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How Does Morphological Novelty Affect the Ecology and Biomechanics of Locomotion in the Namib Day Gecko Rhoptropus afer?

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How Does Morphological Novelty Affect the Ecology and Biomechanics of Locomotion in the Namib Day Gecko *Rhoptropus afer*?

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of the requirements for the degree of

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in

Evolution, Ecology, and Organismal Biology

by

Clint E Collins

December 2016

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Dedication

This dissertation is in memory of William Collins, Sr., Anna Laura Collins, and Bill Jones. I dedicate this dissertation to Ada Carson Collins. You inspire me to be better. I hope your world is more just and more sustainable than mine.

ABSTRACT OF THE DISSERTATION

How Does Morphological Novelty Affect the Ecology and Biomechanics of Locomotion in the Namib Day Gecko *Rhoptropus afer*?

by

Clint E. Collins

Doctor of Philosophy, Graduate Program in Evolution, Ecology, and Organismal Biology
University of California, Riverside, December 2016
Dr. Tim Higham, Chairperson

Movement is a fundamental aspect of animals. Natural and sexual selection act on the ability of an animal to effectively move through the environment in order to find and acquire mates, eat food, and escape from predators. It is generally thought that faster animals sire more offspring, acquire more mates, and evade predators more effectively. Effectively escaping through complex, three-dimensional environments is a principal driver of terrestrial animal biomechanics. However, the integrated, underlying suites of mechanisms including the interacting morphological components by which animals achieve successful performances are varied and contentious. Novel morphological structures increase performance of an ecologically relevant task in a descendent compared to its ancestor, often leading to adaptive radiation. The adhesive toe pad of geckos involves novel morphological structures that permit locomotion on inclined and inverted surfaces. The intricate method by which geckos employ the adhesive system has many cascading trade-offs on locomotion. Using a pad-bearing, secondarily terrestrial gecko from Namibia (Rhoptropus afer), I quantified the intimate relationships between gecko toe pads, habitat use, and the biomechanics of locomotion.

I tested the hypothesis that the adhesive toe pad morphology of R. afer corresponds to the physical structures used during escape behavior in the field. The size of the adhesive toe pad varies in relation to structural habitat use. Second, I brought the same individuals into the laboratory to quantify how they coordinate sprint speeds on ecologically relevant surfaces. Using high-speed video and path analysis, I found that this secondarily terrestrial gecko sprints using an integrated, but behaviorally flexible, suite of muscle group contributions. While many geckos are thought to power locomotion through specialized climbing muscles located near their center-of-mass, I found that R. afer mainly use ankle extensor muscles to power locomotion. Finally, I tested the hypothesis that the adhesive toe pad alters the coordination of locomotion, predicting individuals will alter relative contributions of muscle groups within limb segments to accommodate the toe pad when in use. I found that individuals vary in their use of toe pads, individuals alter limb segment coordination during toe pad use, and toe pads enhance sprint speed on level surfaces.

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CHAPTER 1 - Subdigital adhesive pad morphology varies in relation to structural habitat use in the Namib Day Gecko

Summary

Morphological features that lead to increased locomotor performance, such as faster sprint speed, are thought to evolve in concert with habitat use. The latter depends on available habitat structure and how the animal moves within that habitat. Thus, this behavioral variation will impact how natural selection acts on locomotion and morphology. Quantifying the interplay between escape behavior and locomotor morphology across habitats that vary in structural composition could reveal how selection acts on locomotion at local levels. Substrate features, such as incline and topographical variation, are likely key drivers of morphological and functional disparity among terrestrial animals. I investigated the impact of habitat variation and escape behavior on morphology, including the adhesive system, of *Rhoptropus afer*, a diurnal and cursorial gecko from Namibia. Substrate incline and topographical variation are likely important for this pad-bearing gecko due to the trade-off between adhering and sprinting (i.e. using adhesion results in decreased sprint speed). I corroborate the hypothesis that the adhesive system exhibits the greatest degree of reduction in populations that utilize the flattest terrain during an escape. My findings suggest that the adhesive apparatus is detrimental to rapid locomotion on relatively horizontal surfaces and may thus be counterproductive to the evasion of predators in such situations. A broad scale analysis of geckos would determine whether diversity of adhesive morphology is driven primarily by habitat use. Phenotypic plasticity of the adhesive system and interspecific competition are plausible candidates for driving my results. However, it is unclear whether the differences I observed have a genetic basis. Future work should focus on how variation of the adhesive system impacts downstream locomotor components such as kinematics and mechanics and how the integration of these traits is related to habitat use.

Introduction

Structural habitat complexity may influence the evolution of locomotor traits and performance in several ways. For example, animals must effectively negotiate numerous topographical features (e.g. inclines) that impact such properties as sprint speed. Natural selection should, there- fore, act differentially on locomotor performance and result in morphological features that are particularly suited to increase performance relative to the physical challenges that are encountered (Arnold 1983; Schluter & McPhail 1992; Irschick & Losos 1998; Dickinson et al. 2000; Donohue et al. 2001; Calsbeek 2008). Such morphological changes are sources of functional diversity (e.g. sprinting performance under different physical constraints) and may lead to habitat partitioning and subsequent high rates of diversification (Wainwright 1991, 2007). However, behavior can modulate what structures are employed during locomotion and how they are used. Thus, the physical constraints imposed by the habitat may be accommodated to some degree through modified behavior, thereby altering the trajectory and intensity of morphological adaptation (Smith 1974; Moermond 1979; Kotler 1984; Main 1987; Cooper 1997a,b; Cooper & Wilson 2007). Although this may be so, few studies have teased apart the differential impacts of behavior and morphology in relation to locomotion in terrestrial vertebrates. Quantifying these important, if subtle, impacts is likely critical for an enhanced under- standing of how animals are adapted to the habitats that they occupy.

The interactions of behavioral modulation, habitat use and locomotor morphology may appropriately be observed through a comparison of terrestrial habitats that differ in the magnitude and frequency of inclined substrata (Irschick 2003). During upslope locomotion, for example, gravity resists forward progression, increasing the work required, for the animal to move away from a predator in the case of a predator-prey interaction (Taylor, Rowntree & Caldwell 1972; Preuschoft 1990; Farley & Emshwiller 1996; Roberts & Belliveau 2005; Biewener & Daley

2007). Running downslope requires energy absorption via eccen- tric muscular contraction and thus also increases the cost of locomotion relative to level running (Minetti et al. 2002). In this case, gravity acts to assist the animal in its forward progress, but may also lead to an increase in instability (Minetti et al. 2002; Gabaldon, Nelson & Roberts 2004; Biewener & Daley 2007). Experimental evidence indicates that lizards avoid steep declines during escape maneuvers (Irschick & Losos 1999). However, small lizards potentially use inclines and declines in their escape from larger predators because the relative magnitude of dimensional changes in terrain, and the effects of upslope and downslope locomotion are size-dependent (Birn-Jeffery & Higham 2014). In other words, the locomotion of smaller animals is affected less than large animals when moving upslope or downslope. For example, Cooper & Wilson (2007) found that the relatively small Striped plateau lizard, *Sceloporus virgatus*, runs upslope for shorter distances than it does horizontally or downslope, suggesting that it uses inclines to its advantage. The manner in which such patterns change across habitats could reveal how selection acts, at a local level, on mechanistic links between escape trajectories and the morphological adaptations to the structural features used during the escape (Irschick 2003).

Anti-predator behavior, escape behavior and escape performance are dependent on substrate preference in multiple taxa (Jones, Mandelik & Dayan 2001; Collins et al. 2013; Des Roches et al. 2014). Although substrate–movement interactions are well known for many lizard taxa, little is known about such interactions in geckos, despite their affinity for specialized substrata (Carillo de Espinoza, Daniel Salas & Yehuda Werner 1990; Bauer & Russell 1991; Autumn & Peattie 2002; Autumn et al. 2006; Lamb & Bauer 2006; Russell & Johnson 2007; Johnson & Russell 2009), and the use of their highly specialized adhesive system on these. Interestingly, a study by Cooper & Whiting (2007) found that escape behavior depends on habitat structure in *Rhoptropus boultoni*, a boulder-dwelling gecko equipped with an adhesive system. However, other studies of ancestrally padless geckos are inconclusive (Persaud, Werner & Werner 2003; Werner et al. 2004). Hence, characterizing the interplay between the evolution of the adhesive system and habitat use may reveal consequences of selection on escape behavior, performance and morphology.

