Reproductive Function of the Body and Tail Undulations of *Hynobius leechii* (Amphibia: Hynobiidae): A Quantitative Approach

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Abstract: To clarify the reproductive function of vibration signals in *Hynobius*, which has externally fertilized eggs, we quantitatively analyzed the body and tail undulations of male Korean salamanders (*Hynobius leechii*) in sixteen mating events. One large and one small male, and one female were used in each mating event. We analyzed behaviors recorded over a total of 3 hrs for each mating event; 2 hrs before and 1 hr after female's oviposition. Males touched females using their snouts or body trunk throughout the entire mating periods, but females touched males increasingly more after approximately 1 hr before oviposition. Males conducted body undulations more than 50 times per 10 minutes at a mean frequency of 0.64 Hz. Large males conducted more body undulations than small males, particularly on the tree twig where females attached their egg sacs. Males responded to other males' body undulation throughout the mating period by orienting their head towards, approaching, and touching the undulating male. Females only responded for about 10-20 min before ovipositing, and most responses were directed to the large male's body undulation. Males conducted tail undulations 3.0 times per 10 min at a mean frequency of 1.7 Hz and most tail undulations occurred after one male bit the other male. These results suggest that body undulations function in both male-female and male-male interactions, while tail undulations mainly functions in male-male competition. Also, male *H. leechii* appear to actively attract females, while females respond to the males only at times close to oviposition.

Key words: lateral line, mechanosensory, body undulation, tail undulation, vibration, salamander, urodela

INTORDUCTION

Vibration signals play an important role in the daily lives of

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many animals, and are involved in self, individual, and species recognition, foraging, and reproduction (Vogel and Bleckmann, 1997; Fritzsch and Neary, 1998; Baker and Montgomery, 1999; Hill, 2001; Park et al., 2008). The reproductive function of vibration signals has been studied in several taxa such as rotifers, spiders, and fish. Successful reproduction of a rotifer occurs only when both chemical and vibration cues are available (Joanidopoulos and Marwan, 1999). In spiders, vibration signals are generated by males that initiate mating with females (Maklakov et al., 2003). In salmon, successful mating does not occur when either the male or female vibration system is disrupted (Satou et al., 1994). Even though developmental and morphological aspects and functions of the mechanosensory system used for foraging in amphibians have been intensively studied, the reproductive function of vibration signals is not well understood.

Hynobius leechii, like all hynobiids, uses external fertilization (Salthe, 1967; Houck and Arnold, 2003). Fertilization is preceded by a number of courtship displays (e.g., snout contact, smelling, tail undulation, chin rubbing, fertilization, and post-fertilization; Park and Park, 2000). *Hynobius leechii* uses two different types of tail undulations which generate water currents. In the first type of tail undulation, a male stiffens his body intermittently so that his tail and the hind part of his body move from side to side in a movement termed a body undulation. The body undulation has been reported in several different species such as *H. dunni*, *H. nebulosus*, and *H. leechii* (Usuda, 1993; Park et al., 1996). In both field and laboratory experiments, males and females responded to the water currents that were generated by male body undulations in *H. nebulosus* (Usuda, 1995) and *H. leechii* (Park et al., 2008). During the second type of tail undulation, a male waves only the tip of his tail so that the distal third of the

tail waves, but the hind part of the body does not move. This movement is termed a tail undulation. Tail undulations have been observed in several other species such as *H. takedai* (Tanaka, 1986), *H. nebulosus* (Tanaka, 1989), and *H. leechii* (Park et al., 1996). Males often display this tail undulation before and after attacking other males (Park et al., 1996). In spite of these previous studies, there has been no quantitative description of the body and tail undulations in *Hynobius*, which makes it difficult to clarify the reproductive functions of vibration signals.

In this study, we quantitatively analyzed the body and tail undulations of male *H. leechii*, which generate water currents, and both male and female responses to the male's undulations in sixteen mating events in order to clarify the reproductive function of vibration signals in *Hynobius*.

