

## Relationship of Maximal Take-off Speed to Power and Shortening Velocity of Hindlimb Muscle in Anuran Amphibians

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To learn how maximal locomotory speed of animals is defined in terms of hindlimb structure and muscle contractile function, take-off speed, hindlimb length, thigh muscle mass, shortening velocity and power of the gastrocnemius muscle were measured with one fast species, *Rana nigromaculata* and one relatively slow-responding species, *Bombina orientalis*. Take-off speed ( $\text{m}\cdot\text{sec}^{-1}$ ) was greater in *R. nigromaculata* ( $2.4\pm 0.2\text{SD}$ ,  $n=14$ ) than in the *Bombina* ( $1.6\pm 0.1\text{SD}$ ,  $n=8$ ). Style of the take-off response was a long-jump type in the *Rana* and a short-ranged hopping in the *Bombina*. Faster take-off capacity of the ranid frogs was supported by the longer hindlimb length (relative to body length) and the more massive thigh muscles (relative to body mass), compared to the *Bombina*. Further, the ranids exhibited faster maximal shortening velocity and greater maximal power generation than the *Bombina* [ $V_{\max}$  ( $\text{ML}\cdot\text{sec}^{-1}$ )= $11.79\pm 1.69\text{SD}$  for the *Rana* and  $9.74\pm 1.27\text{SD}$  for the *Bombina*;  $P_{\max}$  ( $\text{W}\cdot\text{kg}^{-1}$ )= $222.42\pm 42.42\text{SD}$  for the *Rana* and  $169.03\pm 34.52\text{SD}$  for the *Bombina*]. With more massive thigh muscles and greater mechanical power, the ranids would generate greater total power and thus higher energy release per unit time to muscle tissues for the burst take-off. As a consequence, biomechanical properties seen in the ranids seem to be more effective for fast take-off than in the *Bombina*.

**KEY WORDS:** *Bombina orientalis*, Hindlimb Structure, Power, *Rana nigromaculata*, Shortening Velocity, Take-off Speed

Speed is an important locomotory capacity of animals for survival in ecological interactions (Hertz, Huey and Nevo 1982; Hildebrand 1988; Jones and Lindstedt 1993). Individuals normally exert their highest speed at a time of escaping predators, pursuing preys, or outrunning competitors. For instance, when a cheetah chases a prey for a short distance, it can attain the maximum speed of nearly  $100\text{ km}\cdot\text{hr}^{-1}$ ; some antelopes are observed to run at  $95\text{ km}\cdot\text{hr}^{-1}$ ; gopher snakes would attain striking speed of  $13.7\text{ km}\cdot\text{hr}^{-1}$  at their preferred body temperature; in some hylid frogs, the maximal jump velocity is

found to reach around  $7.2\text{ km}\cdot\text{hr}^{-1}$  (Greenwald 1974; Hildebrand 1988; Jones and Lindstedt 1993; Choi and Lee 1994; Marsh and John-Alder 1994).

No matter what major locomotory modes of animals are considered, the highest speeds that are generated during burst locomotion thus vary tremendously among individuals. It is however poorly understood how the peak speed of each individual is limited to that level in terms of morphological and physiological factors. General trends rendered in many studies indicate that supportive structures (e.g., limb length, muscle

mass) and contractile rate parameters of muscle (velocity, power) are some major factors limiting the highest speed (Zug 1972; Emerson 1978; Hildebrand 1988; Marsh and John-Alder 1994). These properties essentially determine rate and distance of stride (or take-off) and thus speed of that animal (Hildebrand 1988).

Take-off response seems to be the primary mode among various defence mechanisms in anuran amphibians at a predator's attack (Halliday and Adler 1988; Choi and Lee 1994). For the escape response to be most effective, these animals must take off as rapid as possible before being caught by predators. Take-off behavior of these animals thus provides a good model to test whether the relationship of speed versus structural and contractile properties follows the general observations seen in other animals. I herein present take-off speed, hindlimb morphology, and contractile function of a hindlimb muscle for two anuran species, *Rana nigromaculata* and *Bombina orientalis*. Preliminary observation indicates that take-off speeds of the two species differed significantly. The purpose of this study is particularly focused on testing the relationship of take-off speed to rate properties of muscle contraction (power, shortening velocity) in anuran amphibians. Following is a theoretical basis that is attempted to relate the speed to shortening velocity and mechanical power of hindlimb muscles.