The gekkotan adhesive system, although having originated independently on multiple occasions within this cluster (Gamble et al. 2012), is considered to be an evolutionary innovation, permitting the exploitation of inclined and inverted surfaces (Russell 1975, 1979; Irschick et al. 1996; Johnson & Russell 2009). Adhesion occurs through a finely tuned, hierarchically arranged locomotor system culminating in adhesive toe pads (Russell 1975; Autumn & Peattie 2002). Toe pads are characterized by subdigital scansors that carry highly organized (Johnson & Russell 2009) fields of microfibrillar setae (10–100+ μm in length) that bear branched tips terminating in spatulae (0.2–04 μm wide) that create reversible bonds with the substratum. Adhesion is achieved by a combination of van der Waals forces and frictional loading associated with minute asperities of the locomotor surface (Autumn & Peattie 2002; Autumn et al. 2002; Tian et al. 2006; Johnson & Russell 2009). During loco- motion, the setae are deployed through the unfurling of hyperextended digits subsequent to heel strike. To disengage adhesion, the digits are hyperextended. Engaging and disengaging setae is rapid (~ 20 ms), but this process occupies c. 12.7% of stance time (Russell & Higham 2009). Given that the deployment of the adhesive system takes time, there is a trade-off between adhering (used during climbing) and locomotor speed. In locomotion on horizontal surfaces, pad-bearing geckos routinely hold their digit tips (and thus their adhesive apparatus) in a permanently hyperextended configuration and thus employ only the bases of the digits for purchase and thrust application (Bauer, Russell & Powell 1996; Russell & Higham 2009). In cases where geckos have become increasingly terrestrial, selection has favored the reduction or abandonment of the adhesive system (Bauer, Russell &

Powell 1996; Gamble et al. 2012). Thus, to more fully understand the physical parameters of loco- motion of pad-bearing geckos in relation to potential predator avoidance, it is important to understand the trade-offs that are evident between adhering to a surface and the employment of the fastest possible escape speed when running from a predator.

Despite the now well-understood phenomenon of adhesion in geckos, it is not currently known how the adhesive system differs between species and/or populations in response to habitat structure. Russell & Johnson (2013) found that the theoretical maximum adhesive capacity of all Rhoptropus geckos was tempered by the microtopographical roughness of the rock surfaces that they inhabited, with safety factors falling to within normally recognized biological ranges. Thus, all species of *Rhoptropus* are able to effectively exploit these habitat structures and no clear relationship was found between adhesive morphology and microtopography at the interspecific level, although it was recognized that *R. afer* exhibited reduced adhesive capacity relative to its body size.

Utilizing an intraspecific framework to understand how morphology and patterns of habitat use are related to the manner in which the adhesive system is adapted to habitat structure would be beneficial, due to the lack of phylogenetic history that must be accounted for in multiple species comparisons (Losos & Miles 1994), and would reduce the confounding effects of within-species variation that may obscure comparisons at the interspecific level. Additionally, any differences found among populations of a single species would likely be related to recent changes associated with habitat differences at the local level (Losos & Miles 1994; Herrel, Meyers & Vanhooydonck 2001; Kaliontzopoulou, Carretero & Llorente 2010). To approach the problem in this way, I chose *R. afer* (Fig. 1.1) as my study species.

Rhoptropus afer (Peters 1869) is a member of the well-studied *Pachydactylus* radiation

(FitzSimons 1943; Bauer 1999; Johnson, Russell & Bauer 2005) and is the most distinctive and derived member of its genus (all members of which are diurnal), in terms of both morphology and biology. It differs qualitatively from its congeners in its preference for horizontal sheet rock as a substrate (Bauer, Russell & Powell 1996). It is an accomplished cursor and may run, often for tens of meters, across relatively level terrain, before stopping (Odendaal 1979). Relative to its congeners, *R. afer* exhibits a reduction in the size of its adhesive apparatus and is characterized by elongation of the crus, metatarsals and proximal phalanges of the pedal digits (Higham & Russell 2010). During horizontal sprints, it runs with its adhesive pads hyperextended, with the ventral aspect of the metapodium and the proximal ends of the digits providing contact with the substratum and imparting locomotor thrust. For *Rhoptropus*, therefore, *R. afer* presents a situation for which habitat structure constitutes a compromise between a secondarily cursorial escape style, similar to that of other lizards, and the retention of a functional, albeit reduced, adhesive system (Johnson & Russell 2009).

I quantified the structural composition of the habitat, habitat use during escape, and morphology for four populations of *R. afer* that occupied habitats that appeared to differ in structure (Figs 1.1 and 1.2). If natural selection favors the reduction of the adhesive system in relatively flat habitats, where it would be counterproductive to locomotion, then geckos that use relatively flat terrain during predator evasion should have a relatively smaller adhesive system. I test the hypothesis that the adhesive system exhibits the greatest degree of reduction in populations that rely on the flattest terrain, and therefore escape trajectories, employed. My study integrates the structural composition of the available habitat, the specific structural components of habitat used during escape, and morphology, providing a vehicle for exploring how morphology may respond to, and reflect, environmental demands (Fig. 1.1).

Materials & Methods

I conducted my field study in November 2012 and May 2013 in the Namib-Naukluft and Dorob National Parks, and the Gobabeb Research and Training Centre in Namibia. *R. afer* is a small (3.15–5.2 cm), diurnal, terrestrial gecko that occurs from (20.900833°, 13.533611°) to (.23_557551°, 15.044396°) in the coastal range of Namibia (FitzSimons 1943).

Structural composition of the available habitat

I quantified the structural composition of the available habitat for each population (n = 4) by conducting 2x50 meter transects. I defined the structural composition of the available habitat as the structural composition of the locality that each population occupied. The beginning point of each transect was determined randomly and each 50-m transect was then directed north.

For each transect, substrate type (rock outcrop, gravel or sand) and the incline of the substrate in degrees were determined. Each 50-m transect was subdivided into 10-m segments, demarcated at each end by a vertical indicator. A digital protractor (PRO 360 Digital ProtractorTM; Mitutoyo, Aurora, IL, USA) was leveled at the mid-point of each segment, and each 10-m segment was photographed (Pentax K-x DSLR; Ricoh Imaging Americas Corp, Denver, CO, USA or Nikon D7000 DSLR; Nikon Inc., Melville, NY, USA) from an orthogonal angle with the angle finder in the center of the photograph and the camera lens parallel to the 10-m transect segment.

From the photographs, all inclines (±1°), and the linear distance of the segment occupied by that incline, were measured along the entire transect using IMAGEJ (version 1.46r) (Rasband 1997). The inclinations of the substrate were calibrated and measured in ImageJ using a straight line drawn across the top of the angle finder that represented the 0° horizon.

Two estimates of habitat variability were derived from the absolute values of inclines measured in each photograph: the number of changes >5° for each 10-metre segment and the difference in slope between each successive pair of inclines.

Habitat use: predator simulations and escape observations

Habitat use was defined as the structural composition of each locality that was used by an individual during an escape manoeuver. For each focal locality, random transects were walked by two to three observers. I employed human-simulated predation because of my ability to observe the geckos during their escape movements and to retain consistency with previous studies (e.g. Cooper 1997a,b). Upon sighting an individual (n = 10 per population), predation events were simulated by one observer (CEC, APR, or TEH) facing the animal and walking directly towards it at a steady speed of *c*. 1 ms^{-1}. When the lizard began fleeing, the pursuer stopped and all observers watched it as it ran. This was repeated twice more for each individual in order to fully characterize the structural habitat features used by the geckos during escape. In many instances, the lizard in question ran from an exposed position and took refuge under a rock flake. If this happened, the rock flake was lifted and the distance and trajectory run by the lizard to its next stopping point was observed.

After each escape event, the same methods for measuring structural habitat for the transects (see above) were employed to quantify the features of the terrain used along the escape path. When possible, individuals that were observed in this way in the field were captured. Flagging tape was used to mark each original sighting location and its GPS coordinates recorded. This accomplished three tasks – it: (i) ensured that the same individual was not pursued twice, (ii) allowed us to bring the animals into a laboratory setting laboratory to measure morphology and (iii) allowed the individuals that were not used in further analyses to be released at their original locations.