MATERIALS AND METHODS

Animal collecting and maintenance

For the study, between late February and early March 2008, we collected 50 male and 20 female *H. leechii* from three small ponds (N 37°46'19", E 127°48'56") located in the Research Forests of Kangwon National University in Chuncheon, Kangwon, South Korea using a hand net. Salamanders were kept in refrigerated boxes (5-10°C; 55 cm long, 35 cm wide, 35 cm high) for transportation to the lab. Upon arrival at the laboratory, salamanders were separated on the basis of their sex, determined by the wide tail of males and the presence of eggs in the abdomen of females. Males and females were kept apart in aquaria (33 cm long, 20 cm wide, 25 cm high) which were placed in an environmental chamber containing approximately 25 liters of aged tap water with a density of no more than 10 individuals per tank. Salamanders were fed commercial blood worm, *Limnodrilus gotoi*, and half of the water was changed once every third day. The water temperature of the aquaria was kept between 6 and 8°C and the photoperiod was modeled after the local photoperiod of approximately 12:12 (L:D). Hiding places were provided in the form of wet paper towels and dead leaves collected from field ponds. All males used in this study were in full breeding condition, as shown by swollen cloacae and wide tailfins.

Mating experiments

To study the reproductive function of body and tail undulations, we quantitatively analyzed mating behaviors of sixteen mating events. Each mating event was made by one small male (5.62 cm±0.10 Snout-vent length, 5.39 g± 0.21 Body weight, n=16), one large male (6.43 cm±0.09 SVL, 8.85 g±0.25 BW, n=16), and one female (6.64 cm± 0.08 SVL, 6.61 g±0.24 BW, n=16). In a previous study, two males whose SVL differed by more than 0.5 cm were found to facilitate mating processes (Park and Park, 2000).

Mating experiments in the current study were conducted between March 14th and 19th, 2008. To provide suitable mating conditions, we covered the bottom of a mating aquarium (33 cm long, 20 cm wide, 25 cm high) with a layer of sand with a depth of approximately 3 cm and placed an inverse V-shaped tree twig (42 cm long, 1 cm diameter) between the diagonal corners of the experimental aquarium to provide egg-attachment sites. Approximately half of the twig (at the center) was 1-2 cm above the water surface and the other half (at each corner) was submerged. Tanks were filled with aged tap water to a depth of approximately 4.5 cm. Mating started at 2200 h under <0.1 lux light and was recorded with a low-light B/W camera (Model: 10IR LED, SLCC) and a video recorder (Daewoo) until 0900 h the following morning. No males or females were used more than once in the mating experiments.

Data analysis

All behaviors were analyzed for 2 hrs before female's oviposition and 1 hr after the oviposition in 10 min intervals (for a total of 3 hrs and 18 10-min intervals). When a behavior continued over two 10 min intervals, we considered that behavior to occur in both intervals. However, to calculate the frequency of the behavior, we considered the display to occur only once.

Analyzed behaviors were largely categorized into physical contacts between males and females, male body undulations and both male and female responses to these undulations, male tail undulations and biting, and oviposition and fertilization. For physical contacts, we counted the number of short- and long-touches occurring between males and females. If an individual actively approached the other individual before touching, we considered the individual that was approached to be the subject of the touch. Short-touches were defined as contact between the tip of an individual's snout and the head, body trunk, cloacal region, or tail of the other individual for less than 1 sec. In this case, the tip of the snout of an individual directly and must clearly contact the body parts of the other individual. Long-touches were considered to occur when the body parts (mainly main body trunk) of an individual contacted the body parts of the other individual for more than 10 sec. An individual's snout does not make contact with the body parts of the other individual during this type of touch. Other contacts were not quantified in this study because those contacts often occur when two individuals scrape along so that it is hard to define what constitutes a contact in those cases.

