The Effect of Microhabitat and Body Size on Toe Pad Size in Arboreal, Semi-arboreal, and Torrential Frogs

Authors: Alexis Butefish and Dr. Daniel S. Moen*

Abstract: Frogs can be classified into specialized microhabitats, and for our study, we chose to focus on arboreal (tree dwelling), semi-arboreal (tree and terrestrial dwelling), and torrential (freshwater stream dwelling) species. These species are characterized by their ability to adhere to substrates in their environments, and the adaptations that support these adhesive abilities are enlarged finger and toe pads on the distal tips of each digit and specialized ventral epithelia. A distinction between arboreal and torrential species is that arboreal and semi-arboreal species rely on finger/toe pads alone to adhere, whereas torrential species utilize pads and ventral surfaces. Previous studies have focused on the relationship between morphology and adhesive efficiency, but we sought to analyze the influence of microhabitat and body size on finger/toe pad size. We hypothesized that arboreal species would have the greatest toe pad size in relation to a given body size, followed by torrential and then semi-arboreal with the smallest toe pad area. We measured the body size and toe pad area of 230 specimens, and our results supported our hypothesis. We found that microhabitat and body size have a significant effect on toe pad size.

Keywords: Arboreal, Semi-Arboreal, Torrential, Toe Pads, Morphology, Frogs

Introduction

Adaptive radiation is a form of evolution where a species of one phylogenetic lineage diverges into various species, which is dependent on the presence of ecological opportunity, or a plentiful variety of resources that compels diversification (Losos 2010). This can be reflected in anuran ecology, as frogs have radiated into five specialized microhabitats: arboreal/semi-arboreal, aquatic/semi-aquatic, terrestrial, burrowing, and torrent (Moen et al. 2016). In each microhabitat, frogs converge on similar phenotypical characteristics that are specialized for surviving the different conditions (Moen et al. 2016), and frogs that are distantly related, despite residing in different microhabitats, have evolved similar structures for function such as finger/toe pads on species that live in trees and fast flowing streams (Moen et al. 2013; Moen et al. 2016; Smith et al. 2006a). Arboreal frogs dwell in trees and surrounding ground flora in rainforests (Endlein et al. 2013a; Drotlef et al. 2015). Larger arboreal species are found on the rough bark of tree trunks and twigs, whereas smaller arboreal species usually adhere to leaves in the canopy or shrubs near the floor (Emerson 1991; Barnes et al. 2006). Torrent frogs live near and among fast-flowing freshwater streams and waterfalls, where they adhere to rocks and vegetation in or surrounding the streams (Endlein et al. 2013a; Moen et al. 2016). As other organisms, such as *Anolis* lizards or haplochromine cichlid fishes, display a strong correlation between ecology and morphology (Endlein et al. 2013b; Moen et al. 2016), a defining feature of arboreal and torrent frogs is their ability to cling to substrates using primarily their adhesive toe pads, which are enlarged pads on the distal tips of their digits (Moen et al. 2013; Barnes et al. 2006). In arboreal and torrent species, the toe pad epithelium is characterized by flat-topped, columnar, hexagonal shaped cells (Hanna and Barnes 1991; Drotlef et al. 2015). Ventral epithelia, though not the primary source of adhesion, can display a similar pattern (Endlein et al. 2013a).

Across the epithelium, canal-like channels separate the hexagonal cells, allowing the cells to move as free apices and housing mucous glands

* Faculty Mentor, Department of Integrative Biology

scattered throughout the toe pads (Hanna and Barnes 1991; Endlein et al. 2013a). The glands secrete a watery solution, and the channels allow the drainage of excess fluid from underneath the pads, maximizing the area of contact between toe pad surface and substrate (Endlein et al. 2013a; Drotlef et al. 2015), which is crucial for anuran adhesion ability in wet environments (Drotlef et al. 2015). Recent work supports that arboreal and torrent frogs employ wet adhesion, or capillarity (Emerson 1991; Hanna and Barnes 1991; Barnes et al. 2006; Smith et al. 2006a, b; Endlein et al. 2013a). Capillarity occurs when fluid is drawn towards the pads and the substrate thus forming a meniscus, and surface tension holds separate surfaces together (Barnes et al. 2006; Hanna and Barnes 1991). Adhesion is optimized with the presence of a thin fluid layer underneath the pad, and it ceases when surface tension is lost, emphasizing the importance of the mucous solution and supporting the wet adhesion hypothesis (Hanna and Barnes 1991; Drotlef et al. 2015). Adhesive force of toe pads scales directly with toe pad area, which also aligns with the wet adhesion theory (Barnes et al. 2006; Smith et al. 2006a, b).

