Convergence in the Shape of Frog Toepads

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Abstract

Convergent evolution is the process in which unrelated species have independently evolved to have similar traits as each other despite not having a recent common ancestor. For example, species of anurans are highly diverse, yet there are great resemblances in the structure of toepads amongst unrelated frogs. Many studies have focused on the size of the toepads and adhesion. However, none have studied the actual shape of the toepads. We studied a total of 624 individual museum specimens from 167 different species. We took pictures of the toepads of every specimen and made an outline of the shape. Our analyses showed convergence in the shape of toepads because most species had the same relative shape despite being from different microhabitats. However, there were unique shapes that diverged from the typical shape. The species that had these unique shapes weren't closely related, and therefore independently evolved the unique shapes.

Keywords: Anurans, Macroevolution, Microhabitat use, Phylogeny, Elliptic Fourier Analysis

Introduction

Convergent evolution is the process in which distantly related species have evolved to have similar traits as each other. This observation has been the topic of many papers due to the fact that many characteristics about convergence are still unknown. For example, one study showed that the timescales of evolution could alter the results of convergence for a group. They looked at frogs and found repeated convergence in over 150 million years. (Moen *et al.* 2016). The main question about convergent evolution is if evolution is ever limited, such as if evolution produces the same results every time for specific environmental pressures. Stayton (2006) used morphology to find convergence in herbivorous lizards by looking at their skulls and lower jaws. He found that convergence is not always seen so clearly, and it takes the right comparisons for similarities to appear.

One group that shows frequent convergence is anurans (frogs and toads). Studies have shown that frogs from the same microhabitat have converged to have very similar traits as each other such as toepads and webbing in the feet (Moen *et al.* 2013; Moen *et al.* 2016). Frogs are a fitting group to study because there are several species from multiple microhabitats all over the world. For instance, torrential frogs are found in fast-flowing water streams, terrestrial frogs are found on the forest floor, arboreal frogs are found in the trees, and aquatic frogs are found in the water. This allows us to test for convergence between species, as well as habitats.

Toepads are an appealing trait to look at with convergent evolution in anurans because many species have toepads. Multiple studies have focused on the adhesive forces of toepads and observed how toepad morphology affects adhesion (Emerson *et al.* 1980; Emerson 1991; Hanna *et al.* 1991; McLellan 2003; Smith *et al.* 2006), but such studies have focused exclusively on toepad area. For example, another study indicated that frogs with larger relative toepad surface area are found at greater heights, and the heavier individuals were found closer to the ground (Emerson 1991). However, toepad shape seems to vary between species as well and could potentially affect adhesion in the toepads.

Because many studies have looked at strictly adhesion and surface area of frog toepads, we decided to look at the shape of toepads of several species of frogs, mostly arboreal. We questioned whether all species

*=Mentor

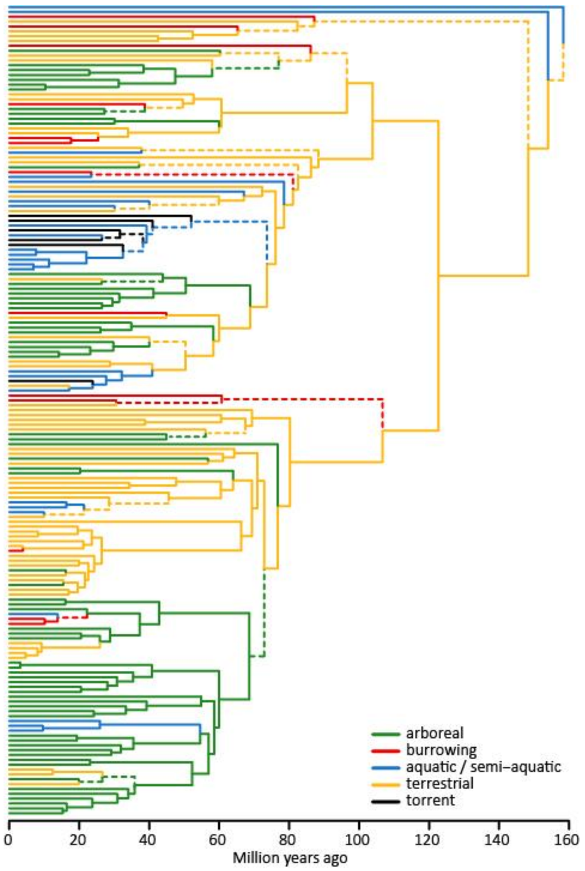

Figure 1 *- Phylogenetic analysis shows that the microhabitat of frogs has converged in different groups.*

converged to have a similar shaped toepad despite not having a recent common ancestor.

Methods

Picture Taking and Outlining

We studied 624 individual specimens from 167 species from museums such as the Smithsonian. We used a Canon Rebel T1i digital SLR camera fitted with a 100 mm macro lens to take pictures of the frogs' toepads. This was done by pressing each individual's left hand and foot against a glass plate to ensure the toepads were flat and as pronounced as possible. To determine the shape of the toe pads, we outlined the margins of toepads and turned them into black and white images in Adobe Photoshop (Figure 2).

Coordinates

Once all of the images were completed, we used the program momocs in R (Bonhomme 2014) to assign XY coordinates to the pixels in the black and white images. It established a center black pixel for each image and aligned all of the images to have the same center point. When needed, it rotated the images to overlap them as much as possible to avoid errors resulting from arbitrary differences in photo orientation or positioning. Toepads were also scaled to uniform size in order to isolate variation in shape across species.