For males, we recorded the number of body undulations and the place where a male undulated. Based on this data, we calculated the frequency of body undulations of males. To measure the responses of both males and females to male body undulations, we counted the number of head

orientations towards an undulating male, the number of approaches within a 7 cm diameter circle centered on the cloaca of the undulating male, and the number of times the undulating male was touched. Head orientation was identified when a test male suddenly turned his head towards an undulating male salamander at more than a 45° angle from the line of his body axis. An approach within the circle was considered to occur when the snout of a response male entered into the circle. Contact with an undulating salamander was defined as a touch between the snout of a test male and an undulating male salamander. To compare the intensity of the response of males and females to male body undulations, we developed a response intensity index by assigning 1 point for a head orientation, 2 points for an approach, and 3 points for a touch because we believed that touching an undulating male is the most intense response that can be made to the body undulation of males. If a male made contact with an undulating male, he received 3 points for the response, while if a male only head-oriented toward the undulating male, he was assigned only 1 point. We also identified the subsequent behaviors of a response male after touching an undulating male as being either avoidance, disturbance, or biting. Avoidance was defined to occur when the response male passed by the undulating male without any distinctive behaviors after touching the undulating male. Disturbance was identified when a response male disrupted the body undulation of the undulating male by nudging, digging in, body-undulating at the same site, or staying close for more than 3 sec while watching the undulating male. Biting was identified when the response male bit any parts of the undulating male.

For tail undulation and biting, we counted the number of tail undulations and also determined if a male bit the other male before and/or after his tail undulation. Based on the data, we also determined the frequency of tail undulations. For biting, we counted the number of bites and also determined which parts of the body (the head, body trunk, tail, or limbs) of the male bit.

For oviposition and fertilization, we counted the number of female approaches to a tree twig and also determined who was on the twig when the female approached. For fertilization, we determined who fertilized the eggs, the number of fertilizations per pair of egg sacs, and the duration of each fertilization display.

Statistical analysis

Since most data did not pass the normality test (Kolmogorov-Smirnov, $P<0.05$), we conducted non-parametric statistical analyses. To determine if the responses of large and small males or female to small and large males were different, we applied a repeated one-way ANOVA test. If the change over the mating period was significant, we compared the data before and after the female's oviposition using a Wilcoxon-signed rank test as a post-hoc test. To test differences in the number and frequency of body and tail undulations and the time of fertilization between large and small males, we used a Mann-Whitney U test. A Chi-square test was applied to determine if large and small males act differently after touching an undulating male and whether or not large males bit small males more frequently. All statistical analyses were two-tailed and were performed using SPSS version 11.0 (SPSS Inc., Chicago Illinois, USA). All values are reported as means±SE.

RESULTS

Touches between males and females

The number of females touched by two males per 10 min interval changed significantly over the mating period ($F_{1,17}=1.81$, $P=0.024$), but the number of large and small males touching a female per 10 min interval were not significantly different before and after the female's oviposition (for large males, the number of touches before oviposition was 2.7 ± 0.44 and after oviposition was 2.34 ± 0.40; $Z=0.957$, $n=16$, $P=0.339$; for small males, the number of touches before oviposition was 1.26 ± 0.24 and after oviposition was 1.14 ± 0.22; $Z=0.078$, $n=16$, $P=0.938$). Large males touched females more often than small males did (for large males, the number of touches per 10 min interval was 2.59 ± 0.40; for small males the value was 1.22 ± 0.22; $F_{1,17}=9.06$, $P=0.005$). Females touched large (0.22 ± 0.05) and small (0.29 ± 0.06) males at a similar rate during 10 min intervals ($F_{1,17}=2.68$, $P=0.112$), but changes over the mating period were significant ($F_{1,17}=2.88$, $P<0.001$, Fig. 1). Females touched both large (before 0.28 ± 0.07, after 0.08 ± 0.05; $Z=2.133$, $n=16$, $P=0.033$) and small (before 0.38 ± 0.09, after 0.10 ± 0.04; $Z=2.847$, $n=16$, $P=0.004$) males more often before oviposition than after oviposition.

Male body undulations and responses of males and females

The number of body undulations per 10 min by large males changed significantly over a mating period ($F_{1,17}=4.31$, $P<0.001$), but that of small males did not ($F_{1,17}=1.20$, $P=0.26$) (Fig. 2). Large males conducted more body undulations per 10 min before female ovipositions than after ovipositions both on tree twigs (before 116.24 ± 21.38, after 63.68 ± 14.20; $Z=2.95$, $n=16$, $P=0.003$) and on the ground (before 50.09 ± 15.40, after 21.53 ± 6.75; $Z=2.53$, $n=16$, $P=0.011$). Large males conducted more body undulations on tree twigs per 10 min than did small males (for large males the number of undulations was 98.72 ± 17.99; for small males, this value was 30.83 ± 14.58; $F_{1,17}=3.21$, $P=0.001$) and also conducted more undulations on the ground (large male conducted 40.57 ± 12.34 undulations; small males conducted 7.19 ± 4.03; $F_{1,17}=3.45$, $P<0.001$). In comparisons between

