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An Integrative Analysis of Gecko Foot Morphology in Relation to the Evolution of the Adhesive System

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An Integrative Analysis of Gecko Foot Morphology in Relation to the Evolution of the Adhesive System

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by

Mingna Zhuang

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DEDICATION

I dedicate this dissertation to my best friend and partner, Kurtis Watanabe and our new life together.

ABSTRACT OF THE DISSERTATION

An Integrative Analysis of Gecko Foot Morphology in Relation to the Evolution of the Adhesive System

by

Mingna Zhuang

Doctor of Philosophy, Graduate Program in Evolution, Ecology and Organismal Biology
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Dr. Timothy E. Higham, Chairperson

The foot is a crucial component to locomotion and is greatly affected by the evolution of new functions. This dissertation examines both functional and morphological aspects of how the evolution of the dry adhesive system is associated with the evolution gecko feet.

In Chapter 1, I obtained obtained 3D movements (with high-speed video) of geckos (*Phelsuma madagascariensis*) running on a range of ecologically relevant inclines to examine in detail, how geckos modulate their feet with the dry adhesive system. We focused on measuring instantaneous within-foot symmetry and foot alignment relative to the body. On smaller perch diameters, geckos inverted their foot

alignment and the hind foot had greater individual digit modulation than the forefoot.

Chapter 2 used a comparative phylogenetic framework to examine how digit morphology and within-foot symmetry changed with the evolution of the adhesive system. I applied geometric morphometric methods to X-rays obtained of both padbearing and padless geckos. Our results confirm that padbearing lineages tend to have shorter digits, greater within-foot symmetry and larger interdigital angles than padless lineages and this suggests repeated shifts to a similar pad-bearing morphology.

In Chapter 3, I obtained microCT scans of padbearing and padless lineages to examine how the evolution of the adhesive system is associated with shape change in the bones (astragalocalcaneum and the fourth distal tarsal) involved in the mesotarsal joint. Our results show that padbearing lineages likely have greater degrees of freedom at the mesotarsal joint as a result of having broader surfaces on the astragalocalcaneum and fourth distal tarsal.

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Introduction

The adaptive radiation of tetrapods into novel habitats is greatly attributed to the morphological diversification of limbs (Shubin, Tabin and Carroll, 1997). Modification of the basic limb structure shared among vertebrates enabled novel forms of locomotion, which permitted the exploration of novel ecological niches. However, morphological modification and variation is not equal across the three regions of the vertebrate limb: the stylopod, zeugopod and autopod (Shubin, 1995; Vorobyeva and Hinchliffe, 1996). Instead, distal structures, such as the foot, a part of the autopod, are more likely to change than more proximal elements of the limb.

Functionally, the tendency for foot morphology to change is important because in animals with limbs that move on land, the foot serves as the first point of contact with the substrate. Deformities in foot morphology in humans can cause a decrease in locomotor performance, as well as changes in gait, since motion is translated upwards from the foot (Ilahi and Kohl, 1998). Therefore, it is no surprise that morphological change at the foot often responds to changes in habitat, as well as changes in function (e.g. Mintsa, Berge and Picard (2009); (Polly, 2008)). The foot also serves as the mechanical unit transmitting force to the ground during locomotion, to absorb shock, to support the weight and stability of the organism, and to play a role in acceleration in the hind limb. Modification to the hind foot is especially important because of its propulsive role. Therefore, changes to the function of the hind foot often must be balanced with its locomotor function as well. Furthermore, given the relationship of form to function, changes to the function of the hind foot is accompanied often by the evolution of morphological novelties.

The definition of an evolutionary novelty is widely debated (Brigandt and Love, 2012; Erwin, 2015; Muller and Newman, 2005; Muller and Wagner, 1991; Shubin, 2002). Muller and Wagner (1991) defines a novelty as a new feature in the observed group that is not present in the outgroup. However, precursors usually exist at some level. In this dissertation, I follow the definition as laid out by Hallgrimsson, Jamniczky, Young, Rolian, Schmidt-Ott et al. (2012): a new trait in which there is a shift between peaks in the adaptive landscape and a developmental constraint has been relaxed. In other words this definition describes that not only do certain morphologies become possible, these morphologies are actively selected for. This definition is broad enough to describe that some morphologies are thought to increase the range of morphological possibilities that are new to a lineage Hall (2008). However, it is unclear if variation in morphology or the possible variation always increases. For example, pharyngeal jaws in fish is associated with an increase in morphological diversity and structural changes (Price, Wainwright, Bellwood, Kazancioglu, Collar et al., 2010). On the other hand, specialization in carnivory has lead to a decrease in morphological diversity in mammals (Holliday and Steppan, 2004). Novelties can also evolve independently across lineages and it is unclear as to whether the repeated evolution of a morphological novelty occurs in the same fashion each time. Furthermore, morphological evolution of the foot in association with novelties is often difficult to study because dramatic changes in foot morphology, such as the loss or fusion of digits, with state transitions are not easily comparable. Therefore, geckos, which for most lineages retain five recognizable digits and comparable bone structures across acquisitions of the adhesive system provides the opportunity to examine how morphological novelties are related to the evolution of morphological diversity.

Study System - Gekkota

Gekkota is a diverse lineage of approximately 1600 species that are found all over the world, in generally warmer climates (Gamble, Greenbaum, Jackman, Russell and Bauer, 2012; Uetz, Freed and Hosek, 2017; Uetz and Stylianou, 2018). As an old lineage, diverging from other squamates 225-180 MY ago, geckos are morphologically diverse and inhabit a wide range of habitats, including arboreal contexts, rocky areas and deserts (Townsend, Larson, Louis and Macey, 2004; Vidal and Hedges, 2009). The fossil record of geckos is sparse, but known from sedimentary and amber-embedded fossils (Arnold and Poinar, 2008; Bauer, Bohme and Weitschat, 2005; Daza, Bauer and Snively, 2014). The oldest gecko embedded in amber found (*Cretaceogekko*), reveals that a dry adhesive system has been present since the Cretaceous period.

The evolution of an unique dry adhesive system in this lineage is recognized as a clear morphological novelty that seemingly has accompanied great morphological diversification (Gamble et al., 2012). Similar adhesive systems have arisen in other taxa such as some skinks and almost all anoles (Ruibal and Ernst, 1965; Williams and Peterson, 1982). However, it is in geckos that the system is most developed. The adhesive system has evolved independently multiple times (approximately 11), is present in approximately 60% of geckos and is therefore ideal for phylogenetic comparative analysis (Gamble et al. 2012). It has been of recent fascination due to its potential biomedical and robotic applications (2009; Autumn, 2006; 2007; Autumn, Buehler, Cutkosky, Fearing, Full et al., 2005; Autumn, Dittmore, Santos, Spenko and Cutkosky, 2006a; Autumn and Gravish, 2008; Autumn, Hsieh, Zesch, Chan, Fearing et al., 1999; Autumn, Liang, Hsieh, Zesch, Chan et al., 2000; Autumn, Niewiarowski and Puthoff, 2014; Autumn and Peattie, 2002; Autumn, Sitti, Liang, Peattie, Hansen et al., 2002; Bauer and Russell, 1991; Peattie, Delannoy, Russell and Full, 2004; Russell, 1975; 1979).

Given the ability of geckos to climb seemingly challenging smooth surfaces, recent studies have focused on understanding the mechanism of the dry adhesive system, its application, and the keratinous structures of the system interacting with the substrate (Autumn, 2007; Autumn et al., 2006a; Autumn et al., 2000; Autumn et al., 2002; Rizzo, Gardner, Walls, Keiper-Hrynko, Ganzke et al., 2006). The gecko adhesive system consists of scansor pads that have rows of hairs known as lamellae. The microscopic hairs in these rows, known as setae, further divide into split ends or “spatulae” (Autumn et al. 2000). Spatulae, which are approximately .28 μm in length, interact with the substrate at the intermolecular level via van der Waals interactions and frictional adhesion (Autumn et al., 2006a; Ruibal and Ernst, 1965). The interaction of several hundred thousand spatulae is able to result in high attachment forces. In order for the adhesive system to engage, the spatula must be loaded parallel to the surface at equal to or less than 30 degrees. Disengagement occurs when the angle between the substrate and the spatula increases above the aforementioned value, allowing the digit to be removed from the surface. These studies demonstrate the importance of geometry and directionality to the adhesive system, suggesting that the adhesive system engagement is best when loaded in the same direction as the spatula orientation.

On smooth substrates, the tokay gecko, *Gekko gecko* is able to produce a clinging force of approximately 20 N with its front feet (Irschick, Austin, Petren, Fisher, Losos et al., 1996; Ruibal and Ernst, 1965). The amount of force produced is well over the amount necessary to support the mass of an average tokay gecko (~50 g) when it is clinging to a vertical surface. However, clinging ability is most often measured when the gecko is static and on smooth substrates, spatulae are able to make maximal contact. Locomotion on more natural substrates and conditions reduces the adhesive forces that can be produced. Natural substrates are much rougher than the smooth glass like substrates used in laboratory experiments and greatly reduce the amount of setal contact that can be made (Russell and Johnson, 2007). Only a tenth of the force possible on smooth substrates may be produced on a rough sandstone surface. Furthermore, geckos may climb on inverted or curved surfaces, such as tree branches, which may further reduce the ability to load each spatula properly (Lammers and Biknevicius, 2004; Wang, Ji, Endlein, Li, Samuel et al., 2014; Wang, Wang, Ji and Dai, 2010; Wang, Wang, Ji, Zhang and Dai, 2011). Padbearing geckos may also not do well in sandy substrates where they have been observed to hyperextend their digits. This behavior is thought to prevent clogging in the adhesive system (Bauer and Russell, 1991; Lamb and Bauer, 2006).

The adhesive system can be effective, but the system impacts locomotion in significant ways. For example, the unfurling of the digit tips slows the speed at which a padbearing gecko may run (Russell and Higham, 2009). Zaaf, Herrel, Aerts and De Vree (1999) suggested that the joint moment for muscles crossing the ankle was smaller in padbearing geckos in comparison to padless geckos, resulting in lower force output in these propulsive muscles. Finally, the directionality of the adhesive system suggests that digits must be placed appropriately during locomotion in order to engage with the substrate.

Kinematic studies have revealed how geckos move with the adhesive system (Autumn, Hsieh, Dudek, Chen, Chitaphan et al., 2006b; Russell, 1975; Russell and Bels, 2000). Unlike typical lizards, the digits are hyperextended before the foot is placed on the substrate at the beginning of each stride. The toepads are unfurled onto the substrate before the crus (leg) moves across the foot. During the propulsive phase, the digits are hyperextended before the foot is removed from the substrate. Although the general behavior of padbearing gecko climbing and level locomotion is understood, detailed foot behavior that would contribute to understanding how the adhesive system is applied is lacking in both geckos and lizards overall (Russell and Bels, 2001).

The basic structure of the padbearing gecko foot shows obvious modifications to the internal skeletal and muscular structure that facilitate the engagement of the adhesive system (Russell, 1975; 1976; 1979; Russell, Bauer and Laroiya, 1997). Branches of a complex musculo-tendinous system insert into each scansor of a toe pad (Russell, 1975). The phalanges are highly irregular, instead of cylindrical and are depressed to enable digital hyperextension. Interdigital angles appear to be increased in many padbearing lineages and change how a digit may be engaged. Although digit arrangement and engagement can be behaviorally modulated, morphology sets the limits of this modulation. Furthermore, there is very little description on how more proximal and complex structures such as the mesotarsal or ankle joint may differ, despite some suggestions that geckos have unique tarsal bones in comparison to other lizard lineages (Schaeffer, 1941).

As a morphological novelty that is linked to dramatic changes in foot morphology, the dry adhesive system in geckos provides an opportunity to study variations in morphological change with the repeated acquisition of a novel trait. The demands of the adhesive system will likely result in the modulation of foot behavior and/or variation in the evolution of foot morphology. In this dissertation, I attempted to not only understand how the adhesive system is used, but also how the major morphological structures of the foot vary with the repeated acquisition of the dry adhesive system.

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Chapter 1

The differential modulation of fore- and hind limb kinematics during arboreal locomotion in day geckos (*Phelsuma madagascariensis*), with an emphasis on foot motion

Mingna V. Zhuang and Timothy E. Higham

Abstract

By using adhesion, geckos can move through incredibly challenging habitats. However, continually changing terrain may necessitate modulation of the adhesive apparatus in order to maximize its effectiveness over a range of challenges. Behaviorally modulating how the adhesive system is applied can occur by altering the alignment of the foot relative to the long axis of the body and/or the angles between the digits (interdigital angle). Given the directionality of the adhesive system, geckos likely vary the application of the system via these mechanisms as they run. We quantified 3D movements (using high-speed video) of the day gecko, *Phelsuma madagascariensis*, running on a range of ecologically relevant inclines (0°, 45°, 90 °) and perch diameters (1.5 cm, 10 cm and broad). We measured the instantaneous sum of interdigital angles and foot alignment relative to the body, as well as other kinematic variables, throughout each stride and across treatments. Modulation of foot alignment at 45° and 90° was similar between the forelimb and hind limb, but differed at 0°, suggesting that *P. madagascariensis* is able to exert an adhesive force using multiple strategies. Both the sum of interdigital angles and alignment in the fore- and hind foot were modulated. Differences in modulation between the limbs are likely related to the underlying morphology. The modulation of interdigital angle and foot alignment suggests that aspects other than the mechanism of adhesion, such as joint morphology, are important for arboreal movement in geckos. Our study of foot usage in arboreal locomotion reveals patterns that may be widespread across pad-bearing lizards. In addition to understanding the constraints exerted by the adhesive

apparatus, we highlight how biomechanical traits may respond to the evolution of novel adaptations and morphologies.

1. Introduction

Patterns of terrestrial locomotion differ considerably among taxonomic, temporal, and spatial scales. Lizards are especially adept at moving on myriad different substrates and have numerous specializations in their feet for doing so. For example, sand-dwelling lizards, such as those from the genus *Uma*, often have toe fringes to maximize surface area (Carothers, 1986; Luke, 1986); chameleons have the ability to grasp small branches and/or rocky projections (Peterson 1984; Higham, Measey, Birn-Jeffery, Herrel, Tolley, 2015); and anoles and geckos often have a dry adhesive system for increasing friction on smooth surfaces (Autumn et al., 2002; Russell, 2002; Irschick, Herrel, and Vanhooydonck, 2006; Higham, Birn-Jeffery, Collins, Hulsey, Russell, 2015). The locomotor behavior of lizards on a variety of substrates including sand, water, and arboreal branches/trunks has been examined in several taxa (Carothers, 1986; Hsieh 2003; Li, Hsieh, and Goldman, 2012; Reilly and Delancey, 1997), and differences among these taxa suggest that lizard locomotion is variable (Russell and Bels, 2001). These dynamic changes in locomotion are constrained by the morphology underlying the locomotor system, especially the foot, which transfers force from the animal to the substrate. Although the variation in kinematics of the more proximal joints has been described, detailed foot kinematics remain relatively understudied.

The foot is the first point of contact with the substrate and is the mechanical unit that transmits force to the ground during locomotion (Scott and Winter, 1993). The foot also maintains locomotor stability (Humphrey and Hemami, 2010) and generates propulsive forces (Alexander, 2003). Although these functions have behavioral lability on varying substrates, morphological modifications determine the limits within which behavior can be modulated. The foot is often a site for morphological modification, resulting in deviations from the typical lizard foot (Laerm, 1974). These changes in foot morphology have consequences for locomotion. Thus, understanding how the foot behaves is a necessary component for understanding the link between morphology and biomechanics (Higham, 2015).

Geckos possess one of the most intricate and complex examples of foot modification. The evolution of a unique directional adhesive system in this lineage is accompanied by a number of morphological changes, as well as the evolution of a digital hyperextension system that fundamentally changes how the foot is deployed and disengaged during locomotion (Russell, 1975; Russell and Higham, 2009). When this active adhesive apparatus is used, the digit tips are the first to disengage with the substrate, instead of being the last. Furthermore, an increase in the interdigital angles and shortening of the digits (Russell, Bauer, and Laroiya, 1997) suggests a morphological departure from the typical lizard foot described by Rewcastle (Rewcastle, 1983), and this difference in morphology may drastically affect locomotion and adhesion. This increase in the overall sum of interdigital angles should allow an increased range of motion of the individual digits in comparison to the typical lizard foot. Given the directionality of the gecko adhesive system (Autumn and Peattie, 2002; Hill, Soto, Peattie, Full, Kenny, 2011; Lepore, Pugno, and Pugno, 2012; Santos, Spenko, Parness, Kim, Cutkosky, 2007), understanding how the foot is oriented during locomotion and how the adhesive system is applied is important.

How the application of the adhesive system is modulated is a key question that has been poorly addressed. Most geckos have a directional adhesive system, meaning that stronger forces of adhesion are achieved when setae are loaded towards the proximal portions of the foot (Autumn and Peattie, 2002; Lepore, Pugno, and Pugno, 2012; Santos, Spenko, Parness, Kim, Cutkosky, 2007). This is especially important for geckos that not only must coordinate the application of adhesion between their limbs, but also modulate adhesion in response to habitat structure. Autumn et al. (Autumn et al., 2006) found differences in the time of adhesive system engagement in the forefoot and hind foot during climbing locomotion. Modulation of the adhesive system may also occur in response to changes in incline. Wang et al. (Wang, Gu, Wu, Ji, Dai, 2010) examined locomotion on vertical and inverted substrates in Tokay geckos and suggested that modulation of the interdigital angles and foot placement ensures that adequate adhesive force is applied in order to counter the effect of gravity. On vertical substrates, digits II, III and IV generate most of the shear forces in the forefoot while digits I, II and III generate most of the shear forces in the hind foot. During static adhesion, geckos modulate digit position so that some of the adhesive system is always aligned in to counter the effect of gravity (Russell and Oetelaar, 2015).

Studies of geckos moving on broad and inclined substrates have revealed the general limb kinematics of gecko locomotion (Russell and Higham, 2009; Autumn et al., 2006; Zaaf, Van Damme, Herrel, Aerts, 2001; Zaaf, Van Damme, Herrel, Aerts, 2001).

However, climbing substrates vary in both diameter and incline. How the gecko adhesive system is applied is likely to change given that these complex surfaces constrain how limbs can be placed, and curved surfaces result in elevated medio-lateral forces (Lammers and Biknevicius, 2004). Therefore, we examined the application of the gecko adhesive apparatus on complex arboreal substrates in order to understand how habitat structure constrains and facilitates locomotion. We examined both forelimb and hind limb kinematics in response to changes in perch diameter and incline, with a focus on the foot kinematics in a clawless arboreal specialist, *Phelsuma madagascariensis.* We hypothesized that foot and limb kinematics would differ depending on perch diameter due to the altered medio-lateral forces (Lammers and Biknevicius, 2004). As a result, geckos should respond to narrower perch diameters by increasing humerus depression and rotation, and decreasing humerus retraction (Foster and Higham, 2012). We also expected increased femur rotation and retraction on narrower perch diameters. Additionally, we hypothesized that geckos would adopt a more sprawled posture on vertical substrates, which would result in decreased femur depression, increased femur protraction and increased ankle extension (Russell and Oetelaar, 2015). Decreased humerus retraction, increased elbow extension, and increased knee flexion were also expected on the vertical substrates.

When stationary on vertical substrates, geckos modulate foot orientation and digit position so that some of the digits directly oppose gravity, ensuring passive loading of the setae (Russell and Oetelaar, 2015). Therefore, with increased inclines, alignment of the foot with the antero-posterior axis should be greater, and the sum of the interdigital angles should decrease in order to increase the number of digits loaded in opposition to gravity.

2. Materials and Methods

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Our protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, Riverside (Protocol Number: A-20110038). We obtained five juvenile *Phelsuma madagascariensis* (mass= 17.53g – 27g; snout-vent length (SVL) = 6.9 cm – 9.3 cm) from commercial suppliers (Gecko Ranch, Durham, NC and Exotic Pets, Las Vegas, NV). This species is either clawless or has vestigial claws and often occupies both broad and narrow surfaces in palm tree environments (Wanger, Motzke, Furrer, Brook, Gruber, 2009).

2. 1. Experimental Procedure

Lizards were marked with white nail polish on the dorsal body, shoulder, hip, elbow, knee, wrist and ankle joints to facilitate digitizing kinematic data (Fig 1.1). Lizards ran on 1.5 cm and 10 cm diameter wooden dowels and a broad wooden trackway made of plywood. Each substrate was inclined at 0°, 45° and 90°. This range of dowels was chosen with respect to a similarly sized species' ecology (Ikeuchi, Mori, and Hasegawa, 2005). The dowel was suspended 1.1 m above ground by a wooden board that rotated on a wood base. Because *P. madagascariensis* can autotomize its skin, precautions to reduce handling were taken by using a black plastic tube with hinged doors on both ends of the tube. The tube was placed at both ends of the setup, with one door open to simulate a dark hiding spot. The lizard was placed in the tube and encouraged to walk onto the top of the dowel with the prod of a thin wooden dowel through the tube. From there, the lizard was encouraged to run by tapping the tail or body lightly. Once the gecko entered the tube at the other end, it was removed and switched with the tube at the other end in order to take additional trials without disturbing the gecko’s skin. After some training, the lizards ran readily across the dowel into the tubes. To prevent lizard escape, the dowel on which the lizard ran was also surrounded by an enclosure of 0.635 cm thick plexiglass. Individuals were run no more than 10 times a day with 2 minutes of rest between trials.

Two high-speed video cameras (Phantom, Wayne, NJ, USA) simultaneously captured the oblique and lateral views at 1600 Hz. This frame rate was required in order to capture adequate digit detail. Video files were downsampled to 800 Hz (Final images per cycle = 111 ± 27 images). For the forelimb and hind limb of each individual, we captured three strides in which the lizard was moving steadily across the top of the perch.

2.2. Kinematics

Digitization of sequences to obtain three-dimensional coordinates of each landmark was performed using DLT DV 3 (Hedrick, 2008). In addition to a variety of points on the body and joints, the surface of the perch was digitized to observe where the limbs moved in relation to the perch. The x-axis of each trial indicated the antero-posterior movement, parallel to the direction of locomotion, positive towards the anterior. The y-axis was perpendicular to the x-axis, vertical to the surface and positive dorsal to the lizard. The z-axis described mediolateral movement and was positive into the view.

Three trials were obtained for each treatment per individual. A total of 134 trials were analyzed. Landmark data were obtained for the following: five markers on the dorsum, right shoulder and hip, knee and elbow, ankle and wrist, and the tips of digits 2-5 in the forefoot and hind foot. Because digit I is reduced in *P. madagascariensis*, it was not digitized and angles involving digit I were not calculated. A spline of $5x10^{-9}$ was applied to all landmark data except for the digit tips. A more conservative spline was applied to the digit tip landmarks of $5x10^{-10}$. Calculations of instantaneous joint and digit angles at footfall, mid stance, and end stance were performed using custom-written code in MATLAB (R2013b, The Mathworks, Natick, MA, USA) (Birn-Jeffery and Higham, 2014). Although we used external landmarks, we assumed that these accurately represented changes in angles between respective bones. For example, depression of the shank will be interpreted as femur depression.

General kinematic variables were calculated using body markers. Speed was calculated using a landmark on the midline of the body (center of the pelvic girdle). The total distance traveled was divided by the duration between frames and was standardized by dividing by SVL. Stride length was the distance traveled on the x-axis in a complete stride cycle, standardized by dividing by SVL. Stride frequency was the number of strides completed per second (Hz). We also digitized the tip of digit III in relation to the top of the substrate, such that a negative number indicates the digit tip is beneath the top of the perch.

Three-dimensional joint angles in the hind limb and forelimb (elbow, knee, wrist and ankle) were calculated using previously published methods (Foster and Higham, 2012; Spezzano and Jayne, 2004; Jayne and Irschick, 1999) (Fig 1.1). Greater flexion is indicated by smaller angles between 0° and 180°. Humerus and femur depression were calculated as the three-dimensional angles between the horizontal plane containing the shoulder/hip joint and the humerus/femur. Positive angles indicate increased depression. Foot depression was the three-dimensional angle between the horizontal plane containing the wrist/ankle joint and the axis of the foot. Humerus and femur retraction was calculated as the two-dimensional angle between the humerus/femur and the line running from the body marker placed between the pectoral/pelvic girdle and the shoulder/hip joint. Positive angles indicate retraction, where the elbow/knee is posterior to the shoulder/hip. Humerus/femur rotation was calculated as the three-dimensional angle between the vertical plane of the humerus/femur and the plane including the humerus/femur and the radius and ulna/ tibia and fibula. Three-dimensional angles between the digits were calculated by measuring the angle between the axes running from the digit tips through the ankle/wrist marker (Fig 1.2). The instantaneous sum of interdigital angles (digit II to V) was then calculated, such that smaller values indicate that the digits are oriented in a similar direction. The vertical tip position of digit III was calculated as the lowest point of digit III during the stride from the top of the perch.

Foot alignment was calculated by measuring the angle between the axis of the foot (digitized by the line from the wrist/ankle to the tip of digit III) and the antero-posterior axis running through wrist or ankle, respectively (Fig 1.2). A larger number indicates that the foot is oriented perpendicular to the body and the alignment with the antero-posterior axis of the body has decreased. Smaller numbers indicate that the foot is positioned more parallel to the antero-posterior axis and therefore, has greater alignment. Negative numbers indicate that the foot is less aligned and inverted. Because of the directional adhesive system and the head-up body position in this study, we interpreted greater alignment as orienting the foot to counteract the effect of gravity.

2.3.Statistical Analysis

Statistical analyses were performed in SPSS Version 22, and we used $p<0.05$ as the threshold for statistical significance. Because speed was affected by perch diameter and incline, we regressed variables against body speed, and took the residuals of variables that had statistically significant relationships ($p<0.1$, one tail). We kept the rest of the variables as they were originally calculated. The raw data was lastly averaged across three strides per treatment and individual for use in further analyses.

