are unamplexed females (amplexed females: 93%, Gerhardt, 1981; 88%, Arak, 1988; 90%, Ryan et al., 1992; unamplexed females: 33%, Martof and Thompson, 1964; 37%, Lopez and Narins, 1991). These studies differed in many ways, however, including the use of different species. In this analysis, we compared both the responsiveness and the preference for different stimuli in female Cricket Frog (*Acris crepitans blanchardi*) captured in amplexus or alone. We analyzed data from previous dichotomous choice experiments with females from two different populations and three different years.

MATERIALS AND METHODS

We collected female *A. c. blanchardi* at night from a population at a permanent pond in a pine forest on Stengl Ranch in Bastrop County,

Texas, between 26 March and 29 June 1992 and between 26 April and 30 June 1993. In 1996, we collected female *A. c. blanchardi* at night at a semipermanent pond in an open grassland on Gill Ranch, Travis County, Texas, between 1 June and 30 June. We recorded whether females were in amplexus when collected.

In 1992 and 1993, females were tested in a sound choice chamber (2 m × 2 m × 1.60 m) under dim red light near the field site. The stimuli were broadcast with a Marantz PMD 420 tape recorder through two ADS L200C speakers. The speakers were placed directly opposite one another on the floor in the middle of the side walls, 2 m apart. The stimuli lasted 8 sec and were presented alternately at an intensity of approximately 75 dB SPL (re: 20 μPa) at approximately 22 C measured at the center of the chamber. Each test lasted 15 min. Before testing, we placed the female under a cone in the middle of the choice chamber and played the stimuli during a 2-min acclimation period. After raising the cone, we observed the female's movement and measured the time until she approached one of the speakers. A female was scored as making a choice if she approached within 10 cm of a speaker within 15 min. Each female was tested once with a stimulus pair. After testing, we measured female body size (SVL) to the nearest 0.5 mm, toe-clipped females and returned them to the site where they were collected.

In 1996, we tested females in a sound choice chamber (1.83 m × 2.70 m × 1.80 m) at the University of Texas. Tests were conducted under dim red light. The stimuli were broadcast from a computer each through a separate channel via an amplifier to two wide-frequency range speakers (Cambridge SoundWorks) placed directly opposite one another on the floor in the middle of the side walls, 2.70 m apart. The stimuli

lasted 8 sec and were presented alternately at an intensity of 80 dB SPL (re: 20 μ Pa) at 24 C measured in the center of the chamber. Each test lasted 20 min. Before testing we placed the female under a cone in the middle of the chamber and played the stimuli for 2 min. After lifting the cone with a remote device, we observed the female's movement and measured the time until she approached one of the speakers. A positive response was recorded when she approached within 20 cm of a speaker within 20 min. Each female was tested once to a stimulus pair. After testing, we measured body size (snout-vent length) and kept females in the lab for other experiments.

In 1992 and 1993, females could choose between a synthetic call from their own population and a synthetic call from the Gill population and combined calls with call parameters from the Gill population and another (Sabine) population. The calls varied in four call parameters: the frequency, the individual call, number of calls, and the call rate. We tested a typical Bastrop call against a Sabine call with the frequency of a Bastrop call, a typical Bastrop call against a call with the frequency and individual call of the Bastrop population and the number of calls and call rate of a typical Sabine call, and a typical Bastrop call against a typical Gill call with the frequency of a Bastrop call. Additionally, in 1993, we tested three Bastrop calls differing in the number of pulse groups and in the number of pulses (one pulse group, five pulses; one pulse group, 11 pulses; two pulse groups, 11 pulses) against each other. In all of these different experiments, we used a similar number of amplexed and unplexed females. On average, we used 19.5 amplexed females and 18.7 unplexed females per experiment (Mann Whitney U -Test $n = m = 7$, $z = -0.77$, $P = 0.43$). Because we were interested in investigating a general difference in phonotactic response of amplexed and unplexed females and because the number of amplexed and unplexed females was similar in our experiments, we combined the data of all amplexed females used in these experiments and data of all unplexed females for our analysis.

