

Study on the function of female calls in *Pelophylax nigromaculatus* and *Pelophylax porosus brevipodus*.

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Introduction

Most anurans show distinct sexual differences in vocalization. Males produce complex calls and attract females, whereas females generally remain silent. Thus, in studies of anuran reproductive behavior, much attention has been paid to male vocalization and female preference in male calls. However, female vocalizations have been reported in several anuran species. Here, I reported novel female calls of Japanese pond frogs, *Pelophylax nigromaculatus* and *Pelophylax porosus brevipodus*, and investigated the function of those calls. I overviewed the functions of other frog species hitherto reported, and proposed a novel function for the female calls of *P. nigromaculatus* and *P. p. brevipodus*. I also discussed a possible role of their female calls in relation to the breeding ecology of the two species.

Materials and Methods

Pelophylax p. brevipodus and *P. nigromaculatus* are pond frogs in Japan. Their

reproductive seasons, which continue from May to June, largely overlap. They coexist in some areas of Japan, and syntopic breeding has been reported. Subjects were collected in the rice field in Kyoto Prefecture, where the two species coexist.

First, encounter experiments were conducted to record female calls of the two species. I recorded a total of 149 calls from 13 females of *P. nigromaculatus* and a total of 80 calls from eight females of *P. p. brevipodus*, and conducted acoustic analysis to compare their acoustic characters.

Second, encounter experiments between males and females of *P. nigromaculatus* were conducted, and behavior of them were recorded. A total of 27 experiments were conducted. After the experiments, I dissected females to observe their ovary and oviduct to examine their reproductive condition. To examine whether female calls affect the probability of success in amplexus by males, behavioral sequences in which males attempted amplexus with females were analyzed in relation to the occurrence of female calls. To clarify the function of female calls, situation in which female calls were emitted was selected, and male responses to these calls were analyzed.

Third, interspecific and intraspecific encounter experiments were conducted to observe female calls and male reaction against female calls in the two species. A total of 52 experiments were conducted; 11 experiments were conducted between male *P.*

nigromaculatus and female *P. p. brevipodus*, 14 experiments between male and female *P. p. brevipodus*, 14 experiments between male and female *P. nigromaculatus*, and 13 experiments between male *P. p. brevipodus* and female *P. nigromaculatus*. In addition, semi-field observations were conducted to observe intraspecific amplexus sequence in the two species.

Results

In acoustic characters, there were no clear differences between the two species. Principal component analysis revealed that female calls of *P. p. brevipodus* were largely overlapped with those of *P. nigromaculatus*.

In *P. nigromaculatus*, no female calls were detected in any of the 19 amplexus sequences. A total of 15 calling females and 32 female calling sequences were observed. The direction of male locomotion was significantly different between before and after female calls. The ratio of females that emitted calls at least once was significantly higher in females without eggs than in those with eggs.

In *P. nigromaculatus*, the number of female calls and the ratio of calling females observed when encountering with heterospecific males were significantly lower than those observed with conspecific males. In contrast, in *P. p. brevipodus*, the number of

female calls and the ratio of calling females observed when encountering with heterospecific males were significantly higher than those observed with conspecific males. Males of *P. nigromaculatus* tended to move away from females of *P. p. brevipodus* after female calls. The intraspecific amplexus sequences were different between the two species.

Discussion and Conclusion

The results of the second experiment, that is, (1) no calls were observed in the amplexus sequence and no amplexus attempt was observed in the female calling sequence, (2) males moved away from females after the females calls, and (3) females without eggs emitted more calls than females with eggs, indicate that female calls of *P. nigromaculatus* work for avoiding sexual coercion from males.

The results of the third experiment demonstrated that the function of female calls were different between *P. nigromaculatus* and *P. p. brevipodus*. In *P. nigromaculatus*, female calls mainly work for intraspecific communication. In contrast, in *P. p. brevipodus*, female calls mainly work through interspecific communication. The interspecific female calls of *P. p. brevipodus* probably have a function to drive approaching males of *P. nigromaculatus* away from the females because males of *P. nigromaculatus* tended to

move away from the females after their calls. I presume that males of *P. nigromaculatus* misrecognize the female calls of *P. p. brevipodus* as those of *P. nigromaculatus* and exhibit the reaction same as that to conspecific female calls, because the acoustic characters of female calls of these species and sub-species are quite similar. In general, there could be strong effects of reproductive interference between closely related species, which could consequently cause displacement of species by related species. The female calls of *P. p. brevipodus* emitted to male *P. nigromaculatus* may enable their sympatric distribution by reducing reproductive interference between them.