In addition to similar morphological adaptations for adhesion, arboreal and torrent species employ both similar and different behaviors to maximize adhesion. Endlein et al. (2013a) found that at low tilt angles, both arboreal and torrent species kept a similar amount of thigh and belly skin in contact with the substrate. As the tilt angle increased, arboreal frogs' ventral contact decreased until all body contact was lost (at approx. 135º) and the frogs adhered by toe pad contact alone. In contrast, torrent species increased thigh and belly—primarily belly—contact with the substrate as the angle of tilt increased. Since adhesive force in toe pads is area-dependent (Barnes et al. 2006; Endlein et al. 2013a), the lower dependence on toe pad adhesion in torrential species in contrast to high dependence in arboreal species could lead to reduced size in torrential toe pad area. Endlein et al. (2013a) found that toe pad area in torrent frog species was about 25% of toe pad area in arboreal frogs of similar size, making ventral contact in torrent frogs essential for adhesion.

Many studies (Emerson 1991; Barnes et al. 2006; Smith et al. 2006a, b) have focused on the effects of size and morphology on adhesive abilities in arboreal and torrent frogs. In general, larger species that display larger toe pads have greater adhesive ability, as adhesive force scales to toepad area (Barnes et al. 2006; Endlein et al. 2013a). This advantage is not enough to overcome the greater mass of larger species, though, due to isometric scaling where toepad area is equal to body length squared and body mass is equal to body length cubed (Barnes et al. 2006; Smith et al. 2006a, b). This results in greater relative adhesive ability in smaller species (Barnes et al. 2006; Smith et al. 2006a, b). Though these studies focus on the consequences of size and morphology, they do not thoroughly analyze the relationships between body size and toe pad size and how these morphological features are influenced by ecology.

In this study, we ask whether frogs that have evolved similar adaptations for adhesion but occupy different microhabitats exhibit similar adhesive surface areas on the limbs. Because of our interest in how microhabitat affects morphology, we focused on arboreal and torrential frog species. Arboreal and torrential frogs utilize toe pad adhesion in different ways to accommodate for opposing ecological conditions of arboreal and torrent microhabitats (Endlein et al. 2013a). When adhering to substrates, arboreal frogs rely on toe pads only, whereas torrent frogs press their bodies to the substrate, increasing ventral contact with the surface (Endlein et al. 2013a). We hypothesized that arboreal species would have greater toe pad area in relation to body size than torrent species due to high dependency upon toe pad adhesion in arboreal frogs, versus dependency on ventral surfaces in addition to toe pads displayed in torrential frogs. To test our hypothesis, we measured the surface area of the finger/toe pads of different species of arboreal, semi-arboreal, and torrential frog species and compared it to body size. We predicted three potential relationships between these morphological features. The rate of increase in toe pad size of frogs in one microhabitat could be dramatically greater than that of the other microhabitat. Another potential outcome is that toe pad sizes in arboreal frogs and torrential frogs will increase at similar rates, but frogs of one microhabitat will have larger toe pads than frogs of the other microhabitat in relation to a single body size. Lastly, there could be no significant difference in toe pad size and the rate of increase between arboreal and torrent frogs.

Methods

For this study, we measured the area of the distal tips of the front and hind digits of 172 arboreal, 27 semi-arboreal, and 31 torrential frog species. The median sample size for each species was 4 specimens. We acquired specimens from the herpetological collections of 21 museums and supplemented with a previously published data set. Using a Canon EOS 6D with a 100mm Canon EF Macrolens and a Canon Macro Twin Lite MT-24 EX flash system (Figure 1a), we photographed the finger/toe pads and webbing by pressing the fore and hind limbs against a glass slide with a millimeter scale, carefully spreading the webbing as much as possible and flattening the finger/toe pads against the glass slide. We used ImageJ (Schneider et al. 2012) to calculate the area in square millimeters of the interdigital webbing, finger/toe pads and the inner/outer metatarsal tubercles (Figure 1b). In ImageJ, we used the line tool to trace the millimeter scale in the photographs and set the scale for the measurements, and we used the polygon tool to trace around each part. To measure body size, we used a Mitutoyo digital caliper and measured the snout-vent length (SVL) of each specimen to the nearest 0.01 mm.