#### Mechanical basis of take-off speed

Anuran amphibians can take off with strong ground reaction force ( $F_g$ ) exerted by two hindlimbs when the flexed joints of the hindlimbs are extended simultaneously by extensor muscles (Hirano and Rome 1984). Burst locomotory power ( $P_b$ ) that enables a frog to take off is proportional to mechanical power ( $P_m$ ) of the extensors during take-off (Marsh and John-Alder 1994). Since power is a product of force times speed (Hildebrand 1988), burst power  $P_b$  equals  $F_g$  times take-off speed ( $v$ ), and muscle power  $P_m$  equals force  $F_o$  times shortening velocity ( $V$ ) of a muscle as seen below.

$$P_b \propto P_m. \quad \text{Eq. (1)}$$

$$(F_g \times v) \propto (F_o \times V). \quad \text{Eq. (2)}$$

$$v \propto (F_o \times V)/F_g = P_m/F_g. \quad \text{Eq. (3)}$$

If ground reaction force  $F_g$  is assumed to be similar among anuran species over a comparable range of body mass, and since maximal tetanic force  $F_o$  is known to be similar among animals within closely related taxa (Alexander and Goldspink 1977), it is highly probable that take-off speed  $v$  is positively correlated with shortening velocity  $V$  and thus mechanical power  $P_m$  of the muscle.

## Materials and Methods

**Animals:** Several frogs of *R. nigromaculata* and *B. orientalis* were collected from streams near rice fields or mountain valleys around Wonju, Republic of Korea, between May and August in 1993 - 1995. Individuals of the two species were kept separately in large-sized aquaria of which temperature was maintained at 23° - 26°C for 5 - 6 days before 'take-off trial' experiments. The animals were fed with house fly larvae *ad libitum* once every morning to keep their body mass.

**Take-off trials:** A jump track (l × h × w = 150 cm × 80 cm × 20 cm) was prepared to conduct take-off experiments. A transparent acrylic plate was put for a long side wall of the track nearest to the camera, and a white acrylic plate for the other long side wall. Dark horizontal and vertical lines were drawn at 10 cm intervals on the white plate as a standard reference. A scouring pad material was placed on the ground to prevent animals from slipping during the jump.

Before experiment, body mass of each animal was measured to the nearest 0.01g. Take-off trials were induced individually at one end of the jump track, and were recorded with a video camera (Sharp Slimcam VL-L50U) at a shutter speed of 1/500 - 1/2000. Animals were startled to take-off with a dark rectangular pad (15 cm × 15 cm) moving suddenly behind them. Five to seven take-offs were induced for each animal and a rest period of about 20 min were given between trials. During the experiment, the chamber

temperature was maintained at 23° - 26°C. Recorded videotapes were analyzed frame by frame using jog-shutter function of a Goldstar 4-head VTR and a 21" near-flat color monitor. Take-off velocity was determined in a series of two consecutive frames of the recording and was calculated from a distance the subject moved between the two frames times the framing rate (30 Hz).

**Muscle contraction:** The gastrocnemius muscle which is one of major extensor muscles in hindlegs was used in this study (Choi and Bakken 1990). Right after take-off experiment, the frog was anesthetized with thiopental sodium (30 mg.g<sup>-1</sup> intraperitoneally). Skins of the thigh and the tibiotarsus were dissected posteriorly to expose the sciatic nerve and the distal tendon of the gastrocnemius muscle. The animal was then held on an acrylic fixture where the knee joint was pinned tightly. The tendon was cut and tied to a fine silver chain that was connected to a Harvard 50-7913 force transducer or a Harvard 50-6360 isotonic transducer. A pair of stainless steel electrodes to points 1 mm apart was attached on the sciatic nerve, and was covered with a petroleum jelly to electrically insulate from other tissue. The electrodes were connected to a Grass S44 stimulator that supplied a 1.0 msec square wave pulse or pulse train. All isometric and isotonic signals were routed through a Harvard Universal Oscillograph, Tektronix 2211 digital storage oscilloscope, A/D converter and then to an IBM AT compatible computer.

