

## Strong Limb Tactics of the Boulenger's Lazy Toad, *Scutigera boulengeri*: Inferred from Limb Muscles

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**Abstract** Theory predicts that well-developed limb musculature can result from sexual selection favoring strong males. We tested for this prediction in the Boulenger's lazy toad (*Scutigera boulengeri*), a species that exhibits inguinal amplexus. As expected, we found that males had more massive forelimbs and hindlimbs than those of females. In addition, amplexant males had relatively more massive hindlimbs than non-amplexant males. This pattern can be explained by sexual selection, as the greater forelimb muscles allow males to hold females more tightly and massive hindlimbs likely confer a locomotor advantage in defending mates. This study contributes to an increasing body of literature clarifying the role of sexual selection in producing sexual dimorphism in anuran limbs.

**Keywords** inguinal amplexus, limb muscles, *Scutigera boulengeri*, sexual dimorphism, sexual selection

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### 1. Introduction

In anurans, as in other vertebrates, the sexes often differ in a variety of traits (Shine, 1979). The relative size of the limbs is one of which that exhibits sexual dimorphism consistently (Lee, 2001; Mi, 2012). Males tend to have more robust limbs, though females are substantially larger in body size (Peters and Aulner, 2000; Lee and Corrales, 2002). The cause of these sexual divergences is generally attributed to sexual selection, because males typically use their forelimbs to hold females and hindlimbs to kick attackers during amplexus (Duellman, 1992; Duellman and Trueb, 1994). Therefore, males with robust forelimbs are not only more able to resist take-over attempts by unpaired males, but are also able to resist females' attempts to dislodge amplexant males by inflating their bodies (Bruning *et al.*, 2010). In addition, the relatively massive hindlimbs of males are believed to enhance mating success in scramble competition (Vargas-Salinas, 2005; Mi, 2013). Many studies have been carried out to investigate this topic in anurans with axillary amplexus, however, none of the studies have been done in species that exhibit inguinal amplexus.

The Boulenger's lazy toad (*Scutigera boulengeri*) is an anuran species endemic to the eastern Tibetan Plateau where it has an extensive altitudinal range (3300 to 5100 m) (Fei *et al.*, 2009). During the mating period (June to August), males clasp females around their waist, exhibiting typical inguinal amplexus. However, little is known so far about the evolution of sexual dimorphism in limb muscles of this toad. The aims of this

study were to assess the nature of sexually dimorphic muscles in limbs. Both forelimbs and hindlimbs were expected to be important in mating success and under sexual selection, thus, we predicted that the limb muscles mass of males significantly exceeded those of females and amplexant males significantly exceeded those of non-amplexant males.

## 2. Materials and Methods

**2.1. Data collection** We examined 37 preserved museum specimens (25 males and 12 females) of *S. boulengeri* collected during the breeding seasons of 2015 at Songduo (20.89°N, 92.45°E, 4351 m elevation), Medro Gongka County of Tibet. The specimens, 12 of which were amplexant and 13 of which were non-amplexant males, had been stored in 10% neutral buffered formalin. The former was characterized as successful maters during breeding season, and the latter was defined as failed ones without regarding the fact that they may have been mated with a female prior to capture. The amplexant pairs were sampled first and then non-amplexant males. Therefore, differences between the two types of males are likely to reflect true variations in a given population. Their body size (snout to vent length, SVL) was measured to the nearest 0.1 mm with digital calipers. Then, five forelimb muscles (pectoralis, deltoideus, triceps branchii, flexor carpi radialis and extensor digitorum) and eight hindlimb muscles (triceps femoris, biceps femoris, semimembranosus, sartorius, adductor longus, gracilis major, gracilis minor and gastrocnemius) were separated from the right side of each specimen and dried for 48 hours to a constant mass using a thermostat drier of 60°C. The dry mass of these muscles was weighted using an electronic balance to the nearest 0.1 mg. We chose these muscles because they may act in clasping females and kicking rivals during scramble competition among anuran species engaging in amplexus.