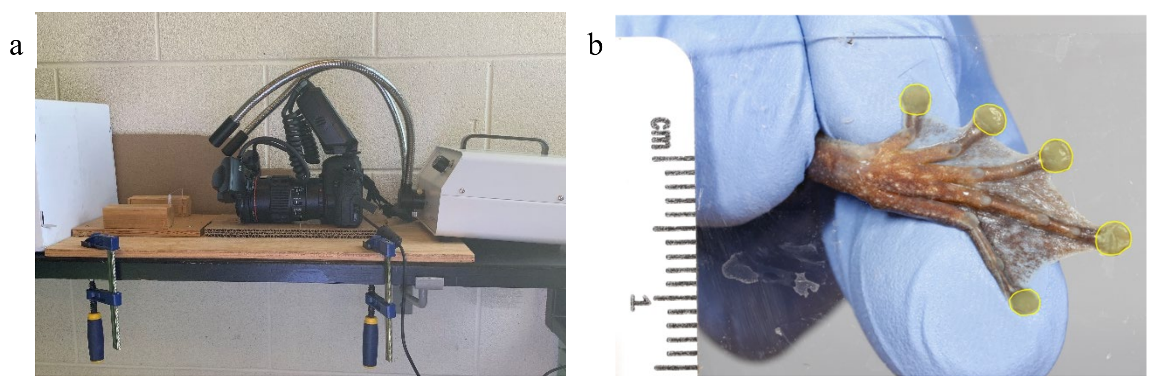

Figure 1: To photograph the finger/toe pads of the frogs, we used a Canon EOS 6D with a 100mm Canon EF Macrolens and a Canon Macro Twin Lite MT-24 EX flash system; photograph by Alexis Butefish (a). We used the polygon tool in ImageJ to trace the circumference and calculate the area of the toe pads. The pictured species is Amolops wuyiensis; photograph by Alexis Butefish (b).

To test our hypothesis that arboreal and torrential frogs have different toe pad areas compared to body size, we analyzed our data using a general linear model as implemented in R version 3.5.2 (R Core Team, 2018) and log transformed the resulting figures. Based on our hypothesis, we predicted three biologically compelling outcomes which we modeled using different combinations of our explanatory variables. First, we ran an interaction model which would show arboreal, semi-arboreal, and torrential toe pad sizes increasing at different rates, suggesting the significant effect of microhabitat. Second, we ran a non-interaction model that would display the same rate of increase in toe pad size, but with different toe pad sizes at any given body size among microhabitats. Third, we ran an SVL only model that would show identical toe pad areas and rates of increase across microhabitats, indicating a correlation between SVL and toe pad area without any influence of microhabitat. We conducted separate analyses on hand pad area, foot pad area, and summed hand and foot pad area but the results were qualitatively similar, so we describe and present the analyses of the summed hand and foot pad area.

Results

When we compared the interaction, non-interaction, and SVL only models, we found that the non-interaction model performed the best (Figure 2). This model explained approximately 80% of the variance in our data ($R^2 = 0.798$). Since the data supported the no interaction model, this indicates that toe pad size can be attributed to a combination of body length and microhabitat influence. The non-interaction model showed that in all three microhabitats, there was a positive relationship between body size and toe pad size, despite that semi-arboreal species had significantly smaller toe pads than arboreal and torrential species at a given body size ($p < 0.001$; Figure 2). For all species, SVL significantly affected finger/toe pad area, as frogs with larger body size had greater finger/toe pad area ($p < 0.001$; Figure 2). This model also showed that microhabitat had a significant effect on finger/toe pad area ($p < 0.001$; Figure 2). There was a significant difference between toe pad area in arboreal and semi-arboreal species ($p < 0.001$; Fig. 2), but no significant difference between arboreal and torrential ($p = 0.161$; Figure 2). SVL and toe pad sizes in arboreal, semi-arboreal, and torrential microhabitats scaled isometrically, as toe pad area scaled with SVL squared (Figure 2).