Experimental temperature was set at 25 ± 1°C by continuous application of the Ringer solution on the gastrocnemius muscle. The Ringer composition (mM) was: NaCl 115.5, KCl 2.0, CaCl<sub>2</sub> 1.8, Na phosphate buffer set at pH 7.0 (Jewell and Wilkie 1958). The muscle temperature was measured with a sensing tip of the 49 AWG duplex thermocouple inserted underneath the gastrocnemius and was read on an Omega HH-73T thermocouple thermometer. In the initiation of each experiment, optimum muscle length ( $l_o$ ), supramaximal stimulus strength, and stimulus frequency were set to generate maximum twitch and tetanus tension ( $F_o$ ) with minimum muscle

fatigue. A rest period of 15 min was allowed for the muscle between stimulations.

To establish a force-velocity relationship, the muscle at  $l_o$  was connected to an isotonic transducer lever that was held by a stop bar, and was stimulated to contract isometrically for the first 200 msec. The muscle was then permitted to shorten when the stop (and thus the isotonic lever) was released quickly by an electromagnetic catch (Jewell and Wilkie 1958). The muscle was shortened with loads ( $F$ ) between 0.05 $F_o$  and 0.85 $F_o$ . Muscle shortening velocity ( $V$ ) was determined from muscle length change divided by a time period between 10 and 20 msec after release. A force-velocity curve was fitted to the data using Hill's equation:  $V = b(F_o - F)/(a + F)$ , where  $a$  and  $b$  are constants associated with the force and velocity.

At the end of each experiment, the muscle was coated in place (at  $l_o$ ) with a 5% gelatin solution for about an hour. The muscle was dissected out, and its  $l_o$  and the diameter of its thickest middle portion were measured to the nearest 0.01 mm with a micrometer. The  $l_o$  was used to normalize shortening velocity; cross-sectional area of the muscle approximated with the muscle diameter was used to normalize tetanic force.

**Morphological parameters:** To see how take-off speed is related with size factors, body length, hindlimb length, and thigh muscle mass were measured for each animal after the muscle contraction experiment. Body length, the distance between snout and vent, was measured to the nearest 0.01 mm. Thigh muscle mass was measured to the nearest 0.01g after non-muscle tissues (e.g., nerves, blood vessels) were carefully removed. Hindlimb length was determined by adding individual lengths of the femur, tibiofibularis, and foot bone measured to the nearest 0.01 mm. Foot bone length was the distance between the posterior tip of the heel and the distal end of the first phalange of the fourth toe.

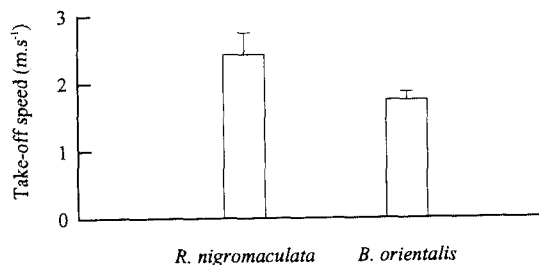
**Data presentation:** All data are presented as mean ± 1 standard deviation, unless otherwise noted. Interspecific differences in means of

morphological and functional parameters were tested with randomized, unpaired *t*-test at  $\alpha = 0.05$ . To control variations in absolute body size of individuals, I used relative values for morphological parameters (e.g., hindlimb length/body length, muscle mass/body mass). All statistical procedures were conducted with SPSS/PC+.