We first performed a principal components analysis (PCA) to reduce dimensionality on the forelimb and hind limb variables separately, including footfall, mid and end stance variables (Table 1.1). We selected principal components that had an eigenvalue of greater than 1. For interpretation of the loadings, we selected variables that loaded above 0.5 on each PC axis. To determine the effects of perch diameter and incline on these axes, we performed a three-way ANOVA with post-hoc Tukey HSD tests ($p<.05$), using PC scores, the first two PCs. For all ANOVAs, we included the effect of the individual as a random factor and incline and perch diameter as fixed factors. Natural log transformations were applied to variables that did not meet the assumption of equal variances. Because we were particularly interested in foot kinematic variables, we conducted separate three-way ANOVAs post-hoc Tukey HSD tests the foot alignment and sum of the interdigital angles.

3. Results

3.1. Effects of perch diameter and incline on P. madagascariensis

Phelsuma madagascariensis decreased speed on the 1.5 cm perch in comparison to the 10 cm and broad perches ($F_{2,8} = 24.79$, $p< 0.001$) and decreased speed on the 90° treatment in comparison to 0° and 45° ($F_{2,8} = 10.19$, $p<0.001$). The effect of perch diameter did not depend on incline ($F_{4,16} = 2.38$, $p =0.07$). In the hind limb, the duty factor averaged 0.46±0.10, 0.51±0.06, 0.52±0.09 for 0°, 45° and 90°, respectively across all perch diameters, and averaged 0.55 ± 0.08, 0.49 ± 0.09, 0.45 ± 0.07 for the 1.5 and 10 cm and the broad perches, respectively across all inclines. Duty factor in the forelimb averaged 0.47 ± 0.09, 0.44 ± 0.07, 0.49 ± 0.11 for 0°, 45° and 90° respectively across all perch diameters and averaged 0.52±0.08, 0.44±0.09, 0.43±0.09 on the 1.5, 10 cm and broad perches.

The first three components of the hind foot PCA explained 54.22% (PC1:25.48%, PC2:15.29%, PC3:13.46%). Higher values on PC1 corresponded with greater long-axis clockwise femur rotation, less femur depression at footfall, midstance and endstance, a lower vertical tip position of digit III and decreased foot alignment at footfall and midstance but increased foot alignment at endstance (Table 1.1). Higher values on PC2 corresponded with greater angles between digits II and III at end stance, digits III and IV at footfall, and IV and V at footfall. Higher values on PC3 corresponded with decreased hind foot alignment at footfall and mid stance, greater ankle joint angles at footfall and midstance, a decreased angle between digits III and IV at end stance and greater stride frequency. Therefore, PC3 represented generally the modulation of more distal hind limb elements.

Geckos exhibited greater femur depression and less long-axis femur rotation (PC1) on the 1.5 cm perch than the 10 cm and broad perch ($F_{2,8}$=9.14, p<.001; Figs 1.3-1.5). Femur motion (PC1) was not significantly affected by incline ($F_{2,8}$=2.75, p=0.08). The interaction term was not statistically significant ($F_{4,16}$=1.03, p=0.41). Modulation of the interdigital angles (PC2) was affected by incline ($F_{2,8}$ = 11.69, p<0.001; Fig 1.4) and perch diameter ($F_{2,8}$ = 16.41, p<0.001). The interaction term was not statistically significant ($F_{4,16}$ = 1.43, p=0.25). Interdigital angles (PC2) were greater on the 10 cm and broad perch than that on the 1.5 cm perch and were greater at the 0° and 45° treatments than that on the 90°. PC2 varied among individuals ($F_{4,32}$ = 3.78, p=0.01). Foot alignment, ankle angles and stride frequency (PC3) were greater on the 0° than at the 45° and 90° treatments ($F_{2,8}$ = 7.18, p=0.003; Figs 1.4-1.6). PC3 was not significantly affected by perch diameter or the interaction term ($F_{2,8}$ = 0.76, p=0.48, $F_{2,8}$ = 1.62, p=0.19; Fig 1.4)

For the forefoot PCA, the first three components explained 48.50% of the variation in kinematics (PC1: 24.75%, PC2:13.39%, PC3:10.35%). Higher values on PC1 corresponded with greater humerus depression at footfall, mid and end stance, long-axis clockwise humerus rotation at footfall and mid stance, increased foot alignment at midstance, and a lower vertical tip position of digit III (Table 1.1). Higher values on PC2 corresponded with greater elbow angles and a lower vertical tip position of digit III. Higher values on PC3 corresponded with greater wrist angles at footfall and mid stance, a smaller angle between digit III and IV at end stance and decreased foot alignment at footfall and mid stance.

Humerus depression, rotation and foot alignment (PC1) were increased with decreasing perch diameter ($F_{2,8}$ = 12.19, p<0.001; Fig 1.4). Humerus motion and foot alignment (PC1) was not significantly affected by incline ($F_{2,8}$=2.07, p=0.14). The interaction term was not statistically significant ($F_{4,16}$=.66, p=0.62 ; Fig 1.5). PC1 varied among individuals ($F_{4,32}$=3.58, p=0.02). Elbow angle (PC2) was greater on the 10 cm and broad perch than that on the 1.5 cm perch ($F_{2,8}$=2.07, p=0.14). Modulation of the elbow joint was not affected by incline or the interaction term, but did vary among individuals ($F_{2,8}$=1.90, p=0.17, $F_{4,16}$=.77, p=0.56, $F_{4,32}$=9.26, p<.001). Foot alignment decreased and wrist angle (PC3) increased with increasing inclines ($F_{2,8}$=25.98, p<0.001). Modulation of the distal elements was not affected by perch diameter ($F_{2,8}$=0.97, p=0.39). The interaction term was not statistically significant ($F_{4,16}$=0.37 p= 0.83).

3.2. Foot kinematics in response to perch diameter and incline

The sum of interdigital angles in the hind foot remained approximately the same throughout stance until the onset of the swing phase, at which time digital spread decreased (Fig 1.3). This sum decreased in the forefoot after mid stance. The instantaneous sum of interdigital angles in the hind foot was usually greater than the forefoot throughout the stride and on average, the sum of interdigital angles in the hind foot was greater than that of the forefoot ($F_{1,4}=248.42$, $p<0.001$; Fig 1.6). Additionally, the effect of incline depended on the limb ($F_{2,8}=5.25$, $p<0.001$). In the hind foot, the sum of interdigital angles was larger at 45° than the 0° and 90°. On the other hand, the sum of interdigital angles in the forefoot decreased with an increase in incline (Fig 1.6). In the forefoot, the angle between digits II and III had the highest coefficient of variation when compared to other interdigital angles across most treatments except on the 10 cm perch at 45° and 90° (Table 1.2). On these latter treatments, the angle between digits III and IV had the greatest amount of variation. In the hind foot, which interdigital angle had the most variation depended on the treatment.

Foot alignment did not differ between the broad and 10 cm perch, but it was significantly less aligned in both of these treatments compared to the 1.5 cm ($F_{2,8}=13.15$, $p<0.001$; Fig 1.6) Foot alignment did not differ between the 0° and 45° treatments, but it was significantly greater in both of these treatments compared to 90° ($F_{2,8}=11.56$, $p<0.001$). The interaction term was not statistically significant ($F_{4,16}=0.604$, $p=0.662$). The foot was directed towards the midline of the body (foot alignment was < 0°) on the 1.5 cm perch at 0°, 45° and 90° and all perches at 0° (Fig 1.6). Hind foot alignment was greater on the 45° treatment relative to the 0° and 90° treatments ($F_{2,8}$=11.30, p<0.001; Fig 1.6). The feet were less aligned on the 1.5 cm perch compared to the 10 cm and broad perches ($F_{2,8}$=4.12, p=0.025; Fig 1.6). The interaction term was not statistically significant ($F_{4,16}$ =0.43, p=0.77). Foot alignment increased or the foot increased inversion with increased humerus/femur depression (r=-0.49, p<0.001, r=-0.77, p<0.001, respectively).

Forefoot depression increased with increasing incline ($F_{2,8}$=30.51, p<0.001), but was not affected by perch diameter ($F_{2,8}$=1.21, p=0.31). The interaction term was not statistically significant ($F_{4,16}$=0.43, p=0.77). Hind foot depression was affected by perch diameter and incline ($F_{2,8}$=5.90, p=0.007, $F_{2,8}$ =8.06, p=0.001, respectively). The effect of perch diameter on hind foot depression depended on incline ($F_{4,16}$=17.05, p<0.001). On the 0° treatment, foot depression increased with decreasing perch diameter. On the 45° treatment, foot depression was greater on the broad and 10 cm perch than that on the 1.5 cm (Fig 1.6).

The sum of the interdigital angles differed among individuals for the forefoot, but not the hind foot ($F_{4,32}$=3.27, p=0.02, $F_{4,32}$=1.28, p=0.30, respectively). The sum of forefoot interdigital angles was less on the 45° and 90° treatments than that on the 0° treatment ($F_{2,8}$=10.10, p<0.001; Fig 1.6). However, the sum was not affected by perch diameter and the interaction term was not statistically significant ($F_{2,8}$=2.75, p=0.08, $F_{4,16}$=.98, p=0.43, respectively) The sum of interdigital angles in the hind foot was greater on the 0° and 45° treatments than that at 90° ($F_{2,8}$=4.96, p=0.01). Neither perch diameter, nor the interaction term affected the sum of interdigital angles in the hind foot ($F_{2,8}$=0.34, *p*=0.71, $F_{4,16}$=.83, *p*=0.52, respectively; Fig 1.6). In both limbs, the sum of interdigital angles decreased as alignment decreased (*r*=-.654, *p*<.001 *r*=-0.33, *p*=0.029, respectively).

4. Discussion

The directional adhesive system of geckos is an innovation that permits the exploitation of smooth vertical surfaces. Geckos can overcome these climbing challenges by loading the adhesive system passively with the digits oriented in opposition to the force of gravity and/or actively by pulling the digits towards the midline of the body (Autumn et al., 2006; Russell and Oetelaar, 2015). Both of these require that the feet and digits be oriented to maximize the utility of the apparatus (Russell, 2002; Hill, Soto, Peattie, Full, Kenny, 2011, Autumn et al., 2000). In our study, *Phelsuma madagascariensis* modulated the positions and motions of the forefoot and hind foot in response to changes in perch diameter and incline by altering foot alignment and digital spread. The modulation of foot alignment in both limbs was similar on more inclined surfaces. However, at 0°, the forefoot was inverted and the hind foot was everted. Differences in hind foot and forefoot kinematics suggest differences in contributions to stability and adhesion during locomotion at 0°, which may be related to digital configurations of the forefoot and hind foot, as well as constraints imposed by more proximal elements of the limb (Fig 1.2).

Russell and Oetelaar (Russell and Oetelaar, 2015) observed the limb and digital modulation of *Chondrodactylus bibronii* during stationary adhesion in several orientations (head-up, head-down, laterally facing to the left, and laterally facing to the right). By measuring interdigital angles and the alignment with the digits to the vector of gravity, they found that modulation of digit orientation during stationary adhesion on a vertical substrate allows the passive loading of at least several digits. The large resting sum of interdigital angles (almost 180°) within the limbs of *C. bibronii* likely facilitates adhesive application in any body orientation via increasing the potential for passive loading. However, variation in the digital arrangement among gecko lineages potentially has different kinematic consequences (Russell, Bauer, and Laroiya, 1997). Our study supports the conclusions about the benefits of a wide digital spread, but also suggests potential benefits to narrower, albeit symmetrical, digital spreads.

4.1.Foot kinematics in response to perch diameter and incline

When moving uphill, animals can only move forward by overcoming the counteracting force of gravity. This is achieved, to some extent, by increasing the frictional forces between the animal and the substrate (Cartmill, 1974). A single digit could support the weight of *Phelsuma madagascariensis* on glass, suggesting that, like Tokay geckos, the adhesive system is “overbuilt” (Russell and Oetelaar, 2015; Gilman et al., 2015). However, engaging more digits to the substrate may be necessary on rougher substrates and/or during dynamic motion due to the limited area of contact (Russell and Oetelaar, 2015). Geckos, which have a friction-based adhesive system, are expected to arrange their digits in direct opposition to gravity via modulation of the digital spread and foot alignment with increasing demands on adhesion (Russell and Oetelaar, 2015).

In our study, forefoot and hind foot alignment responded to perch diameter and incline similarly, except for the 0° treatment.

We initially predicted that, with increasing incline, foot alignment should increase and the sum of interdigital angles should be small in order to increase the number of digits effectively oriented in opposition to gravity. The first part of the prediction was only upheld on the broad perch diameters at the 45° incline when compared to the 0° treatment for the hind foot and forefoot (Fig 1.6). This finding suggests that passive loading via gravity was used for attachment on these treatments and there was a greater reliance on digit III. Although increased alignment appeared to occur on the 90° incline at the 1.5 cm perch, greater foot depression on this treatment indicated that the foot was oriented away from the body and likely wrapped around the perch (Fig 1.6). As a result, the foot was positioned so that the digits can be engaged via pulling the foot towards the midline of the body. If not loaded by gravity (i.e. oriented more parallel to the antero-posterior axis), the adhesive apparatus of geckos may be loaded by pulling the feet towards the midline of the body when the tips of the digits are more abducted than the proximal portions of the digits. For example, *Hemidactylus garnotii* pulls its limbs toward the midline during vertical climbing (Autumn et al., 2006). This behavior likely contributes to both propulsion and adhesion (Autumn et al., 2006). This alternative way of employing the adhesive apparatus likely helps stabilize the animal during locomotion, in addition to increasing the effectiveness of adhesive system application. Although Autumn, Hsieh (Autumn et al., 2006) suggests that this strategy of adhesive engagement should occur on inclined surfaces in order to generate greater forces to engage the adhesive system, it is likely that the demands due to gravity are lower at 45° than at 90°. Therefore, loading the adhesive system by orienting the digits in opposition to gravity was sufficient to engage the adhesive system at 45°. On the 1.5 cm perch at 0°, the forefoot was inverted and the hind foot was everted during locomotion (Fig 1.6). For this treatment, inversion of the forefoot allows gravity to facilitate the attachment of the adhesive system. On the other hand, eversion of the hind foot positions the foot to be engaged via pulling the foot towards the midline. Inversion of the forefoot is likely facilitated by the relatively upright posture of the forelimb in comparison to the sprawled posture of the hind limb. Given the challenges of locomotion on narrow perches, these results suggest that both limbs can counteract elevated mediolateral forces associated with small perch diameters and contribute to stability during locomotion by employing different strategies. Our findings also suggest that greater reliance on digit II in the hind foot occurs on smaller perch diameters, given that digit II was better positioned to be inverted than digit III due to the large morphological interdigital angles of the hind foot (Fig 1.2). This differentiation in foot alignment modulation indicated multiple strategies for employing adhesion to alter stability and propulsion in the forefoot and hind foot.

We expected decreased foot alignment on the smallest perch diameters in order to allow some digits to counteract the effect of the medio-lateral force experienced when the foot is not placed on the top of the perch (Lammers and Biknevicius, 2004). This prediction was supported in both the forefoot and hind foot (Fig 1.6). The foot was rarely aligned with the antero-posterior axis. The forefoot was inverted on the small perch diameter at 0° and 45°. Inversion of the hind foot also occurred on the 1.5 cm perch at the

45° incline. On the 1.5 cm at 90°, decreased space to place the limb due to the narrow perch, resulted in greater humerus/femur depression, increased height of the center of mass and therefore less stability on this treatment than that on broader perches. On this treatment, the foot was positioned along the long axis of the body, but oriented perpendicular to the long axis of the body, allowing the gecko to counteract toppling forces in addition to gripping the substrate (Autumn et al., 2006; Cartmill, 1979) Likewise, the orientation of the foot on broader perches may have been driven, in part, by more proximal elements. As a result of the sprawled posture that decreased the distance between the center of mass and the substrate, decreased humerus/femur depression on these broader perches in comparison to that on the 1.5 cm perch at 90° oriented the foot away from the body (Rewcastle, 1983).

Meldrum (Meldrum, 1991) qualitatively observed that, arboreal cercopithecine species (*Cercopithecus pogonias*, *C. nictitans* and *Lophecebus albigena*), orient the foot so that digits IV and V are positioned perpendicular to the perch in order to facilitate grasping. On broad and level substrates, the foot aligns with the antero-posterior axis. In these arboreal primates, foot modulation facilitates grasping to counteract mediolateral forces when traveling on small perch diameters and facilitates propulsion for forward locomotion on broad perches. We observed a similar strategy in *P. madagascariensis*, but we also observed the geckos employing an inverted foot posture as an alternate strategy.

Based on anatomical studies of the Tokay geckos, *Gekko gecko*, the forefoot of geckos possesses a unique tendon pattern that includes a reduced flexor plate and absence of a sesamoid. This morphology allows a greater capability for grasping than lizards such as *Pogona vitticeps,* which possess a common pattern of tendinous connections that has one or two embedded sesamoids (Abdala, Manzano, Tulli, Herrel, 2009). This morphology may facilitate the deployment of the adhesive apparatus and may facilitate its role in maintaining stability while locomoting on smaller perch diameters via a combination of grasping and adhesion. In *P. madagascariensis*, grasping in the hind foot was observed during stationary holding on small perch diameters or when falling off the perch. However, Digits IV and V of the hind foot, which were more likely capable of grasping the perch during locomotion, often remained hyperextended on the 1.5 cm perch. This indicates that *P. madagascariensis* relies less on grasping for increased lateral stability during forward locomotion. An examination of tendon morphology is needed to examine the differences in grasping ability of the forelimb and hind limb.

Although foot kinematics are often neglected in studies of locomotor biomechanics, a few studies in several species of terrestrial lizard have examined the orientation of the foot relative to the antero-posterior axis (Reilly and Delancey, 1997; Fieler and Jayne, 1998; Irschick and Jayne, 1999). *Dipsosaurus dorsalis* increases foot alignment so that the fourth digit is aligned with forward locomotion at higher running speeds (Fieler and Jayne, 1998). Several other species (*Callisaurus draconoides*, *Cnemidophors tigris* and *Phrynosoma platyrhinos*) orient their hind feet approximately 10° away from the plane of forward locomotion at footfall, (Irschick and Jayne, 1999). This increase in foot alignment allows a greater proportion of the forces produced by plantarflexion to contribute to forward propulsion (Fieler and Jayne, 1998). However, *Uma scoparia* decreases its foot alignment by approximately 10° more than the other species examined, suggesting inter-specific differences. *Sceloporus clarkii* also does not change its hind foot orientation with changes in speed, although maximum speed was not elicited (Reilly and Delancey, 1997). Russell and Bels (Russell and Bels, 2001) proposed that the inter-specific differences in the modulation of foot orientation may be the result of differences in the anatomy of the mesotarsal joint. Neither forefoot (r=-0.39, p=0.65) nor hind foot alignment (r=-0.11, p=0.22) was affected by speed in *P. madagascariensis,* but was affected by perch diameter and incline. However, a full range of speeds may not have been observed. Our results suggest that modulation of foot orientation is not only beneficial for enhancing propulsion, but also for modulating the application of adhesion in geckos. Modulation of foot orientation should be facilitated in geckos due to the modified astragalocalcaneum, which is suggested to have a greater range of motion about the mesotarsal joint than that of the typical lizard (Russell, 1975). An examination of mesotarsal and mesocarpal joints in geckos would reveal how they have evolved in concert with the evolution of the adhesive system.

4.2. Consequences of differences in the sum of interdigital angles

The sum of the interdigital angles differentiates between situations where the digits are oriented in multiple directions and those in which the digits are in the same direction. We take this as a proxy of how much of the adhesive system is recruited in the direction of foot orientation. Although the absolute distance between digit II and digit V may be small when wrapped around a small perch, the digits are not oriented in the same direction. Therefore, a large sum of interdigital angles on broader perches will be interpreted similarly to that of a large value on a small perch.

Although increases in the sum of interdigital angles may potentially be attributed to the modulation of a single interdigital angle, we found that the same interdigital angle was not always responsible for the variation in the sum of interdigital angles (Table 1.2). However, modulation of interdigital angles differed between the forefoot and hind foot. In the forefoot, the angle between digits II and III was the most variable across treatments except for the 10 cm perch at 45° and 90°. For these treatments, the angle between digits III and IV was the most variable. For the hind foot, the angle between digits II and III was most variable on the 10 cm perch across all inclines. The angle between digits III and IV was most variable on the 1.5 cm at 45° 90° and the broad perch at 0°. The angle between digits IV and V was most variable on the 1.5 cm perch, at 0° and broad perch, at 45°. Given the greater resting sum of interdigital angles in the hind foot (Fig. 1.2), modulation of the sum of interdigital angles may be attributed to anatomical differences between feet.

We predicted that more digits would align in a similar direction when the geckos ran on inclines greater than 0° and broader perch diameters (10 cm and broad). This prediction was upheld with regards to incline in the forefoot and the hind foot. At 0°, digits in the forefoot were not recruited in a similar direction. Although a large interdigital sum of angles on the broader perches at 0° may not be relevant to adhesion because digital hyperextension occurs at 0° (Russell and Higham, 2009), the large sum of interdigital angles on the 1.5 cm perch is important given that geckos must both propel forwards in addition to counteracting the lateral pull on smaller perch diameters. On this perch treatment, the forefoot and hind foot was inverted, some digits were likely dedicated to counteracting the effect of the medio-lateral forces experienced and some digits were likely dedicated to propulsion. In both feet, the sum of interdigital angles decreased with increasing inclines as a result of gravity becoming opposed to the direction of forward locomotion, which then elevated the demand on the gecko in terms of adhesion. Significant decreases in the sum of interdigital angles with increases in incline occurred at 45° in the forefoot and 90° in the hind foot. Thus, shallower angles led to the digits of the forelimb, and therefore the adhesive system, becoming aligned with gravity, whereas it took steeper angles before the same was true for the hind limb.

Modulation of forefoot and hind foot motion involved both changes in the foot alignment and the instantaneous sum of interdigital angles. The differences in the patterns of modulation between limbs may reflect morphological differences. Although not studied in *P. madagascariensis*, Russell (Russell, 1975) described the myological and osteological differences between the forelimb and the hind limb in *Gekko gecko*. Unlike *G. gecko*, which has a similar resting sum of interdigital angles in the forelimb and hind limb, *P. madagascariensis* possesses a smaller resting sum of interdigital angles in the forelimb than that in the hind limb (Fig 1.2). As a result, the sum of interdigital angles at midstance in the hind foot almost always greater than that observed in the forefoot. The sum of interdigital angles in the hind foot ranged from approximately 100° to 150°, whereas the sum of interdigital angles in the forefoot was restricted to a smaller range of approximately 70°-100° (Fig 1.6). In general, the sum of interdigital angles was more variable in the hind foot than in the forefoot. These results suggest that a smaller resting sum of interdigital angles is reflective of its instantaneous sum of interdigital angles during locomotion. The forefoot, which has a sum of inter-metatarsal angles of approximately 87°, is limited in its modulation of interdigital angles and direction of adhesive system recruitment. Modulation of adhesion in the forefoot is therefore more reliant on the modulation of foot alignment. The greater resting sum of the interdigital angles in the hind foot, which has a sum of inter-metatarsal angles of approximately 101°, permits the use of a larger range of interdigital angle modulation. However, fewer digits are then recruited in the direction of foot orientation, potentially reducing the contribution of hind foot adhesion to locomotion overall.

In order to facilitate the operation of the adhesive system, interdigital angles in the hind feet of pad-bearing lineages are thought to be greater than that of padless lineages (Russell, Bauer, and Laroiya, 1997). However, studies examining the digital configuration in the forelimb of gecko lineages has not been examined, although variation seems to exist. For example, *C. bibronii* not only has a nearly 180° sum of interdigital angle configuration in the hind limb, but also in the forelimb (Russell and Oetelaar, 2015). It is evident that *P. madagascariensis* does not possess such a digital configuration in the forelimb (Fig 1.2). Whereas the 180° range in the forelimb and hind limb of *C. bibronii* may allow the gecko to adhere regardless of body orientation, *P. madagascariensis* may require more modulation to maintain adhesive system engagement in the same body orientations or may favor some body orientations over others. This might also reflect an arboreal habitat as compared to a rocky habitat that includes more flat surfaces.

4.3.Forelimb and hind limb kinematics of arboreal locomotion

Narrower perches reduce the amount of space on which the foot can be placed and, as a result, the alteration of the proximal limb elements is crucial for accommodating these conditions (Spezzano and Jayne, 2004). Locomotion of *P. madagascariensis* was affected more by perch diameter than incline. Furthermore, proximal limb elements appeared to be more important than more distal elements for locomotion, especially in the forelimb (Table 1.1). These results suggest that there are significant upstream effects of proximal limb elements on more distal limb elements.

In response to decreasing perch diameter, hip and shoulder height decreased in *P. madagascariensis*. Decreased hip/shoulder height may have occurred via a number of kinematic changes. Although greater femur and humerus depression occurred on these treatments, the decrease in hip height and shoulder height was likely due to the increased elbow and knee flexion. This response is consistent with previous studies, in which animals responded to smaller perch diameters by lowering the center of mass to increase stability (Foster and Higham, 2012; Spezzano and Jayne, 2004; Foster and Higham, 2014; Franz, Demes, and Carlson, 2005; Schmitt, 1994; Schmidt and Fischer, 2010). Like *Anolis carolinensis*, geckos increased long-axis humerus rotation and decreased femur rotation with decreasing perch diameter (Foster and Higham, 2012) (Fig 1.5, Table 1.1). Additionally, greater femur rotation was associated with decreased femur depression.

Greater femur rotation may be attributed to the sprawling posture of the hind limb, which requires femur rotation as a mechanism of decreasing rotation necessary at the mesotarsal joint, and during femur retraction, maintaining the knee joint axis perpendicular to the parasagittal plane (Rewcastle, 1983).

Far more studies have examined the effects of incline on locomotor kinematics in lizards than perch diameter (Higham, Measey, Birn-Jeffery, Herrel, Tolley, 2015; Foster and Higham, 2012; Birn-Jeffery and Higham 2014; Jayne and Irschick, 1999; Higham and Jayne, 2004; Irschick and Jayne, 1998; Russell and Higham, 2009; Russell and Oetelaar, 2015; Krause and Fischer, 2013; Spezzano and Jayne, 2004; Wang et al., 2015; Birn-Jeffery and Higham, 2016). As incline increases, the impact of gravity acts to pull the animal down the slope. Thus, during vertical locomotion, the vertical component of the ground reaction force acts perpendicular to the force of gravity and does not contribute to substrate attachment. The effect of gravity at 90° directly opposes forward locomotion, which causes slipping if there is no increase of friction or engagement of an adhesive system (Cartmill, 1985). *Phelsuma madagascariensis* increased wrist extension and decreased forefoot alignment with increasing inclines. Depending on the perch diameter, *P. madagascariensis* modulated hind foot alignment and ankle angle. Additionally, unlike *Tarentola mauritanica*, which engages the adhesive system at inclines greater than 10° (Russell and Higham, 2009), *P. madagascariensis* often did not engage the hind foot adhesive toepads of digits IV and V on steeper inclines and narrower perches. Due to their orientations on these treatments, digits IV and V were not likely to contribute to forward locomotion if engaged and instead, remained hyperextended.