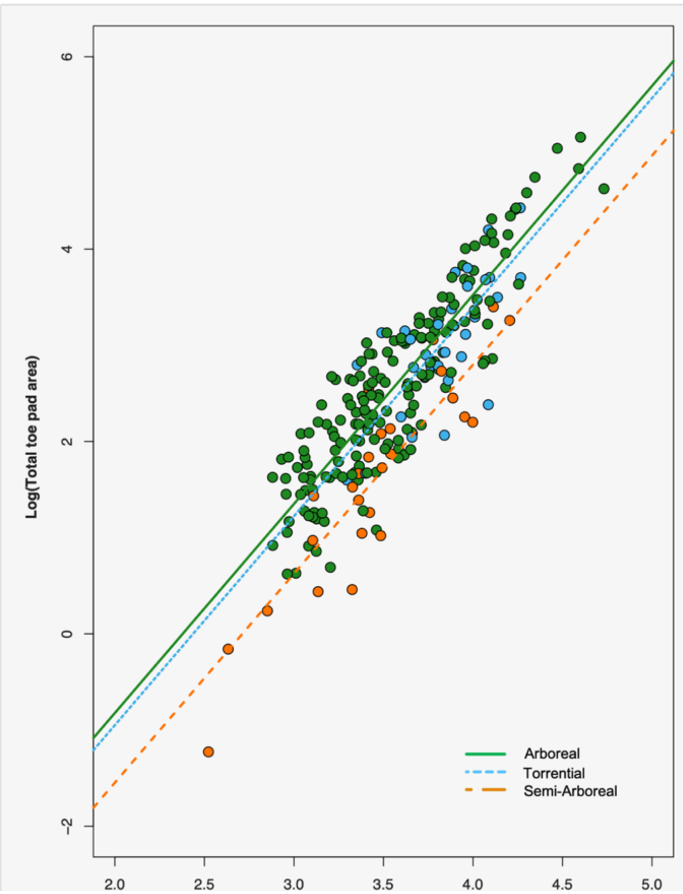

Figure 2: The non-interaction model shows a positive correlation between body size and toe pad area in arboreal, semi-arboreal, and torrential frogs (R^2 = 0.0798). There was a significant difference in toe pad size between arboreal and semi-arboreal ($p < 0.001$) and there was no significant difference between arboreal and torrential ($p = 0.161$).

Discussion

Previous studies (Emerson 1991; Barnes et al. 2006; Smith et al. 2006a, b) have analyzed how morphology affects function and performance in arboreal and torrential frogs. Few studies, however, consider the relationship between specific morphological features, such as body size and toe pad size, and how those relationships interact with ecology. In this study, we sought to understand the relationship between microhabitat and toe pad size in frogs that utilize adhesion as a crucial part of survival. We asked the question of how toe pad size scales to body size in arboreal, semi-arboreal, and torrential species of frogs. For our study, we measured the area of finger/toe pads and plotted the data against SVL to analyze the significance of the effects of body size and microhabitat on toe pad area. We ran a general linear model and found that arboreal species had larger toe pads than torrential species when compared to a given body size, as expected, but there was no significant difference between the toe pad areas of arboreal and torrential species.

Torrential frogs employ different behavioral mechanisms than arboreal frogs; as the substrate becomes more inclined, arboreal species depend on toe pad adhesion alone whereas torrential species depend on ventral surfaces and toe pads to cling to the substrate (Endlein et al. 2013a). We predicted that because torrential frogs employ this unique behavioral mechanism and depend on a combination of toe pads and ventral surfaces, they would display lesser toe pad area in comparison to those found in arboreal species. We now hypothesize that because both arboreal and torrential frogs heavily depend on adhering to substrates in their respective habitats, having large toe pads is essential. Because torrential frogs adhere to rocks in flowing streams, sometimes with pads completely submerged (Endlein et al. 2013a), this provides an additional obstacle in torrential habitats that frogs in arboreal habitats do not have to overcome, making it possible that frogs in torrential microhabitats need the adhesive force from large toe pads in addition to ventral surfaces to sufficiently cling to substrates.

Though there was no significant difference between arboreal and torrential toe pad areas, semi-arboreal species displayed significantly smaller toe pads than arboreal species (Figure 2). It was not unexpected that toe pads of semi-arboreal species were not as greatly enlarged as arboreal or torrential species because semi-arboreal species spend substantial amounts of time both in the trees and on the ground (Hedges et al. 2004; Hedges et al. 2010). Microhabitat has a significant effect on toe pad size, resulting in smaller toe pads in semi-arboreal species (Figure 2). Another possible explanation for these smaller toe pads is that enlarged toe pads such as those in arboreal and torrential species could hinder functions in terrestrial life such as crawling through leaf litter or escaping terrestrial predators.