**2.2. Statistical analyses** The body size between the sexes and between amplexant and non-amplexant males were examined using independent-samples *t* test. The differences of muscle mass between the sexes and between the two types of males were assessed by general linear models with muscles mass as dependent variables, sexes/male mating category as a fixed factor, and SVL as a covariate. The total muscle mass of both sexes and for the two type males were regressed on SVL. In addition, we tested the significance of differences in adjusted means by analysis of covariance (ANCOVA). Before analysis, all morphometric variables were transformed to their natural logarithm to correct for normality and improve homogeneity of variances. All probabilities were two-tailed, and the significance level was set at  $P = 0.05$ . Data are presented as mean  $\pm$  SD.

## 3. Results

**3.1. Comparison between males and females** On average, females significantly exceeded males in SVL (females,  $(62.50 \pm 6.12)$  mm,  $n = 12$ ; males,  $(52.61 \pm 3.70)$  mm,  $n = 25$ ;  $t = 6.12$ ,  $df = 35$ ,  $P < 0.001$ ). Four forelimb muscles (pectoralis, deltoideus, triceps branchii and flexor carpi radialis) and the total forelimb muscle mass differed significantly between the sexes when the influence of SVL was controlled, but the mass of the extensor digitorum muscle did not differ between the sexes (Table 1). Linear regression of total forelimb muscle mass on SVL was highly significant for both sexes (Figure 1A, males:  $r^2 = 0.157$ ,  $F = 4.28$ ,  $P = 0.050$ ; females:  $r^2 = 0.671$ ,  $F = 20.39$ ,  $P = 0.001$ ). The differences between the adjusted means of males and females are highly significant by ANCOVA for the size of four muscles and for the total forelimb mass (muscle pectoralis:  $F_{1,34} = 5.27$ ,  $P = 0.028$ ; muscle deltoideus:  $F_{1,34} = 10.24$ ,  $P = 0.003$ ; muscle triceps branchii:  $F_{1,34} = 5.54$ ,  $P = 0.024$ ; muscle flexor carpi radialis:  $F_{1,34} = 44.38$ ,  $P < 0.001$ ; total forelimb muscle mass,  $F_{1,34} = 22.13$ ,  $P < 0.001$ ).

Regarding hindlimbs, the gender differences presented in seven muscles (triceps femoris, biceps femoris, semimembranosus, adductor longus, gracilis major, gracilis minor, and gastrocnemius) and the total hindlimb muscle mass when body size was controlled, and only one muscle (sartorius) mass did not differ between the sexes (Table 1). The total hindlimb muscle mass regressed significantly on SVL within each sex (Figure 1B, males:  $r^2 = 0.261$ ,  $F = 8.12$ ,  $P = 0.009$ ; females:  $r^2 = 0.655$ ,  $F = 19.02$ ,  $P = 0.001$ ). By ANCOVA analysis, the adjusted means of males significantly exceeded those of females for the size of seven muscles and for total hindlimb mass when the influence of SVL was controlled (muscle triceps femoris:  $F_{1,34} = 8.34$ ,  $P = 0.007$ ; muscle biceps femoris:  $F_{1,34} = 8.68$ ,  $P = 0.006$ ; muscle semimembranosus:  $F_{1,34} = 25.41$ ,  $P < 0.001$ ; muscle adductor longus:  $F_{1,34} = 10.81$ ,  $P = 0.002$ ; muscle gracilis major:  $F_{1,34} = 30.37$ ,  $P < 0.001$ ; muscle gracilis minor:  $F_{1,34} = 17.34$ ,  $P < 0.001$ ; muscle gastrocnemius  $F_{1,34} = 28.06$ ,  $P < 0.001$ ; total hindlimb muscle mass,  $F_{1,34} = 31.72$ ,  $P < 0.001$ ).

For 11 of the 13 muscles (both forelimbs and hindlimbs) examined, the adjusted means of males significantly exceeded those of females. A broad range of dimorphism existed in both the forelimb and hindlimb muscles (35%–108%), and the flexor carpi radialis and the gastrocnemius were the most dimorphic muscles in the forelimb and hindlimb, respectively (Table 1).