5. Conclusion

We explored the function of feet in geckos, an understudied aspect of vertebrate locomotion. *Phelsuma madagascariensis* modulated not only proximal limb elements, but also distal limb elements, in response to changes in perch diameter and incline. Modulation of these elements differed between the forelimbs and hind limbs in ways consistent with observations from previous studies. Furthermore, we identified certain unique behaviors of *P. madagascariensis* that may be relevant to its morphology and/or the evolution of the dry adhesive system. Limitations in the range of motion of the individual digits in the forefoot may be related to greater reliance on the modulation of overall foot alignment in comparison to the hind foot. This potential morphological constraint necessitates further examination of foot morphology in concert with kinematic studies examining foot modulation and confirms that digit placement is context dependent during locomotion. Overall, studying foot kinematics in concert with adhesion during locomotion in geckos is critical for revealing potential constraints or ways in which constraints are circumvented. This will help illuminate the evolution of the gekkotan adhesive apparatus.

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Tables and Figures

Table 1.1 Loadings from a principal component (PC) analysis of kinematic variables performed separately on the forelimb and hind limb

Forelimb				Hind Limb			
Variable	PC1	PC2	PC3	Variable	PC1	PC2	PC3
Stride frequency*	0.28	0.15	0.07	Stride frequency*	-0.26	-0.05	**0.53**
Stride length*	-0.19	0.47	-0.05	Stride length*	0.13	0.14	-0.01
Humerus depression (ff)	**0.87**	0.05	-0.03	Femur depression (ff)*	**-0.57**	-0.07	-0.37
Humerus depression (ms)	**0.91**	0.10	-0.03	Femur depression (ms)	**-0.91**	-0.01	-0.31
Humerus depression (es)*	**0.79**	0.27	-0.29	Femur depression (es)	**-0.80**	0.01	-0.18
Humerus retraction (ff)	-0.07	-0.06	0.17	Femur retraction (ff)	0.07	-0.25	0.15
Humerus retraction (ms)*	0.14	-0.16	-0.08	Femur retraction (ms)*	0.14	-0.05	-0.04
Humerus retraction (es)	0.23	0.10	-0.06	Femur retraction (es)*	-0.33	-0.06	-0.09
Humerus rotation (ff)	**0.79**	-0.31	-0.03	Femur rotation (ff)	**0.89**	0.15	0.03
Humerus rotation (ms)	**0.81**	-0.38	-0.20	Femur rotation (ms)	**0.93**	0.09	0.01
Humerus rotation (es)*	0.41	-0.40	0.19	Femur rotation (es)	**0.89**	-0.15	-0.02
Elbow angle (ff)*	-0.04	**0.72**	0.25	Knee Angle(ff)*	0.09	0.01	0.10
Elbow angle (ms)*	-0.11	**0.72**	0.22	Knee Angle (ms)	0.11	0.13	0.14
Elbow angle (es)*	0.06	**0.83**	-0.16	Knee Angle (es)	-0.21	0.14	-0.09
Wrist Angle (ff)*	-0.05	0.02	**0.76**	Ankle angle (ff)	0.40	0.20	**0.67**
Wrist Angle (ms)*	-0.02	-0.10	**0.90**	Ankle angle (ms)	0.09	-0.08	**0.88**
Wrist angle (es)	-0.14	0.03	0.32	Ankle angle (es)	-0.07	0.10	0.39
IDA digits II and III (ff)	0.21	-0.03	-0.22	IDA digits II and III (ff)	-0.31	0.01	-0.30
IDA digits II and III (ms)	0.11	-0.09	-0.44	IDA digits II and III (ms)	-0.39	0.31	0.18
IDA digits II and III (es)	0.06	-0.14	-0.25	IDA digits II and III (es)*	0.04	**0.94**	-0.03
IDA digits III and IV (ff)	0.47	0.01	-0.21	IDA digits III and IV (ff)*	0.08	**0.96**	-0.05
IDA digits III and IV (ms)*	0.02	0.04	-0.14	IDA digits III and IV (ms)	0.00	0.26	-0.34
IDA digits III and IV (es)	0.35	-0.11	**-0.53**	IDA digits III and IV (es)	-0.14	0.27	**-0.63**
IDA digits IV and V (ff)*	-0.15	0.05	-0.08	IDA digits IV and V (ff)*	0.06	**0.97**	-0.01
IDA digits IV and V (ms)*	-0.05	0.07	-0.02	IDA digits IV and V (ms)	-0.15	0.42	-0.20
IDA digits IV and V (es)	-0.28	0.01	-0.19	IDA digits IV and V (es)	0.18	-0.11	-0.07
Foot alignment (ff)	-0.48	0.48	**0.56**	Foot alignment (ff)	**0.60**	0.06	**0.55**
Foot alignment (ms)	**-0.53**	0.36	**0.55**	Foot alignment (ms)	**0.65**	-0.04	**0.59**
Foot alignment (es)*	0.14	-0.16	0.12	Foot alignment (es)*	**-0.51**	-0.12	-0.22
Digit III vertical tip distance †*	**-0.69**	**0.54**	-0.23	Digit III vertical tip distance†*	**0.73**	0.01	-0.15

Loadings with a magnitude ≥ .5 are in bold

ff, footfall; ms, midstance; es, endstance

* Variable affected by speed

† Digit III vertical tip position is measured from the lowest position of the digit tip to the top of the substrate

Table 1.2. Averages and coefficients of variation (CV) of interdigital angles in the forelimb and hind limb in response to 1.5 cm, 10 cm and broad perches at 0°, 45° and 90°

Limb	Interdigital Angle	Incline (Deg)	1.5 cm		10 cm		Broad	
			Mean	CV	Mean	CV	Mean	CV
Forelimb	Angle II-III	0	30.09	0.22	35.71	0.18	34.70	**0.21**
		45	30.75	0.18	25.40	0.24	30.20	0.23
		90	26.90	0.18	23.57	**0.31**	23.20	0.22
	Angle III-IV	0	36.78	0.31	26.47	0.27	23.07	0.20
		45	36.39	0.17	23.99	**0.31**	21.01	0.26
		90	31.47	0.16	20.05	0.19	25.55	**0.28**
	Angle IV-V	0	27.37	**0.34**	33.17	**0.28**	38.05	0.20
		45	22.84	**0.42**	29.60	0.19	33.00	**0.28**
		90	28.38	**0.19**	30.17	0.26	31.85	0.22
Hind limb	Angle II-III	0	31.93	**0.43**	30.27	**0.25**	31.45	0.21
		45	28.70	***0.33***	29.84	**0.30**	25.33	**0.34**
		90	25.51	0.24	22.59	0.25	18.64	**0.40**
	Angle III-IV	0	41.60	0.22	56.76	0.20	57.67	**0.28**
		45	59.10	0.18	52.94	0.22	50.94	0.33
		90	49.28	0.17	30.90	**0.38**	39.17	0.28
	Angle IV-V	0	48.69	0.35	53.26	0.20	57.47	0.25
		45	52.80	0.30	60.94	0.21	67.14	0.21
		90	46.35	**0.28**	57.81	0.30	61.04	0.20

The highest coefficient of variation per treatment is in bold

Figure Legends

Fig. 1.1. Oblique and lateral views of *Phelsuma madagascariensis* at footfall (A), midstance (B) and endstance (C) on the 1.5 cm perch diameter inclined at 45°. The gecko is moving at approximately 0.71 ms^{-1}. Joint angles calculated (yellow arrows) include humerus/femur retraction (a), knee/elbow angle (b), humerus/femur depression (c), wrist/ankle angle (d), humerus/femur rotation (e) and vertical tip position of digit III (f). Landmarks (red dots) used in the calculations of joint angles are as follows: midline of the pectoral girdle (1), shoulder (2), elbow (3), wrist (4) tip of digit III of the forefoot (5), middle of pelvic girdle (6), hip (7), knee (8), ankle (9), and tip of digit III of the hind foot (10)

Fig. 1.2. Dorsal images of the forefoot (A) and hind foot (B) of *P. madagascariensis*. The scale bar is 10 mm. Foot alignment (a) is calculated as the two-dimensional angle between the axis of the foot and the antero-posterior axis. Interdigital angles (b-d) are calculated as the angle between the line connecting the wrist/ankle joint to the tip of the digit and the line connecting the wrist/ankle joint to the adjacent digit tip.

Figure 1.3. Representative joint angles for the forelimb (A and B) and hind limb (C and D) and sums of interdigital angles (IDA) for the small perch diameter inclined at 90° (A, C and E) (speed = 0.71 ms^{-1}) and the broad perch at 0° (B, D and F) (speed = 1.13 ms-1) of the same individual. The shaded regions (A-D) indicate the stance phase and solid vertical lines (E, F) indicate the end of stance phase for the forelimb (blue) and hind limb (pink). The x-axis represents the fraction of the stride. For joint angles, smaller values along the y-axis indicate greater flexion.

Figure 1.4. Principal component analysis (PCA) results for the kinematics of the forelimb (A-C) and hind limb (D-F). The first two principal components are plotted with the percent of variance explained by each component. Separate plots are presented for the 0° (A and D), 45° (B and E) and 90° (C and F) treatments. Each point represents the mean value of an individual per condition. For the hind limb, femur rotation and depression loaded strongly on PC1. On PC2, foot alignment and ankle flexion loaded strongly. For the forefoot, humerus depression, vertical tip position of digit III and foot alignment loaded strongly on PC1. Foot alignment and wrist extension loaded strongly on PC2.

Fig. 1.5. Mean values for several variables that loaded strongly in the principal component analyses for the forelimb and hind limb at mid stance. These include humerus and femur depression, humerus and femur rotation, wrist and ankle angle, angle (IDA) between digits IV and V in the forelimb and the vertical digit tip distance of digit III. Error bars indicate SEM.

Fig 1.6. Mean value plots for the sum of interdigital angles (A and B) foot alignment (C and D) and foot depression (E and F) at midstance. Results are shown for the forefoot (A, C and E) and hind foot (B, D and F). Note that decreased values of foot alignment with the z-axis indicate increased alignment with the long-axis of the body. Negative values indicate that the foot is inverted and oriented medially. See methods for a detailed explanation. Error bars indicate SEM.

Fig. 1.1. Distribution of digitized points and kinematic variables calculated

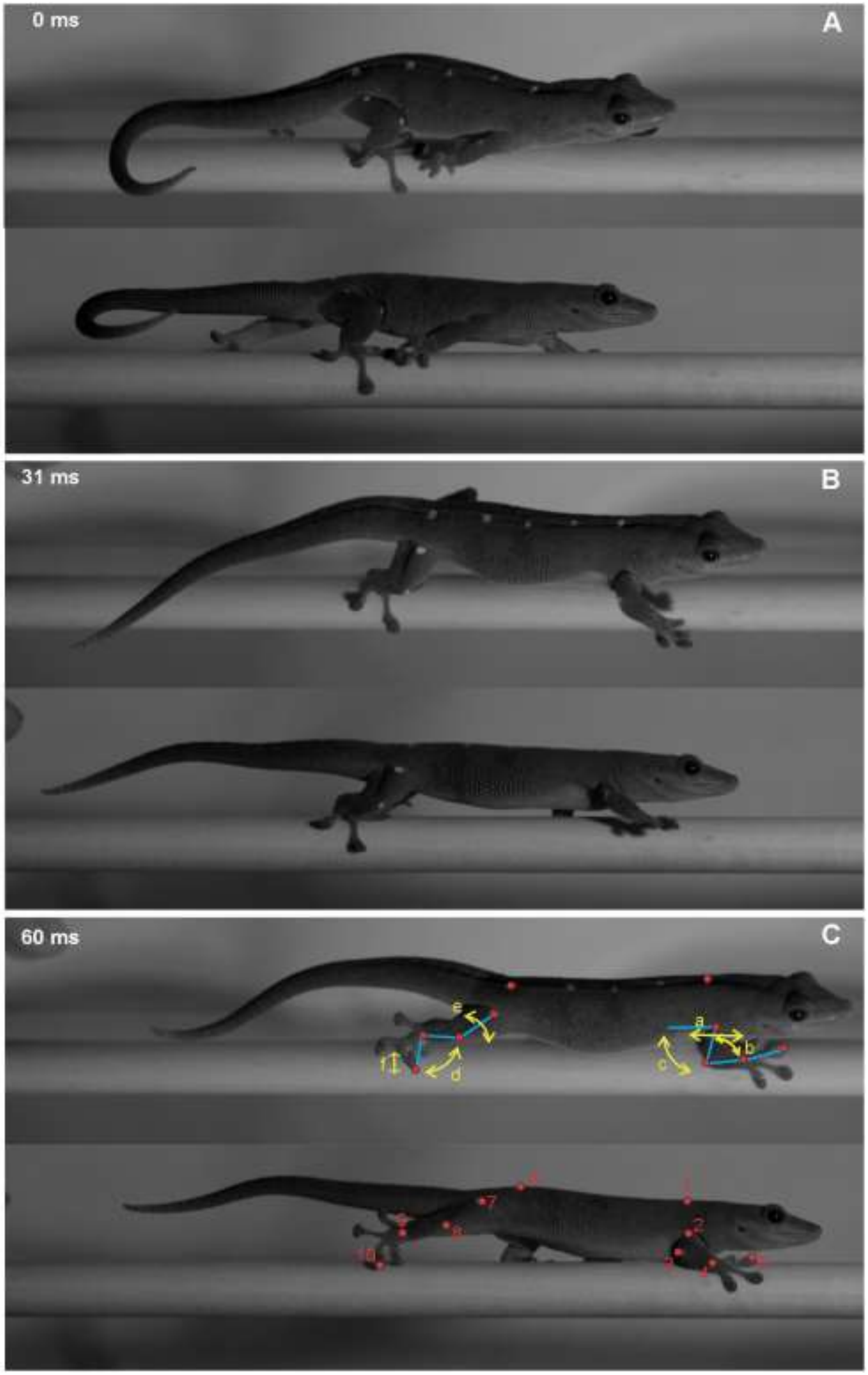


Fig 1.2. Variables calculated to describe foot behavior

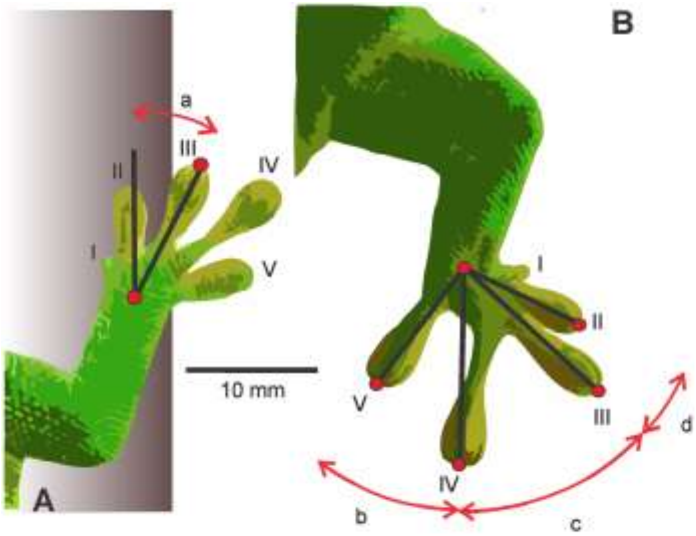


Fig. 1.3. Selected trials of kinematic variables

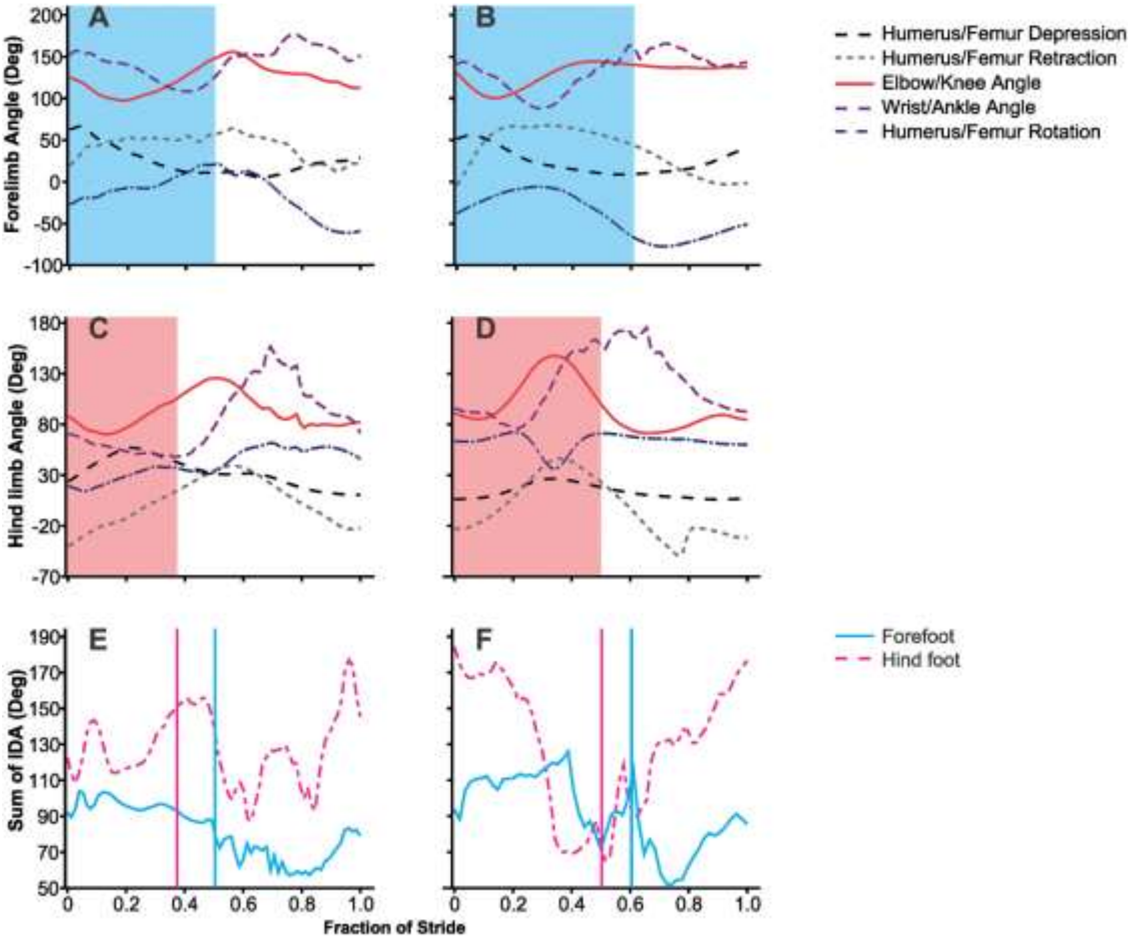


Fig. 1.4. Principal component analysis of kinematic variables in the forelimb and hind limb

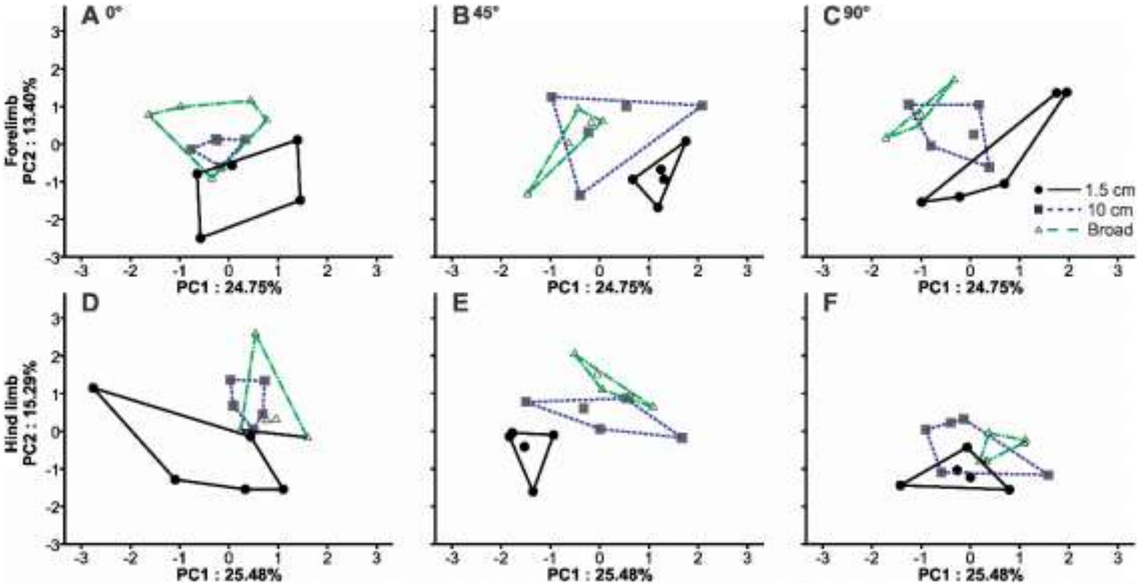
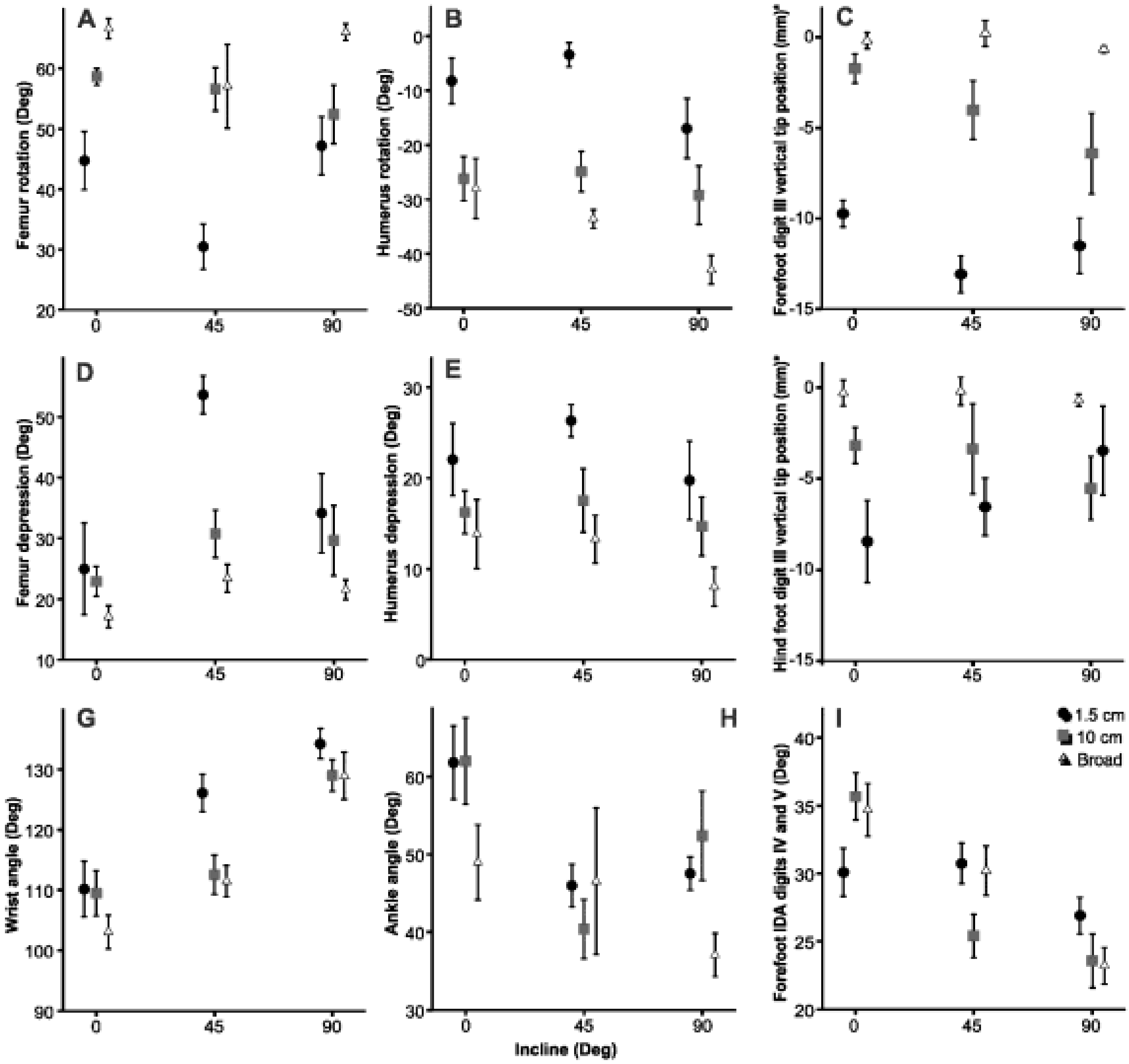
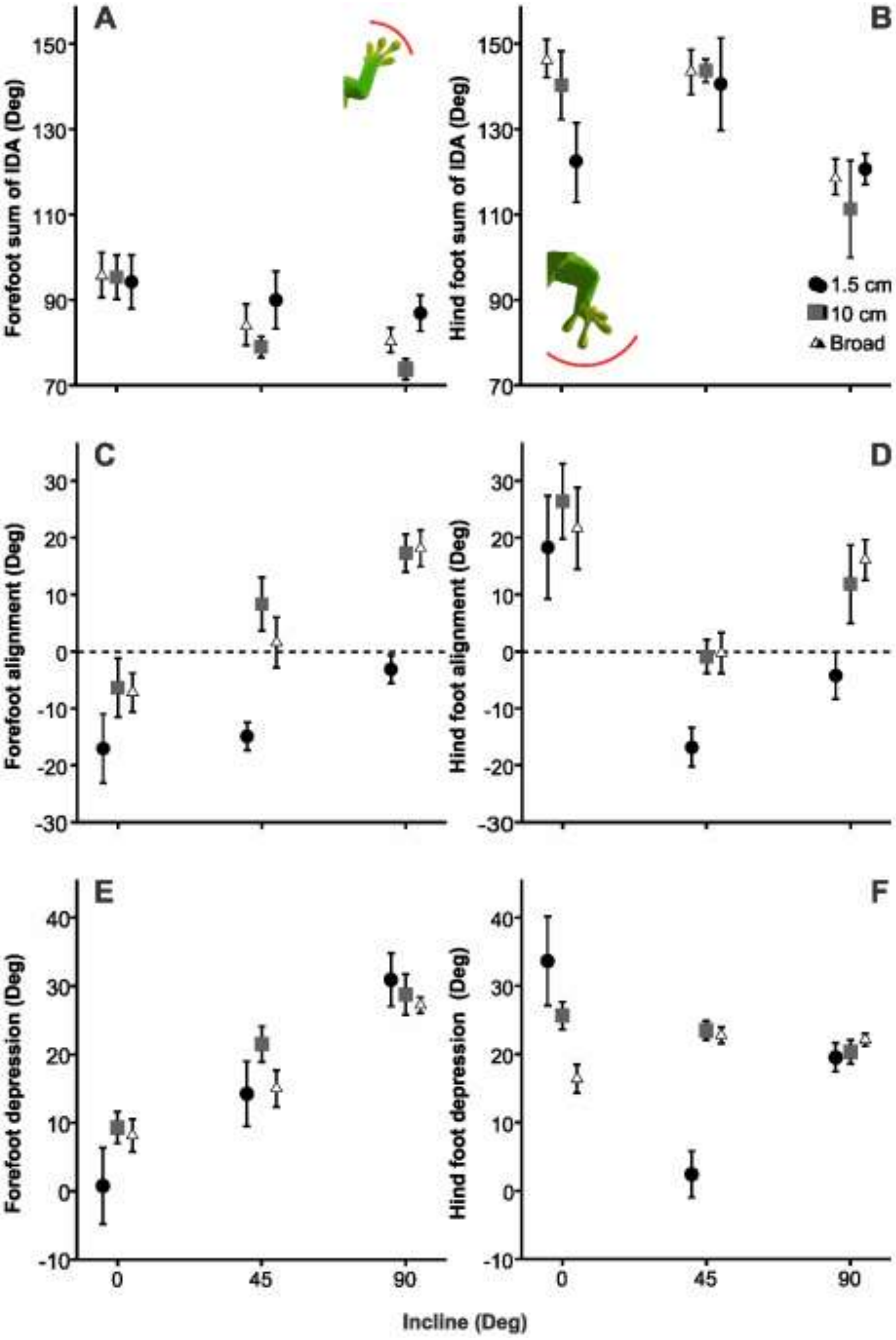


Fig. 1.5. Means of selected kinematic variables on different inclines and perch diameters



*Vertical tip position, the lowest position of the digit tip as measured from the top of the substrate.