The scope of this study could be greatly expanded by implementing our results into an evolutionary context. In the future, we could examine phylogenetic relatedness of the species and analyze how phenotypically similar they are to evaluate the influence of microhabitat on evolution versus lineage on evolution. Are closely related species that reside in different microhabitats more phenotypically similar, or are distantly related species that reside in the same habitat more phenotypically similar? To answer this question, it would likely be more sufficient to analyze species across all five microhabitats instead of only arboreal/semi-arboreal and torrential. Morphological features of interest would likely be toe pad size, interdigital webbing size, metatarsal tubercle size, body size, and limb length. Considering the relationship among these features in an evolutionary context would provide great insight into the significance of the effects of lineage versus microhabitat in anuran species.

Another potential study is to observe performance and behavior in semi-arboreal species and analyze the significance on toe pad size. We can observe how much time that certain semi-arboreal species spend in trees and how much time they spend on the ground, and compare that to toe pad size. Do species that spend more time on the ground than in the trees have significantly smaller toe pads than those that spend more time in the trees than on the ground? Analyzing this relationship could further contribute to our understanding of the effect of microhabitat and behavior on morphology.

In conclusion, for species in arboreal, semi-arboreal, and torrential microhabitats, there is a significant effect of both body size and microhabitat on toe pad area. Though arboreal and torrential species do not have significantly different toe pad areas, semi-arboreal species display significantly smaller toe pads. This indicates the importance of microhabitat and warrants further investigation of the effects of behavior, lineage, and microhabitat on morphology in semi-arboreal species.

Literature Cited

Barnes, W. J. P. and C. Oines, and J. M. Smith. 2006. Whole animal measurements of shear and adhesive forces in adult tree frogs: insights into underlying mechanisms of adhesion obtained from studying the effects of size and scale. Journal of Comparative Physiology A 192:1179-1191.

Drotlef, D. M., E. Appel, H. Peisker, K. Dening, A. del Campo, S. N. Gorb, and W. J. P. Barnes. 2015. Morphological studies of the toe pads of the rock frog, *Staurois parvus* (family: Ranidae) and their relevance to the development of new biomimetically inspired reversible adhesives. Interface Focus 5:1-11.

Emerson, S. B. 1991. The ecomorphology of Bornean tree frogs (family Rhacophoridae). Zoological Journal of the Linnean Society 101:337-357.

Endlein, T., W. J. P. Barnes, D. S. Samuel, N. A. Crawford, A. B. Biaw, and U. Grafe. 2013a. Sticking under wet conditions: the remarkable attachment abilities of the torrent frog, *Staurois guttatus*. PLOS ONE 8:1-12.

Endlein, T., A. Ji, D. Samuel, N. Yao, Z. Wang, W. J. P. Barnes, W. Federle, M. Kappl, and Z. Dai. 2013b. Sticking like sticky tape: tree frogs use friction forces to enhance attachment on overhanging surfaces. Journal of the Royal Society Interface 10:1-11.
Hanna, G. and W. J. P. Barnes. 1991. Adhesion and detachment of the toe pads of tree frogs. Journal of Experimental Biology 155:103-125.
Hedges, B., R. Thomas, and R. Powell. 2004. *Eleutherodactylus amadeus*. The IUCN Red List of Threatened Species 2004.
Hedges, B., R. Thomas, and R. Powell. 2010. *Eleutherodactylus thorectes*. The IUCN Red List of Threatened Species 2010.
Losos, J. B. 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. American Society of Naturalists 175:623-639.
Moen, D. S., D. J. Irschick, and J. J. Wiens. 2013. Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in frogs. Proceedings of the Royal Society B 280:1-9.
Moen, D. S., H. Morlon, and J. J. Wiens. 2016. Testing convergence versus history: convergence dominates phenotypic evolution for over 150 million years in frogs. Society of Systematic Biologists 65:146-160.
Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9: 671-675.
Smith, J. M., W. J. P. Barnes, J. R. Downie, and G. D. Ruxton. 2006a. Structural correlates of increased adhesive efficiency with adult size in the toe pads of hylid tree frogs. Journal of Comparative Physiology A 192:1193-1204.
Smith, J. M., W. J. P. Barnes, J. R. Downie, and G. D. Ruxton. 2006b. Adhesion and allometry from metamorphosis to maturation in hylid tree frogs: a sticky problem. Journal of Zoology 270:372-383.