Morphological measurements

A subset of five individuals from each locality were euthanized, fixed in 10% neutral buffered formalin and preserved in 70% ethanol. These were transported to the University of California, Riverside, and the following standard morphological measurements recorded using digital calipers accurate to the nearest 0_01 mm: snout to vent length (SVL), intergirdle distance (from shoulder to hip), femur (from hip to knee) length, shank (from knee to ankle) length and pes length (from ankle to the tip of the longest toe).

The toe pad on the ventral side of the longest digit of the right or left pes was photographed using a Leica MA FlII SPOT Pursuit camera attached to Leica MZIII Pursuit Stereo Scope. From the resulting image, the total pad area was measured using ImageJ. As in other studies (Russell & Johnson 2007, 2013; Johnson & Russell 2009), the scansors (bearing setae) were included in the measurements of pad area, but the more proximal lamellae were not.

Subsequently, the tip of each digit for which pad area was recorded was removed and sectioned sagittally under a dissecting microscope. The two resulting sections, with their cut face uppermost, were affixed using double stick, electrically conductive car- bon tape, to a stub that was secured into the custom tilt stage of a Hitachi TM-1000 Tabletop Scanning Electron Microscope (in the Institute for Integrative Genome Biology at UCR) and viewed at a 90° angle. Each complete section and each scansor were photo- graphed, saved and opened in ImageJ, from which the following measurements were taken: setal length, setal diameter and setal density. Setal length was measured along the midline of each seta from its base to its tip. Setal diameter was measured at a height of c. 5–15 µm from its base. Setal density was calculated by first counting the number of setae along a 32 µm length of each scansor, squaring this number and then multiplying by 1000 to obtain a setal density per mm2 (Russell & Johnson 2007, 2013; Johnson & Russell 2009).

Statistical analysis

A series of discriminant function analyses (DFA) were used to characterize the relationships between structural habitat composition and habitat use during escape and to explore differences among the localities in escape and behavior. First, the variables for structural habitat composition were entered into a DFA, with each locality serving as an independent variable. Secondly, the structural habitat composition variables from each locality were pooled and compared to the habitat structures used during escape by entering all variables into a DFA with 'availability' and 'use' set as independent variables. Thirdly, a DFA was used to explore the differences among populations in habitat use patterns during escape. Then, a DFA was used to characterize and describe the differences among populations in the suite of morphological traits measured for each individual. Because all but one of the morphological variables measured were predicted by body size ($P < 0.05$), prior to analysis, they were regressed against SVL, and the residuals were used as size-free morphological components in subsequent analyses. Wilks' Lamda, relative eigenvalues, and the misclassification rate were used to determine the power of each DFA to discriminate between the indicated independent variables. I interpreted the canonical loadings by comparing the positive and negative values to the raw data for each locality.

Post-hoc comparisons were made for the strongest DFA independent variables to elucidate the differences among populations, as follows: t-tests between the percentage of upslope vs. downslope escapes; a one-way analysis of variance (ANOVA) on the percentage of habitat exhibiting a pitch of >10° for each population; a one- way ANOVA on the first set of canonical axis scores derived from the morphological DFA; finally, a two-way ANOVA on the size-corrected raw morphological data that were most informative in separating the groups in the DFA. All statistical analysis was conducted using JMP® (Version 10 for Mac. SAS Institute Inc., Cary, NC, USA, 1989–2007).

Results

My initial observation that the four localities varied in incline and steepness was verified by examining plots of the percentage of habitat with a pitch of >10° available within each locality vs. that which was used during escape (Fig. 1.3). A t-test indicated that lizards constituting the Beach & Boulders population (Fig. 1.2a), but no others, used 10° inclines significantly less than would be expected relative to their availability ($P < 0.05$) (Fig. 1.3).

Differences between structural habitat composition and habitat use

The DFA successfully distinguished between the habitat structures used during escape and the availability of habitat structures as measured in transects. The habitat variables that best distinguished between use and availability data for each locality were differences between successive inclines and the percentage of distance with a pitch >10° incline (Table 1.1). The overall misclassification rate was 22%. For each locality, *R. afer* consistently used substrates during escapes that traced flatter and less heterogeneous courses than would be predicted from the average lie of the terrain.

Differences among localities in habitat structural composition and habitat use

The DFA for the structural habitat composition of each locality revealed differences in the structural composition of available habitat at each locality. The features that best distinguished among habitats along canonical axis one were the percentage of habitat pitched at >10° incline, habitat variability and the rockiness of each habitat (Table 1.2, Fig. 1.4a). The second canonical function accounted for 19% of the data, and the most powerful variables were habitat variability and maximum incline (Table 1.2, Fig. 1.4a). The structural composition of the Granite Mountain (Fig. 1.2b) locality exhibited the rockiest, most variable, and most inclined structural habitat composition relative to the other three localities ($P < 0.001$) (Table1. 2, Fig. 1.4a). The Beach & Boulders (Fig. 1.2a) locality exhibited relatively horizontal and less variable substrates ($P < 0.001$) (Table 1.2, Fig. 1.4a). The Gravel Plain (Fig. 1.2c) locality was misclassified as Sheetrock (Fig. 1.2d) at a rate of 50%, thus I infer that these two localities were similar in their structural composition (Fig. 1.4a).

The structural habitat features used during escape varied among localities. A DFA on habitat use successfully classified *R. afer* populations at a rate of 68%. The first canonical axis accounts for 74% of the variation between localities and the most powerful predictors were the percentage of habitat pitched at >10° incline, and the percentage of habitat composed of rock outcrops (Table 1.3, Fig. 1.4b). Canonical axis two accounts for 24% of the variation between localities, with the most powerful predictors being the percentage of habitat pitched at >10° incline, and habitat variability (Table 1.3, Fig. 1.4b). *Rhoptropus afer* at the Beach & Boulders locality were subject to the least misclassification – only one individual was misclassified from this locality, whereas 2–7 individuals were misclassified from other habitats. A t-test revealed that at the Granite Mountain locality, the majority of *R. afer* escapes occurred on upslope substrates, rather than downslope or level ones ($P < 0.01$). Additionally, the percentage of escapes at this locality pitched at greater than a 10° incline was greater than would be predicted based upon the average availability of habitat pitched at greater than a 10° incline, although this was not statistically significant. For all other localities, there were no statistical differences between upslope vs. downslope escape trajectories.

Morphology

The DFA for the morphological measurements revealed no significant differences (Wilks' Lambda, P = 0.4). However, a 25% misclassification rate indicated that this DFA, although not statistically significant, performed moderately well and indicated important distinctions between each population (Fig. 1.4c). Canonical axis one accounted for 74% of the variation among populations and discriminated among groups, with the strongest coefficients being for pad area and foot length. Canonical axis two accounted for 19% of the variation among populations and most strongly discriminated among populations for maximum seta length and shank length (Table 1.4, Fig. 1.4c). The Beach & Boulders population was not misclassified, yet all of the other populations exhibited at least one misclassification.

Because trends in morphological differentiation were evident in the DFA, a two-way ANOVA with Tukey–Kramer post hoc tests on size-corrected subdigital pad area (P < 0.02) and pes length (P = 0.06) was used to clarify the differences among populations. The Beach & Boulders population exhibited smaller toe pads relative to the other three populations (Table 1.5, Fig. 1.5).

Discussion

Adhesion is an evolutionary innovation that enables some geckos to exploit a variety of substrata, including inverted surfaces, that are not generally accessible to other tetrapods (Ruibal & Ernst 1965; Autumn et al. 2006; Russell & Johnson 2007, 2013). My findings suggest that the employment of the adhesive apparatus is inefficient for locomotion on relatively horizontal surfaces and may thus be counterproductive to the evasion of predators in such situations. The geckos in my study used habitat topography in non-random ways when assessed against availability among and within each locality. In escape sprints, *R. afer* avoided steep inclines and declines and also heterogeneous substrates, thus selecting escape routes that were circuitous rather than direct. Avoiding steep inclines was the principal mode of differentiation between structural composition of the available habitat and escape trajectory in the Beach & Boulders population. *R. afer* in this locality also exhibited reduction of the area and structure of the adhesive toe pads beyond that which typifies the species as a whole (Johnson & Russell 2009) and relative to the patterns shown by conspecifics at the other locations examined in this study. Thus, my study directly associates variation in morphology with variation in the habitat structures used during escape, something not previously observed for geckos. My study also reinforces the idea that information about morphological variation should be combined with escape behavior to understand the ways in which species are adapted to local habitat structures (Vitt et al. 1997; Herrel, Meyers & Vanhooydonck 2001; Kaliontzopoulou, Carretero & Llorente 2010).