Fig. 1.6 Means of foot alignment and sum of interdigital angles in the forefoot and hind foot on varying perch diameters and inclines



Chapter 2

Evolution of digital morphology in relation to the acquisition of the adhesive system in geckos

Mingna V. Zhuang[1], Anthony P. Russell[2], Timothy E. Higham[1]

Abstract

Morphological diversity is often influenced by the evolution of a functional innovation, which permits novel morphological arrangements. Among geckos, the evolution of the adhesive system is associated with several morphological changes in the hind feet that are thought to facilitate the application of the adhesive apparatus. We applied geometric morphometrics analysis from morphological data collected from X-Ray scans in order to examine patterns of morphological evolution in association with the gain and loss of adhesive capabilities, as well as transitions in habitat. Pad-bearing gecko lineages tend to have shorter digits and larger inter-digital angles than padless lineages. Digit arrangement is affected by both habitat and the presence of adhesive toe pads. These results suggest repeated shifts to a similar pad-bearing morphology, with some modifications that are dependent on environmental context. We demonstrate that functional innovation and habitat can operate on different components of foot morphology.

1. Introduction

Novel functions are often associated with morphological change and, therefore, are integral to the generation and maintenance of morphological diversity (Hunter, 1998; Price, Wainwright, Bellwood, Kazancioglu, Collar et al., 2010; Wainwright, 2007). Morphological diversity is inherently constrained by a number of factors, including phylogeny and function (McGhee, 2006). However, constraints can also be relaxed by morphological and/or functional innovations, which permit an increased range of possible morphologies (e.g. Higham, Birn-Jeffery, Collins, Hulsey and Russell, 2015). Therefore, innovation is often associated with morphological and ecological diversification (Losos, 2010; Price et al., 2010; Wainwright, 2007). In this new adaptive landscape, tradeoffs are imposed by the innovation and may shape an organism's morphology (Price et al., 2010). For example, flight is considered a functional innovation in birds that is associated with a diversity of wing morphologies (Prum, 2005). However, constraints related to flight (inherent mechanical constraints) restrict the range of possible morphologies. Bird bones tend to be hollow and reduce the amount of lift necessary for take-off. Simultaneously, hind limb morphology can vary because they are no longer the only mechanism for propulsion (Gatesy and Middleton, 1997).

Several studies suggest that the evolution of a novel function may not always lead to an increase in morphological diversity or even morphological change. In parrotfish, rates of morphological change increase with modifications to the pharyngeal jaw (Price et al., 2010; Wainwright and Price, 2016). However, morphological diversity does not appear to increase as a result of the novel trait alone. In contrast, specialization in carnivory in mammals is associated with a decrease in morphological diversity, potentially due to increased integration of the entire feeding apparatus (Holliday and Steppan, 2004). The evolution of the hypocone, an additional cusp in the molars of therian mammals, is associated with increased taxonomic diversity, but is not associated with much morphological change (Hunter and Jernvall, 1995). An increase in rates of morphological change in secondarily padless species of gecko suggests that constraints on foot morphology are relaxed as a result of the loss of the adhesive system (Higham et al., 2015). Although this study suggests that the function of the adhesive system imposes constraints on foot morphology and therefore, should be associated with subsequently decreased morphological diversity, it is unclear whether constraints are imposed on the entire foot complex. Furthermore, it is unclear how subsequent changes in morphology and morphological diversification are associated with shifts in ecology.

Several lineages of gecko possess an adhesive system that permits locomotion on smooth surfaces, such as glass, leaves and bamboo (Autumn, Liang, Hsieh, Zesch, Chan et al., 2000). Setae, or hairs on the ventral side of the toepad, engage with the substrate using a combination of van der Waals interactions and frictional adhesion (Autumn, Hsieh, Zesch, Chan, Fearing et al., 1999). Although setae are the main structures that adhere to the substrate, large scale morphological changes associated with the evolution of the gecko adhesive system occur throughout the foot, including changes in the skeleton, musculotendinous systems and toepad shape (Russell, 1975; 1979; Russell and Bauer, 1988; Russell, Bauer and Laroiya, 1997; Zaaf, Herrel, Aerts and De Vree, 1999). Modifications to the penultimate phalanx permit the hyperextension of each digit except in digit I. At the footfall of each stride, digits are hyperextended and continue to be hyperextended until the foot contacts the substrate (Russell, 1975). The toes then unfurl, allowing the toepads to interact with the substrate. Towards the end of the stride, disengagement via hyperextension occurs. As a result, the toepads disengage with the substrate before the rest of the foot. Morphological changes associated with the evolution of the adhesive system are thought to facilitate the engagement and disengagement of the adhesive system (Russell, 1975; 1979; Russell et al., 1997).

The gecko adhesive system has originated up to 11 times (Gamble, Greenbaum, Jackman, Russell and Bauer, 2012; 2017) and, therefore, this system provides the opportunity to examine if these suites of morphological changes are convergent on a similar pad-bearing morphology. Previous studies have proposed how the morphology of the gecko feet is expected to change with the evolution of the adhesive system (Russell, 1975; 1979; Russell et al., 1997). In addition to changes in the shape of the penultimate phalanx, digits are likely to be shorter and digital spread should be increased in pad-bearing lineages. A large digital spread provides enough space for the individual toepads to prevent overlap and ensures that each digit is oriented in a different direction, therefore permitting the engagement of the adhesive apparatus in multiple directions simultaneously (Russell and Oetelaar, 2015). These changes appear to precede the evolution of the gecko adhesive system and are exaggerated following its acquisition (Russell et al., 1997). In *Gonotodes*, further digital shortening with the acquisition of the adhesive system is observed (Russell, Baskerville, Gamble and Higham, 2015). These morphological changes are not absolutely necessary for the evolution of the adhesive apparatus, as evidenced by their absence in taxa that have a similar dry adhesive apparatus (e.g. anoles, certain skinks). However, the dry adhesive apparatuses in other taxa are relatively weak and simple in comparison to the adhesive apparatus of geckos (Autumn, Dittmore, Santos, Spenko and Cutkosky, 2006; Dalla Valle, Nardi, Bonazza, Zuccal, Emera et al., 2010).

The arrangement of the digits within the hind feet is likely a key determinant of function, especially the application for forces on the substrate. In non-gekkotan lizards, the hind feet approaches asymmetry, characterized by the increase in length from digits I -IV, a short fifth digit and a large interdigital angle between the fourth and fifth digits (Brinkman, 1980; Rewcastle, 1983; Russell et al., 1997; Schaeffer, 1941). The gradual increase in length in the first three digits creates a metatarsophalangeal line, which is important in directing locomotion along the anterior-posterior axis (Rewcastle 1983). This line is often broken in geckos, who have larger interdigital angles between all four digits and hind feet that are more bilaterally symmetrical (Russell et al. 1997). Although there may be a cost to locomotion because that the hind foot may not be oriented optimally for propulsion, a symmetrical foot ensures equally distributed toepads and, therefore, the ability the adhere in any body orientation (Russell and Oetelaar 2015). On the other hand, asymmetry returns in some gekkotan lineages, such as *Phelsuma*, although large interdigital angles may be retained (Russell et al., 1997). If asymmetry is associated with propulsion, we expect this feature to be retained or return in cursorial, terrestrial geckos.

We used a phylogenetic framework to understand not only the principles of how gecko feet evolve and how morphology might be associated with the evolution of the adhesive system, but also how habitat might impact the expression of this pad-bearing morphology. We combined data from Russell et al. (1997) and data collected from museum specimens to test predictions from Russell et al. (1997) about the differences between pad-bearing and padless geckos, namely that interdigital angles are larger, digits are shorter and greater symmetry is present in pad-bearing geckos. We also predicted that morphospace occupation and rates of morphological change in pad-bearing geckos would be smaller due to constraints imposed by the adhesive apparatus (Higham et al., 2015). Finally, we applied a geometric morphometrics framework to capture shape evolution to better assess the evolution of symmetry.

2. Materials and Methods

2.1. Phylogeny

We pruned a tree from Zheng and Wiens (2016) and added additional species. (Fig. 2.1). *Aristelliger* species and *Rhoptropus taeniostictus* were added based on taxonomic and morphological information (Bauer and Good, 1996). The following species and genera were added to the tree by combining trees from Bauer and Good, (1996); Bauer, Good and Branch (1997a); Jacobsen, Kuhn, Jackman and Bauer (2014) Pyron, Burbrink and Wiens (2013); Wood, Heinicke, Jackman and Bauer (2012) *Cyrtodactylus, Goggia*, *Pseudothecadactlus*, *Rhacodactylus*, *Hoplodactylus pacificus*.

2.2 Sampling

We used data from Russell et al. (1997) and collected additional data from 67 specimens (55 species) from X-rays. The original data included 137 specimens and a total of 100 species. Additional specimens were obtained from the California Academy of Sciences, the Museum of Vertebrate Zoology, La Sierra University and private collections from Tony Russell and Raul Diaz. Specimens were flattened between two slides of glass before being x-rayed to avoid parallax and only specimens that could be flattened were used so that each digit could be viewed clearly. We obtained measurements for the individual interdigital angles, digit lengths and metatarsal lengths following descriptions in Russell et al. (1997) (Fig. 2.2).

To obtain the sum of phalangeal lengths within each digit, we subtracted the metatarsal length from the digit lengths. Interdigital angle data were collected by extending axes along the metatarsals until they intersected with the neighboring axis (Russell et al., 1997). Three measurements to describe the spread of the digits were used, which were the sum of the first three interdigital angles (IDA I-IV), the sum of total interdigital angles (Sum of IDA I-V) and the angle between the first and fifth digit (IDA I-V) (Fig. 2.2). We used three measures to account for errors in a single measure. The first four metatarsals are sheathed in a common sac so that the axes of these metatarsals intersect at a single point. However, the position of the fifth metatarsal is more flexible and therefore, the last interdigital angle may depend on the specimen’s preparation. Because of the potential unreliability of the last interdigital angle between digits IV and V (Russell et al., 1997), the sum of the first three interdigital angles (IDA I-IV) were calculated in addition to the sum of all of the interdigital angles. Because the axis of the fifth metatarsal does not often intersect the axes bifurcating the other metatarsals, an additional measure to describe the digital spread was quantified by measuring the interdigital angle between digits I and V. To obtain the bilateral asymmetry of the angles, we subtracted the sum of the angles between digits I and III from the sum of the angles from digits III-V. Then, we took the absolute value of this measure.

Padstate was coded as padless or pad-bearing. Secondarily padless geckos were coded as padless. Toepad arrangement is not uniform across geckos. A leaf-toed morphology, characterized as having divided and expanded scansors, has evolved several times throughout the phylogeny (Gamble et al. 2012). Because leaf-toed morphology differs significantly from the more “basal” pattern of toepad, we initially coded leaf-toed geckos separately. Information about the presence of leaf-toes was also collected from the literature (Gamble et al., 2012; Higham et al., 2015). Habitat data were collected from the literature and geckos were defined as arboreal, saxicolous or terrestrial (See Table 2.9)

2.3 Data collection for geometric morphometrics analysis

Eleven landmarks describing the arrangement of the metatarsals were digitized on each X-ray image (Fig. 2.2; Table 2.1) using tpsDig2 (Rohlf, 2005). Because digit position can depend on the quality of the preservation or the ability of a prepator to properly lay out digits, landmarks were only placed on the metatarsals, which are more constrained in position by muscle and tissue (Russell et al., 1997). Landmarks were then superimposed using General Procrustes Analysis (Rohlf and Slice, 1990) in the geomorph package (Adams and Otarola-Castillo, 2013) and an mean per species was calculated for further analysis. For this analysis, centroid size was highly correlated with body size (r2=0.71, df=102, p=0.001 with phylogenetic correction).

Bilateral asymmetry of the shape is calculated by measuring the Procrustes distance from the original shape of a species and its symmetric consensus (Klingenberg, Barluenga and Meyer, 2002). In this study, we measured the bilateral asymmetry of the sole shape by reflecting the landmarks collected across an axis created by landmarks 1 and 9 (third digit). Greater values of bilateral asymmetry indicate larger asymmetry, whereas smaller values indicate an object that has greater within-object symmetry.

2.4 Statistical analysis

We performed a principal components analysis using size corrected measures of all of the sums of phalangeal lengths, metatarsal lengths, interdigital angles, the sum of the interdigital angles and the bilateral asymmetry measure. To correct for size and examine the effect of size on the variables, we regressed each length variable against body size (snout-vent-length) using a linear model regression and phylogenetic generalized least squares regression (PGLS). We then applied equation 1.1, where b was the slope of the regression line from PGLS (Blomberg, Garland and Ives, 2003). Raw interdigital angles and the bilateral asymmetry measures were not transformed because these variables were not improved by log transformation. For most of the analyses, we found that geckos with the leaf-toe morphology did not significantly differ from geckos that had the basal pattern of toepad shape and we therefore, pooled these data together as pad-bearing.

$$\log\left(\frac{variable}{SVL^b}\right) \tag{1.1}$$

In order to assess the effect of padstate and habitat, we performed two-way ANCOVAs, using type III sum of squares, on variables that loaded highly in the principal components analysis. For these analyses, we used the car and nlme packages. If the interaction effect was not significant, we repeated the model with type II sum of squares and without the interaction effect. We first performed the analyses assuming Brownian motion, then took the residuals from the model and fitted the residuals to both Brownian motion (BM) models and the Ohrlein-Uhlensteinbeck (OU) models in the Geiger package (Revell, 2010). We then chose a model based on the lowest Akaike information criterion unless the difference was less than 4. If the difference was less than 4, the Brownian motion model was used. If the OU model was a better fit, we repeated the analysis with the OU model. Phylogenetic correction was only used if the residuals of the PGLS model had a significant phylogenetic signal. If we found a significant interaction effect or effect of habitat, we performed a tukey posthoc test using the lsmeans package in R.

Because some data were missing from the original data set, we used a subset of the total data, which resulted in 100 species. The phylogeny was then projected onto the morphospace to create a phylomorphospace using the phytools package.

2.4.1.1 Geometric morphometric analysis

All statistical analyses pertaining to the geometric morphometric data were performed using a combination of the geomorph and phytools packages (Adams and Otárola-Castillo, 2013; Revell, 2012). We performed a principal components analysis on the landmarks and then conducted two-way ANOVAs on the first two PC axes, as well as the bilateral asymmetry measure, using padstate and habitat as the independent variables. To understand the evolution of foot shape, we fitted the shape data to single rate Brownian motion, and OU, using the fitContinuousMV function in geiger (Harmon, Weir, Brock, Glor and Challenger, 2008).

3. Results

3.1. Principal components analysis

A total of 105 species were used in the principal components analysis (PCA). The first three principal component axes (PC) explained 74.83% of the data (Fig. 2.3). The first PC axis (40.80%) described changes in lengths within the digit (Table 2.2). Higher scores on the first PC axis corresponded to longer metatarsal and sums of the phalangeal lengths (Table 2.3). The second PC axis (23.38%), described changes in interdigital angles between digits II and V and angular descriptions, such that higher scores on the second PC axis corresponded to lower bilateral asymmetry, smaller interdigital angles (III-IV and IV-V) and a smaller sum of total interdigital angles. Finally, the third PC axis (10.65%) described changes in the interdigital angles of the medial digits (I-II and II-III), the last interdigital angle and bilateral asymmetry. Higher scores on the third PC axis represented larger angles between digits I and II and between digits III and IV and a smaller interdigital angle between digits IV and V, as well as lower bilateral asymmetry. With the exception of the last interdigital angle (IV-V), all traits had a phylogenetic signal (IV-V) (Table 2.4). Angular bilateral asymmetry had a weak phylogenetic signal. Body size (SVL), and the rest of the interdigital angles, angular measurements, and the sum of phalangeal lengths had moderate signals (.3< lambda <.6). Metatarsal lengths, digit lengths and the sum of angles between digits I and IV had strong phylogenetic signals (lambda >.6).

The most ancestral lineages, the carphodactylids, occupied a region of the morphospace described by longer digits (Fig. 2.3). The pad-bearing diplodactylids, sister to the carphodactylines, occupied the region closest to the mean morphology and the leaf-toed lineages of the diplodactylids separated from those with a basal pattern of toepads. Within the diplodactylids, the *Strophurus* genus appeared to have shorter digits than the Rhacodactyline clade. Within Phylodactyllidae, pad-bearing lineages occupied a region of morphospace described by large interdigital angles. Within the *Cyrtodactylus* group, the shift to pad-bearing represented a transition to the lower quadrant of the morphospace, described by shorter digits, but not necessarily larger interdigital angles. Instead *Cyrtodactylus*, a highly arboreal, but padless genus was characterized by having the largest interdigital angles (III-IV and IV-V) of the group. Finally, within Gekkonidae, there were several shifts to a pad-bearing morphology. When secondarily padless species were identified separately from ancestrally padless geckos in the morphospace, it was clear that they were different from their ancestrally padless counterparts. However, because that there were only seven secondarily padless geckos in this dataset, these were not analyzed separately. Many of the species remained in a region of morphospace that was characteristic of pad-bearing lineages. However, *Ptenopus kochi* and *Rhoptropus afer*, secondarily padless geckos, shared a region of the morphospace with the ancestrally padless lineages. *Gehyra* and *Uroplatus* represented had the largest interdigital angles of the gekkonids.

The effect of habitat on digit lengths depended on padstate (PC1) (Table 2.2; Table 2.5; Fig. 2.6). In arboreal and saxicolous habitats, pad-bearing lineages had shorter digit lengths than padless lineages ($p<.05$, *tukey posthoc test*). Within pad-bearing lineages, geckos from arboreal habitats had shorter digits than those in saxicolous habitats and pad-bearing arboreal lineages differed from any padless lineage (Fig. 2.4).

Neither habitat nor padstate affected the spread of interdigital angles (PC2; Fig. 2.5; Table 2.5).

3.2 Geometric morphometric analysis

The first three PCs described (77.24%) of the data. The first PC (45.91%) described changes in the interdigital angles, shortening of the metatarsals, and an increase in the space between the heads of the metatarsals (Fig. 2.7). Positive scores on the first PC axis described shapes in which the metatarsal heads were closer together and the interdigital angles were smaller. The second PC axis (19.73%) similarly described changes in the interdigital angles, the spacing of the metatarsal heads and changes in the relative length of the fourth metatarsal. Higher scores on the second axis corresponded to larger spaces between the metatarsal heads, larger interdigital angles, as well as a relatively longer fourth metatarsal in comparison to the other metatarsals. Foot shape had a relatively strong phylogenetic signal (Blomberg's K=0.853, p=0.001).

Padstate separated on the first PC axis, but habitat did not (Table 2.7). The effect of habitat depended on padstate on PC2. Only arboreal pad-bearing species had a more asymmetrical shape and larger interdigital angles than arboreal padless species. Within padless species, arboreal species had greater scores on PC2 than saxicolous species. Within pad-bearing species, arboreal species occupied higher scores on PC2 than terrestrial species but were no different from saxicolous species. Finally, saxicolous pad-bearing species had higher scores on PC2 than saxicolous padless species (p<.05, posthoc tukey test).

Centroid size was a good indicator of size (SVL) (r2=0.71, df=102, p=0.001 with phylogenetic correction). Therefore, we examined changes in shape with size by using the centroid size. Shape was not affected by size (t=1.152, df=102, p=0.35 with phylogenetic correction). Foot size was not affected by padstate or habitat (X2(2,5)=-0.99, p=0.325 with phylogenetic correction and X2(2,5)=1.42, p=0.163 with phylogenetic correction, respectively).

When centroid size is considered in the analysis as a covariate, pad-bearing species were generally different from padless lineages (Procrustes ANCOVA: F(2,5)=4.56, p=0.001). Pad-bearing species had more equally distributed interdigital angles, Foot shape also differed depending on habitat (F(2,5)=3.69, p=0.001). A posthoc test at p<.005 showed that saxicolous and arboreal species were not significantly different from each other, but both were different from terrestrial lineages. Rates of morphological evolution were not different between padstates (sigma ratio=1.07, p=0.64)

When centroid size is considered in the analysis as a covariate, pad-bearing lineages had lower degrees of asymmetry than padless lineages and a posthoc test showed that saxicolous lineages had greater degrees of asymmetry than arboreal or terrestrial species, as measured using geometric morphometric methods (F(2,5)=7.3232, p=0.000 and F(2,5)=7.43, p=0.002, respectively, posthoc Tukey test p<.05). However, arboreal pad-bearing species have approximately the same degree of symmetry as saxicolous pad-bearing species and there are few terrestrial pad-bearing species in our sample. Therefore, padless lineages may be driving the observable differences in symmetry among arboreal, saxicolous and terrestrial lineages. Furthermore, PC1 was not correlated with asymmetry (r2(1,53)=.01, p<.451). PC2 scores were negatively correlated with asymmetry (r2(1,53)= .4553, p<1.61*10^-8) but only explained 6% of the variation.

3.3 Effect of size

With the exception of bilateral asymmetry and the angles between digits III and V, lengths within the digit and angle measurements increased with body size with scaling exponents ranging from 0.87 to 1.0 (Table 2.4).

3.4 Effect of padstate and habitat on angle measures

Angular measurements and digital spread were usually greater in pad-bearing lineages than padless lineages (Table 2.5). The digital spread variables (IDA I-V, IDA I-IV and sum of total interdigital angles) were not affected by habitat. Bilateral asymmetry was not affected by padstate, but was greater in arboreal species than saxicolous species (F(2,4)=5.82, p=0.00394, posthoc Tukey test p<.05). Each interdigital angle was greater in pad-bearing lineages than in padless lineages. The effect of padstate depended on habitat for interdigital angle (III-IV).

3.5 Effect of padstate on digital morphology.

Overall, digits were shorter in pad-bearing lineages than in padless lineages (Fig. 2.6; Table 2.6; Table 2.5). Only the first two metatarsals were shorter in pad-bearing lineages than padless lineages. However, all metatarsals, with the exception of the fifth, were affected by habitat. The effect of habitat depended on padstate for the first and third metatarsals. The sum of the phalangeal lengths within each digit was shorter in pad-bearing lineages than in padless lineages, but was not affected by habitat. In all digits, the total length was shorter in pad-bearing lineages than in padless lineages.

3.6 Predictors of angular measurements

Angular bilateral asymmetry and digital spread were not determined by a single interdigital angle. Bilateral asymmetry increased with an increase in the interdigital angles between digit III and V (r(1,117)0.49, $p<0.05$ and t(1,117)=22.81, $p<0.05$, respectively). Bilateral asymmetry was not affected by changes in interdigital angles between the medial digits (I-III) (t(1,117)=-0.36, p=0.721, r=0.07, df=101, p=0.483). Bilateral asymmetry increased with an increase in digital spread (Digital Spread (I-IV): t(1,117)=2.4, p=0.018). All interdigital angles increased with an increase in digital spread as measured by IDA I-5 (t=2.4 to 12.64, df=101, $p<.001$). All interdigital angles were intercorrelated with the exception of the last interdigital angle (IV-V), which was only correlated with interdigital angle (III-IV) (Table 2.8).

4. Discussion

We expected the morphology of gecko feet to be impacted by the functional constraints imparted by the adhesive system, as well as the demands of the habitat in which they live. We used a phylogenetic framework and geometric morphometrics to characterize the morphology of the gecko feet in species that never had an adhesive system, have a well-developed adhesive system, and in species that have secondarily lost adhesive capabilities. Both habitat and the presence or absence of an adhesive system influence how the foot is shaped. Thus, we provide support for the idea that morphological novelty can evolve along different trajectories that are dependent on ecology.