In 1996, the tests were originally designed to determine the threshold at which females are able to exhibit phonotaxis to the advertisement call in a noisy environment. The stimuli were white noise created with the software program SIGNAL Version 2.2[®] (Engineering Design, Belmont, MA) and three natural male call groups from the Gill population. The white noise and the call group used in one test had the same average energy (measured as the av-

erage root mean square of a signal) and had been sampled at a rate of 15625 Hz. Each test had three parts. First, we played white noise out of one speaker and a natural call group alternately out of the other speaker. If the female chose the natural call group, we presented white noise and a natural call group embedded in white noise in different call/noise ratios in the second part. If a female did not respond to the call group in the second part, we repeated part one (white noise against the natural call group) to determine whether the female was still motivated to exhibit phonotaxis. For testing the responsiveness of amplexed females and unplexed females, we analyzed only the first part (white noise vs a natural call group from the Gill population) of this experiment. Using data from the experiments described above, we measured the number and proportion of amplexed and unplexed females responding to male advertisement calls, their preference when responding, and the latency to exhibit such a choice.

RESULTS

Initially, it was important to determine that unplexed females at the pond were mature females and neither juveniles nor males. In *A. c. blanchardi*, females and juveniles lack a disintended and pigmented vocal sac, as did all the individuals we classified as females. Also, as in most anuran species (Shine, 1979), female *A. c. blanchardi* are much larger than males and juveniles. Table 1 shows that unplexed females were of similar size to amplexed females in both populations in all three years. Thus we assume that the individuals classified as unplexed females were indeed mature females.

A significantly higher proportion of amplexed females versus unplexed females responded in phonotaxis choice tests in both populations and in all three years (Table 2). When unplexed females responded, however, their phonotactic preference did not differ from that of amplexed females. In 1996, all five amplexed females chose the natural call. Among unplexed females, two females approached the white noise, and four females approached the natural call (Fisher's exact test: $P = 0.454$). In 1992 and 1993, amplexed and unplexed females showed the same preferences to the stimuli as well (Fisher's exact test: 1992, $P = 1.0$; 1993, $P = 0.67$). Finally, amplexed and unplexed females did not differ in their latency to respond to different call variants (Table 3). All amplexed females of the Gill Ranch population oviposited the night following collection. In

TABLE 1. AVERAGE BODY SIZE ($\bar{x} \pm SD$) IN MILLIMETERS OF UNAMPLEXED AND AMPLEXED FEMALES FROM THE BASTROP POPULATION AND THE GILL POPULATION IN THREE DIFFERENT YEARS (n = SAMPLE SIZE). Mann-Whitney U-test; all P-values are two-tailed.

Site	SVL (mm) unamplexed females	SVL (mm) amplexed females	P-value
Bastrop, 1992	25.6 \pm 1.83 (n = 73)	26.2 \pm 1.58 (n = 28)	0.18
Bastrop, 1993	24.79 \pm 1.32 (n = 31)	24.79 \pm 1.5 (n = 36)	0.9
Gill Ranch, 1996	24.32 \pm 2.26 (n = 44)	24.18 \pm 1.76 (n = 6)	0.9

contrast, none of the unamplexed females oviposited during the two weeks after capture.

DISCUSSION

In *A. c. blanchardi*, amplexed females tend to be significantly more responsive in phonotaxis choice tests than unamplexed females. Unamplexed females that exhibited phonotaxis, however, showed preferences similar to amplexed females. In phonotaxis experiments such as these, the use of unamplexed females does not seem to bias any interpretation of preferences but would increase the number of females. These results were consistent over the three field seasons under two somewhat different experimental procedures and several pairs of test stimuli. The consistency suggests that responsiveness and preferences of unamplexed females was not an artifact of one particular experimental situation or pair of test stimuli. It is possible, however, that amplexus might influence preferences to other stimuli not tested here.

We defined "amplexed" and "unamplexed" solely by the state of the female at the time of capture. We did not obtain data on the reproductive history of the females prior to capture, and therefore we cannot exclude the possibility that our unamplexed females had experienced amplexus earlier in the breeding season. In 1992, we recaptured seven females. Two were in amplexus both times with a period of 14 and 16 days between captures. Thus *A. c. blanchardi* can reproduce several times within a season, and it is possible that some of our females had mated

previously. However, we have no reason to believe that a previous mating was more likely for unamplexed or amplexed females.