Elliptic Fourier Analysis (EFA)

Using these coordinates, we performed an EFA. An EFA takes data points from an image and uses the trigonometric functions of sines and cosines to create ellipses with the points from the curves of the images. The curve can have any shape and many intersections (Ferson *et al.* 1985). EFA is used for describing shapes of two-dimensional images that do not contain definitive, similar landmarks and maps the distance from an indicated origin of the contour with a polar coordinate function. This function is then expressed as a Fourier series with its series of harmonics. The coefficients from the harmonics are used as data (Shen *et al.* 2009). Following Bonhomme et al. (2014), we used the minimum number of harmonics to capture 99% of the variation. This resulted in six harmonics. Since each harmonic has four coefficients, the EFA quantified our data into 24 dimensions (Shen *et al.* 2009).

Principal Component Analysis (PCA)

Because two dimensions are easier to visualize than 24, we used a PCA. The analysis evaluated a multivariate line of best fit of the data from the 24 dimensions. Once we obtained this information, we plotted the PC points onto a graph, overlaid the toepad shape images to visualize what each PC represented, and overlaid

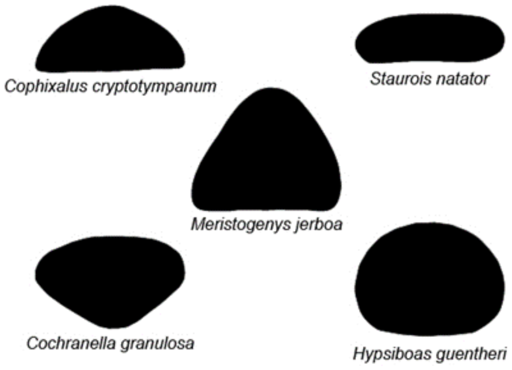

Figure 2 *- Toepad outlines demonstrate the diversity of toepads from various species.*

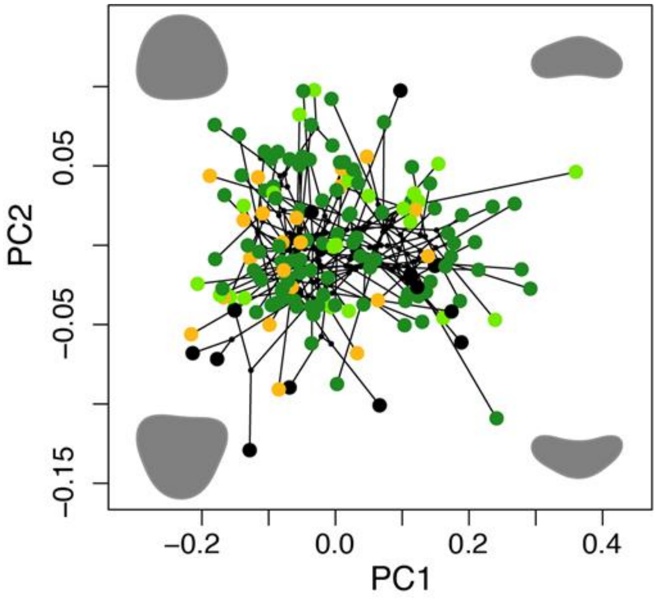

Figure 3 *- Mapping of phylogeny onto toepad shape space shows no relation between the two. Grey shapes show the toepad shapes associated with the four corners of PC space. Point colors are as in Figure 1, but with semi-arboreal as bright green. PC1 stretched and compressed the toepad shape, while high values of PC2 made the tip of the toepad thinner at the top and then the tip of the toepad wider at the bottom, whereas low values showed the opposite pattern.*

the phylogeny on the points to visualize the evolution of toepad shape (Figure 3).

<u>*MANOVA*</u>

We used a phylogenetic MANOVA to test if toepad shape is different in different microhabitats. The MANOVA related toepad shape (PC scores of the harmonic coefficients) to microhabitat use. The MANOVA incorporated the phylogeny of the species through generalized least squares (Martins and Hansen 1997).

Results

Principal components analysis showed that components 1 and 2 together described 95.6% of the variation in toepad shape. By overlaying the phylogeny on PC space, we found that toepads are diverse across species, and that closely related species are not necessarily similar in toepad shape, which suggests convergence (Figure 3). Most of the species converged to an oval-shaped toepad, and our MANOVA test showed that there is no difference among frogs that inhabit different microhabitats ($P = 0.137$).

Discussion

<u>*Convergence*</u>

Toepad shape among various species is diverse, and convergence in shape is not a result of microhabitat use. Many species from different microhabitats have a more oval-shaped toepad, which shows the possibility that this shape is typically well suited for most environments. However, there may also be an advantage to the unique shapes that we found because the species were distantly related. Other studies have looked at toepads in other species such as anole lizards (Macrini *et al.* 2006). They found that anole lizards typically found at higher heights had larger sized toepads than anoles found closer to the ground, showing that toepad size correlates directly with habitat use in anole lizards. This corresponds with what Emerson’s (1991) study found with frogs. Because our study scaled all of the toepads to the same size, this urges the question of whether bigger species have differently shaped toepads than smaller frogs because they have smaller toepads relative to their size than that of the smaller frogs.

Future Studies

Knowing the general shape of toepads, we can perform many other studies. For example, we can test how the shape affects adhesion. The oval-shaped toepad may be better adapted for adhesion than the other extreme shapes because several species converged to have this same shape. We can also examine how the shape relates to body mass and toepad surface area, such as looking for any differences in shape between bigger frogs and smaller frogs. While we used PCA, it isn't a statistical analysis. We attempted to use a common method for observing convergence called SURFACE that uses PC values, but it would not configure our data. Therefore, other studies could attempt using this method for a complete statistical value. This other phylogenetic analysis has been done with lizards and is an accurate way of determining convergence (Ingram and Mahler 2013; Mahler *et al.* 2013).

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