## Results

**Take-off speeds:** The highest speed in each jump by individual frogs was usually seen at the moment of take-off occurring at about 100 msec (or on the third frame) after the first take-off motion. The framing rate of 30 Hz in this study was generally satisfactory since take-off motions over the first three frames were repeatable in each individual jump. Figure 1 illustrates a comparison of take-off speeds ( $\text{m}\cdot\text{sec}^{-1}$ ) for the two anuran species. As previously noticed, *R. nigromaculata* generated a significantly faster speed ( $2.4 \pm 0.2\text{SD}$ ,  $n = 14$ ) than the *Bombina* ( $1.6 \pm 0.1\text{SD}$ ,  $n = 8$ ) (unpaired *t*-test,  $P < 0.001$ ). Take-off style differed remarkably between the two species, i.e., that of *R. nigromaculata* was a long-jump type whereas that of the *Bombina* was a short-hopping type. Body mass did not affect take-off speed within each species (regression slope  $b = -0.065$  for *R. nigromaculata* and  $0.02$  for *B. orientalis*,  $P > 0.05$ ).

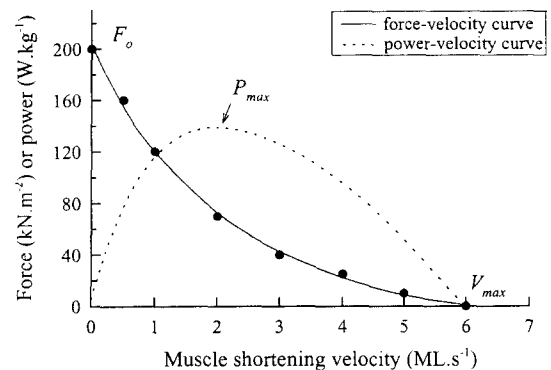
**Muscle power and velocity:** The gastrocnemius



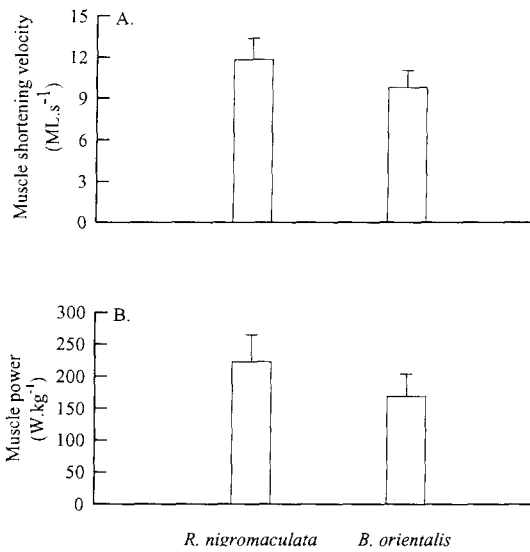
**Fig. 1.** Comparison of take-off speed between *Rana nigromaculata* (body mass =  $9.2 \text{ g} \pm 3.3\text{SD}$ ,  $n = 14$ ) and *Bombina orientalis* ( $6.5 \text{ g} \pm 0.8\text{SD}$ ,  $n = 8$ ) examined at  $25^\circ\text{C}$  which is the most active field body temperature of these animals. Vertical error bars on histogram express one standard deviation.

muscle of both species was fairly thick for the study of muscle power, and often barely sustained an initial force for the entire experimental period. This may be caused by muscle fatigue over repeated maximal stimulations before the muscle was fully recovered from previous contractions. I thus included in subsequent analyses only those data that showed 90% or above the initial force at the end of each experiment. Figure 2 shows a typical example of a force-velocity curve and a power-velocity curve from one *B. orientalis*, with each point of maximal isometric tetanic force ( $F_o$ ), maximal power ( $P_{max}$ ), and maximal shortening velocity ( $V_{max}$ ). In Figure 3, muscle power and shortening velocity were compared for the two anuran species. *R. nigromaculata* exhibited greater power and shortening velocity than *B. orientalis*, although interspecific difference for each variable was statistically indistinguishable (unpaired *t*-test,  $P > 0.05$ ). Greater sample size may however result in significant differences in these parameters between the two species, as a larger sampling and hence a smaller standard error of the mean reduces a chance of committing type II error in the *t*-test.

**Hindlimb morphology:** Ratios of thigh muscle mass to body mass ( $HL/BL$ ) and hindlimb length to body length ( $TM/BM$ ) is illustrated in Figure 4.