Effectively evading predators often includes accelerating and sprinting at high velocities (Arnold 1983; Zehr & Sale 1994; Irschick & Losos 1998; Miles 2004; Dayton et al. 2005; Husak 2006; Calsbeek & Irschick 2007). Greater velocity is achieved through increasing stride length, increasing stride frequency or a combination of both. Relative to its congeners, *R. afer* exhibits much longer hind- limbs and toes, and these have evolved in concert with a cursorial lifestyle (Higham & Russell 2010). Hence, greater stride length is achieved through morphological modification (Johnson, Russell & Bauer 2005; Higham & Russell 2010). Increasing stride frequency is complex and involves many integrated suites of physiological and morphological traits, such as greater muscle contraction rates and mass reduction in distal limb elements (Biewener 1989; Fieler & Jayne 1998; McElroy & Reilly 2009). Stride frequency in pad-bearing geckos is further limited by the time it takes to deploy and detach the adhesive system (Autumn et al. 2006; Russell & Higham 2009). Deployment of the adhesive system is triggered at inclines of about 10° (Russell & Higham 2009), and it is not recruited on horizontal surfaces. This is important because sprint speed becomes limited on inclined surfaces above 10° as a result of the time taken to insert the attachment and detachment phases of adhesion into the step cycle. This response is reflexive and is seemingly controlled by feedback from the vestibular system (Russell & Higham 2009). Furthermore, the relative (and absolute) size of the adhesive apparatus will influence the absolute amount of time taken to engage and disengage setae during the step cycle, thereby increasing stride frequency through morphological modification. Thus, I suggest that the adhesive apparatus of geckos is both morphologically and behaviorally phenotypically plastic and that it is responsive to the demands of habitat topography and heterogeneity encountered during escape. I have shown that this is the case for localized populations within the confines of a single species. However, common garden experiments are necessary to rule out genetic differences as opposed to phenotypic plasticity between populations.

The adhesive apparatus is reduced via subdigital pad area reduction where flatter escape trajectories are used. In addition to reducing the time taken for setal attachment and detachment, reduced subdigital pad area is also advantageous in this context because it allows for more toe area to be dedicated to generating friction during escape (Russell & Bels 2001; Russell & Higham 2009). Because longer digits confer sprint speed and stability advantages, exhibiting a reduced adhesive system would allow the R. afer from the Beaches & Boulder site to use a longer toe to increase its running speed while carrying less of the digit in a hyperextended state (Russell & Bels 2001; Russell & Higham 2009). The proximal components of the pedal digits are relatively elongated in R. afer (Bauer, Russell & Powell 1996), demonstrating that there is a trade-off between proximal and distal digit length associated with the reduction of the adhesive system in this taxon. Future work detailing the mechanics of locomotion will reveal whether this advantage leads to more effective force transmission.

The sizes and shapes of gecko toe pads and setae vary tremendously between species, and this variation is tenuously linked to the occupancy of different structural habitats (Russell 1975, 1979; Carillo de Espinoza, Daniel Salas & Yehuda Werner 1990; Bauer & Russell 1991; Autumn & Peattie 2002; Harmon & Gibson 2006; Lamb & Bauer 2006; Gamble et al. 2008, 2011; Johnson & Russell 2009; Sistrom et al. 2012). It is possible that the differences in subdigital pad area in *R. afer* represent intraspecific adaptive phenotypic plasticity because *R. afer* sprints away from potential predators (FitzSimons 1943; Johnson & Russell 2009; Higham & Russell 2010). Hence, relaxation of the physical constrains requiring adhesion and avoidance of inclines of >10°, that would trigger its deployment, may lead to further reduction of the size of the adhesive apparatus through the decrease of subdigital toe-pad area. This suggests that the reduction of adhesive capacity, long considered a key innovation, may be beneficial for the enhancement of cursoriality in geckos.

Competition is an important driver of habitat partitioning, structural niche realization, and speciation (Schluter 1994, 2001; Stamps, Losos & Andrews 1997). *Anolis* lizards in the Caribbean likely radiated to occupy their ecomorphological niches as a result of increased competition typical of insular ecosystems (Losos 1990, 1992; Irschick *et al.* 1997; Beuttell & Losos 1999; Glor *et al.* 2004). In cases in which phenotypic plasticity is evident, character displacement may occur as a result of competitive exclusion. Given a three-dimensional structural habitat available to a population (species A), a competitor (species B) that occupies an exclusive part of this habitat would relegate species A to a smaller realized niche (Schluter 1994, 2001). *Rhoptropus afer* is syntopic with the closely related *R. bradfieldi* at the Beach & Boulders locality and may compete with it. *Rhoprtopus bradfieldi* exclusively occupies the large boulders, which are the principal sources of inclination at this locality. Although the ecological mechanics of the potential competition between these two species is unknown, it may have been a causal factor in driving the more horizontal escape trajectories used by *R. afer*. If this is the case, then competition may have provided the impetus for the adaptive phenotypic plasticity observed in the adhesive system. In other localities included in this study, *R. afer* is the only diurnal gecko present.

Despite the possession of reduced toe-pad size in the Beach & Boulders population of *R. afer*, the carriage of the digits in a hyperextended posture during horizontal sprinting imposes a physical disadvantage – reduced traction through a lessened area of contact. Epidermal spinules just proximal to the seta-bearing scansors on the subdigital pads of *R. afer* may enhance friction (Russell 2002; Russell, Johnson & Delannoy 2007) on level surfaces, while the digits are carried in hyperextension (Russell 2002). Because *Rhoptropus* is characterized as being pad-bearing but clawless, it is likely that the epidermal spinules enhance frictional interactions with the substratum during level locomotion (Lamb & Bauer 2001; Johnson, Russell & Bauer 2005; Johnson & Russell 2009). The relatively elongated proximal portions of the pedal digits in *R. afer* would provide for increased area for frictional interactions. Future experiments should characterize the role of the spinules and setae in ground-dwelling geckos (Khannoon *et al*. 2014), as well as internal morphological features such as tendon and muscular patterns that may enhance or constrain context – dependent performance (Abdala *et al*. 2009).

References

Abdala, A., Manzano, A.S., Tulli, M.J. & Herrel, A. (2009). The tendinous patterns in the palmar surface of the lizard manus: functional consequences for grasping ability. *The Anatomical Record* **292**, 842–853.

Arnold, S.J. (1983). Morphology, performance, and fitness. *American Zoologist* **23**, 347–361.

Autumn, K. & Peattie, A.M. (2002). Mechanisms of adhesion in Geckos. *Integrative and Comparative Biology* **42**, 1081–1090.

Autumn, K., Sitti, M., Liang, Y.A., Peattie, A.M., Hansen, W.R., Sponberg, S. (2002). Evidence for van der Waals adhesion in gecko setae. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 12252–12256.

Autumn, K., Hsieh, S., Dudek, D., Chen, J., Chitaphan, C. & Full, R. (2006). Dynamics of geckos running vertically. *Journal of Experimental Biology* **209**, 260–272.

Bauer, A.M. (1999). Evolutionary scenarios in the Pachydactylus group geckos of southern Africa: new hypotheses. *African Journal of Herpetology* **48**, 53–62.

Bauer, A.M. & Russell, A.P. (1991). Pedal specializations in dune-dwelling geckos. *Journal of Arid Environments* **20**, 43–62.

Bauer, A.M., Russell, A.P. & Powell, G.L. (1996). The evolution of locomotor morphology in *Rhoptropus* (Squamata: Gekkonidae): functional and phylogenetic considerations. *African Journal of Herpetology* **45**, 8–30.

Beuttell, K. & Losos, J.B. (1999). Ecological morphology of Caribbean anoles. *Herpetological Monographs* **13**, 1–28.

Biewener, A.A. (1989). Mammalian terrestrial locomotion and size: mechanical design principles define limits. *BioScience* **39**, 776–783.

Biewener, A.A. & Daley, M.A. (2007). Unsteady locomotion: integrating muscle function with whole body dynamics and neuromuscular control. *Journal of Experimental Biology* **210**, 2949–2960.

Birn-Jeffery, A.V. & Higham, T.E. (2014). The scaling of uphill and downhill locomotion in legged animals. *Integrative and Comparative Biology* **Icu015**.

Calsbeek, R. (2008). An ecological twist on the morphology - performance - fitness axis. *Evolutionary Ecology Research* **10**, 197–212.