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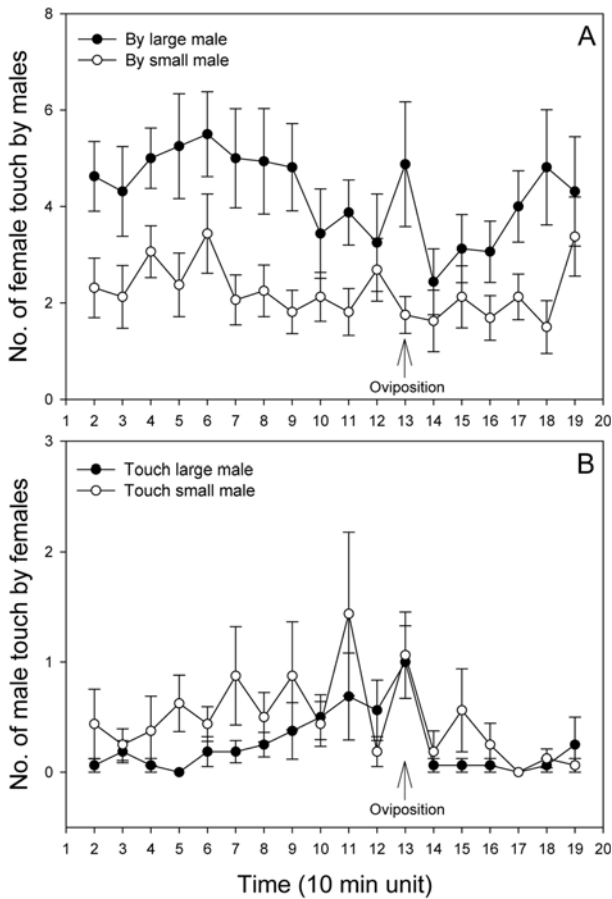
Fig. 1. Number of physical contacts between males and females: female contact by large and small males (A); and male contact by females (B). Contacts were divided into short- and long-touches. A short touch occurred when the tip of snout of an individual contacted body parts of the other individual for approximately 1 sec. A long touch occurred when body parts of two individuals contacted more than 10 sec.

Fig. 2. The number of body undulations by males is presented based on male size (large and small) and the location at which males conducted body undulations (one the tree twig and on the ground).

the places where males conduct body undulations, large males were found to conduct more body undulations per 10 min interval on tree twigs than on the ground ($F_{1,17}=7.10$, P $=0.012$), but the number of undulations was not different in small males ($F_{1,17}=2.44$, P=0.13). For each body undulation display, large males (n=990) undulated their bodies a mean of 40.04±1.91 times and small males (n=251) a mean of 51.46 ± 7.13 times (Z=1.60, n=1241, P=0.110). The mean frequency of body undulations of large and small males was similar (0.66±0.02 Hz, n=990 for large males and 0.62 ±0.03 Hz. n=251 for small males), respectively (Z=1.12, n =1241, P=0.265). On tree twigs, large males stayed 3.6 times longer than small males (for large males, 47,315 sec, n=294; for small male 13,141 sec, n=78).