Murphy and Gerhardt (1996) investigated whether female *Hyla gratiosa* in amplexus discriminate between meaningful stimuli in mate choice tests as well as females before entering amplexus. They tested 20 females before and after entering amplexus. Six other unamplexed females, however, did not respond in the first test and were therefore not used in the second test. Their results indicate that *H. gratiosa* females are slightly less responsive before amplexus than after amplexus. This is similar to what we found in *A. c. blanchardi*. In the 20 females that were tested twice, Murphy and Gerhardt (1996) found no difference in the ability to discriminate between the given stimuli. This indicates that in *H. gratiosa* amplexus does not affect the ability to discriminate between given stimuli in phonotaxis experiments. This is also similar to what we found in *A. c. blanchardi*. In *A. c. blanchardi*, all unamplexed females that responded in tests chose a stimulus after a similar amount of time as did amplexed females, and we found no difference in the preference for a stimulus between amplexed and unamplexed females. Thus, the most important difference between amplexed and unamplexed cricket frog females is the difference in their responsiveness to male advertisement calls in phonotaxis experiments.

Our results suggest that there is a physiological difference between amplexed and unamplexed females related to motivation, but there is no difference in how females perceived the

TABLE 2. RESPONSIVENESS OF UNAMPLEXED AND AMPLEXED FEMALES IN PHONOTACTIC EXPERIMENTS. Shown are number of females responding to given stimuli and the total number of females used in an experiment. In parentheses is the proportion of females responding in percent. Fisher's exact test; all P-values are two-tailed.

Site	Unamplexed females	Amplexed females	P-value
Bastrop, 1992	19 of 73 (26.03)	18 of 28 (64.2)	0.0003
Bastrop, 1993	12 of 31 (38.7)	24 of 36 (66.66)	0.028
Gill Ranch, 1996	6 of 48 (12.5)	5 of 6 (83.33)	0.003

TABLE 3. AVERAGE LATENCY ($\bar{x} \pm SD$; MIN) OF RESPONSE TO ADVERTISEMENT CALLS IN UNAMPLEXED AND AM-
PLEXED FEMALES. Mann-Whitney *U*-test; all *P*-values are two-tailed.

Site	Unplexed females	Amplexed females	<i>P</i> -value
Bastrop, 1992	7.66 \pm 3.32 (n = 19)	7.26 \pm 3.34 (n = 18)	0.67
Bastrop, 1993	8.31 \pm 2.93 (n = 12)	7.17 \pm 4.25 (n = 24)	0.32
Gill Ranch, 1996	11.64 \pm 6.7 (n = 4)	5.57 \pm 5.24 (n = 5)	0.14

signals used in these tests. Cause and effect is not clear, however. Amplexus might be a sign of a female who was motivated to mate. Alternatively, the male clasping the female might induce physiological change that causes amplexed females to be more responsive in phonotaxis experiments. Amplexus has been ascribed an especially important role in stimulating ovulation in frogs. In several frog species, ovulation occurs only when the female has been clasped by the male (Bragg, 1941). Therefore, amplexed females that have lost their mate before fertilizing eggs have to find another mate in the same night; otherwise they will lose their nonfertilized eggs. The fact that all of the amplexed females but none of the unplexed females lost their eggs further suggests that amplexed and unplexed females were indeed in different physiological conditions, although we do not know precisely how these females differed. It is well known that hormones like prostaglandin influence phonotactic responses in females (Schmidt, 1984, 1985). It is also possible to increase significantly phonotactic responses in nongravid females by injection of progesterone or arginine vasotocin (AVT) or both, as Schmidt (1985) has shown in *Bufo americanus*. Boyd (1994) showed that AVT injection influenced call phonotaxis in female Bullfrogs (*Rana catesbeiana*). The AVT injection significantly decreased the time required for females to reach a call source as well as the latency of females to leave the starting position during call playback. Harvey et al. (1997) found that the concentration of sexual hormones significantly changed throughout the reproductive season in females of the Desert Spatefoot Toad *Scaphiopus couchii*. Plasma testosterone and estradiol (E_2) were greatest in prebreeding and amplexed females, whereas progesterone and dihydrotestosterone (DHT) were highest only in unplexed females. Unreceptive females of *Rana pipiens* emit a release call when clasped by a sexually active male. This release call can be inhibited by injection of prostaglandin and arginine-8 vasotocin (Diakow and Nemiroff, 1981). This result suggests that both vasotocin and prostaglandin may be

involved in the manifestation of female mating behavior in this species.