4.1 Evolution of digit morphology

We predicted that pad-bearing species would express digit morphologies that facilitate the function of the adhesive system. Specifically, we expected that, in comparison to padless lineages, pad-bearing lineages would have shorter digits and a greater digital spread, features that would prevent the overlapping of expanded toepads. This would not only maintain a wide range of possible orientations for the foot, but also allow each toe to adhere simultaneously (Russell et al., 1997; Russell and Oetelaar, 2015; Webster, Johnson and Russell, 2009). Our results support this prediction, but we found considerable overlap in morphology between pad-bearing and padless lineages. Padless lineages within the overlapping region belong to more ancestral lineages, the carphodactylids and suggests that padless lineages may have a greater range in digit length than is possible in pad-bearing lineages.

We identified multiple trajectories to a pad-bearing morphology by tracing the phylogenetic branches (Bergman, 2015). Among the carphodactylines, diplodactylids (padbearing) do not have increased interdigital angles and primarily differ from carphodactylids by having shorter digits. The simultaneous decrease in digit lengths and increase in digital spread occurs in Gekkonidae (containing padbearing and padless) and Phyllodactylidae (padbearing). Finally, within the *Hemidactylus* group, *Hemidactylus* overlaps greatly with the padless lineages in its clade, but has relatively shorter digits. *Hemidactylus* is the only pad-bearing lineage of its clade and is sister to *Cyrtodactylus (padless)*, a genus characterized by not only relatively long digits, but also relatively large interdigital angles when compared to many padless species. The lack of change in the digital spread and interdigital angle could be attributed to the existing presence of larger interdigital angles in the clade. Therefore, the change in morphology with the acquisition of the adhesive system in this instance is characterized by change in digit lengths rather than interdigital angles. In each of these instances, there were shifts in a similar direction, indicating convergence in digit morphology. However, changes in the interdigital angles were not in the same direction. These results identify changes in digit length as a key and common component of the gecko adhesive system, whereas changes in the interdigital angles appear to vary among pad-bearing lineages.

The changes in morphological variation among pad-bearing lineages is likely impacted by the function of the gecko adhesive system (Higham et al., 2015). When examined on a broad scale, however, there appear to be multiple solutions to acquiring an adhesive system. This might explain the large region of morphospace being occupied by pad-bearing lineages and lack of difference in the rates of morphological change between pad-bearing and padless lineages. In contrast, rates of morphological change in the foot of a climbing salamander genus, *Hydromantes* were lower than in the foot of a nonclimbing salamander (Adams, Korneisel, Young and Nistri, 2017). However, this study had only two genera and it remains to be seen if this is a pattern repeated among climbing and nonclimbing salamanders. Within the *Pachydactylus* radiation of geckos from Southern Africa, the rate of morphological and kinematic evolution increases following the secondary loss of adhesion and the reversion to a cursorial lifestyle (Higham et al., 2015). One explanation for the comparable rates of morphological change in found in our study is that morphological change in the gecko foot with the acquisition of the adhesive system is characterized by a shift in the centroid of possible morphologies rather than an expansion. In other words, although certain morphologies become unavailable or less likely (extremely long digits) to pad-bearing geckos, new ranges of morphology do become available (shorter digits). The finding of increased rates of morphological change in secondarily padless lineages then might be attributed to the relaxation of functional constraints in one direction of morphological change, instead of all directions. This study suggests then that understanding how morphological novelties relate to morphological evolution requires the comparison of the region of morphospace explored to the region of morphospace conserved. Both basic functional constraints of the adhesive system and multiple solutions to acquire the adhesive system explains the lack of decrease in morphological diversity but shifts in morphology in pad-bearing geckos.

The finding that functional constraints on foot morphology are relaxed in secondarily padless lineages may be clade specific. Therefore, closer examination of morphological evolution within a clade may reveal repeated instances of constraints on the morphological diversity of pad-bearing lineages as a result of the adhesive system, whereas this may not be true for other clades. Although we did not find evidence for the relaxation of functional constraints, it is apparent that secondarily padless gekkonids may be driven back to a morphology similar to ancestrally padless lineages. This is most obvious in *Ptenopus kochi* and *Rhoptropus afer*. The latter diverges from the rest of its genus by having relatively longer digits, but is also an unusual gecko in that it is highly cursorial (Collins, Russell and Higham, 2015; Higham and Russell, 2010; Johnson, Russell and Bauer, 2005). Other secondarily padless lineages or lineages with highly reduced adhesive systems remain in a region of morphospace that overlaps with both padless and pad-bearing lineages. These findings suggest that secondarily padless lineages are somewhat different from ancestrally padless lineages in digit morphology and arrangement. They may retain shortened digits despite losing the adhesive system. Features associated with the adhesive system that are retained among secondarily padless lineages also include modification to the phalanges to permit the function of the hyperextension system. This pattern may be further reflected in the skeletal, muscular and external levels of morphology (Russell and Rewcastle, 1979).

Leaf-toe geckos do not have a significantly different internal morphology from those with the basal pattern, despite very apparent changes in the toepad morphology. This suggests that external morphology may evolve prior to modifications of internal form. However, large scale changes in the vasculature and neural control systems the digits in leaf-toe geckos are evident (Russell, 1979; Russell and Delaugerre, 2017), so perhaps the skeletal elements lag behind soft tissue differentiation. It should be noted that within Diplodactylidae, the *Strophrurus* genus appear to separate from nonleaf-toed geckos within that family, but this was not a pattern repeated in every family. Alternatively, differences between leaf-toe lineages and the pad-bearing lineages with the basal pattern may not be captured by the measurements from this analysis. Instead, widths and shapes of the penultimate and ultimate phalanges may be the important aspects that are modified and are furthermore, an indicator that modifications to articular and subtler changes of phalanges shape occurs before lengths and interdigital angles. Future studies should survey the differences in soft tissue variation together with changes in skeletal structure to better understand how leaf-toed morphology may differ from the basal pattern.

However, in this study, leaf-toe lineages are an example of the decoupling of external and internal levels of morphology.

Unlike more proximal elements of the limb, the foot interacts directly with the substrate and is likely impacted by its functional demands (Higham, 2015). Foot width, which may serve as a proxy for the digital spread measured in this study, is found to be greater in arboreal than terrestrial lineages, such as squirrels and several lineages of frog (Mintsa, Berge and Picard, 2009; Simons, 2008). In squirrels, foot length is also shorter in the arboreal ecomorph. According to our analyses using traditional morphometrics, within pad-bearing lineages, arboreal geckos had shorter digits than other geckos. However, saxicolous species had a larger spread. Terrestrial geckos also appeared to have greater variance in interdigital angles. The results of other studies of gecko lineages are somewhat consistent with our findings. Differences between climbers and nonclimbers are not clear in the *Pachydactylus* radiation (Johnson et al., 2005). However, among neotropical geckos, climbing geckos have shorter digits and a shorter fifth metatarsal when compared to nonclimbing lineages (Rothier, Brandt and Kohlsdorf, 2017). These latter results are congruent with our findings and suggest that this pattern is repeated across the phylogeny. With the exception of *R. afer*, *Rhoptropus* groups together in the morphospace. However, this group does not appear to differ greatly from the rest of the *Pachydacytlus* radiation, which is in contrast to Johnson et al. 2005 and (Higham et al., 2015; Johnson et al., 2005). This difference may be attributed to the inclusion of interdigital angle data and slightly different length measurements, especially given the fact that the number of phalanges varies across the gecko phylogeny. In other words, this variation may wash out any variation within radiations of geckos. A consistent pattern, however, is that the lengths of the phalanges describes most of the variation in foot morphology among geckos. We therefore propose this to be a key aspect of functional divergence, which can be addressed in future studies.

In some aspects of the foot, the expressions of pad-bearing or padless morphologies depend on the context in which the adhesive system evolved. Within pad-bearing lineages, arboreal species tend to have shorter digits than saxicolous species. How an evolutionary novelty is expressed is thought to be modulated in association with changes in habitat due to the intense selection pressure imposed by habitat (Hunter, 1998). Furthermore, within padless lineages, arboreal geckos had a different morphology than saxicolous species. Therefore, it is likely that the change across this transition may be exaggerated with the evolution of the adhesive system.

4.2 Evolution of sole shape

Results from the geometric morphometric analyses echoed many of the findings from the traditional morphometrics approach and, furthermore, were able to describe the shape of the foot encompassing the metatarsals and the interdigital angles simultaneously. Most importantly, it identified changes in the distance between the articulation of the mesotarsal joint and the divergence in the metatarsal heads with changes in padstate and habitat. An increased distance suggests that the third and fourth distal tarsal may be larger in pad-bearing and arboreal lineages in comparison to padless and terrestrial lineages. Change to the shape of the fourth distal tarsal is especially important because of its involvement in the mesotarsal joint (Brinkman, 1980; Rewcastle, 1983). A relatively enlarged fourth distal tarsal could provide a larger articular surface for the astragalocalcaneum and, therefore, increase the possible range of motion possible at the joint by providing a larger surface across which the astragalocalcaneum could translate. Spacing of the metatarsal heads could also change as a result of changes in the fourth distal tarsal and be the mechanism for increased interdigital angles. Increased distance between the metatarsal heads is also a potential indicator of the expansion of the metatarsal heads, which may be an additional mechanism for increasing interdigital angles in pad-bearing lineages and the mechanism for increasing the area of muscle or tendon insertion. For example, the plantar aponeuroses, responsible for supinating the limb and controlling scansors on digit five, has one branch that inserts onto the head of the fifth metatarsal (Russell, 1975). Further analyses on the shape of both the metatarsal heads and bones involved in the mesotarsal joint are needed to clarify this.

Geometric morphometrics also provide a more comprehensive measure of symmetry that not only takes into account the interdigital angles, but also the lengths of the metatarsals. This is important because symmetry within the foot can be created by changing the angles or the lengths of the digits. Therefore, bilateral angular asymmetry may not be the best measure to describe the distribution of the toepads overall and, instead, is only a measure of how symmetrical the radiation of the digits may be. As a result of these methods, we found support for our prediction that pad-bearing species have a greater degree of symmetry than padless lineages. These results also support suggestions by (Russell et al., 1997) that trends toward symmetry are present in more basal padless lineages.

We found that PC2 correlates negatively with our symmetry measure. However, this does not necessarily indicate that species located on the positive end of PC2 have symmetrical sole shapes. Instead, our analysis revealed several ways that asymmetry can be achieved. Species located at the negative end of PC2 achieved asymmetry by having a large last interdigital angle, whereas on the positive side of PC2, asymmetry, which was lower in value, was created by a longer fourth digit on the positive side of this axis (Fig 2.7). This latter region of the morphospace was occupied mostly by arboreal pad-bearing lineages, indicating secondary asymmetry.

The observation that secondary asymmetry occurs in some lineages, such as *Phelsuma*, may be the product of an adhesive system operating in the arboreal environment (Russell et al., 1997). This secondary asymmetry is different than those of padless lizards in that interdigital angles are much larger than the ancestral foot morphology and may also be the result of the reduction in the first digit (Russell and Bauer, 1990; Russell et al., 1997). Asymmetry, produced by the last interdigital angle being the largest of the interdigital angles and a long fourth digit, has had several proposed functions, including permitting grasping between the first four digits and the last digit, facilitating propulsion and positioning the fifth metatarsal, such that it can function like the calcaneal tuber in mammals (Robinson, 1975; Russell and Rewcastle, 1979; Zhuang and Higham, 2016).

In lizards with an asymmetrical foot, the distal tips of metatarsals I-III are aligned such that they form the metatarsophalangeal line (Rewcastle, 1983). This line serves as an important contact with the substrate at the end of the propulsive phase during locomotion and is oriented perpendicular to the long axis of the body. Although symmetry within the foot reduces the number of digits involved with the metatarsophalangeal line, secondary asymmetry may increase the number of distal tips involved, thereby increasing propulsive forces. Therefore, secondary asymmetry may facilitate propulsion on relatively broad substrates that are characteristic of many arboreal microhabitats, such as tree trunks. *Phelsuma madagascariensis* in particular, is an arboreal ambush predator who may need higher acceleration forces facilitated by hind feet asymmetry. On the other hand, the arboreal habitat can also be complex if it is composed of varying branch diameters and inclines (Cartmill, 1985). Grasping on smaller perch diameters may be facilitated by a longer fourth digit and an asymmetrical foot. Given that symmetry of the foot is ultimately ancestral, these patterns reveal multiple shifts between relatively symmetrical and asymmetrical hind feet, which may have multiple functions depending on behavior and habitat (Russell et al., 1997).

Asymmetry created by a shorter fourth digit and larger interdigital angles appears to be characteristic of more saxicolous lineages among both pad-bearing and padless species. This finding implies that the more symmetrical sole shapes, characteristic of saxicolous pad-bearing lineages, are achieved mainly through the shortening of the fourth digit. Although it is true that arboreal pad-bearing and saxicolous pad-bearing lineages had similar values of asymmetry, the similar values were achieved via differing pathways as mentioned above. Our results do indicate that the symmetry measure must be interpreted along with examining the morphospace because similar values can be achieved in multiple ways. It should also be noted that the geometric morphometrics only takes into account the arrangement of the metatarsals and not the digit tips. Therefore, the shape analyzed is a descriptor of the sole shape rather than the whole foot shape.

4.3 The effect of size

We identified a positive relationship between digital spread, mediated by the increase in the first two interdigital angles (I-II and II-III), and body size. As body size increases, body mass and the forces acting on a lizard are expected to increase disproportionately due to geometric similarity (Biewener, 2005). Therefore, a greater digital spread, corresponding to a larger surface area for which adhesive and clinging mechanism can be applied, would facilitate counteracting gravity in climbing situations. Increased surface area of the foot through ontogeny occurs in several species of salamanders that use wet adhesion (Adams and Nistri, 2010). However, unlike wet adhesion, the gecko adhesive system is directional (Autumn et al., 2000). Therefore, an increased surface area of the overall foot does not necessarily equate to an increased surface area that is engaged at one time (i.e. the functional area). Instead, for pad-bearing lineages, a greater digital spread may increase the potential area into which a toepad may expand into without overlapping with its neighboring digits. Thus, the digital spread permits the ability to increase the size of an individual toepad (Webster et al., 2009). Additionally, the importance of the digital spread for pad-bearing lineages is apparent, since it permits the engagement of the adhesive apparatus at a variety of positions simultaneously (Birn-Jeffery and Higham, 2014; Russell and Oetelaar, 2015; Zhuang and

Higham, 2016). Larger species may need greater support from toepads engaged in opposing directions or to facilitate changing directions quickly.

This relationship was also found among padless lineages, suggesting that a relationship between digital spread and body size may be characteristic of gekkotans overall. One possible explanation is that the interdigital angles, which are affected by the shape of metatarsal heads, may change with size due to the expansion of the metatarsal heads. If this is true, we would expect a similar relationship to be found in non-gekkotans. Interdigital angles in non-gekkotans have not been measured or documented outside of this study with a few exceptions, and also in fossil specimens (e.g. (Kubo, 2010). Its relationship to size is also not documented. Padless lineages rely primarily on claws when climbing, which are also a directional albeit with an interlocking clinging mechanism (Cartmill, 1985). A greater digital spread would reduce the amount of support that the lizard could bear, but this problem could be compensated by the lizard's behavioral modulation of the digits, so that all digits were oriented in the same direction.

As demonstrated by the geometric morphometric analysis, arboreal padless species, such as *Saltuarius salebrosus,* often have a highly symmetrical foot despite lacking toepads. A study examining ontogenetic growth in such species, with the simultaneous measurement of metatarsal head shape and interdigital angle measurement could help determine the mechanisms behind changes in the interdigital angles. Additionally, behavioral analysis, similar to Russell and Oetelaar (2015), would reveal how such a gecko uses its claws in combination with a large digital spread.

5. Conclusion

Morphological evolution of gecko feet is shaped by a number of factors. We demonstrated not only repeated shifts to a pad-bearing morphology that depended on environmental context, but also several cases of shifts to a secondarily padless morphology. This study also characterized important principles of foot morphology and demonstrated that symmetry and digital spread are important components of the pad-bearing morphology. Geometric morphometric analysis further revealed potential changes in more proximal elements of the foot that are highly relevant to locomotion in pad-bearing lineages and affect more distal elements of the foot. These results provide evidence of not only changes at the proximal end of the foot that may affect the morphology of distal elements, but also the link between internal skeletal structure and external morphology. We focused on museum specimens in this study, but it is possible that geckos can, to some extent, actively modulate symmetry. However, future studies are needed to determine the extent of this ability.

Specimens:

RD1, NAM40, AMB184, AMB228, AMB237, AMB240, AMB259, AMB290, AMB316, AMB317, AMB318, AMB320, AMB321, AMB325, AMB327, AMB420, AMB432, AMB442, AMB444, APR111, APR115, APR120, APR16, APR53, TEHangul, CAS138984, CAS140554, CAS148556, CAS157760, CAS159786, CAS172379, CAS179786, CAS248337, CAS74738, CordH, LL1,

LSU7716, LSU9245, LSU9981, AMB328, NAM23, NAM29, No8, R102608, R102613, R102614, RD3, RD4, TEH1, TEH2, TG1702, TG1708, TG1729, Yel1, AMB429, CAS167545, CAS76250, LSU10023, LSU11269, LSU12234, LSU9285, MVZ243535, NAM25, NAM66

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Tables and Figures

Table 2.1. Definition of landmarks for geometric morphometric analysis

Landmark #	Description
1	The most central point between the articulation of the fourth distal tarsal and the astragalocalcaneum
2	The most proximal point of the first metatarsal
3	The most proximal point of the second metatarsal
4	The most proximal point of the third metatarsal
5	The most proximal point of the fourth metatarsal
6	The most proximal point of the fifth metatarsal
7	The most distal point of the first metatarsal
8	The most distal point of the second metatarsal
9	The most distal point of the third metatarsal
10	The most distal point of the fourth metatarsal
11	The most distal proximal point of the fifth metatarsal

Table 2.2. Results of ANOVAs performed on variables that loaded highly on the first 2 PCs

Principal Component	Effect	*df*	*F*	*p*	*F**	*p**
PC 1	Padstate	1,3	25.30	**0.00000**	5.11	***0.02***
	Habitat	2,3	0.21	0.81	2.96	*0.23*
	Interaction effect				6.82	***0.03***
PC 2	Padstate	1,3	2.60	0.11	3.96	**0.047**
	Habitat	2,3	3.08	0.05	4.48	0.11
PC 3	Padstate	1,3	1.20	0.28	1.15	0.28
	Habitat	2,3	7.96	**0.0006**	9.19	**0.01**

* phylogenetically corrected results

Italicized results indicate where phylogenetic correction was necessary. Significant results at p<.05 are bolded

Table 2.3. PCA Loading Scores

Variable	PC1	PC2	PC3
Interdigital angle (I-II)	-0.05	-0.27	**0.51**
interdigital angle (II-III)	-0.09	**-0.31**	**0.44**
interdigital angle (III-IV)	-0.01	**-0.39**	-0.07
interdigital angle (IV-V)	-0.08	**-0.41**	**-0.34**
Bilateral Asymmetry	-0.05	**-0.36**	**-0.52**
Sum of interdigital angles	-0.08	**-0.47**	0.06
Metatarsal I	**0.34**	-0.06	-0.08
Metatarsal II	**0.33**	-0.01	-0.18
Metatarsal III	**0.32**	0.06	-0.20
Metatarsal IV	0.28	0.20	0.01
Metatarsal V	0.28	0.10	0.15
Phalanges of digit I	0.29	-0.10	0.10
Phalanges of digit II	**0.32**	-0.13	0.08
Phalanges of digit III	**0.36**	-0.14	0.03
Phalanges of digit IV	**0.31**	-0.13	0.09
Phalanges of digit V	0.28	-0.19	0.12

Table 2.4. Results of linear regression of variables against the effect of size and phylogenetic signal

Variable	df	*r^2*	*p*	*Exp*	*r^2**	*p**	*Exp**	λ
Sum of IDA(I-IV)	115	0.10	0.00		0.10	**4.42E-05**	0.01	0.66
Bilateral Asymmetry	113	0.00	0.99		0.00	0.97	0.00	0.66
Sum of IDA	114	0.07	**3.44E-03**		0.07	**0.01**	0.00	0.59
IDA (I-V)	109	0.09	**1.21E-03**		0.09	**1.63E-03**	0.00	0.69
IDA (I-II)	112	0.14	**3.97E-05**	11.26	0.14	**2.26E-05**	0.01	0.63
IDA (II-III)	116	0.06	**0.01**	6.56	0.06	**2.88E-03**	0.01	0.65
IDA (III-IV)	115	0.01	0.21	4.40	0.01	0.08	0.00	0.63
IDA (IV-V)	116	0.02	0.14	14.23	0.02	0.35	0.00	0.64
Metatarsal I	116	0.70	**3.23E-32**	0.94	8.90	**9.02E-40**	0.74	0.92
Metatarsal II	118	0.69	**9.08E-32**	0.90	2.62	**5.13E-44**	0.80	0.97
Metatarsal III	118	0.69	**7.97E-32**	0.87	23.29	**4.00E-42**	0.84	0.93
Metatarsal IV	118	0.74	**6.50E-36**	0.91		**1.12E-42**	0.84	0.95
Metatarsal V	117	0.68	**8.35E-31**	0.92	2.73	**3.09E-31**	0.73	0.75
Digit I	111	0.71	**1.10E-31**	1.01	5.98	**1.52E-37**	0.69	0.90
Digit II	115	0.74	**3.25E-35**	0.99		**9.74E-44**	0.76	0.93
Digit III	115	0.73	**1.25E-34**	0.96	10.13	**1.59E-45**	0.75	0.97

Digit IV	113	0.75	**4.52E-36**	0.95	2.27	**7.67E-44**	0.79	0.83
Digit V	115	0.69	**7.99E-31**	1.00		**3.34E-32**	0.70	0.71
Phalanges of digit I	111	0.58	**2.08E-22**	1.07	8.22	***1.98E-25***	0.51	0.78
Phalanges of digit II	115	0.68	**4.79E-30**	1.08	2.90	***1.80E-35***	0.64	0.88
Phalanges of digit III	115	0.70	**6.66E-32**	1.02		***5.23E-40***	0.65	0.93
Phalanges of digit IV	113	0.66	**1.72E-28**	0.96	15.48	**1.58E-32**	0.69	0.73
Phalanges of digit V	114	0.58	**3.56E-23**	1.03	2.06	**3.35E-23**	0.56	0.60

* phylogenetically corrected results

Underlined results indicate where phylogenetic correction was necessary. Significant results at p<.05 are bolded

Table 2.5. Results of ANCOVAs performed on variables that loaded highly on the first 2 PCs

Principal Component	Effect	*df*	*F*	*p*	*F**	*p**
Metatarsal I	Padstate	1,116	18.75	**3.21E-05**	4.62	**0.03**
	Habitat	2,116	1.67	0.19	9.38	**0.01**
	Interaction effect				6.43	**0.04**
Metatarsal II	Padstate	1,116	16.18	**1.03E-04**	6.72	**0.01**
	Habitat	2,116	5.40	**0.01**	6.26	**0.04**
						-
Metatarsal III	Padstate	1,117	9.04	**3.24E-03**	0.76	0.38
	Habitat	2,117	6.49	**2.13E-03**	21.95	**0.00**
					8.90	**0.01**
Metatarsal IV	Padstate	1,118	7.88	**0.01**	2.62	**0.11**
	Habitat	2,118	11.60	**2.56E-05**	23.29	**0.00**
	Interaction effect					**-**
Metatarsal V	Padstate	1,117	5.25	**0.02**	2.73	**0.10**
	Habitat	2,117	3.12	**0.05**	5.98	0.05
						-
Phalanges of Digit 1	Padstate	1,118	10.13	**1.90E-03**	10.13	**0.00**
	Habitat	2,118	1.14	**0.32**	2.27	**0.32**
Phalanges of Digit II	Padstate	1,118	21.72	**8.71E-06**	8.22	***0.00***
	Habitat	2,118	3.93	**0.02**	2.90	*0.23*
	Interaction effect					**-**

Phalanges of Digit III	Padstate	1,119	15.48	**1.44E-04**	15.48	**0.00**
	Habitat	2,119	1.03	0.36	2.06	0.36
Phalanges of Digit IV	Padstate	1,117	16.02	**1.14E-04**	8.41	**0.00**
	Habitat	2,117	2.48	**0.09**	2.36	**0.31**
						-
Phalanges of Digit V	Padstate	1,117	10.82	**1.35E-03**	4.07	***0.04***
	Habitat	2,117	2.89	0.06	2.63	*0.27*
	Interaction effect					-
Sum of interdigital angles	Padstate	1,117	9.07	**3.22E-03**	10.00	**1.57E-03**
	Habitat	2,117	0.61	0.54	0.93	0.63
						-
Interdigital angle (II-III)	Padstate	1,117	11.52	**9.47E-04**	11.52	**6.88E-04**
	Habitat	2,117	0.96	**0.39**	1.92	**0.38**
Interdigital angle (III-IV)	Padstate	1,117	0.83	**0.36**	1.56	***0.21***
	Habitat	2,117	1.33	0.27	2.50	*0.29*
	Interaction effect		5.40	**0.01**	8.61	***0.01***
Interdigital angle (IV-V)	Padstate	1,116	9.63	**2.41E-03**	9.63	**1.91E-03**
	Habitat	2,116	2.32	0.10	4.65	0.10
Interdigital angle (I-V)	Padstate	1,116	6.97	**0.01**	7.67	**0.01**
	Habitat	2,116	2.79	**0.07**	5.56	**0.06**
Bilateral asymmetry	Padstate	1,116	1.01	**0.32**	1.13	***0.29***
	Habitat	2,116	5.86	**3.80E-03**	9.04	***0.01***
	Interaction effect					

	Padstate	1,116	7.74	**0.01**	7.75	***0.01***
Sum of IDA (I-IV)	Habitat	2,116	0.50	0.61	0.80	*0.67*
	Interaction effect					

* phylogenetically corrected results

Underlined results indicate where phylogenetic correction was necessary. Significant results at p<.05 are bolded