There was no difference in the response intensity (the sum of the number of head orientations, approaches, and contacts with an undulating male) per 10 min between large and small males to body undulations made by other males ($F_{1,17}=1.30$, P=0.263) and there was no significant change
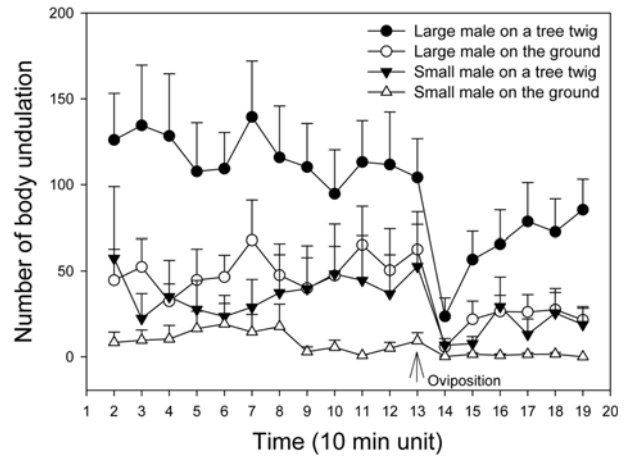
in the response intensity over a mating period ($F_{1,17}=1.06$, P=0.387; Fig. 3). The frequency of avoidance, disturbance, or biting behaviors of large males after touching small undulating males was different than that of small males when they had touched undulating large males ($X^2=10.48$, df=2, P=0.005, Fig. 4). In particular, large males bit small males more often than small males bit large males ($X^2=$ 9.35, df=1, P=0.002), but the frequency of subsequent avoidance and disturbance behaviors was similar between large and small males (Chi-square test, P>0.05 for both cases). The response intensity of females to the body undulations by large males was greater than that to the body undulations by small males ($F_{1,17}=11.56$, P=0.002) and changes in females' response intensity over a mating period was significant ($F_{1,17}=6.25$, P<0.001) (Fig. 3). Female response intensity to the body undulations by both large and small males were greater before female oviposition than after female oviposition (for large males, Z=2.48, n= 16, P=0.013; for small males, Z=2.52, n=16, P=0.012).

Tail undulation and biting

Large males conducted more tail undulations per 10 min intervals than small males did (for large male, 5.03±1.50; for small male, 1.04±0.77; $F_{1,17}=5.76$, P=0.023), but changes in the number of tail undulations per 10 min interval over the mating period were not significant ($F_{1,17}=1.05$, P= 0.397; Fig. 5). For each tail undulation display, large males undulated their tails a mean of 7.55±0.30 times (n=194) and small males undulated a mean number of 5.74±0.45 times (n=47) (Z=0.723, n=241, P=0.470). The mean frequency of tail undulations by large and small males was similar (1.67±0.03 for large males and 1.73±0.06; Z= 0.744 for small males, n=241, P=0.455). Of the total 241 tail undulations, 137 occurred before and/or after one male bit the other male. Males conducted tail undulations in 114
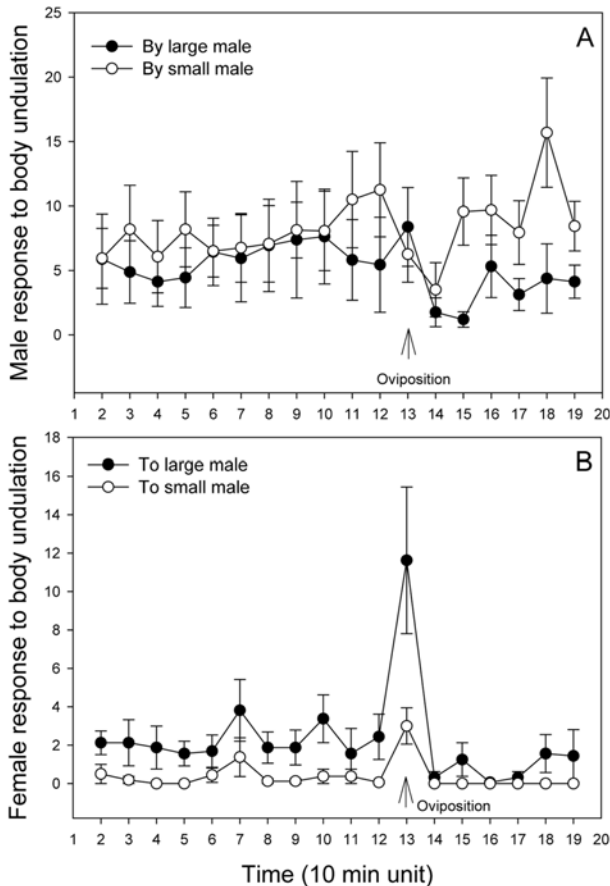
Fig. 4. Comparison of behaviors of large and small males after touching an undulating male on a tree twig. Large males bit other males more often than small males, while small males avoided other males more than large males did.