Calsbeek, R. & Irschick, D.J. (2007). The quick and the dead: correlational selection on morphology, performance, and habitat use in island lizards. *Evolution* **61**, 2493–2503.

Carillo de Espinoza, N.R., Daniel Salas, A. & Yehuda Werner, L. (1990). Radiation and convergence among desert geckos: *Phyllodactylus* species resembling both *Ptyodactylus* and

Stenodactylus. *Amphibia – Reptilia* **11**, 1–13.

Collins, C.E., Russell, A.P. & Higham, T.E. (2015). Data from: Subdigital adhesive pad morphology varies in relation to structural habitat use in the Namib Day Gecko, *Rhoptropus afer*. Dryad Digital Repository. http://doi.org/10.5061/dryad.r20tr

Collins, C.E., Self, J.D., Anderson, R.A. & McBrayer, L.D. (2013). Rock- dwelling lizards exhibit less sensitivity of sprint speed to increases in substrate rugosity. *Zoology* **16**, 151–158.

Cooper, W.E. (1997a). Escape by a refuging prey, the broad-headed skink (*Eumeces laticeps*). *Canadian Journal of Zoology* **75**, 943–947.

Cooper, W.E. (1997b). Factors affecting risk and cost of escape by the broad-headed skink (*Eumeces laticeps*): predator speed, directness of approach, and female presence. *Herpetologica* **00**, 464–474.

Cooper, W.E. & Whiting, M.J. (2007). Universal optimization of flight initiation distance and habitat-driven variation in escape tactics in a Namibian lizard assemblage. *Ethology* **113**, 661–672.

Cooper, W.E. & Wilson, D.S. (2007). Beyond optimal escape theory: microhabitats as well as predation risk affect escape and refuge use by the Phrynosomatid lizard *Sceloporus virgatus*. *Behavior* **144**, 1235–1254.

Dayton, G.H., Saenz, D., Baum, K.A., Langerhans, R.B. & DeWitt, T.J. (2005). Body shape, burst speed, and escape behavior of larval anurans. *Oikos* 111, 582–591.

Des Roches, S., Torresdal, J., Morgan, T.W., Harmon, L.J. & Rosenblum, E.B. (2014). Beyond black and white: divergent behavior and performance in three rapidly evolving lizard species at White Sands. *Biological Journal of the Linnean Society* **111**, 169–182.

Dickinson, M.H., Farley, C.T., Full, R.J., Koehl, M.A.R., Kram, R. & Lehman, S. (2000). How animals move: an integrative view. Science, 288, 100–106.

Donohue, K., Pyle, E.H., Messiqua, D., Heschel, M.S. & Schmitt, J. (2001). Adaptive divergence in plasticity in natural populations *of Impatiens capensis* and its consequences for performance in novel habitats. *Evolution* **55**, 692–702.

Farley, C.T. & Emshwiller, M. (1996). Efficiency of uphill locomotion in nocturnal and diurnal lizards. *Journal of Experimental Biology* **199**, 587–592.

Fieler, C.L. & Jayne, B.C. (1998). Effects of speed on the hindlimb kinematics of the lizard *Dipsosaurus dorsalis*. *Journal of Experimental Biology* **201**, 609–622.

FitzSimons, V.F.M. (1943). The lizards of South Africa. Pretoria, South Africa: *Transvaal Museum, Memoir* 1. pp xv + 1-528 + plates I- XXIV.

Gabaldon, A.M., Nelson, F.E. & Roberts, T.J. (2004). Mechanical function of two ankle extensors in wild turkeys: shifts from energy production to energy absorption during incline vs. decline running. *Journal of Experimental Biology* **207**, 2277–2288.

Gamble, T., Bauer, A.M., Greenbaum, E. & Jackman, T.R. (2008). Out of the blue: a novel, trans-Atlantic clade of geckos (Gekkota, Squamata). *Zoologica Scripta* **37**, 355–366.

Gamble, T., Bauer, A.M., Colli, G.R., Greenbaum, E., Jackman, T.R., Vitt, L.J. (2011). Coming to America: multiple origins of New World geckos. *Journal of Evolutionary Biology* **24**, 231–244.

Gamble, T., Greenbaum, E., Jackman, T.R., Russell, A.P. & Bauer, A.M. (2012). Repeated origin and loss of adhesive toe pads in geckos. *PLoS ONE* **7**, e39429.

Glor, R.E., Gifford, M.E., Larson, A., Losos, J.B., Schettino, L.R., Lara, A.R.C. (2004). Partial island submergence and speciation in an adaptive radiation: a multilocus analysis of the Cuban green anoles. *Proceedings of the Royal Society of London B* **271**, 2257–2266.

Harmon, L.J. & Gibson, R. (2006). Multivariate phenotypic evolution among island and mainland populations of the ornate day gecko, *Phelsuma ornata*. *Evolution* **60**, 2622–2632.

Herrel, A., Meyers, J.J. & Vanhooydonck, B. (2001). Correlations between habitat use and body shape in a phrynosomatid lizard (*Urosaurus ornatus*): a population-level analysis. *Biological Journal of the Linnean Society* **74**, 305–314.

Higham, T.E. & Russell, A.P. (2010). Divergence in locomotor performance, ecology, and morphology between two sympatric sister species of desert-dwelling gecko. *Biological Journal of the Linnean Society* **101**, 860–869.

Husak, J.F. (2006). Does speed help you survive? A test with collared lizards of different ages. *Functional Ecology* **20**, 174–179.

Irschick, D.J. (2003). Measuring performance in nature: implications for studies of fitness within populations. *Integrative and Comparative Biology* **43**, 396–407.

Irschick, D.J. & Losos, J.B. (1998). A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution* **52**, 219–226.

Irschick, D.J. & Losos, J. (1999). Do lizards avoid habitats in which performance is submaximal? The relationship between sprinting capabilities and structural habitat use in Caribbean anoles. *American Naturalist* **154**, 293–305.

Irschick, D.J., Austin, C.C., Petren, K., Fisher, R.N., Losos, J.B. & Ellers, O. (1996). A comparative analysis of clinging ability among pad-bearing lizards. *Biological Journal of the Linnean Society* **59**, 21–35.

Irschick, D.J., Vitt, L.J., Zani, P.A. & Losos, J.B. (1997). A comparison of evolutionary radiations in mainland and Carribean Anolis lizards. *Ecology* **78**, 2191–2203.

Johnson, M.K. & Russell, A.P. (2009). Configuration of the setal fields of *Rhoptropus* (Gekkota: Gekkonidae): functional, evolutionary, ecological, and phylogenetic implications of observed pattern. *Journal of Anatomy* **214**, 937–955.

214, 937–955.Johnson, M.K., Russell, A.P. & Bauer, A.M. (2005). Locomotor morphometry of the Pachydactylus radiation of lizards (Gekkota: Gekkonidae): a phylogenetically and ecologically informed analysis. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **83**, 1511–1524.

Jones, M., Mandelik, Y. & Dayan, T. (2001). Coexistence of temporally partitioned spiny mice: roles of habitat structure and foraging behavior. *Ecology* **82**, 2164–2176.

Kaliontzopoulou, A., Carretero, M. & Llorente, G. (2010). Intraspecific ecomorphological variation: linear and geometric morphometrics reveal habitat related patterns within *Podarcis bocagei* wall lizards. *Journal of Evolutionary Biology* **23**, 1234–1244.

Khannoon, E.R., Endlein, T., Russell, A.P. & Autumn, K. (2014). Experimental evidence for friction-enhancing integumentary modifications of chameleons and associated functional and evolutionary implications. *Proceedings of the Royal Society B: Biological Sciences* **281**, 2013–2334.

Kotler, B.P. (1984). Risk of predation and the structure of desert rodent communities. *Ecology* **65**, 689–701.

Lamb, T. & Bauer, A.M. (2001). Mitochondrial phylogeny of Namib day geckos (*Rhoptropus*) based on cytochrome b and 16S rRNA sequences. *Copeia* **273**, 775–780.

Lamb, T. & Bauer, A.M. (2006). Footprints in the sand: independent reduction of subdigital lamellae in the Namib-Kalahari burrowing geckos. *Proceedings of the Royal Society B: Biological Sciences* **273**, 855–864.

Losos, J.B. (1990). A phylogenetic analysis of character displacement in Caribbean *Anolis* lizards. *Evolution* **44**, 558–569.

Losos, J.B. (1992). The evolution of convergent structure in Caribbean *Anolis* communities. *Systematic Biology* **41**, 403.