**3.2. Comparison between amplexant and non-amplexant males** The mean body size did not differ significantly between amplexant and non-amplexant males (amplexant males,  $(53.05 \pm 3.41)$  mm,  $n = 12$ ; non-amplexant males,  $(52.20 \pm 4.04)$  mm,  $n = 13$ ;  $t = 0.57$ ,  $df = 23$ ,  $P = 0.576$ ). When controlling for the influence of body size, only muscle deltoideus differed

significantly between the amplexant and non-amplexant males (Table 2). Linear regression of the total forelimb muscle mass on SVL was highly significant for non-amplexant males, but not for amplexant males ( $r^2 = 0.485$ ,  $F = 10.37$ ,  $P = 0.008$ ;  $r^2 = 0.012$ ,  $F = 0.12$ ,  $P = 0.735$ ; respectively). The ANCOVA analysis revealed that the differences between the adjusted means of amplexant and non-amplexant males are highly significant for muscle deltoideus ( $F_{1,22} = 7.91$ ,  $P = 0.010$ ).

When controlling for the influence of body size, three hindlimb muscles (triceps femoris, semimembranosus, and gracilis major) and the total hindlimb muscle mass differed significantly between the amplexant and non-amplexant males (Table 2). Linear regression of the total hindlimb muscle mass on SVL was significant for non-amplexant males, but not for amplexant males ( $r^2 = 0.401$ ,  $F = 7.36$ ,  $P = 0.020$ ;  $r^2 = 0.110$ ,  $F = 1.24$ ,  $P = 0.292$ ; respectively). The ANCOVA analysis revealed that the adjusted means of amplexant males significantly exceeded those of non-amplexant males for the mass of three muscles and for the total hindlimb mass when the influence of SVL was controlled (ANOVA: muscle triceps femoris:  $F_{1,22} = 10.09$ ,  $P = 0.004$ ; muscle semimembranosus:  $F_{1,22} = 5.64$ ,  $P = 0.027$ ; muscle gracilis major:  $F_{1,22} = 4.97$ ,  $P = 0.036$ ; total hindlimb muscle mass,  $F_{1,22} = 5.23$ ,  $P = 0.028$ ).

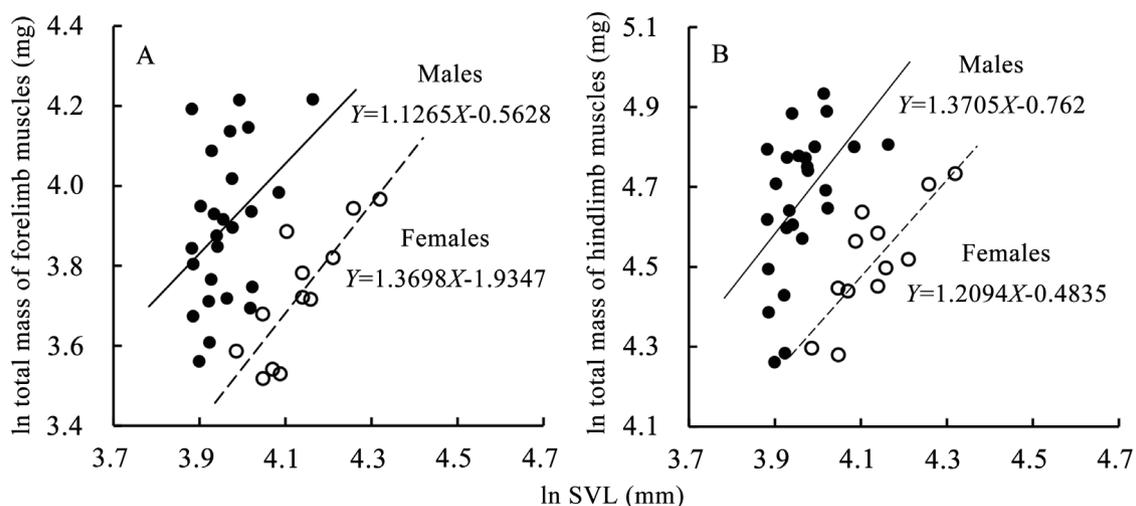
#### 4. Discussion

The results of this study demonstrate that adult males of *S. boulengeri* significantly exceed adult females in the mass of both forelimb and hindlimb muscles, though females are larger than males. This is consistent with previous works that have

shown that male anurans have relatively more robust limbs than female anurans under sexual selection. In addition, the total mass of hindlimb muscles between amplexant and non-amplexant males differed significantly in *S. boulengeri*.