**Fig. 2.** A typical example of a force-velocity curve and a power-velocity curve obtained from one *B. orientalis* at muscle temperature of  $25^\circ\text{C}$ . Note each point of maximal isometric tetanic force ( $F_o$ ), maximal power ( $P_{max}$ ), and maximal shortening velocity ( $V_{max}$ ). Regressions in force-velocity and power-velocity relations were fitted using the traditional Hill's equation.

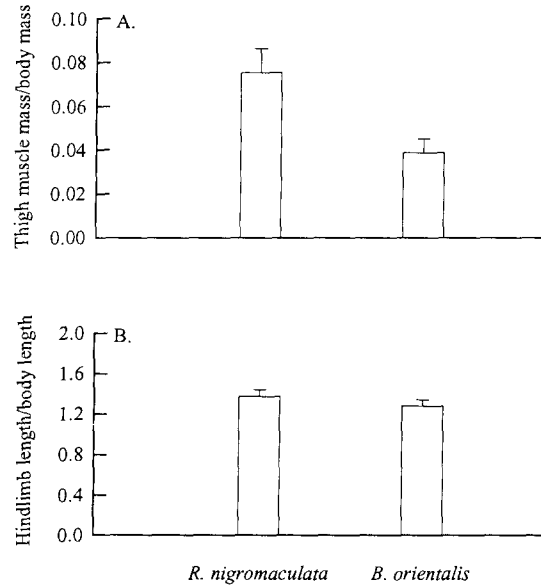


**Fig. 3.** Comparison of shortening velocity (A) and power generation (B) between *R. nigromaculata* and *B. orientalis* measured at 25°C. Vertical error bars represent one standard deviation (n = 4 for both species for both variables).

The ranid frogs showed more effective hindlimb structure for faster take-off. Both  $HL/BL$  and  $TM/BM$  were significantly greater in *R. nigromaculata* than in *B. orientalis* (unpaired *t*-test,  $P < 0.05$  for both parameters).

## Discussion

**Locomotor speed versus physiological function:** Preliminary observation indicated that *R. nigromaculata* exhibited a very hasty defensive response with the capacity of a long and rapid take-off, whereas *B. orientalis* showed less alert responsiveness, often displaying a turn-over behavior at predators' repeated attacks. The results of this study clearly demonstrate such contrasting behavioral responses between the two species: the former could take off about 1.4 times faster than the latter (Figure 1), and this difference is reflected by variation in structural and functional capacity associated with the hindlimbs. Relative hindlimb length ( $HL/BL$ ) and relative thigh muscle mass ( $TM/BM$ ) of the *Rana* is 1.1-fold and 2.5-fold the respective variables of the *Bombina*



**Fig. 4.** Comparison of thigh muscle mass relative to body mass (A) and hindlimb length relative to body length (B) between *R. nigromaculata* (n = 14) and *B. orientalis* (n = 8). Vertical error bars represent one standard deviation.

(Figure 4). Interspecific comparison of mechanical power and shortening velocity also supported the relationship of speed versus functional capacity of the extensor muscle described in the equation (3) in Introduction. The ranids generated 1.3 times greater muscle power and 1.2 times faster shortening velocity than the *Bombina* (Figure 3).

Similar results presented by Emerson (1978) show that the relative hindlimb length is around 1.6 - 1.9 for (fast) jumping species but is only 1.1 - 1.5 for (slower) walking/burrowing species. From the high-speed cine film analyses, Marsh and John-Alder (1994) found that hylid frogs with faster take-off could generally produce greater muscle power. For instance, take-off speed ( $m.sec^{-1}$ ) of *Osteopilus septentrionalis*, *Hyla cinerea*, *H. squirella*, and *Acris gryllus* is 2.43, 1.69, 1.51, and 2.05, respectively; muscle power ( $W.kg^{-1}$ ) of these species in the same order is 37.7, 22.4, 21.9, and 60.6.