Fig. 3. Responses of males (A) and females (B) to the body undulations of other males. Response intensity is the sum of the weighted times of head orientation towards, approach to, and touching the undulating male. A head orientation received 1 point, an approach received 2 points and a touch received 3 points in the weighting process.

Fig. 5. Large males conducted more tail undulations than small males.

cases after biting the other male (83.2%), in 19 cases before biting (13.87%), and in 4 cases both before and after biting (2.92%).

Large males bit small males in 319 cases, while small males bit large males in 210 cases out of total 529 bites, and this difference was significant (X^2=4.66, df=1, P=0.0309). However, both the difference in the average number of bites per 10 min interval between large and small males (for large males the number of bites was 1.11±0.23, while for small male the number of bites was 0.70±1.56; $F_{1,17}$=1.05, P=0.404) and the change in the number of bite per 10 min interval over a mating period were not significant ($F_{1,17}$= 1.41, P=0.126). Males bit the tail of the other male in 186 cases (35.77%), the head in 122 cases (23.46%), the main body trunk in 122 cases (23.46%), and the limbs in 90 cases (17.31%).

Female approaches to a tree twig and fertilization

Out of the total 75 times that females approached a tree twig, including 16 approaches in which oviposition occurred,
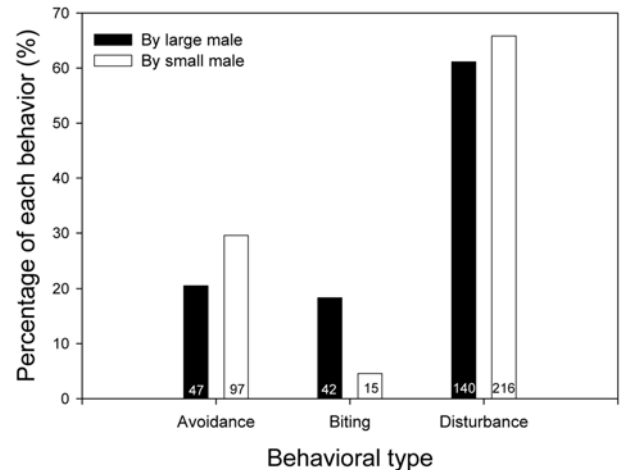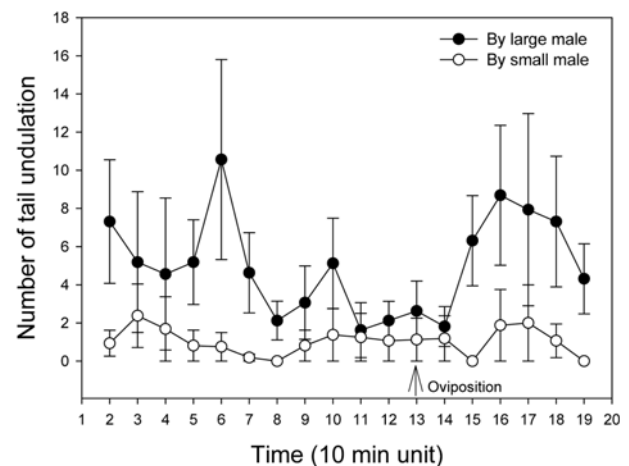
body-undulating large males were on the tree twig in 39 cases (52%, oviposition occurred in 9 cases), body-undulating small males in 4 cases (5.33%, oviposition did not occur), both body-undulating large and small males in 8 cases (10.66%, oviposition occurred in 3 cases), and no males were on the tree twig in 24 cases (32%, oviposition occurred in 4 cases). Females approached tree twigs more often when body-undulating large males were present than when body-undulating small males were present (X^2= 28.49, df=1, P<0.001). Most females approached the tree twigs about 10-20 min before oviposition.

In the 16 fertilization cases, large males alone fertilized eggs in 4 cases and in the remaining 12 cases, both large and small males fertilized eggs in each egg sac from a pair of egg sacs. Males fertilized eggs over a mean of 4.14± 0.61 times, and produced fertilizing displays over a mean of 79.14±9.50 sec (n=28). Times and time used for fertilization

by large and small males were not significantly different (for times, Z=0.05, n=28, P=0.982; for time, Z=0.42, n= 28, P=0.677).