Life-history models suggest that many factors can influence the evolution of sexual dimorphism, such as mating behavior (Roff, 2002). Amplexus is a common reproductive behavior in anurans and the male-biased sex ratio is normal, so males often engage in scramble competition for mates (Wells, 2010; Loman and Madsen, 2010; Liao and Lu, 2012). Under such context, the robust forelimbs can allow males to retain a firm grip on the female in amplexus and the relatively larger hindlimbs likely confer a locomotor advantage in defending mates (Lee, 2001; Clark and Peters, 2006). So, males with massive limbs can function efficiently, and thus the dimorphism emerges (Lee and Corrales, 2002; Liao *et al.*, 2012; Mi, 2013). These predictions were confirmed by our study. In addition, the extent of dimorphism is an important employee in functional roles (Andersson, 1994). In *S. boulengeri*, a broad range of dimorphism existed in both forelimb and hindlimb muscles. Muscles involved in the clasping action and fighting among males were predicted to be the most dimorphic of the muscles (Warburton *et al.*, 2013; Richards *et al.*, 2015). Indeed, the flexor carpi radialis and the gastrocnemius are the most dimorphic muscles in the forelimb and hindlimb, respectively. The former is a primary forelimb flexor and its essential function is to flex the wrist. The latter is a premier muscle crossing the knee and it plays an important role in biomechanics. These results suggest that these muscles are likely important in inguinal amplexus.

In vertebrate animals, muscle mass has significant positive



**Figure 1** Linear regression of the total mass of forelimb (A) and hindlimb muscles (B) on SVL for males (close circles, solid line) and females (open circles, broken line) in the Boulenger's lazy toad *S. boulengeri* in eastern Tibetan Plateau.

**Table 1** Comparisons of mass of male and female limb muscles in *S. boulengeri* using general linear models (GLMs) analysis.

Muscle type	Males (n = 25)	Females (n = 12)	Female/male *100%	General linear models
<b>Forelimbs</b>				
Pectoralis (mg)	14.4 ± 4.1	12.4 ± 2.8	86	$F_{1,34} = 5.27, P = 0.028$
Deltoides (mg)	10.5 ± 2.8	9.6 ± 1.8	91	$F_{1,34} = 10.24, P = 0.003$
Triceps branchii (mg)	11.0 ± 1.2	10.6 ± 1.8	96	$F_{1,34} = 5.54, P = 0.024$
Flexor carpi radialis (mg)	8.4 ± 3.0	2.9 ± 0.8	35	$F_{1,34} = 45.38, P < 0.001$
Extensor digitorum (mg)	6.0 ± 3.0	6.5 ± 1.4	108	$F_{1,34} = 0.002, P = 0.964$
Total forelimb muscles (mg)	50.2 ± 9.7	41.9 ± 6.7	84	$F_{1,34} = 23.15, P < 0.001$
<b>Hindlimbs</b>				
Triceps femoris (mg)	29.0 ± 5.9	26.7 ± 3.6	92	$F_{1,34} = 8.34, P = 0.007$
Biceps femoris (mg)	6.0 ± 1.3	5.7 ± 0.9	95	$F_{1,34} = 8.68, P = 0.006$
Semimembranosus (mg)	20.1 ± 3.6	16.9 ± 3.0	84	$F_{1,34} = 25.41, P < 0.001$
Sartorius (mg)	7.6 ± 2.3	7.7 ± 1.3	101	$F_{1,34} = 1.17, P = 0.287$
Adductor longus (mg)	5.5 ± 2.0	4.3 ± 0.9	78	$F_{1,34} = 10.81, P = 0.002$
Gracilis major (mg)	8.9 ± 2.0	6.6 ± 1.1	74	$F_{1,34} = 30.37, P < 0.001$
Gracilis minor (mg)	6.0 ± 1.4	4.5 ± 0.7	75	$F_{1,34} = 17.34, P < 0.001$
Gastrocnemius (mg)	26.2 ± 4.4	18.4 ± 2.0	70	$F_{1,34} = 28.06, P < 0.001$
Total hindlimb muscles (mg)	109.5 ± 16.7	91.2 ± 12.2	83	$F_{1,34} = 31.72, P < 0.001$

**Table 2** Comparisons of mass of amplexant and non-amplexant male limb muscles in *S. boulengeri* using general linear models (GLMs) analysis.