Table 2.6. Ranges of measured values

	Pad state			
	Pad-bearing		Padless	
Variable	Mean	Range	Mean	Range
SVL (mm)	67.48	24 - 197.17	69.56	28 - 128.25
Sum of IDA (degrees)	78.39	29 - 156.19	53.75	23.5 - 97.27
Bilateral asymmetry (degrees)	30.67	1.5 - 85.7	22.56	0.24 - 54.97
I-II (degrees)	12.81	2.5 - 26.7	7.51	1- 16
II-III (degrees)	12.77	2 - 24.5	8.92	2 -20
III-IV (degrees)	14.54	3 - 30.64	11.64	0.5 - 36.25
IV-V (degrees)	40.60	11 - 92.03	25.68	5.36 - 59.43
Digital Spread (I-V)	86.16	24.5 - 207	61.42	24.58 - 149
Digital Spread (I-IV	40.12	14 - 71.53	28.07	8.5 - 58.3
Metatarsal I (mm)	2.84	0.99 - 8.57	3.80	1.3 - 7.63
Metatarsal II(mm)	3.75	1.27 - 10.5	4.90	1.8 - 8.95
Metatarsal III(mm)	4.02	1.33- 10.55	5.16	1.6 - 9.19
Metatarsal IV (mm)	3.29	1.02 - 9.32	4.28	1.3 - 7.61
Metatarsal V (mm)	1.69	0.61 - 5.48	2.03	0.75 - 4.09
Digit I (mm)	5.54	1.84 - 19.88	7.19	2.48 - 16.51
Digit II (mm)	7.80	2.41 - 28.5	10.18	4.03 - 21.92
Digit III (mm)	9.43	2.84 - 29.72	11.97	4.16 - 24.89
Digit IV (mm)	9.32	2.52 - 22.61	11.78	4.53 - 24.27
Digit V (mm)	6.83	2.04 - 19.95	8.62	3.24 - 20.38

Table 2.7. Results of ANOVAs performed on variables that loaded highly on the first 2 PCs of the geometric morphometric analysis

Principal Component	Effect	*df*	*F*	*p*	*F**	*p**
PC 1	Padstate	1,3	8.54	**6.32E-04**	18.53	***9.47E-05***
	Habitat	2,3	5.23	0.03	2.61	*0.11*
PC 2	Habitat	1,3	16.16	4.07E-06	22.86	***1.08E-05***
	Padstate	2,3	17.59	1.14E-04	8.49	**3.57E-03**
	Interaction effect	1,3	4.66	**1.40E-02**	7.92	**0.02**

* phylogenetically corrected results

Italicized results indicate where phylogenetic correction was necessary. Significant results at $p<.05$ are bolded

Table 2.8. Intercorrelations of interdigital angles

Interdigital Angle	IDA (I-II)	IDA (II-III)	IDA (III-IV)
IDA (I-II)			
IDA (II-III)	0.32***		
IDA (III-IV)	0.09***	0.1***	
IDA (IV-V)	0.04	0.06	0.15***

* p<.05, ** p<.005, ***p<.005

Table 2.9. Habitat classification of species used

Species	Species Author	Habitat classification	Citation
Phyllurus platurus	SHAW In WHITE 1790	saxicolous	Cogger 2014
Carphodactylus laevis	Günther 1897	arboreal	Cogger 2014; Wilson 2012; Peeters et al. 2014
Nephrurus levis	Sauvage 1879	terrestrial	Pianka and Pianka 1976; Cogger 2000
Bavayia sauvagii	Boulenger 1883	arboreal	Bauer 1990; Sadlier and Bauer 1997
Rhacodactylus leachianus	Cuvier 1829	arboreal	Bauer and Sadlier 2001; Werner and Seifan 2006; Henkle 1991; Bauer and Vindum 2001
Bavayia cyclura	Günther 1872	arboreal	Bauer and Sadlier 1994
Rhacodactylus auriculatus	Bavay 1869	arboreal	Bauer and Sadlier 2001; Bauer and Vindum 1990
Rhacodactylus chahoua	Bavay 1869	arboreal	Bauer 1985; Bauer and Sadlier 2000
Naultinus elegans	Gray 1842	arboreal	Pawson et al. 2010; Taylor 1976
Oedura marmorata	Gray 1842	arboreal	Cogger 2014; Pianka 1972
Oedura monilis	De Vis 1888	arboreal	Vanderduys et al. 2012; Cogger 2014
Strophurus strophurus	Duméril & Bibron 1836	arboreal	Cogger 2014
Strophurus ciliaris	Boulenger 1885	arboreal	Cogger 2014
Strophurus rankini	Storr 1979	arboreal	Cogger 2014
Coleonyx variegatus	Baird 1858	terrestrial	Parker and Pianka 1974; Miller and Stebbins 1964; Klauber 1945; Mosauer 1935
Eublepharis macularius	Blyth 1854	terrestrial	Khan 1999; Minton 1966
Aristelliger lar	Cope 1861	arboreal	Schwartz 1980; Schwartz and Hendereson 1991
Aristelliger praesignis	Hallowell 1856	arboreal	Schwartz and Henderson 1991; McCranie 2015; Bauer and Russell 1993
Teratoscincus scincus	Schlegel 1858	terrestrial	Anderson 1999; Seligmann et al. 2006

Asaccus griseonotus	Dixon & Anderson 1973	saxicolous	Parsa et al. 2009; Dixon and Anderson 1973
Tarentola mauritanica	Linnaeus 1758	saxicolous	Loveridge 1947; Johnson et al. 2005; El Din 2006; Frankenberg 1978
Gekko gecko	Linnaeus 1758	arboreal	Brown et al. 2011; Yu et al. 2010; Bobrov 1993; Barbour 1912
Ptychozoon sp.	Boulenger 1899	arboreal	Taylor 1963
Gehyra marginata	Boulenger 1887	arboreal	Tanner 1950
Gehyra punctata	Fry 1914	saxicolous	Storr and Harold 1985; Storr and Hanlon 1980; Withers and Edward 1997; Dougherty et al. 2011
Alsophylax pipiens	Pallas 1827	terrestrial	Belousova 2014; Anajeva et al. 2010
Cnemaspis kendallii	Gray 1845	saxicolous	Werne and Chou 2002
Cnemaspis littoralis	Jerdon 1854	arboreal	Beddome 1962; Lewis et al. 2010; Cyriac and umesh 2013; inger et al. 1984
Bunopus blanfordii	Strauch 1887	terrestrial	Haas et al. 1959; Farag and Banaja 1980
Agamura persica	Duméril 1856	saxicolous	Anderson 1999; Minton 1966; Blanford 1876
Hemidactylus platyurus	Schneider 1797	arboreal	Bansal 2010
Hemidactylus giganteus	Stoliczka 1871	arboreal	Rao et al. 2005; Srinivasulu and Das 2008; Giri et al. 2003
Ailuronyx seychellensis	Duméril & Bibron 1836	arboreal	Blackmore et al. 2011; Gardnere 1986
Narudasia festiva	Methuen & Hewitt 1914	saxicolous	Methuen and Hewitt 1914; Loveridge 1947
Cnemaspis spinicollis	Müller 1907	saxicolous	Sura 1987; LeBreton et al. 2003; Gonwuo et al. 2010; Joger 1981; Leache 2005; Segniabeto et al. 2015; Chiro and lebreton 2007; Joger 1981
Afrogecko porphyreus	Daudin 1802	arboreal	Jacobsen and Randall 2013; Cooper et al. 1999; Fitsmons 1943; Rose 1952
Cryptactites peringueyi	Boulenger 1910	arboreal	Branch and Bauer 1994

Geckolepis polylepis	Boettger 1893	arboreal	Lemme et al. 2013; Raxworthy and Vencs 2010
Blaesodactylus boivini	Duméril 1856	arboreal	Metcalf et al. 2007
Rhoptropus bradfieldi	Hewitt 1935	saxicolous	Johnson et al. 2005
Rhoptropus afer	Peters 1869	terrestrial	Johnson et al. 2005
Rhoptropus boultoni	Schmidt 1933	saxicolous	Johnson et al. 2005
Rhoptropus barnardi	Hewitt 1926	saxicolous	Johnson et al. 2005
Rhoptropus biporosus	Fitzsimons 1957	saxicolous	Johnson et al. 2005
Chondrodactylus angulifer	Peters 1870	terrestrial	Johnson et al. 2005
Pachydactylus laevigatus	Fischer 1888	saxicolous	FitzSimons 1978; Loverridge 1947; Werner 1977
Colopus kochii	Fitzsimons 1959	saxicolous	Bauer et al. 1992; Fitzmons 1959
Pachydactylus namaquensis	Sclater 1898	saxicolous	Brown 2013; Methuen and Hewitt 1913; Loveridge 1947; Branch et al. 1996
Pachydactylus austeni	Hewitt 1923	terrestrial	Johnson et al. 2005
Lygodactylus capensis	Smith 1849	arboreal	Simbotwe 2013; Pianka and Huey 1978; Branach et al. 2005; Stewart 1969
Phelsuma sundbergi	Rendahl 1939	arboreal	Noble et al. 2011; Hagan et al. 2013; Whitaker 1987
Phelsuma astriata	Tornier 1901	arboreal	Noble et al. 2011; Murphy and Myers 1996
Pseudothecadactylus cavaticus	Cogger 1975	saxicolous	Cogger 2014
Rhoptropus taeniostictus	Laurent 1964	saxicolous	REDO?
Goggia microlepidota	Fitzsimons 1939	terrestrial	Loveridge 1947; Todd 2012; Mouton and Van Wyk 1981
Goggia essexi	Hewitt 1925	saxicolous	Branch 1998; Lovereidge 1947
Goggia rupicola	Fitzsimons 1938	saxicolous	Branch et al. 1996; Fitsmons 1938
Cyrtodactylus khasiensis	Jerdon 1870	arboreal	Das et al. 2016

Phyllodactylus ventralis	O'shaughnessy 1875	arboreal	Ugueto et al. 2013; Dixon 1962
Naultinus gemmeus	Mccann 1955	arboreal	Wilson and Cree 2003; Jewell and mcQueen 2007; Knox 2010;
Uroplatus fimbriatus	Schneider 1797	arboreal	Raxworthy 1988
Uroplatus ebenaui	Boettger 1879	arboreal	Raxworthy et al. 2011
Uroplatus phantasticus	Boulenger 1888	arboreal	Ratsoavina, F., Glaw, F., Ramanamanjato, J.-B., Rabibisoa, N. & Rakotondrazafy, N.A. 2011. e.
Uroplatus sikorae	Boettger 1913	arboreal	Raselimana 2010
Hemitheconyx caudicinctus	Duméril 1851	terrestrial	Trape et al. 2012
Gehyra spheniscus	Doughty, Palmer, Sistrom, Bauer & Donnellan 2012	saxicolous	Cogger 2014
Homopholis walbergii	Smith 1849	arboreal	Greenbaum et al. 2007
Strophurus williamsi	Kluge 1963	arboreal	Cogger 2014
Tarentola annularis	Geoffroy-St-Hilaire 1827	saxicolous	Din 2006
Phyllopezus pollicaris	Spix 1825	saxicolous	Recoder et al. 2012; Righi et al. 2012
Phelsuma nigristriata	Meier 1984	arboreal	Lever 2003
Ptyodactylus hasselquistii	Donndorff 1798	saxicolous	Perry and Brandeis 1992
Goniurosaurus luii	Grismer, Viets & Boyle 1999	terrestrial	Grismer 1999
Chondrodactylus bibronii	Smith 1846	saxicolous	Meyer and Mouton 2007; Branch 1998

Phelsuma madagascariensis	Gray 1831	arboreal	Hawlitschek et al. 2011
Nactus pelagicus	Girard 1858	terrestrial	Cogger 2000
Diplodactylus bilybara	Couper, Pepper & Oliver 2014	unknown	Oliver et al. 2014
Strophurus elderi	Stirling & Zietz 1893	saxicolous	Cogger 2014
Saltuarius salebrosus	Covacevich 1975	arboreal	Cogger 2014
Nephrurus asper	Günther, 1876	terrestrial	Cogger 2014
Stenodactylus petrii	Anderson 1896	terrestrial	Din 2006
Bunopus tuberculatus	Blanford 1874	terrestrial	Minton 1966; Minton and Anderson 1992
Perochirus ateles	Duméril 1856	arboreal	Sabath 1981
Gehyra oceanica	Lesson 1830	arboreal	Fritts et al. 1990; Sabath 1981
Lepidodactylus lugubris	Duméril & Bibron 1836	arboreal	Cogger 2000
Ptychozoon kuhli	Stejneger 1902	arboreal	Taylor 1963
Cnemaspis psychadelica	Grismer, Ngo & Grismer, 2010	saxicolous	Grismer 2010a
Dixonius siamensis	Boulenger 1899	terrestrial	Pauwels et al. 2003; Chan et al. 2015
Cyrtodactylus macrotuberculatus	Grismer & Ahmad 2008	generalist	Grismer 2011
Cyrtodactylus astrum	Grismer, Wood Jr, Quah, Anuar, Muin, Sumontha, Ahmad, Bauer, Wangkulangkul, Grismer & Pauwels 2012	saxicolous	Grismer et al. 2012
Cyrtodactylus consobrinus	Peters 1871	arboreal	Onn et al. 2010
Cyrtodactylus quadrivirgatus	Taylor 1962	arboreal	Onn et al. 2010
Cyrtopodion scabrum	Heyden 1827	saxicolous	Ibrahim 2013; Minton and Anderson 1992
Pachydactylus rangei	Andersson 1908	terrestrial	Branch 1998
Ptenopus	Smith, 1849	terrestrial	Branch 1998

Pachydactylus punctatus	Peters 1854	terrestrial	Pianka 1971
Homonota	Burmeister, 1861	terrestrial	Frederickson et al. 2003
Underwoodisaurus milii	Bory De Saint-Vincent, 1823	saxicolous	Cogger 2014
Stenodactylus sthenodactylus	Lichtenstein 1823	terrestrial	Din 2006
Strophurus taenicauda	De Vis 1886	arboreal	Cogger 2014
Gonatodes ocellatus	Gray 1831	saxicolous	Seifan 2002
Hemidactylus mercatorius	Gray 1842	saxicolous	D'cruze and Sabel 2005; Lever 2003
Coleonyx mitratus	Peters 1863	terrestrial	Cope 1879; Klauber 1945
Holodactylus africanus	Boettger 1893	terrestrial	Sprawls 2006
Nephrurus laevissimus	Mertens, 1958	terrestrial	Henle 1991; Pianka and Pianka 1976; Coggere 2014
Correlophus ciliatus	Guichenot 1866	arboreal	Mayer 2011
Eurydactylodes vieillardi	Bavay 1869	arboreal	Bauer et al. 2009; Roux 1913
Hoplodactylus duvaucelii	Duméril & Bibron 1836	arboreal	Hicks et al. 1975; Hoare et al. 2007; Melzaer and Bell 2014
Hoplodactylus pacificus	Gray 1842	arboreal	Whitaker 1973; 1987; Parish and Pierce 1989; Towns 1972; Benson 1976
Hoplodactylus granulatus	Gray 1845	arboreal	Whitaker and Lyall 2003
Aeluroscalabotes felinus	Günther 1864	arboreal	Zaaf and Damme 2001
Tarentola ephippiata	O'shaughnessy 1875	saxicolous	Salvador 1975; Loveridge 1947; Trape and Chirio 2012; Epps et al. 2007

Gehyra mutilata	Wiegmann 1834	arboreal	Rocha et al. 2009; Grismer 2001; Wiles t al. 1990; Lagat 2011; Grismer et al. 2006; Bazzano 2007; Shahirza et al.; Cogger 2014; Brown et al. 1996; on et al. 2009
Gehyra variegata	Duméril & Bibron 1836	arboreal	Bustard 1969; Bustard 1968; Michael et al. 2015
Stenodactylus haasi	Haas 1957	terrestrial	Modry et al. 2004; Disi 2011; Gardner 2009
Hemidactylus brookii	Gray 1845	arboreal	Minton 1966; Mitchell and Zug 1975; Tsetan and Ramnibai 2011; Schwartz and Henderson 1991
Afroedura hawequensis	Mouton & Mostet, 1985	saxicolous	Branch et al. 1996; Mouton and Mostert 1985
Lygodactylus picturatus	Peters 1870	arboreal	Western 1974; Schmidt et al. 1919; Malonza et al. 2005; Loveridge 1947
Pseudothecadactylus australis	Günther 1877	arboreal	Cogger 2000; Cogger 1975
Aristelliger cochranae	Grant 1931	arboreal	Gifford et al. 2000; Schwartz 1980; Thomas 1966
Aristelliger barbouri	Noble & Klingel 1932	arboreal	Noble and Klingel 1932; Bauer and Russell 1993
Alsophylax przewalskii	Strauch, 1887	arboreal	Blanford 1875
Mediodactylus kotschyi	Steindachner 1870	saxicolous	Mollov 2011; Ajtic 2014; Petrov 2007
Geckolepis maculata	Peters 1880	arboreal	Lehtinen 2002

Figure legends

Fig. 2.1. Phylogenetic tree of geckos used for statistical analyses. The tree was trimmed from the phylogeny by Zheng and Wiens (2016). Sampling covered at least five origins of the adhesive system and included ancestrally padless geckos (black), pad-bearing (green), leaf-toed pad-bearing (blue) and secondarily padless lineages (red).

Fig. 2.2 Landmarks describing the sole shape of the hind feet was digitized on 2D X-rays of the left hind feet primarily (A). In addition to data from Russell et al. (1997), measurements were taken from the 2D X-Rays following the methods of Russell et al. 1997 (B).

Fig. 2.3. Principal components analysis (PCA) on 16 variables describing digit morphology. The phylogeny (grey) was projected onto the morphospace. Minimum convex polygons outline ancestrally padless (red), pad-bearing (green), leaf-toed (blue) and secondarily padless lineages (pink).

Fig. 2.4 Box plots of the first two principal component axes (A and B respectively) relative to habitat use (arboreal, saxicolous and terrestrial) for pad-bearing (green) and padless (grey) lineages. Stars indicate that the bracketed groups are significantly different based on a tukey posthoc test ($p<.05$).

Fig. 2.5. Box plots of the sum of total interdigital angles (A), bilateral asymmetry (B), digital spread (I-V) (C) and digital spread (I-IV) (D). Error bars indicate one standard error (SE).

Fig. 2.6. Box plots of relative metatarsal lengths (A), interdigital angles (B), sum of phalangeal lengths (C) and total digit length (D) for each digit (white, grey, green, orange and blue for digits I-V, respectively and white, grey, green and orange for interdigital angles I-II, II-III, III-IV and IV-V, respectively) in padless and pad-bearing lineages.

Fig. 2.7. PCA performed on 11 landmarks on the left hind feet, including 55 species of padless (grey) and pad-bearing (green) lineages. Minimum convex polygons group the padless and pad-bearing lineages. Warp grids on each axis illustrate the shape of the minimum and the maximum PC score of that axis. A sample X-Ray is shown to indicate the placement of the landmarks and the orientation of the hind feet in the warp grids.

Fig. 2.8. Scaling of the sum of total interdigital angles with size (log(SVL)) for ancestrally padless (black), pad-bearing (green), leaf-toed (blue) and secondarily padless (red) lineages.

Fig. 2.1. Phylogenetic tree of geckos used for statistical analyses

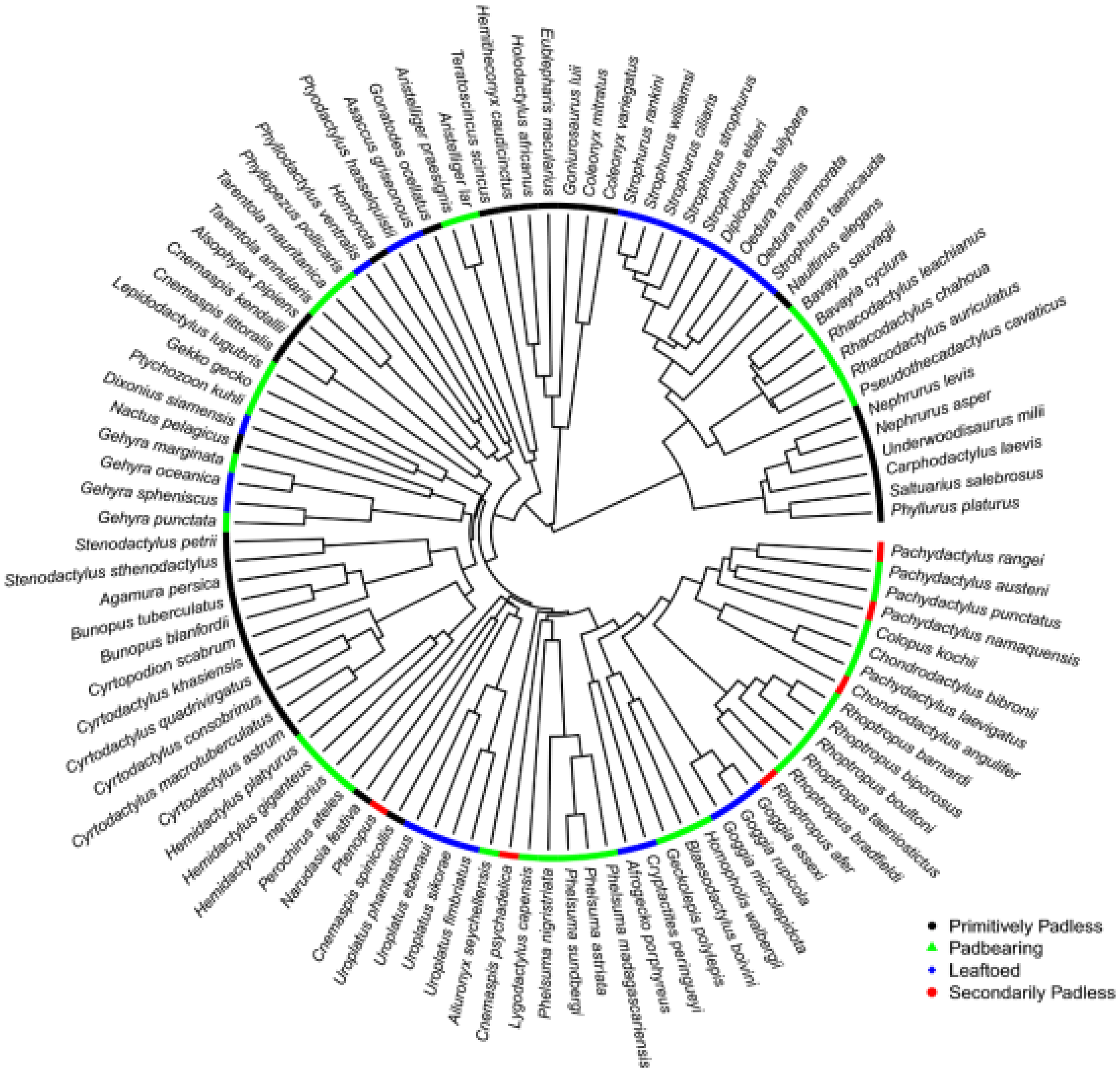


Fig. 2.2. Landmarks describing the sole shape of the hind feet was digitized on 2D X-rays of the left hind feet primarily

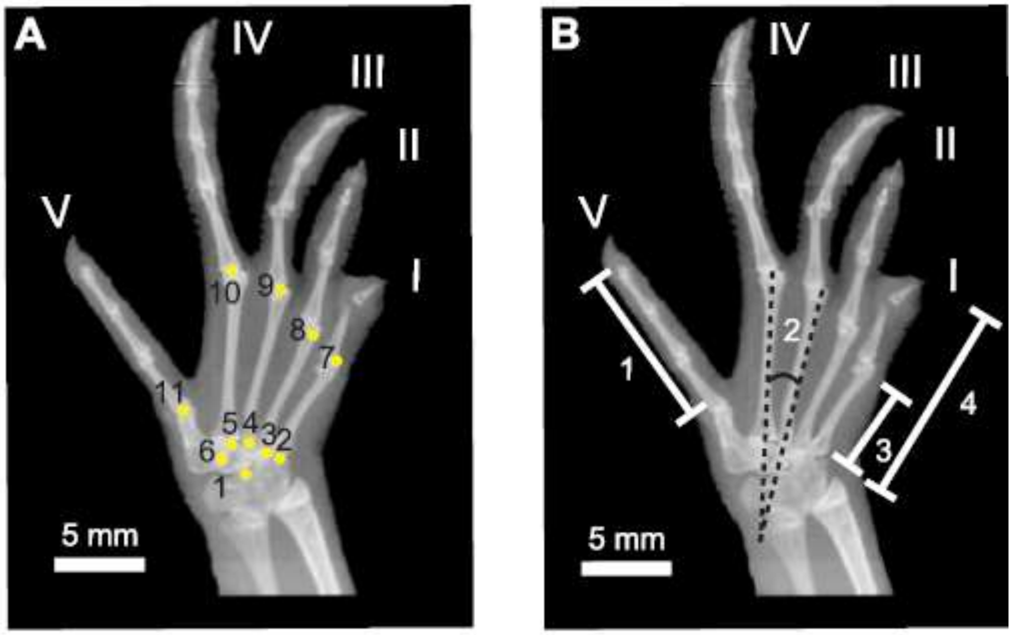


Fig. 2.3. Principal components analysis (PCA) on 16 variables describing digit morphology