Combined effect of the hindlimb structure and muscle function on speed should be considerable in the two species. From a biomechanical point of view, take-off speed is primarily a result of gear

ratio of the out-lever arm versus the in-lever arm (Hildebrand 1988). The gear ratio must be greater in the *Rana* than in the *Bombina* because the former has a greater *HL/BL* and the in-lever arm is almost negligible in both species. In addition to high gear ratio, the ranid frogs would also have a greater functional capacity of the hindlimb muscles. With more massive muscles, these frogs would generate greater total power ( $W \cdot kg^{-1} \times$  mass), which provides the frogs with greater energy release per unit time than the *Bombina* at take-off (see Lutz and Rome 1994).

**Variability in defence mechanisms and its fitness value:**

Variation in take-off speed seems quite large in anuran amphibians. However, this type of variation is only one of many phenotypic traits for defence mechanisms of these animals (e. g., the abdominal color pattern, mucous toxin secretion, camouflage, mimicry) (Kang and Yoon 1975). The variability in defensive traits would ultimately influence their survival success in the natural habitat. Hence, it is crucial to address the phenotypic variation from ecological and evolutionary perspectives and that (a) defence mode(s) adopted by each species may be viewed as a product of specialized adaptation for high fitness (Putnam and Bennett 1981; Hertz *et al.* 1982). It is impressive to observe that the take-off response is diverged between the two anuran species, i.e., a rapid, long-jump in the ranid versus a relatively slow, short-hopping in the *Bombina*. Since the slower frogs also thrive very well in their habitat, we may see the *Bombina* possess completely different solutions, such as the toxin secretion and warning coloration, in escaping predation. The ranid frogs who have few alternatives other than jumping may have to rely on rapid flight at take-off. To test this hypothesis and to draw a generality for the relationship of locomotory capacity and defence mechanisms, a full set of experiments on coloration, mucous toxicity, and other phenotypic traits should be addressed simultaneously for a broad range of anuran species.

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무미양서류의 도약속도와 다리근육의 동력 및 수축속도와와의 관계  
최인호(연세대학교 문리대학 생명과학과)

동물의 최대운동속도가 다리구조와 근육기능 면에서 어떻게 제한되는 지를 알기 위해 빠른 반응을 보이는 참개구리(*Rana nigromaculata*)와 상대적으로 느린 동작을 보이는 무당개구리(*Bombina orientalis*)의 도약속도와, 다리길이, 대퇴부 근질량, 비복근의 근수축속도 및 동력을 측정하였다. 도약속도( $m \cdot sec^{-1}$ )는 참개구리( $2.4 \pm 0.2SD$ ,  $n = 14$ )가 무당개구리( $1.6 \pm 0.1SD$ ,  $n = 8$ )보다 훨씬 빨랐다. 도약 모습을 비교해보면 참개구리는 멀리 점프하는 동작을 보이는 반면 무당개구리는 짧게 합핑하는 동작을 보였다. 참개구리가 무당개구리보다 더 빠른 속도로 도약할 수 있는 것은 뒷다리가 몸길이에 비해 더 길다는 점과 대퇴부 근질량이 체질량에 비해 더 크다는 점과 연관되었다. 더구나 참개구리의 최대근수축속도( $V_{max}$ )와 최대동력( $P_{max}$ )은 무당개구리의 그것보다 더 크게 나타났다(참개구리의  $V_{max}$  ( $ML \cdot sec^{-1}$ ) =  $11.79 \pm 1.69SD$  그리고 무당개구리의  $V_{max}$  =  $9.74 \pm 1.27SD$ ; 참개구리의  $P_{max}$  ( $W \cdot kg^{-1}$ ) =  $222.42 \pm 42.42SD$  그리고 무당개구리의  $P_{max}$  =  $169.03 \pm 34.52SD$ ). 근질량과 근동력이 클 경우 총 근동력은 더 커지기 때문에 참개구리는 도약과정에서 단위 시간당 근수축에 더 많은 에너지를 낼 수 있을 것으로 여겨진다. 따라서 참개구리의 생체역학적인 특질들이 무당개구리의 그것들보다 빠른 도약에 더 효과적일 것으로 사료된다.