Losos, J.B. & Miles, D.B. (1994). Adaptation, constraint and the comparative method: phylogenetic issues and methods. *Ecological Morphology: Integrative Organismal Biology* (eds P.C. Wainwright & S.M. Reilly), **pp. 60–98**. University of Chicago Press, Chicago.

Main, K.L. (1987). Predator avoidance in seagrass meadows: prey behavior, microhabitat selection, and cryptic coloration. *Ecology* **68**, 170–180.

McElroy, E.J. & Reilly, S.M. (2009). The relationship between limb morphology, kinematics, and force during running: the evolution of locomotor dynamics in lizards. *Biological Journal of the Linnean Society* **97**, 634–651.

Miles, D.B. (2004). The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. *Evolutionary Ecology Research* **6**, 63–75.

Minetti, A.E., Moia, C., Roi, G.S., Susta, D. & Ferretti, G. (2002). Energy cost of walking and running at extreme uphill and downhill slopes. *Journal of Applied Physiology* **93**, 1039–1046.

Moermond, T. (1979). The influence of habitat structure on *Anolis* foraging behavior. *Behavior* **70**, 147–167.

Odendaal, F.J. (1979). Notes on the adaptive ecology and behavior of four species of *Rhoptropus* (Gekkonidae) from the Namib Desert with special reference to a thermoregulatory mechanism employed by *Rhoptropus afer*. *Madoqua* **11**, 255–260.

Persaud, D., Werner, N. & Werner, Y.L. (2003). Foraging behavior of three sphaerodactyline geckos on Trinidad and Tobago (Sauria: Gekkonomorpha: Sphaerodactylini: *Gonatodes*). *Journal of Natural History* **37**, 1765–1777.

Peters, W. (1869). Uber neue Gattungen und Arten von Eidechsen. Koniglich-Preußische Akademie der Wissenschaften, 1869, 57–66.

Preuschoft, H. (1990). Gravity in primates and its relation to body shape and locomotion. *Human Evolution* **5**, 559–578.

Rasband, W.S. (1997). ImageJ. US National Institutes of Health, Bethesda, Maryland, USA.

Roberts, T.J. & Belliveau, R.A. (2005). Sources of mechanical power for uphill running in humans. *Journal of Experimental Biology* **208**, 1963– 1970.

Ruibal, R. & Ernst, V. (1965). The structure of the digital setae of lizards. *Journal of Morphology* **117**, 271–293.

Russell, A.P. (1975). A contribution to the functional analysis of the foot of the Tokay, *Gekko gecko* (Reptilia: Gekkonidae). *Journal of Zoology* **176**, 437–476.

Russell, A.P. (1979). Parallelism and integrated design in the foot structure of gekkonine and diplodactyline geckos. *Copeia* **1979**, 1–21.

Russell, A.P. (2002). Integrative functional morphology of the gekkotan adhesive system (Reptilia: Gekkota). *Integrative and Comparative Biology* **42**, 1154–1163.

Russell, A.P. & Bels, V. (2001). Biomechanics and kinematics of limb-based locomotion in lizards: review, synthesis, and prospectus. *Comparative Biochemistry and Physiology-Part A* **131**, 89–112.

Russell, A.P. & Higham, T.E. (2009). A new angle on clinging in geckos: incline, not substrate, triggers the deployment of the adhesive system. *Proceedings of the Royal Society B: Biological Sciences* **276**, 3705–3709.

Russell, A.P. & Johnson, M.K. (2007). Real-world challenges to, and capabilities of, the gekkotan adhesive system: contrasting the rough and the smooth. *Canadian Journal of Zoology* **85,** 1228–1238.

Russell, A.P. & Johnson, M.K. (2013). Between a rock and a soft place: microtopography of the locomotor substrate and the morphology of the setal fields of Namibian day geckos (Gekkota: Gekkonidae: *Rhoptropus*). *Acta Zoologica* **95**, 299–318.

Russell, A.P., Johnson, M.K. & Delannoy, S.M. (2007). Insights from studies of gecko-inspired adhesion and their impact on our understanding of the evolution of the gekkotan adhesive system. *Journal of Adhesion Science and Technology* **21**, 1119–1143.

Schluter, D. (1994). Experimental evidence that competition promotes divergence in adaptive radiation. **Science** *266*, 798–801.

Schluter, D. (2001). Ecology and the origin of species. *Trends in Ecology & Evolution* **16**, 372–380.

Schluter, D. & McPhail, J.D. (1992). Ecological character displacement and speciation in sticklebacks *The American Naturalist* **140**, 85–108.

Sistrom, M., Edwards, D., Donnellan, S. & Hutchinson, M. (2012). Morphological differentiation correlates with ecological but not with genetic divergence in a *Gehyra* gecko. *Journal of Evolutionary Biology* **25**, 647– 660.

Smith, J.N. (1974). The food searching behavior of two European thrushes II: the adaptiveness of the search patterns. *Behavior* **49,** 1–61.

Stamps, J.A., Losos, J.B. & Andrews, R.M. (1997). A comparative study of population density and sexual size dimorphism in lizards. *American Naturalist* **149**, 64–90.

Taylor, C.R., Rowntree, V.J. & Caldwell, S.L. (1972). Running up and down hills - some consequences of size. *Science* **178**, 1096–1097.

Tian, Y., Pesika, N., Zeng, H., Rosenberg, K., Zhao, B., McGuiggan, P. (2006). Adhesion and friction in gecko toe attachment and detachment. *Proceedings of the National Academy of Sciences United States of America* **103**, 19320–19325.

Vitt, L.J., Caldwell, J.P., Zani, P.A. & Titus, T.A. (1997). The role of habitat shift in the evolution of lizard morphology: evidence from tropical *Tropidurus*. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 3828–3832.

Wainwright, P.C. (1991). Ecomorphology: experimental functional anatomy for ecological problems. *American Zoologist* **31**, 680–693.

Wainwright, P.C. (2007). Functional vs. morphological diversity in macroevolution. *Annual Review of Ecology, Evolution, and Systematics* **38**, 381–401.

Werner, Y., Takahashi, H., Yasukawa, Y. & Ota, H. (2004). The varied foraging mode of the subtropical Eublepharid gecko *Goniurosaurus kuroiwae orientalis*. *Journal of Natural History* **38**, 119–134.

Zehr, E.P. & Sale, D.G. (1994). Ballistic movement: muscle activation and neuromuscular adaptation. *Canadian Journal of Applied Physiology* **19**, 363–378.

Table 1: Summary of DFA of structural habitat features used during escape and available at each locality. Standardized canonical coefficients greater than 0.8 are boldfaced.

Canonical Axis	Eigenvalue	%	Total	Appx. F	P > F	>10	Max Incline	Avg. Difference	Max Difference
1	0.46	100	100	6.48	0.0001	**0.859**	-0.48	**1.64**	**-0.71**

Table 2: Summary of DFA of structural habitat features available by locality. Standardized canonical coefficients greater than 0.8 are boldfaced.

Canonical Axis	Eigenvalue	%	Total	Appx. F	P > F	>10	Max Incline	Avg. Difference	Max Difference	% Rock
1	1.84	80	80	3.22	0.0001	**0.92**	0.39	**-1.76**	**1.47**	**1.45**
2	0.45	19	99	1.36	0.22	0.02	**1.18**	**-1.77**	**1.35**	-0.41
3	0.01	1	100	0.12	0.97	-0.19	-0.41	2.03	-0.88	-0.04

Table 3: Summary of DFA of habitat used during escape by locality.
Standardized canonical coefficients greater than 0.8 are boldfaced in the far right column.

Canonical Axis	**Eigenvalue**	**%**	**Total**	**Appx. F**	**P > F**	**>10**	**Max Incline**	**Max Rise**	**AvgDiff Prev**	**MaxDiff Prev**	**%Rock**
1	1.70	75	75	2.48	0.0021	**2.83**	0.19	0.05	-0.32	0.20	**1.00**
2	0.55	24	99	1.24	0.28	**3.03**	0.23	0.48	0.37	0.43	-0.07
3	0.03	1	100	0.19	0.96	2.69	-0.54	0.49	0.65	-0.38	0.18

Table 4: Summary of DFA of morphology.
Standardized canonical coefficients greater than 0.8 are boldfaced in the appropriate column.