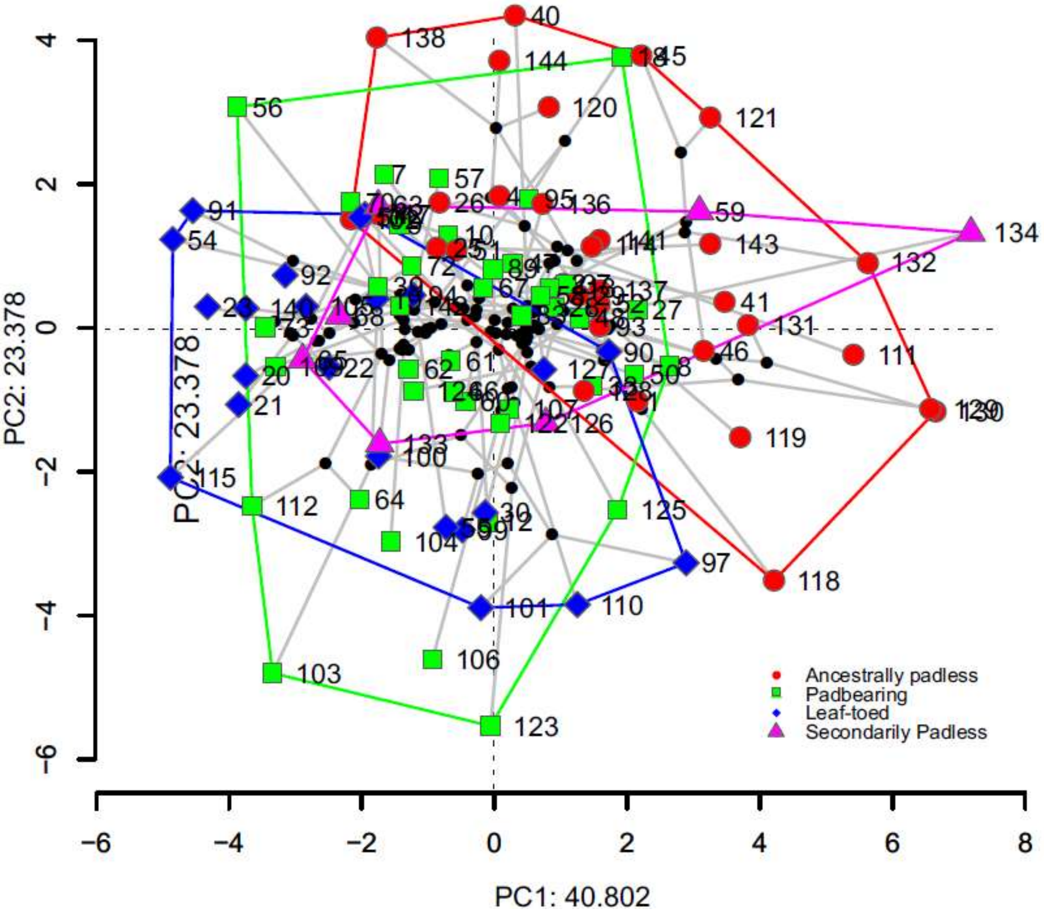


Fig. 2.4. Box plots of the first two principal component axes (A and B respectively) relative to habitat use (arboreal, saxicolous and terrestrial) for pad-bearing (green) and padless (grey) lineages

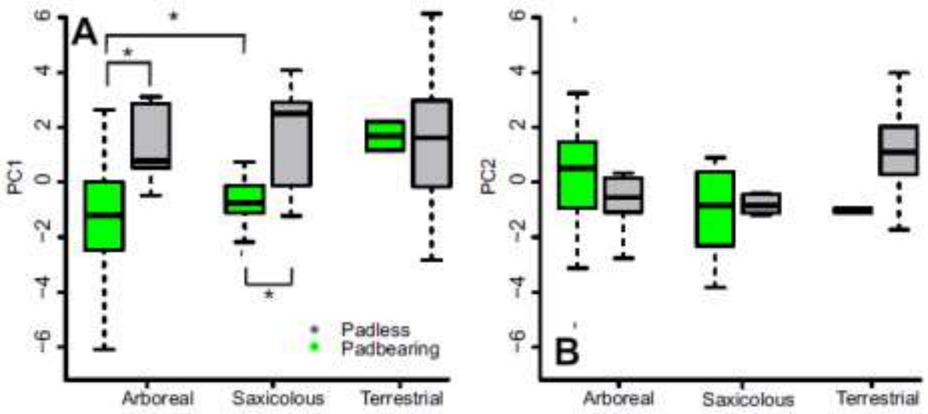


Fig. 2.5. Box plots of the sum of total interdigital angles (A), bilateral asymmetry (B), digital spread (I-V) (C) and digital spread (I-IV) (D)

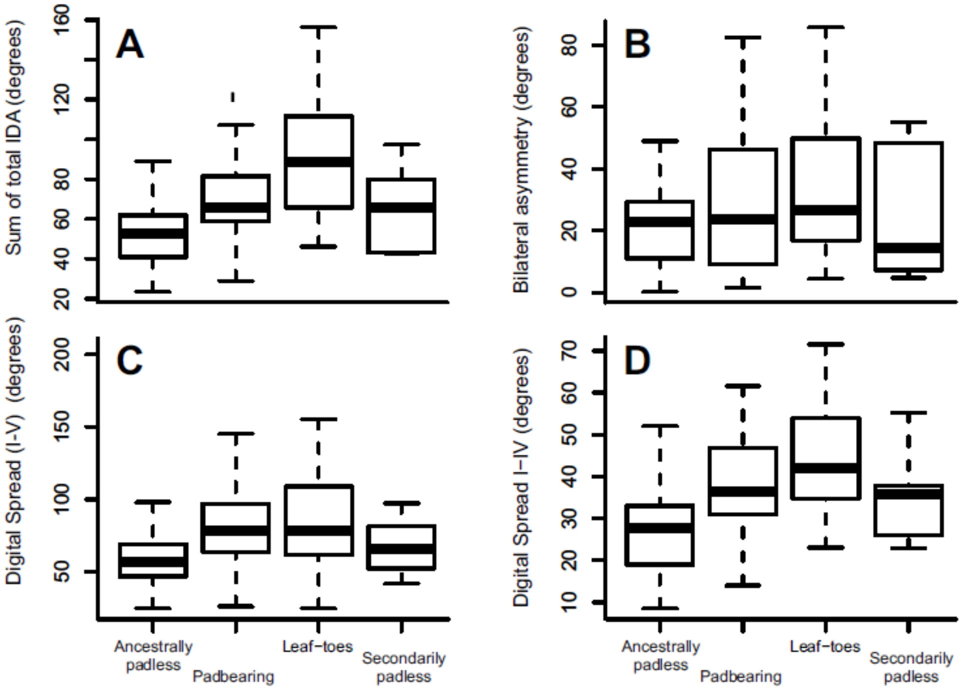


Fig. 2.6. Box plots of relative metatarsal lengths (A), interdigital angles (B), sum of phalangeal lengths (C) and total digit length (D) for each digit (white, grey, green, orange and blue for digits I-V, respectively and white, grey, green and orange for interdigital angles in padless and pad-bearing lineages

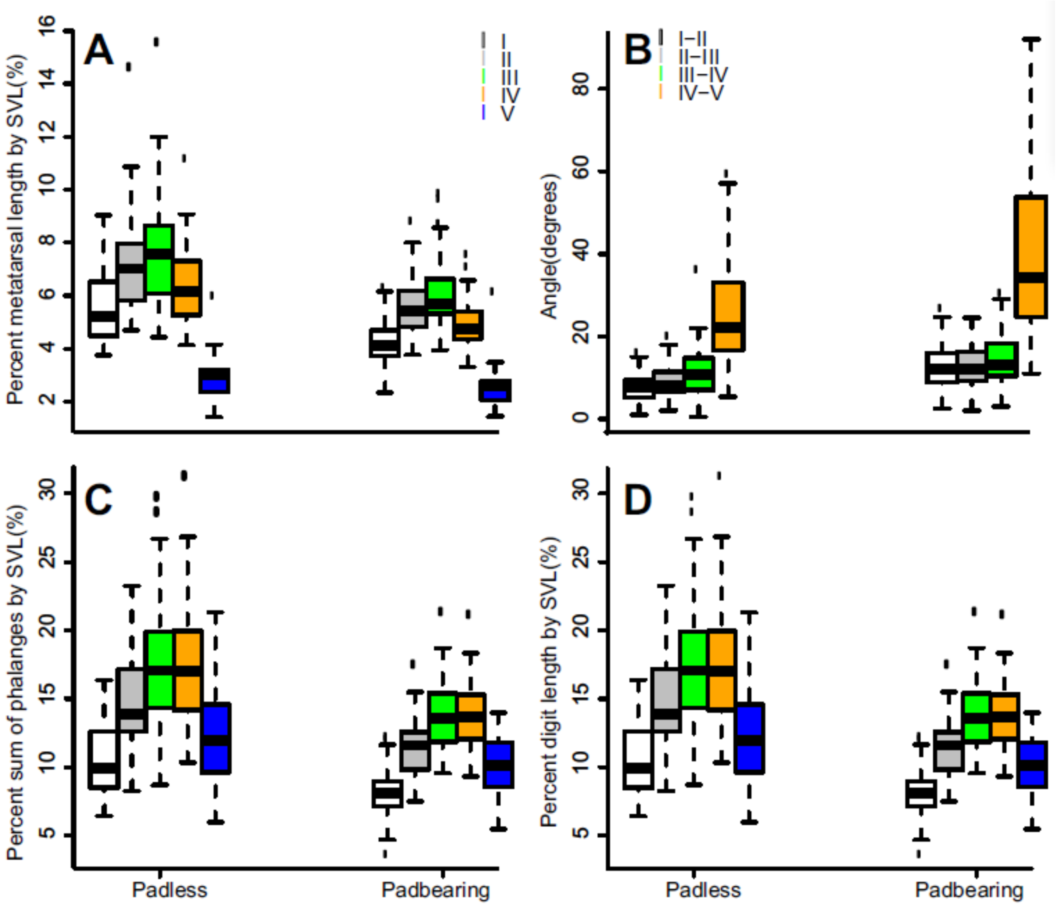


Fig. 2.7. PCA performed on 11 landmarks on the left hind feet, including 55 species of padless (grey) and pad-bearing (green) lineages

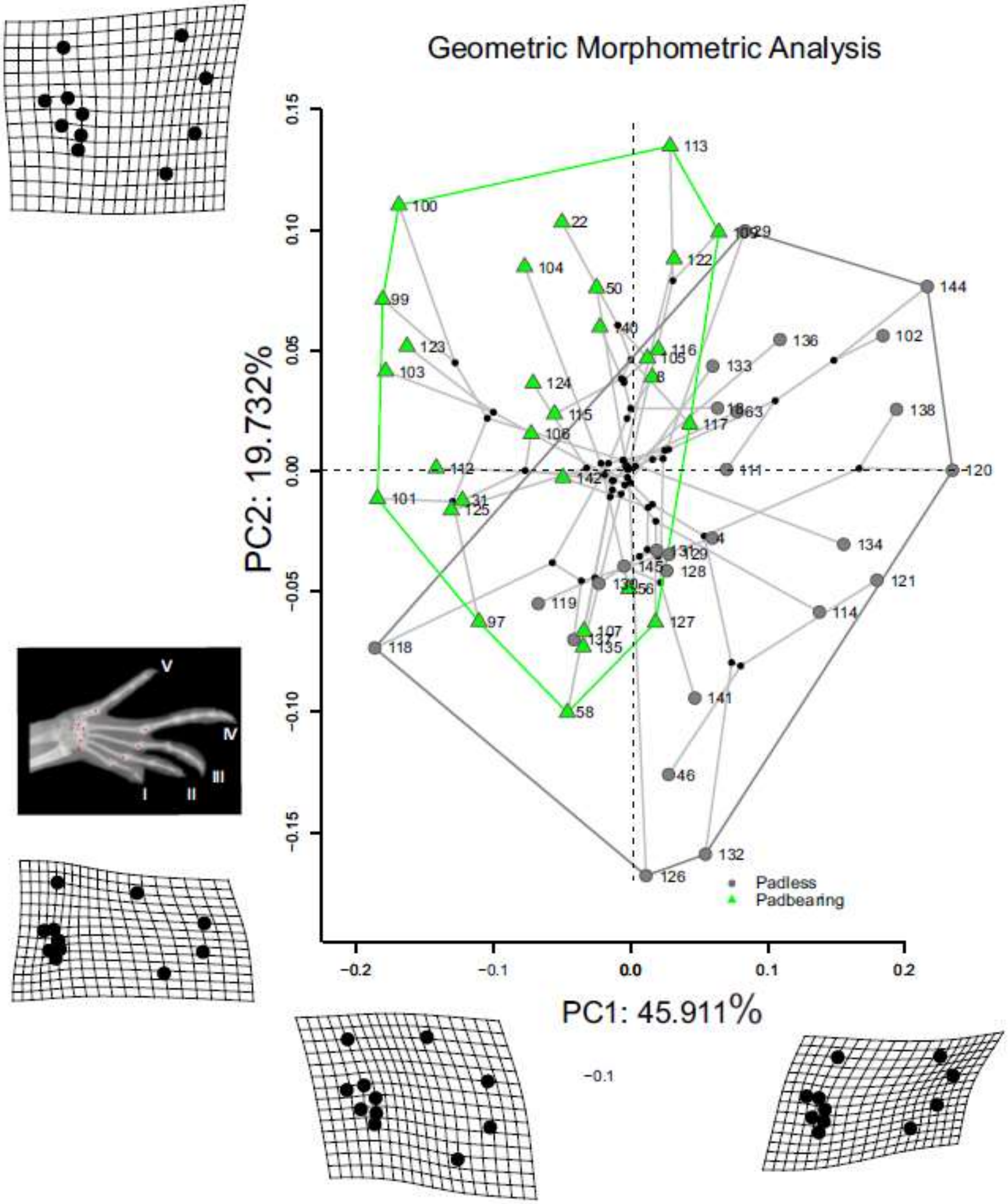
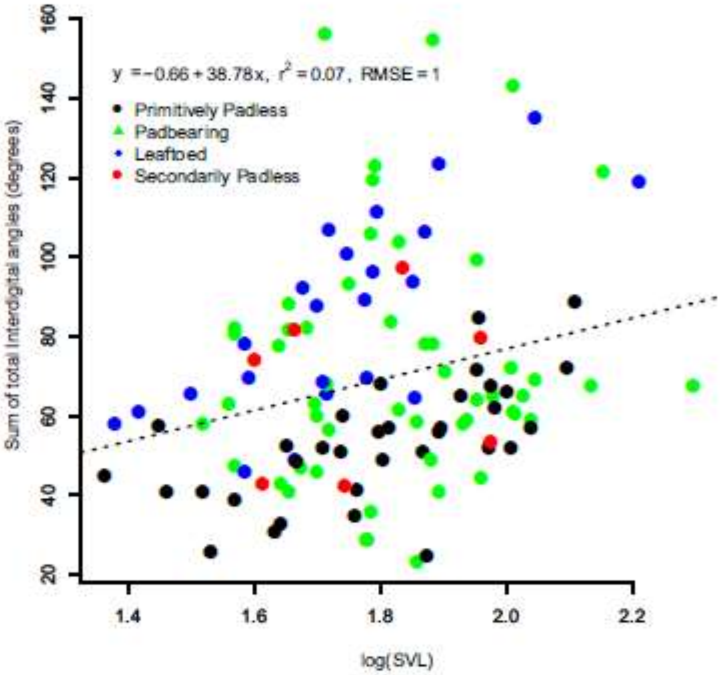


Fig. 2.8. Scaling of the sum of total interdigital angles with size (log(SVL)) for ancestrally padless (black), pad-bearing (green), leaf-toed (blue) and secondarily padless (red) lineages



Chapter 3

Evolution of digital morphology in relation to the acquisition of the adhesive system in geckos

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Abstract

Geckos that have an adhesive system (pad-bearing) have a dramatically different pattern of locomotion. We used geometric morphometric analysis from morphological data collected from microCT scans to examine whether the gain of the adhesive system was associated with changes in the mesotarsal joint. Pad-bearing geckos have joint surfaces that appear to be shallower than those of ancestrally padless geckos, suggesting that dorsiflexion and rotation is decoupled at the mesotarsal joint in pad-bearing lineages. These results suggest that morphology in more proximal elements in the hind limb than previously suggested, are modified to facilitate the engagement of the adhesive system. The evolution of the adhesive system likely impacts locomotion not only at the contact with the substrate, but also increases the degrees of freedom for the mesotarsal joint at the propulsive phase.

1. Introduction

The hind foot in lizards and many other animals is important for propulsion. Both minor and dramatic changes to limbs may affect how an animal moves and locomotes. For example, tarsal and metatarsal length correspond with a longer stride length and higher speeds in several measured lizards (Irschick and Jayne, 1999). Morphological adaptations clearly change the function and kinematics of the hind foot during locomotion (i.e. webbed feet, hoofs, specialized feet in chameleons).

Most lizards differ from mammals due to their lack of an upright posture and instead, have a sprawled posture (Rewcastle, 1983). As a result, those that have a sprawled posture do not experience a decrease in distance between the hip and the knee, which characterize the kinematics of those with an upright posture. Support of the body is comparatively lower. Secondly, in a typical sprawling posture, the feet of a lizard are placed lateral to the axis of the body (Rewcastle 1983). The consequences of this are that the foot is oriented somewhat perpendicular to the long axis of the body. Thus, the foot must be oriented parallel to the body to increase the amount of anterolateral force contributing to forward locomotion. This is achieved during the propulsive phase in which the crus (the tibia and the fibula) both extends and rotates on the foot (Rewcastle, 1980; 1983). The foot elevates to a digitigrade-like posture as the foot rolls onto the medial metatarsals before moving to the distal phalanges and into the swing phase.

Geckos that have a dry adhesive system locomote differently than typical lizards and those that lack the system. On level surfaces, digits remain hyperextended during the entire stride to protect the toepads (Russell and Higham, 2009). When locomoting on inclined surfaces, the toe pads disengage from the substrate by hyperextending first before the rest of the foot (Russell, 1975). Zaaf et al. (1999) suggested that the joint moment across the mesotarsal joint was smaller in pad-bearing geckos, so that ankle extension and forward propulsion might result in decreased performance. Given the importance of the mesotarsal joint to lizard locomotion and fast running, this fundamental change in the pattern of lizard locomotion is likely to be reflected in the shape of the bones that influence propulsion and extension.

This typically simultaneous motion of extension and rotation is made possible by the mesotarsal joint, which is complex and is made up of two bones: the astragalocalcaneum and the fourth distal tarsal (Fig 3.1). The astragalocalcaneum articulates with the fibula and the tibia proximally by the fibular and tibial facets and the fourth distal tarsal distally. This latter articulation forms the mesotarsal joint by way of the oblique ridge, which is a protrusion on the distal side of the astragalocalcaneum and a deep concave groove on the fourth distal tarsal. The proximal joint is held fast by ligaments (Russell and Bels, 2001). Therefore, the mesotarsal joint is where most rotation occurs (Rewcastle, 1983). Distally and ventrally, is the disto-mesial notch, in which the peg of the fourth distal tarsal articulates. The lateral process (Fig 3.1) is a structure that protrudes prominently from the lateral side of the bone in lizards such as *Varanus,* but not *Iguana iguana* (Brinkman, 1980; Rewcastle, 1980; Schaeffer, 1941).

The fourth distal tarsal articulates proximally with the astragalocalcaneum and distally with the third and fourth metatarsals and the third distal tarsal (Russell 1975; Rewcastle 1983). Laterally, the fourth distal tarsal articulates with the fifth metatarsal. A groove on the proximal side of the fourth distal tarsal rises from the medial side to the lateral side of the bone. A more thorough description of the bones is expanded on by Rewcastle (1980).

The mesotarsal joint is a complex saddle joint that permits concurrent flexion and extension and rotation of the crus (tibia and fibula) on the pes. In other words, the action of flexion-extension is tightly coupled with rotation occurring at the joint. Rewcastle (1980) described the motion at the joint as similar to a nut being screwed onto a bent wood screw in an attempt to describe that the joint moves on two helical axes. One axis runs perpendicular to the tarsal facet and the other is an axis that runs from the ventral side to the dorsal side of the fourth distal tarsal. As the astragalocalcaneum translates across the groove of the fourth distal tarsal, it rotates on the second axis. Concurrently, the astragalocalcaneum rotates on the first described axis to produce flexion or extension and rotation of the crus on the pes. The motion can be described somewhat similarly to how a person crosses a spiraling staircase in which the person translates across and up a staircase while simultaneously spinning. These motions are made concurrent by the complex control surfaces of the joint. Therefore, it is possible that deviation from the shape of these surfaces may result in decoupling of the flexion/extension and rotation actions.

Repeated evolution of the adhesive system, makes geckos an ideal system to understand how suites of morphological changes are associated with the evolution of the adhesive system (Gamble et al., 2012). Although there have been a few studies that have documented morphological differences between pad-bearing and padless geckos (Russell, 1979; Russell et al., 1997), none have addressed extensively how tarsal elements such as the bones in the mesotarsal joint vary. The lack of these studies is partially due to the highly three-dimensional nature of the tarsal bones, which is remedied by the increase in availability of microCT scanning technology. Several studies outside of the study of reptiles and amphibians have examined the variation in tarsal elements (Harcourt-Smith, Tallman, Frost, Wiley, Rohlf et al., 2008; Polly, 2008; Turley and Frost, 2013).

Orientation of the feet is thought to be important for adhesive system engagement because of the directional nature of the adhesive system (Autumn et al., 2000). Functional studies suggest orientation of the feet as an important factor for propulsion and adhesive system engagement (Birn-Jeffery and Higham, 2014; Russell and Oetelaar, 2015; Zhuang and Higham, 2016). We might expect then that the evolution of the adhesive system may be facilitated by the ability to have a greater range of rotation at the mesotarsal joint, which may be reflected by shallow surfaces in the groove of the fourth distal tarsal and the oblique ridge of the astragalocalcaneum. Shallower surfaces in the mesotarsal joint may lead to a greater range of motion, but may also result in decoupling of the concurrent actions of rotation and dorsiflexion.

Given that joint moments for the muscles crossing the mesotarsal joint may be smaller in pad-bearing geckos than padless geckos and typical lizards and that later toepad disengagement during the stance phase of a stride may increase adhesive contact, we predict pad-bearing geckos may have greater rotation than flexion, decoupling the two motions. This decoupling will be reflected by shallower surfaces that make up the mesotarsal joint, namely a broader oblique ridge on the astragalocalcaneum and a shallow groove on the fourth distal tarsal, as well as a less prominent peg. We used a phylogenetic framework and obtained microCT data to test predictions of morphology that reflects our current understanding of the functional constraints.

2. Methods

2.1. Phylogeny

We pruned a tree obtained from (Zheng and Wiens, 2016) and added additional species (Fig 3.2). *Aristelliger* species were added based on taxonomic and morphological information (Bauer and Good, 1996). The following species and genera were added to the tree by combining information from trees from (Bauer and Good, 1996; Bauer et al., 1997a; Jacobsen et al., 2014; Pyron et al., 2013; Wood et al., 2012): *Cyrtodactylus* and *Rhacodactylus*

2.2. Sampling

We selected species of 31 species of Gekkonidae of one specimen each, including three origins of the adhesive system for a total of 18 pad-bearing and 11 padless species. Specimens were obtained from the California Academy of Sciences, the Museum of Vertebrate Zoology, La Sierra University and private collections from Anthony P. Russell and Raul Diaz.

For each specimen, we measured snout-vent-length and obtained scans of the left hind foot of each gecko specimen. Where a left hind foot was unavailable, a right hind foot was scanned and reflected after the segmentation process. Each specimen was scanned using the Scanco 35 and Scanco 40 at 5 um resolution in the MicroCT Laboratory at the University of Calgary. Specimens were wrapped in cheesecloth, lightly soaked with ethanol and sandwiched between Styrofoam to prevent movement, rotation and ethanol evaporation in the sample chamber during long scanning periods, which were approximately an hour for each foot. After the scans were processed, the foot was volumized and the astragalocalcaneum and the fourth distal tarsal of the foot were segmented in 3D Slicer. Padstate data was obtained from Gamble et al. (2012).

Because of the lack of easily identifiable homologous landmarks on the fourth distal tarsal and the astragalocalaneum, we used auto3dgm, an auto alignment program in R to obtain 300 pseudolandmarks enveloping each bone. The program does so by aligning pairs of bone model surfaces with unique features before placing landmarks (Boyer, Puente, Gladman, Glynn, Mukherjee et al., 2015). Because this process is automated, the landmarks are not based on definitions for landmarks or semi-landmarks as outlined by Zelditch Swiderski and Sheets (2012) and are named “pseudolandmarks” because they are positioned based on an algorithm that is consistent.

2.3. Statistical analysis

We performed separate principal components analyses on both the fourth distal tarsal and the astragalocalcaneum, using a covariance matrix calculated from the standardized Procrustes coordinates. Landmark sets were standardized with Procrustes superimposition (Adams and Otarola-Castillo, 2013). We regressed log-transformed centroid size against snout-vent length to test whether centroid size was a good proxy for body size. To test whether shape covaried with size, we regressed the superimpositioned Procrustes coordinates against the log-transformed centroid size. We then created a phylomorphospace for each bone by projecting the tree onto the morphospace in order to assess the morphospace occupation between groups, using the phytools and geomorph packages (Adams and Otarola-Castillo, 2013; Revell, 2009).

Statistical analyses were primarily performed in geomorph and phytools. In order to assess the effect of padstate, we performed a one-way ANOVA, using type III sum of squares on the first two principal component axes for each principal component analysis where padstate was the independent variable. We also performed a one-way Procrustes ANOVA on each bone with padstate as the independent variable. We first performed the analyses assuming Brownian motion, then took the residuals from the model and fitted the residuals to both Brownian motion (BM) models and the Ohrlein-Uhlensteinbeck (OU) models in the Geiger package (Revell, 2010). We then chose a model based on the lowest Akaike information criterion unless the difference was less than 4. If the difference was less than 4, the Brownian motion model was used. Otherwise, we repeated the analysis with the OU model. Phylogenetic correction was only reported if the residuals of the PGLS model had a significant phylogenetic signal.

3. Results

3.1.Principal component analysis

3.1.1. Astragalocalcaneum

29 specimens were used in the principal components analysis. The first three principal components (PC) explained 49.07%. The first PC axis (26.32%) described changes to the length of the astragalocalcaneum (Fig 3.3). Higher scores on the first PC axis corresponded to a shorter astragalocalcaneum, a broader oblique ridge and a ventrally oriented lateral process. The second PC axis (13.96%) described changes to the tarsal facet of the astragalocalcaneum. Higher scores corresponded to a taller tarsal facet and broader facet.

3.1.2. Fourth distal tarsal

31 species were used in the principal components analysis of the fourth distal tarsal data. The first three principal components (PC) explained (47.22%). The first PC axis (27.5%) described changes to the prominence of the lateral process of the fourth distal tarsal and the height of the peg (Fig 3.4). Higher scores on the first PC axis

corresponded to a taller peg, a deeper groove and a larger lateral process. The second PC axis (11.17%) described changes to the height of the fourth distal tarsal.