We do not know how the amplexed and unplexed cricket frog females we tested differed in their hormonal state, but it seems obvious that they were in different states. Understanding how female responses to mating signals covary with physiological state would contribute to a deeper understanding of mechanisms guiding female mate choice.

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LITERATURE CITED

- ARAK, A. 1988. Female mate selection in the natterjack toad: active choice or passive attraction? *Behav. Ecol. Sociobiol.* 22:317-327.
- BODNAR, D. A. 1996. The separate and combined effects of harmonic structure, phase, and FM on female preferences in the barking treefrog (*Hyla gratiotosa*). *J. Comp. Physiol. A* 178:173-182.
- BOYD, S. K. 1994. Arginine vasotocin facilitation of advertisement calling and call phonotaxis in bullfrogs. *Horm. Behav.* 28:232-240.
- BRAGG, A. N. 1941. Observations on the ecology and natural history of Anura. 8. Some factors in the initiation of breeding behavior. *Turtox News* 19:10-12.
- DIAKOW, C., AND A. NEMIROFF. 1981. Vasotocin, prostaglandin, and female reproductive behavior in the frog, *Rana pipiens*. *Horm. Behav.* 15:86-93.
- GERHARDT, H. C. 1981. Mating call recognition in the barking treefrog (*Hyla gratiotosa*): responses to synthetic calls and comparisons with the green treefrog (*Hyla cinerea*). *J. Comp. Physiol.* 144:17-25.
- HARVEY, L. A., C. R. PROPPER, S. K. WOODLEY, AND M. C. MOORE. 1997. Reproductive endocrinology of the explosively breeding desert spadefoot toad, *Scaphiopus couchii*. *Gen. Comp. Endocrinol.* 105:102-113.
- LOPEZ, P. T., AND P. M. NARINS. 1991. Mate choice in

- the Neotropical frog, *Eleutherodactylus coqui*. *Anim. Behav.* 41:757–772.
- MARTOF, B. S. 1961. Vocalization as an isolating mechanism in frogs. *Am. Midl. Nat.* 65:118–126.
- , AND E. F. THOMPSON JR. 1964. A behavioral analysis of the mating call of the chorus frog, *Pseudacris triseriata*. *Ibid.* 71:198–209.
- MURPHY, C. G., AND H. C. GERHARDT. 1996. Evaluating the design of mate-choice experiments: the effect of amplexus on mate choice by female barking treefrogs, *Hyla gratiosa*. *Anim. Behav.* 51:881–890.
- RYAN, M. J., S. A. PERRILL, AND W. WILCZYNSKI. 1992. Auditory tuning and call frequency predict population-based mating preferences in the cricket frog, *Acris crepitans*. *Am. Nat.* 139:1370–1383.
- SCHMIDT, R. S. 1984. Mating call phonotaxis in the female American toad: induction by hormones. *Gen. Comp. Endocrinol.* 55:150–156.
- . 1985. Prostaglandin-induced mating call phonotaxis in female American toad: facilitation by progesterone and arginine vasotocin. *J. Comp. Physiol. A* 156:823–829.
- SHINE, R. 1979. Sexual selection and sexual dimorphism in the Amphibia. *Copeia* 1979:297–306.
- (KW, MJR) DEPARTMENT OF ZOOLOGY, UNIVERSITY OF TEXAS, AUSTIN, TEXAS 78712-1064; (WW) DEPARTMENT OF PSYCHOLOGY, UNIVERSITY OF TEXAS, AUSTIN, TEXAS 78712-1064; AND (K-CC) DEPARTMENT OF BIOLOGY, NEW YORK UNIVERSITY, NEW YORK, NEW YORK 10003. PRESENT ADDRESS: (KW) LEHRSTUL FÜR VERHALTENSFORSCHUNG, UNIVERSITÄT BIELEFELD, POSTFACH 100131, 33501 BIELEFELD, GERMANY. E-mail: (KW) klaudia.witte@biologie.uni-bielefeld.de. Send reprint requests to KW. Submitted: 22 Oct. 1998. Accepted: 11 May 1999. Section editor: A. H. Price.