Canonical Axis	**Eigenvalue**	**%**	**Total**	**Appx. F**	**P > F**	**Pad Area**	**Intergirdle**	**Femur**	**Shank**	**Foot**	**Setae Length**	**Setal Width**
1	1.85	74	74	1.05	0.44	**0.80**	-0.57	0.68	-0.74	**1.16**	-0.60	-0.22
2	0.44	17	91	0.60	0.81	0.45	0.24	-0.33	0.58	-0.22	0.61	0.23
3	0.22	9	100	0.54	0.74	-0.78	0.31	0.21	0.63	0.02	-0.12	0.36

Table 5: Summary of one-way ANOVA of morphology canonical axis scores 1 (left) and Tukey-Kramer multiple post-hoc comparisons (right). Localities connected by the same letter are not significantly different.

Source	DF	SS	MS	F	P > F	Locality	SE	Mean	Letters Report
Model	3	29.60	9.87	9.87		Beaches & Boulder	0.45	-1.86	**A**
Error	16	16	1			Sheetrocks	0.45	0.01	**B**
Total	19	45			0.0006	Granite Mtn	0.50	0.18	**B**
						Gravel Plains	0.41	1.42	**B**

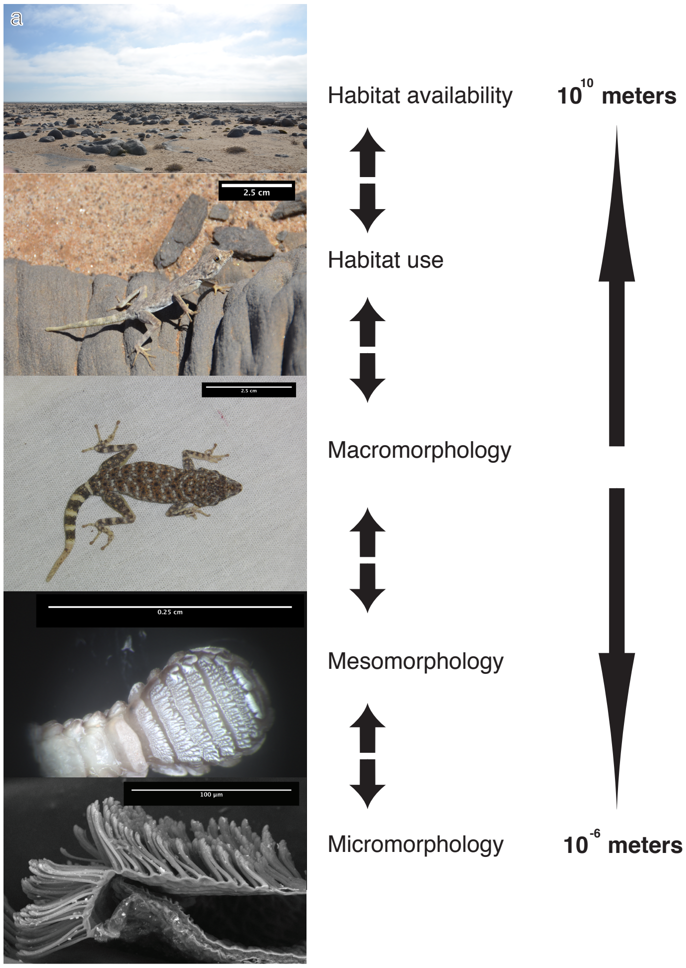


Figure 1.1. The 8 orders of magnitude spanned by this study. From top: (a) structural composition of the available habitat, (b) structural composition of each locality that was used by an individual during an escape manoeuver, (c) gross locomotor morphology, (d) subdigital pad area and (e) setal morphology.

Figure 1.2. Depictions of localities of *R. afer* examined in this study. (a) The Beach & Boulders locality is located on the Atlantic coast of Namibia and is characterized by sand and gravel with larger boulder outcrops strewn throughout. (b) The Granite Mountain habitat is located adjacent to Ro€ssing Mountain and consists of large granite outcrops adjacent to sandy substrata, either as isolated patches (fore- ground) or as the lower reaches (midground) of more continuous granitic outcrops of greater elevation (background). (c) The Gravel Plains habitat is located east of the Gobabeb Research and Training Centre and consists of low-lying, but undulating gravelly substrata interspersed among low-elevation rock outcrops. (d) The Sheetrocks habitat located northeast of Dune 7 in the Namib-Naukluft Park consists of sandy substrate interspersed with low-lying sheetrock outcrops.

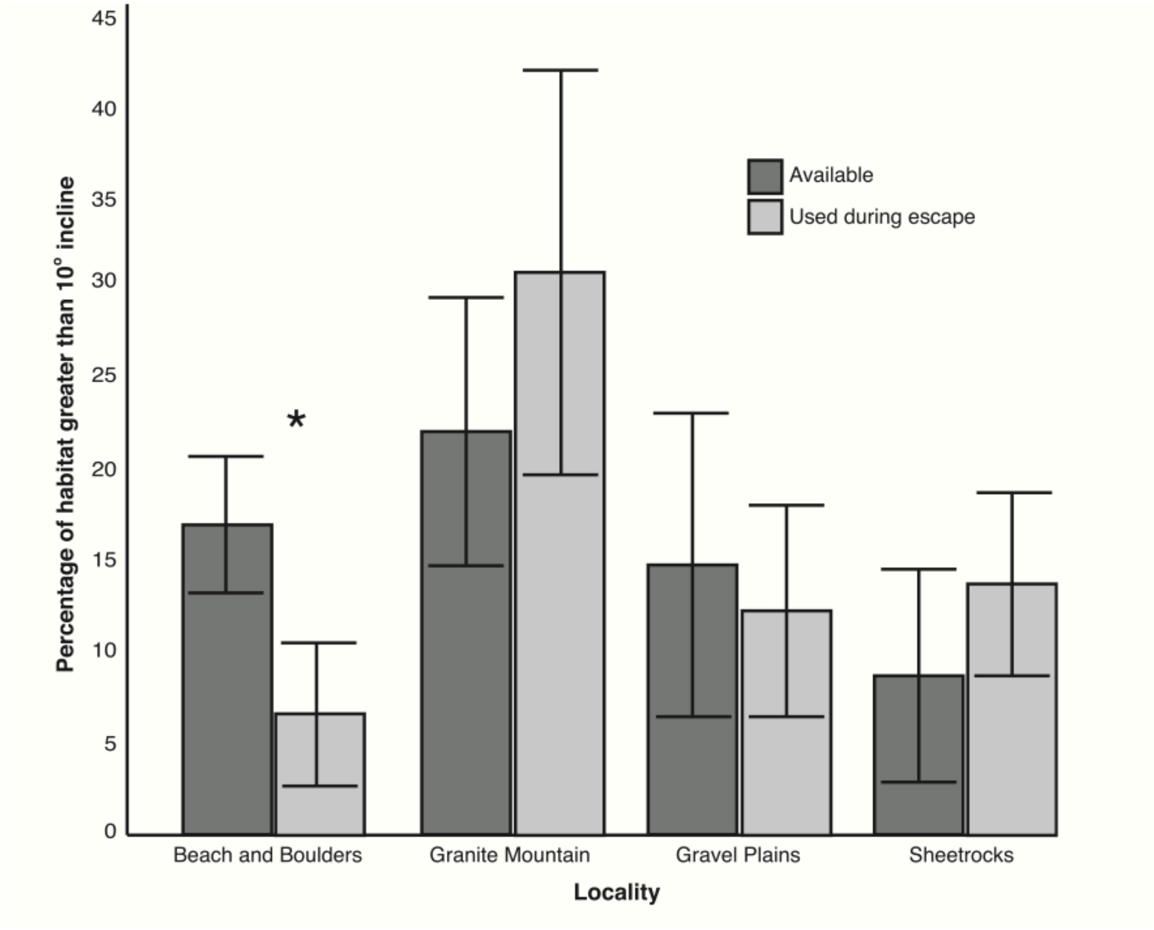


Figure 1.3. Bar graph representing the percentage of habitat with a pitch of >10° supports the initial observation that the each locality varied in its structural habitat composition. An asterisk indicates a significant difference between the structural composition of available habitat and habitat use (t-test, $P < 0.05$). The R. afer at the Beach & Boulders locality used significantly fewer 10° inclines relative to the terrain available to it compared to R. afer examined at other localities.