3.2.Effect of padstate

For the astragalocalcaneum, padstate separated on the first, but not the second principal component (F(1,28)=7.17, p=0.01248, and F(1,27)=0.61, p=0.434, with phylogenetic correction, respectively). Pad-bearing geckos tended to have a more laterally oriented lateral process, a broader oblique ridge and a longer astragalocalcaneum than padless geckos. For the fourth distal tarsal, padstate separated on the first but not the second PC axis (F(1,30)=9.63, p=0.002, with phylogenetic correction and F(1,30)=2.16, p=0.15196, respectively. Pad-bearing geckos had shorter pegs, a shallower groove on the fourth distal tarsal and a less prominent lateral process. Overall, padstate affected both the astragalocalcaneum and the fourth distal tarsal (Procrustes ANOVA: F(1,28)=2.3, p=0.015 and F(1,30)=101.85, p=0.001 respectively) in ways that were consistent with the above described direction of variation. Neither the astragalocalcaneum nor the fourth distal tarsal were affected by size (F(1,28)=0.81, p=0.643 and F(1,30)=101.85, p=0.001 respectively.

4. Discussion

Functional constraints imparted by the adhesive system are likely to affect bones involved in a critical joint: the mesotarsal joint. We used a phylogenetic framework and 3D geometric morphometrics to characterize the bones involved in the mesotarsal joints of geckos with and without a well-developed adhesive system. Padstate influenced the shape of the astragalocalcaneum and fourth distal tarsal in functionally relevant ways.

4.1.General variation in the astragalocalcaneum

The astragalocalcaneum in geckos primarily differs from outgroups by the presence of a prominent lateral process in most species (Brinkman, 1980; Schaeffer, 1941). *Varanus* however, is one outgroup that has a prominent lateral process (Sullivan, 2010). This finding suggests that a prominent lateral process has been gained and lost several times throughout the squamatan phylogeny. Our results also revealed variation in the orientation of the lateral process from being laterally oriented to being ventrally oriented. The orientation and length of the lateral process likely affects the peroneus longus muscle, which has a tendon that wraps around the head of the lateral process (Brinkman, 1980; Russell, 1975). A more broadened survey outside of gekkotans with a larger sample size and measuring the length, orientation and presence absence of the lateral process would explore in what contexts the lateral process varies.

The general structure of astragalocalcanea is similar to outgroups and within gekkotans. However, in the most ancestral lineages, such as *Saltuarius*, the astragalocalcaneum takes a notably different N like shape and lacks a notable lateral process. Similarly, astragalocalcanea were often taxonomically diagnostic down to the genus level and genera often grouped together in the morphospace. For example, specimens of *Uroplatus* were easily identifiable by the shortened lateral process and square like lateral rim. *Rhoptropus* is another group that is easily identifiable by its elongated astragalocalcaneum. These diagnostic characters likely are associated with changes in kinematics at the mesotarsal joint and therefore, provide support for a future comparative kinematic study like Higham et al. (2015) among genera of gecko.

4.2.Effect of padstate on the astragalocalcaneum

Larger and shallower joint surfaces are often associated with larger ranges of motion (Gnanasekaran, 2012). Thus, because we expected pad-bearing geckos to have a larger range of motion to facilitate the engagement of the adhesive system, we expected that the astragalocalcaneum would have a broader oblique ridge and the fourth distal tarsal would have a shallow groove and shorter peg. The shape of this joint articulation would decouple the simultaneous flexion and rotation that would typically occur in the typical lizard joint. Our results demonstrate that not only do the bones of pad-bearing geckos reflect our predictions, but we also found that the lateral process was oriented more laterally in pad-bearing geckos than in padless geckos. The change of these features also explained most of the variation among the geckos.

The astragalocalcanea among pad-bearing geckos look relatively similar to each other, except for *Gehyra* species (Fig 3.2). *Gehyra* instead, was associated with a relatively ventrally oriented lateral process and a deeper astragalocalcaneum. The separation of the *Gehyra* genus from the rest of pad-bearing lineages in our data set indicates that there may be multiple shapes of astragalocalcanea that may fit the functional constraints of the adhesive system. Likewise, among padless geckos, *Cyrtodactylus astrum*, a generalist, greatly deviated from the rest of the padless geckos and its congener in our data set, *C. astrum*, is a cave gecko (Grismer, Wood, Tri and Murdoch, 2015) and likely encounters smooth surfaces, which may play a role in its pad-bearing like morphology. A larger sample of *Cyrtodactylus* would confirm if the differences seen here are typical of each ecomorph.

The variation in the lateral process of the astragalocalcaneum is significant because of its association with the peroneus longus (Russell, 1975). The more laterally oriented the process and the longer it is, the greater the distance between the mesotarsal joint and the muscle, increasing the length of the moment arm. These results suggest that force output is relatively greater in pad-bearing geckos than padless geckos. The lateral process is suggested to act as a calcaneal heel and facilitate the function of the peroneus longus in dorsiflexion and propulsion (Sullivan, 2010). In *Varanus*, simulations suggest that the lateral process increases propulsive output. Thus, our results suggest that the peroneus longus may output more force in pad-bearing geckos padless geckos, who have either a shortened lateral process or are ventrally directed. However, Zaaf et al. (1999) suggested that pad-bearing geckos had shorter joint moments over the mesotarsal joint, resulting in less propulsive forces than in padless geckos. In that study however, joint moments were measured on a 2D plane, which may have underestimated joint moments of muscles that involve not only flexion and extension, but also rotation. It may be the combination of a laterally oriented lateral process and shallow articular surfaces that result in greater rotation instead of flexion/extension due to the decoupling of these two actions. In arboreal contexts that are often associated with pad-bearing geckos, this decoupling and reduction in flexion/extension may be enabled by forelimb taking an equal role in propulsion (Cartmill, 1985). More detailed kinematic study of how the crus rotates on the pes is needed to understand the role of the lateral process.

4.3. General comments on the fourth distal tarsal

In contrast to the astragalocalcaneum, the fourth distal tarsals are not easily distinguishable from each other with the exception of the fourth distal tarsal of *Saltaurius salebrosus,* which had a cube like fourth distal tarsal with defined corners. Observable occurred in the distance between the lateral edge of the fourth distal tarsal and the peg, the shallowness of the groove and the articular facet for the fifth metatarsal.

4.4.Effect of padstate on the evolution of the fourth distal tarsal

Our predictions that the fourth distal tarsal would have a shallower groove were also supported by our data. Pad-bearing geckos tend to have shallower grooves and shorter pegs. Again, the major axis of variation that differentiates padstates also explains most of the variation in the dataset. The lateral process of the fourth distal tarsal also was found to be more prominent in padless geckos. This feature articulates with the fifth metatarsal. The fifth metatarsal can be shaped in primarily one of two ways (a hook shape typical of nongekkotan lizards (Robinson, 1975) or a short square shape (as illustrated in Russell et al. (1997). Hook shaped metatarsals fit into the surface of the lateral process of the fourth distal tarsal. The axis of its articulation is perpendicular to the axis of the peg whereas the square shaped fifth metatarsal, often observed in pad-bearing geckos is aligned more parallel with the peg. Therefore, the prominence of the lateral process of the fourth distal tarsal is diagnostic of the shape of the fifth metatarsal and thus an indicator of the spread of the digits, since a square shape of the fifth metatarsal creates a greater angle between the fourth and fifth digits then the hooked shape. The fifth metatarsal itself is functionally important to locomotion as many of the muscles that perform dorsal flexion insert on this bone (Russell, 1975; Russell and Rewcastle, 1979). Russell and Rewcastle (1979) observed that in many lizards that had a reduced fifth digit retained the fifth metatarsal. Changes in shape of the fifth metatarsal may imply changes in muscular insertion. Our results demonstrate a quick way of identifying the shape of the fifth metatarsal based on the fourth tarsal alone.

Our study also demonstrates that unlike the astragalocalcaneum, the fourth distal tarsal of ancestrally padless lineages are largely constrained and limited to a small area in the morphospace. In comparison to padless geckos, pad-bearing geckos appear to occupy a much larger portion of the morphospace, suggesting that functional constraints imparted by the adhesive system were relaxed. Secondarily padless geckos occupy a region that is closer to that of ancestrally padless geckos, but still appear retain that characteristic of having a larger variation in shape. These results are reflected by several previous papers suggesting that secondarily padless geckos would retain some features that facilitated the adhesive system (Russell 1979). It should also be noted that *Dixonius simaensis*, is a pad-bearing gecko that overlaps in morphospace with ancestrally padless geckos. This may be due to its ground-dwelling habits that is generally characteristic of padless geckos. Again, a larger sample size and habitat data may begin to parse out the effects of habitat and padstate.

5. Conclusion

The mesotarsal joint is a highly functionally relevant joint that is closely tied with both locomotion and adhesion in pad-baring geckos. We not only characterized the mesotarsal joints in geckos, but also demonstrated that padstate influences even proximal elements, such as the bones involved in the mesotarsal joint. We also identified that the adhesive system appears to relax functional constraints on the fourth distal tarsal to permit greater exploration of the morphospace. The shallower grooves in the fourth distal tarsal and the astragalocalcaneum in pad-bearing gecko permits future studies to examine if movement in the joints of pad-bearing geckos and padless geckos are different.

6. Specimens

RD1, NAM40, AMB184, AMB228, AMB237, AMB240, AMB259, AMB290, AMB316, AMB317, AMB318, AMB320, AMB321, AMB325, AMB327, AMB420, AMB432, AMB442, AMB444, APR111, APR115, APR120, APR16, APR53, TEH 1, CAS138984, CAS140554, CAS148556, CAS157760, CAS159786, CAS172379, CAS179786, CAS248337, CAS74738, CordH, LL1, LSU7716, LSU9245, LSU9981, AMB328, NAM23, NAM29, No8, R102608, R102613, R102614, RD3, RD4, TEH1, TEH2, TG1702, TG1708, TG1729, Yel1, AMB429, CAS167545, CAS76250, LSU10023, LSU11269, LSU12234, LSU9285, MVZ243535, NAM25, NAM66

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Figures

Figure legends:

Fig. 3.1. MicroCT scans of the astragalocalcaneum of *Gekko gecko* using the Scanco 35. The structures listed following are shown in the following views: A (proximal), B (distal), C (ventral) D. (distal). OR – oblique ridge, lp – lateral process, tf – tarsal facet, p – peg, g – groove of the fourth distal tarsal, dmn – disto-mesial notch

Fig.3.2. The phylogeny of species used in this study are shown above. At least three origins of the adhesive system are included in the sample. Pad-bearing (green) and padless (black) species are all from Gekkonidae with the exception of *Saltuarius salebrosus*, which is from Carphodactyldae

Fig. 3.3. Principal components analysis (PCA) of 300 pseudolandmarks describing the shape of the astragalocalcaneum. The phylogeny (grey) was projected onto the morphospace. Minimum convex polygons outline ancestrally padless (grey), pad-bearing (green).

Fig. 3.4. Principal components analysis (PCA) of 300 pseudolandmarks describing the shape of the fourth distal tarsal. The phylogeny (grey) was projected onto the morphospace. Minimum convex polygons outline ancestrally padless (grey), pad-bearing (green).

Fig. 3.1. MicroCT scans of the astragalocalcaneum and fourth distal tarsal of *Gekko gecko*

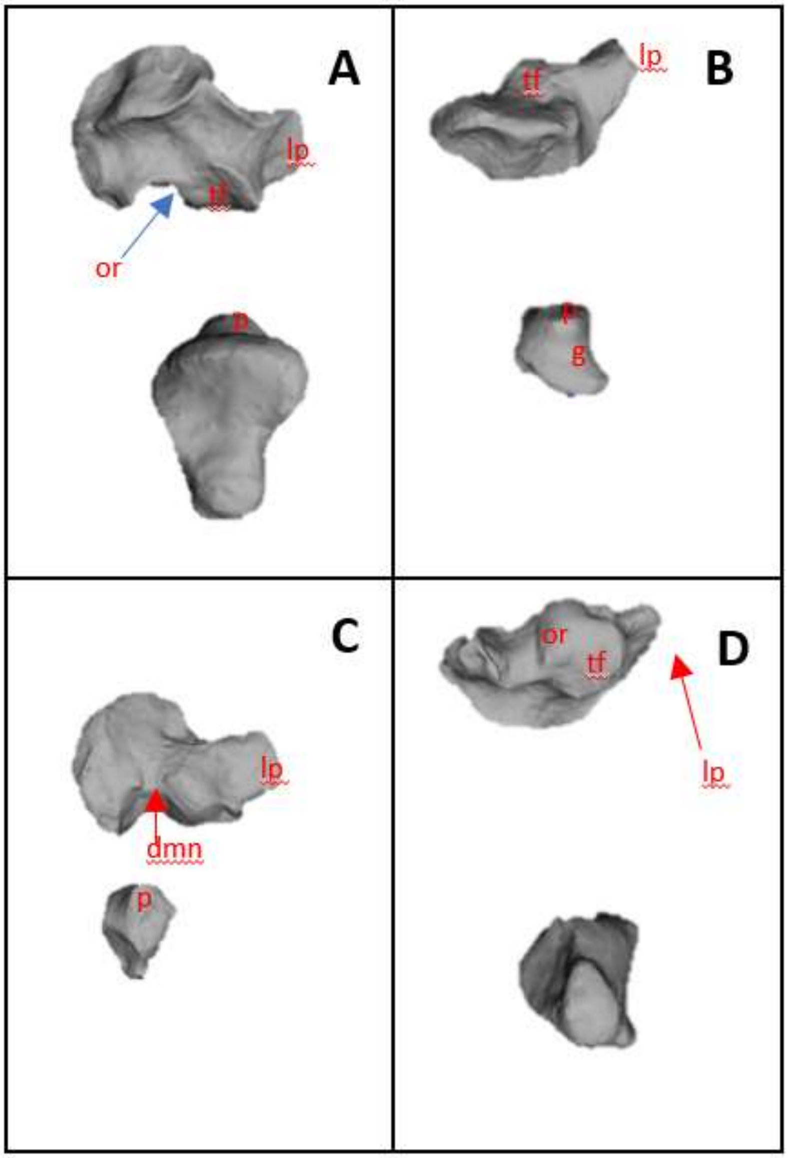


Fig. 3.2. Phylogeny of pad-bearing and padless species used

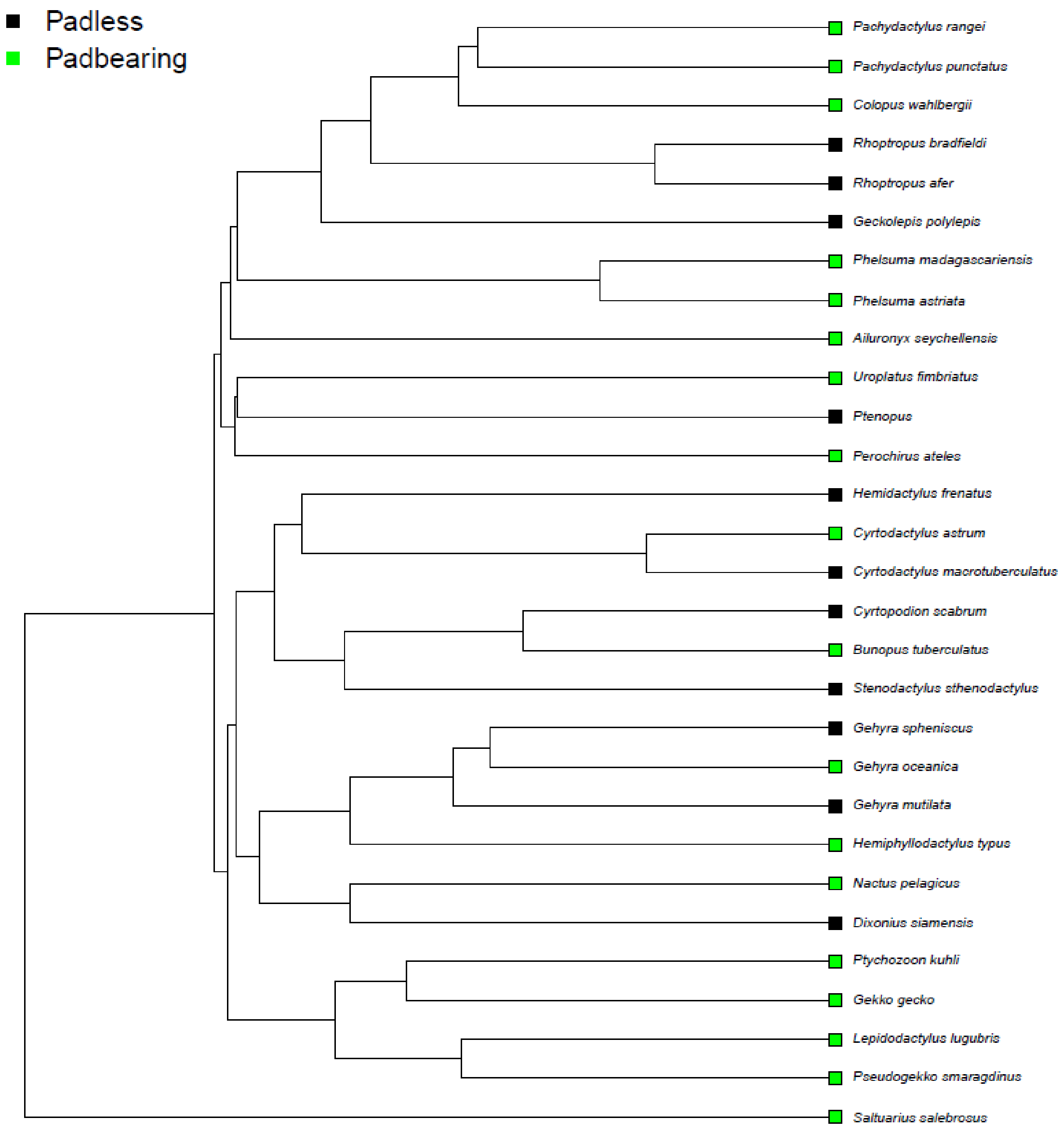


Fig. 3.3. Principal components analysis (PCA) of 300 pseudolandmarks describing the shape of the astragalocalcaneum

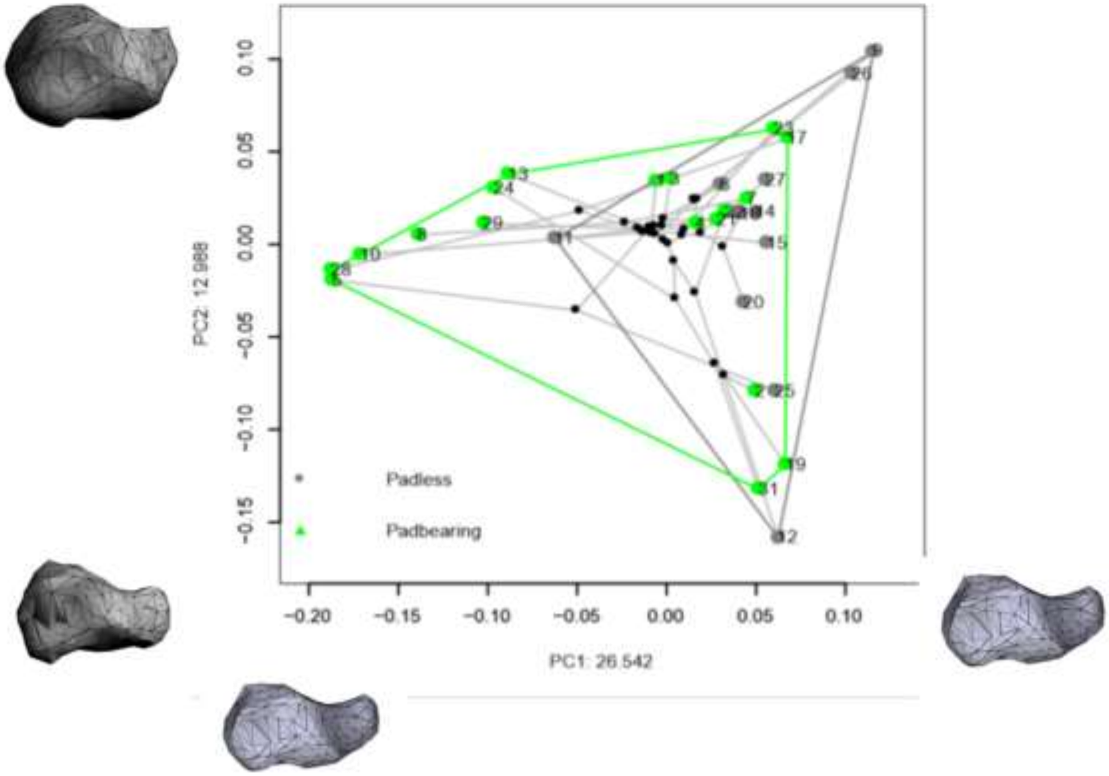
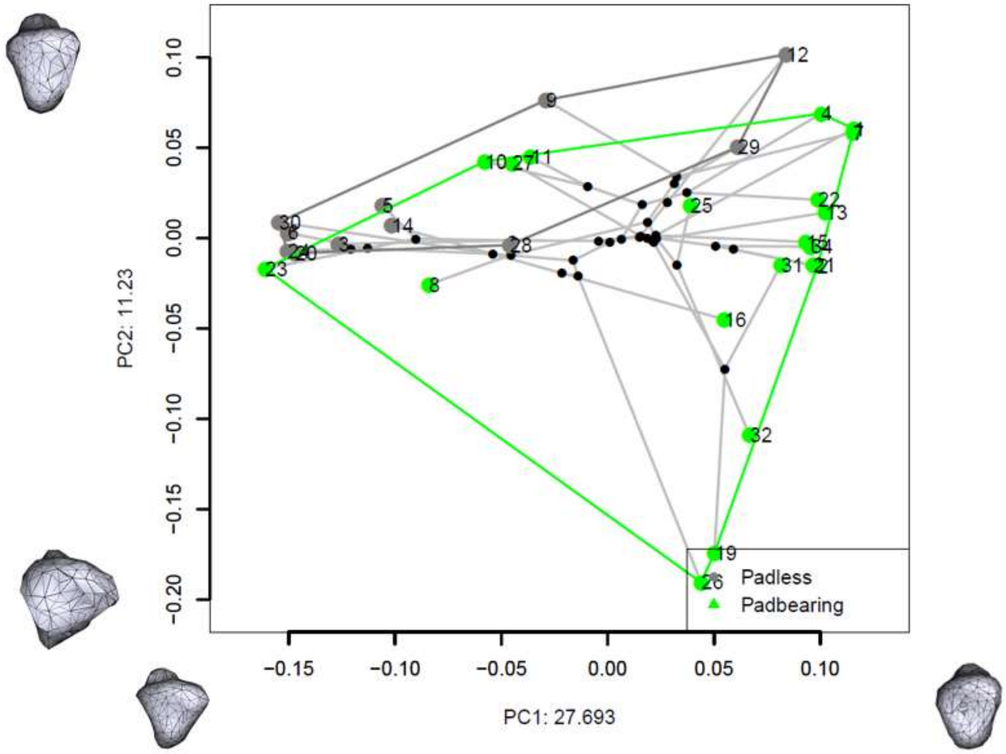


Fig 3.4. Principal components analysis (PCA) of 300 pseudolandmarks describing the shape of the fourth distal tarsal



Conclusions

In previous years, study of the gecko dry adhesive system has produced over many studies examining the mechanism of adhesion. However, very few have examined how geckos use their adhesive system (Birn-Jeffery and Higham, 2014; Russell and Oetelaar, 2015; Wang, Gu, Wu, Ji and Dai, 2010). In this dissertation, I examined the evolution of the skeletal structures of the foot underlying the adhesive system and likely affecting locomotion. This dissertation therefore, identifies important factors of the adhesive system that impact gecko locomotion and considerations of how the adhesive system functionally constrains foot morphology.

Chapter 1 revealed that geckos modulate their individual digits as well as the alignment of their forefeet and hind feet in response to changes in perch diameter and incline, in a fashion that is consistent with engaging the adhesive system appropriately. Phelsuma madagascariensis invert their feet on smaller perch diameters, which facilitates adhesive system engagement in challenging arboreal contexts. Individual digit modulation was more important in the hind foot, given the limited range of motion in the forefoot. This study examined usage in one species of gecko with an unusual asymmetrical hind foot morphology and limited forefoot morphology. This study also determined that geckos do modulate limbs in similar ways to previously examined lizards. Future studies should compare the movements of this gecko with those with more symmetrical foot shapes, such as *Gekko gecko* or *Chondrodactylus bibronii* as well as padless geckos, such as *Cyrtodactylus* to confirm the differences in pad-bearing and padless locomotor behavior and the effect of the distribution of digits in different contexts.

The last two chapters examine components that affect the results of the first chapter across geckos. The second chapter confirms that a greater sum of digits is important to shame of the gecko foot and is not mediated by the increase in the last interdigital angle. In fact, the last interdigital angle was often greater in pad-bearing lineages than padless lineages. Furthermore, this study reveals that the digit arrangement of *Phelsuma* is an extreme that does occur several times in which the foot acquires secondary asymmetry. However, this secondary asymmetry is not similar to the asymmetry observed in padless geckos. Future studies examine foot use in both padless geckos and other gecko genera that have secondary asymmetry would confirm the function of foot use.

Finally, in the third chapter, we found increased degrees of freedom in the mesotarsal joints of pad-bearing lineages. The mesotarsal joint is highly functionally relevant. The increased degrees of freedom in the mesotarsal joint potentially implies that there is increased instability at this joint because of the lack of concurrent action of flexion and rotation. At the same time, the adhesive system can remain engaged in a greater amount of orientations without modulation of the more proximal elements (the humerus). Functional studies examining the range of motion at this joint will confirm if the morphological implications can be carried out. This study further more suggests that the acquisition of the adhesive system not only is associated with morphological change in the distal elements (digit morphology), but also more proximal elements (the mesotarsal joint).

The function of the adhesive system can not only be attributed to the engagement of the setae, but also the placement. This dissertation reveals the underlying structural principles that are likely important to the engagement of the adhesive system.

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