DISCUSSION

To clarify the reproductive function of vibration signals in *Hynobius*, we quantitatively analyzed the body and tail undulations of male *H. leechii*, which generate water currents. Males actively conducted body and tail undulations throughout the mating period. In particular, large males conducted most body undulations on a tree twig where females attached their egg sacs. Males responded to other male's body undulations throughout the mating period, while females responded for only about 20 min before ovipositing, and their response was mostly directed to the large males' body undulation. The tail undulations of males usually occurred after biting other males. These results suggest that body undulation has functions in both male-female and male-male interactions, while tail undulation has functions in male-male competition. In addition, these results indicate that male *H. leechii* actively attract females using vibration signals, while females respond to the males only close to oviposition.

Males displayed body undulations throughout the mating period and both females and males responded to water currents generated by the body undulations. Male salamanders conducted body undulations about 50 times per 10 min interval at a frequency of approximately 0.64 Hz. Males responded to undulating males throughout the mating period, but females only responded for 20 min before their oviposition. Body undulations of male salamanders has been reported in several *Hynobius* species such as *H. nebulosus* (Hayashi, 1980), *H. dunni* (Mashiba, 1969), and *H. leechii* (Park et al., 1996; Park and Park, 2000; Park et al., 2008). In previous studies, both females and males responded to water currents generated by the body undulations (Usuda, 1995; Park et al., 2008). These results indicate that male body undulations in *Hynobius* functions in both female-male and male-male interactions. On the other hand, results from this study did not clarify whether males actively send water currents by the body undulation to both females and males or whether males might receive the water current signals generated for females and then respond.

Large males appeared to defend sites where females can attach their egg sacs. The distance between males and ovipositing females is an important factor in determining a male's mating success in a species which externally fertilize eggs. In Usuda's study (1997), *H. nigrescens* males that stayed at the site where females attached egg sacs fertilized about 50% of oviposited eggs, while males that approached the site from other areas after oviposition fertilized less than

20% of oviposited eggs. In this study, large males stayed much longer on tree twigs where females attached egg sacs and conducted most of their body undulations on these twigs. Also, large males attacked small males more frequently than small males attacked large males. Defending a site exclusively where females attach egg sacs has also been reported in *H. takedai* (Tanaka, 1986), *H. nebulosus* (Tanaka, 1989), and *H. nigrescens* (Hasumi, 1994; Usuda, 1997). In addition, in this study, females approached tree twigs more frequently when large males stayed there and in fertilization, large males fertilized more pairs of egg sacs than small males. These results suggest that large males actively defended the site where females attached egg sacs. On the other hand, both large and small males also conducted some body undulations on the ground. At the moment, it is not clear why males display body undulations on the ground where females can not attach egg sacs. It is possible that water currents generated by body undulations might stimulate female reproductive activities such as the process of oviposition, and/or that body undulation itself may increase the release of chemical substances from the male cloaca which provides chemical signals. Additional studies are needed to clarify these possibilities.

Male salamanders conducted tail undulations which are similar to body undulations and generated some water currents. Although both undulations look similar, tail undulations may function only in male-male interactions. Male tail undulations have also been reported in several *Hynobius* species such as *H. nebulosus* (Hayashi, 1980; Tanaka, 1989) and *H. takedai* (Tanaka, 1986). Both Sato (1992) and Park et al. (1996) considered the tail undulation to be a type of aggressive behaviors in *H. retardatus* and *H. leechii*, respectively although they did not describe patterns of the tail undulation in detail. In our study, more than 80% of tail undulations occurred after a male bit another male, which suggests that tail undulations function as an aggressive display. Female responses to male tail undulations were not detected at all. In a previous study, large *H. nigrescens* males often exclusively monopolized egg sacs to fertilize during scramble competition of several large and small males (Hasumi, 1994). Considering that large *H. leechii* males bit small males more often and also conduct more tail undulations, large *H. leechii* males might put more effort into aggressive interactions since large males occupy and defend a tree twig where females attach their egg sacs, which would result in a higher mating success for large males.