Muscle type	Amplesant males (n = 12)	Non-amplexant males (n = 13)	General linear models
<b>Forelimbs</b>			
Pectoralis (mg)	14.0 ± 4.4	14.8 ± 4.0	$F_{1,22} = 0.52, P = 0.479$
Deltoides (mg)	11.9 ± 2.3	9.1 ± 2.6	$F_{1,22} = 7.91, P = 0.010$
Triceps branchii (mg)	11.4 ± 1.44	10.6 ± 0.9	$F_{1,22} = 2.70, P = 0.115$
Flexor carpi radialis (mg)	8.5 ± 3.7	8.3 ± 2.3	$F_{1,22} = 0.19, P = 0.668$
Extensor digitorum (mg)	5.5 ± 3.2	6.5 ± 2.7	$F_{1,22} = 1.79, P = 0.194$
Total forelimb muscles (mg)	51.3 ± 8.5	49.3 ± 11.0	$F_{1,22} = 0.18, P = 0.677$
<b>Hindlimbs</b>			
Triceps femoris (mg)	32.6 ± 4.0	25.6 ± 5.4	$F_{1,22} = 10.09, P = 0.004$
Biceps femoris (mg)	6.4 ± 0.8	5.6 ± 1.6	$F_{1,22} = 2.27, P = 0.146$
Semimembranosus (mg)	21.7 ± 2.5	18.6 ± 3.9	$F_{1,22} = 5.64, P = 0.027$
Sartorius (mg)	7.9 ± 2.7	7.3 ± 1.8	$F_{1,22} = 0.01, P = 0.988$
Adductor longus (mg)	5.9 ± 2.6	5.0 ± 1.4	$F_{1,22} = 0.30, P = 0.590$
Gracilis major (mg)	27.1 ± 3.3	23.0 ± 3.8	$F_{1,22} = 0.57, P = 0.458$
Gracilis minor (mg)	6.3 ± 1.3	5.7 ± 1.5	$F_{1,22} = 1.04, P = 0.319$
Gastrocnemius (mg)	9.8 ± 1.8	8.1 ± 1.8	$F_{1,22} = 4.97, P = 0.036$
Total hindlimb muscles (mg)	117.6 ± 13.5	102.1 ± 16.2	$F_{1,22} = 5.23, P = 0.028$

associations with physical activity (Baumgartner *et al.*, 1999; Nakao *et al.*, 2006). For anurans, the positive relationship between the tetanic force and muscle mass has been confirmed, and certain limb muscles of males were less fatigable than those of females (Peters and Aulner, 2000; Navas and James, 2007). So, the greater limb muscle masses attained by males can allow them to hold and defend mates more firmly and extendedly. For *S. boulengeri*, the pattern of sexual dimorphism in limb musculature was recapitulated by comparing the limb muscles of amplexant and non-amplexant males, and amplexant males have relatively more robust hindlimbs than non-amplexant males. Proximate reasons for this were mainly due to the relatively more robust thigh muscles in amplexant males. This finding is generally true in anuran species, indicating that sexual selection is operating on males.

In conclusion, the results of our study revealed that the

sexual dimorphism in limbs musculature was evident in *S. boulengeri*, with males having more massive limbs than females. To verify the conclusion, more studies are required to explore the inter-sexual differences in limb musculature of other species with inguinal amplexus. In addition, studies that compare sexual dimorphism in musculature for species that exhibit different types of amplexus and that reveal functional attributes of limb muscles in living anurans can shed lights on this issue. The prediction that the intensity of sexual selection positively covaries with the degree of sexual dimorphism in anuran limbs remains to be tested by future research efforts.