In several salamander species which internally fertilize eggs, it has been known that male salamanders often vibrate their tail in various ways, called as tail vibration. For example, male *Triturus* lashes their tails against their sides to send a wave of water towards the female's snout (Halliday, 1977). Male fire-bellied newts, *Cynops pyrrhogaster*,

vigorously vibrate their tails while touching a female (Toyoda et al., 1993). This tail vibration has been suggested to deliver chemical cues to a female mate (Toyoda et al., 1993 Halliday, 1977) and is comparable to the body and tail undulation in *H. leechii* in several ways. Tail vibration functions between males and females (Toyoda et al., 1993; Halliday, 1977) as do the body undulations of *H. leechii*, although the body undulation of *H. leechi* also plays roles in male-male interactions. Tail vibration does not compromise the movements of body parts, like the tail undulation of *H. leechii*, although body undulation does. Tail vibration occurs when males and females are close (Toyoda et al., 1993) unlike body undulation which occurs far from females or sometimes out of sight of females. Considering that fertilizing systems have evolved from external to internal fertilization, further studies investigating the signal pathways and related control mechanisms of body and tail undulations in *H. leechii* could provide valuable insight into which undulations are more closely related to the tail vibrations found in internally-fertilizing salamanders.

Females responded to male body undulations only when they were about to oviposit, and mostly responded to large male body undulations. More pairs of egg sacs were fertilized by large males than by small males. These results indicate that a female may actively select a male who will fertilize her eggs. Usuda (1995) reported that female *H. nigrescens* tended to only respond to water currents produced by body undulation within 2 hrs of ovipositing. For successful fertilization in a species which uses external fertilization, females only need to pay attention to males when they are close to ovipositing. In our study, the finding that the rate of females who touched males increased gradually from 1 hr before oviposition suggests that females might actively try to confirm the existence of males who will fertilize their eggs or to let males know their condition (i.e., that they are close to ovipositing). Previous studies have reported that *Hynobius* females often approach the site where males stay and where they attach their egg sacs (Sato, 1992; Hasumi, 1994; Usuda, 1995, 1997). In this study, *H. leechii* females approached the tree twigs where males were present in more than 68% of the total number of oviposition cases. In particular, large *H. leechii* males were present in 56% of the cases that females approached tree twigs, while females did not approach tree twigs when only small *H. leechii* males were present. Even though large males stayed at the twigs longer than small males did, female *H. leechii* approached large males at a much higher rate than smaller males, while the difference in time spent at the twigs between large and small males was relatively small. These results strongly suggest that female *H. leechii* actively approach the site where large males are present and that females might actively choose large males.

Our study confirmed that large males are more successful at mating and that multiple fertilization occurs frequently. Out of 16 pairs of egg sacs, four pairs were fertilized by large males and the remaining 12 pairs were simultaneously fertilized by both large and small males, resulting in multiple fertilization. The higher success of large males in mating may be caused by their higher ability in male-male competition such as occupying a site for egg attachment and biting small males, and also by the female's increased frequency of approach to large males when close to oviposition. In previous *H. leechii* studies, multiple fertilization occurred about 50% of the time (Park and Park, 2000). The higher rate of multiple fertilization in this study (approximately 75%) could be caused by the use of smaller mating aquaria compared to the aquaria used in the previous study. Multiple-fertilization has also been observed in several different *Hynobius* species such as *H. dunni* (Mashiba, 1969) and *H. nigrescens* (Hasumi, 1994). In addition, post-fertilization was observed in this study as Park and Park (2000) had previously reported.

Although it has been known that vibration signals play an important role in moving and foraging in amphibians (Fritzch and Neary, 1998), the reproductive function of vibration signals was not well understood. Our study showed that male *H. leechii* uses body undulations for male-female and male-male interactions, and that female salamanders respond strongly to the body undulations of large males when they are close to oviposition. Also, results indicate that males conduct most tail undulations after biting other males, and thus these undulations may play a role in male-male interactions. Further studies of this system could provide valuable insight into the function of vibration signals in urodele reproduction along evolutionary changes from external to internal fertilizing systems.

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