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## References

- Andersson M. 1994. Sexual selection. Princeton, United States: Princeton University Press
- Baumgartner R. N., Waters D. L., Gallagher D., Morley J. E., Garry, P. J. 1999. Predictors of skeletal muscle mass in elderly men and women. *Mech Ageing Dev*, 107(2): 123–136
- Bruning B., Phillips B. L., Shine, R. 2010. Turgid female toads give males the slip: A new mechanism of female mate choice in the Anura. *Biol Lett*, 6(3): 322–324
- Clark D. L., Peters S. E. 2006. Isometric contractile properties of sexually dimorphic forelimb muscles in the marine toad *Bufo marinus* Linnaeus 1758: Functional analysis and implications for amplexus. *J Exp Biol*, 209: 3448–3456
- Duellman W. E. 1992. Reproductive strategies of frogs. *Scient Am*, 267(1): 80–87
- Duellman W. E., Trueb L. 1994. Biology of Amphibians. Baltimore, United States: John Hopkins University Press
- Fei L., Hu S. Q., Ye C. Y., Tian W. S., Jiang J. P., Zhong S. X., Wang Y. S. 2009. Fauna Sinica, Amphibia Vol. 3, Anura Ranidae. Beijing, China: Science Press (In Chinese)
- Lee J. C. 2001. Evolution of a secondary sexual dimorphism in the toad, *Bufo marinus*. *Copeia*, 2001(4): 928–935
- Lee J. C., Corrales A. D. 2002. Sexual dimorphism in hind-limb muscle mass is associated with male reproductive success in *Bufo marinus*. *J Herpetol*, 36(3): 502–505
- Liao W. B., Lu X. 2012. Variation in mating patterns in the Andrew's toad *Bufo andrewsi* along an elevational gradient in southwestern China. *Ethol Ecol Evol*, 24(2): 174–186
- Liao W. B., Wu Q. G., Barrett K. 2012. Evolution of sexual dimorphism in the forelimb muscles of Andrew's toad (*Bufo andrewsi*) in response to putative sexual selection. *Anim Biol*, 62(1): 83–93
- Loman J., Madsen T. 2010. Sex ratio of breeding common toads (*Bufo bufo*)-influence of survival and skipped breeding. *Amphib Reptil*, 31(4): 509–524
- Mi Z. P. 2012. Sexual dimorphism in the forelimb muscles of the Asiatic toad *Bufo gargarizans*. *Herpetol J*, 22(4): 219–224
- Mi Z. P. 2013. Sexual dimorphism in the hindlimb muscles of the Asiatic toad (*Bufo gargarizans*) in relation to male reproductive success. *Asian Herpetol Res*, 4(1): 56–61
- Nakao H., Yoshikawa T., Mimura T., Hara T., Nishimoto K., Fujimoto, S. 2006. Influence of lower-extremity muscle force, muscle mass and symmetry in knee extension force on gait ability in community-dwelling elderly women. *J Phys Ther Sc*, 18(1): 73–79
- Navas C. A., James R. S. 2007. Sexual dimorphism of extensor carpi radialis muscle size, isometric force, relaxation rate and stamina during the breeding season of the frog *Rana temporaria* Linnaeus 1758. *J Exp Biol*, 210(4): 715–721
- Peters S. E., Aulner D. A. 2000. Sexual dimorphism in forelimb muscles of the bullfrog, *Rana catesbeiana*: A functional analysis of isometric contractile properties. *J Exp Biol*, 203(23): 3639–3654
- Richards H. L., Grueter C. C., Milne N. 2015. Strong arm tactics: Sexual dimorphism in macropodid limb proportions. *J Zool*, 297(2): 123–131
- Roff D. A. 2002. Life history evolution. Sunderland, England: Sinauer Associates
- Shine R. 1979. Sexual Selection and Sexual Dimorphism in the Amphibia. *Copeia*, 1979(2): 297–306
- Vargas-Salinas F. 2005. *Bufo marinus* (Cane toad). Amplexus displacement. *Herpetol Rev*, 36(4): 431–432
- Warburton N. M., Bateman P. W., Fleming P. A. 2013. Sexual selection on forelimb muscles of western grey kangaroos (Skippy was clearly a female). *Biol J Linn Soc*, 109(4): 923–931
- Wells K. D. 2010. The Ecology and Behavior of Amphibians. Chicago, United States: University of Chicago Press

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