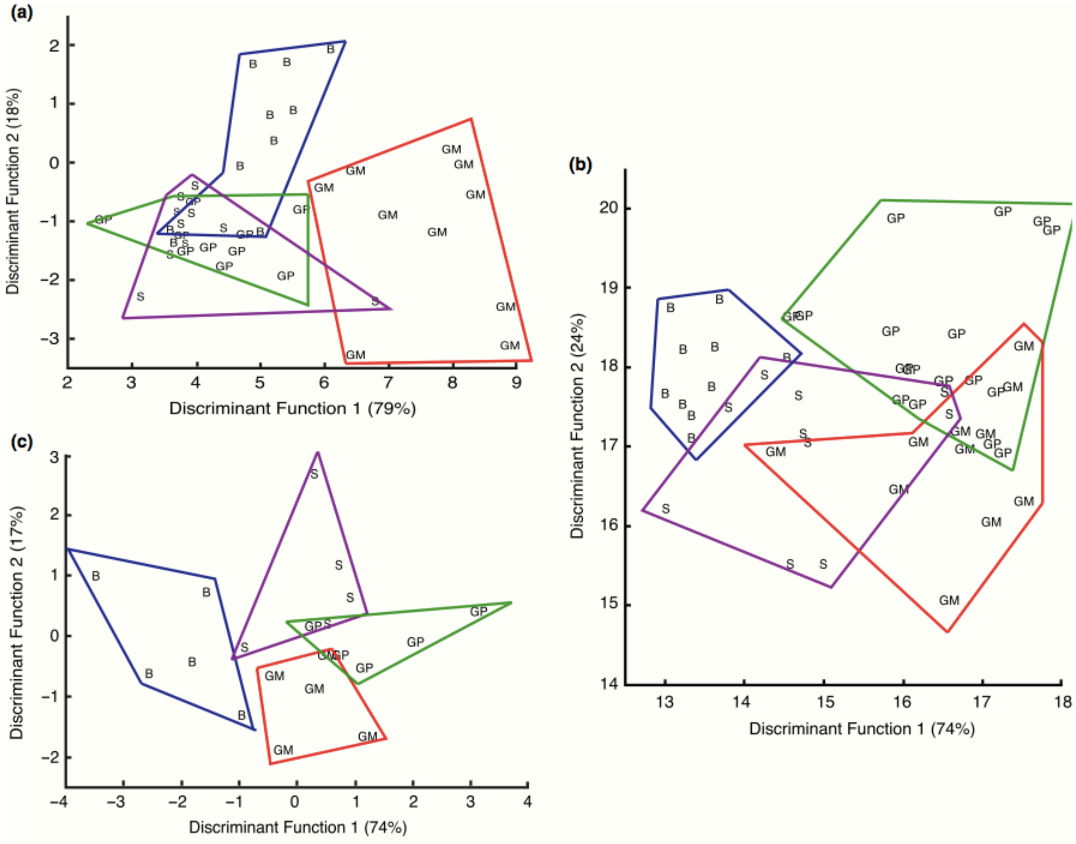


Figure 1.4 (a) Discriminant function plot of scores from transects through each locality indicating differences among populations in the structural habitat features available. Positive loadings on canonical axis one describe rockier, more inclined substrata and large differences between successive incline changes. Positive loadings on canonical axis two describe more inclined substrata and large differences between successive incline changes. (b) Discriminant function plot of scores from the habitat structures used during escape by each population of R. afer. Positive loadings on canonical axis one describe rockier and more inclined substrata. Positive loadings on canonical axis two describe more horizontal habitats. (c) Discriminant function plot of scores from morphological measurements indicate separation among populations in two directions. Positive loadings on canonical axis one indicate greater toe-pad area and longer pes. There were no loadings equal to or >0.80 on canonical axis two but the strongest, seta length, indicated that positive loadings on canonical axis two described longer setae. Abbreviations: B – Beach & Boulders; GP – Gravel Plains; GM – Granite Mountains; S – Sheetrocks.

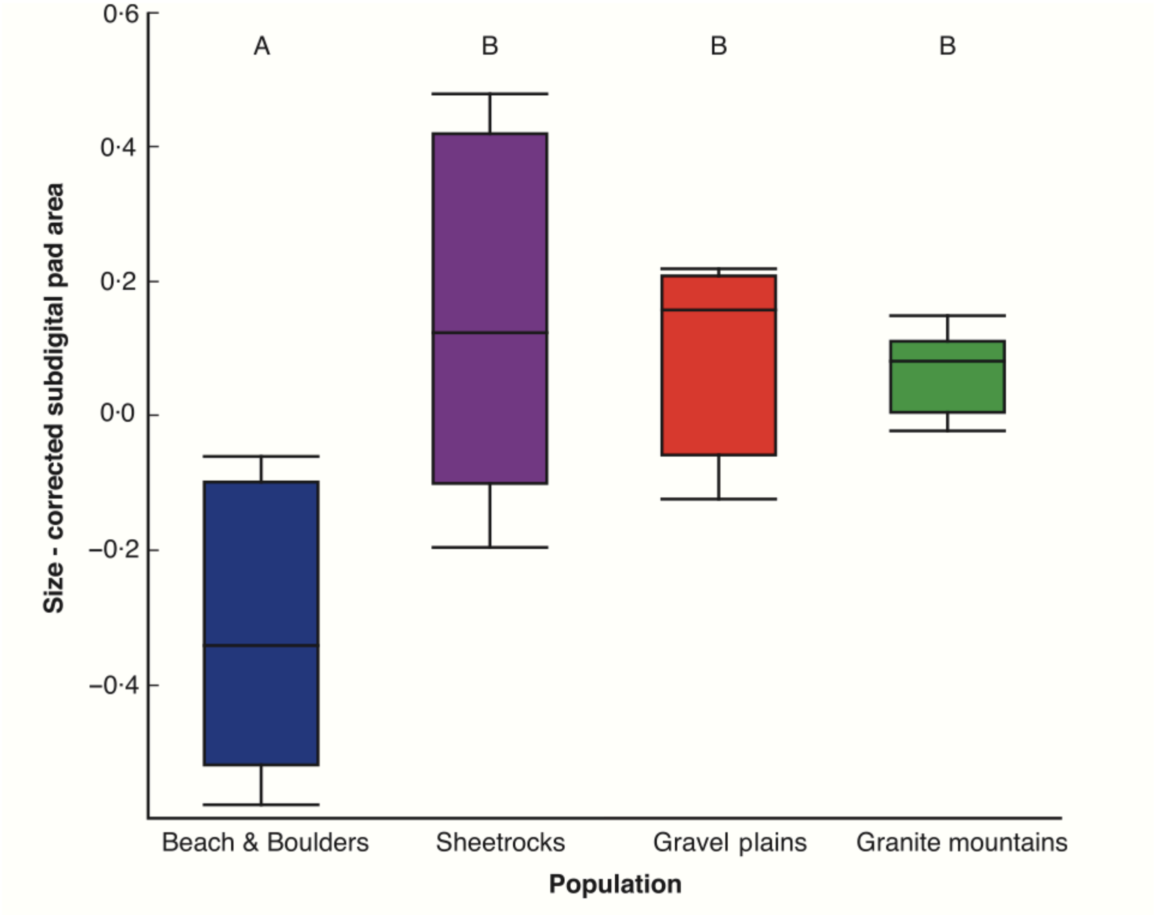


Figure 1.5. Box & whiskers plot showing the morphological differences between populations of R. afer. The Beach & Boulders population exhibited significantly smaller subdigital pad area than the other three populations (P = 0.005). Localities connected by the same letter are not significantly different.

CHAPTER 2: The Namib Day Gecko, *Rhoptropus afer,* overcomes habitat constraints by altering the relative contributions of joint motions to locomotion.

Summary:

Locomotion is a fundamental aspect of animals. Morphological features leading to increased locomotor performance, such as faster sprint speed, are thought to evolve in concert with habitat use. Fast sprint speeds results from dynamic combinations of behavioral and morphological components. The evolution of fast maximal sprint speed has evolved several times in lizards. However, the underlying mechanisms by which lizards coordinate high speed still await discovery in many evolutionary trajectories. This chapter explores how sprint speed is coordinated in *R. afer* with special consideration of its habitat use. I found that how sprint speed is determined is different between level and inclined surfaces.

Introduction:

Animal performance is molded by natural selection to meet the functional demands of the physical environment (Arnold 1983; Darwin, 1859; Losos 2010; Ricklefs and Miles 1994). These performances are emergent properties of an integrated suite of functional traits including muscles, bones, tendons, and the cardiovascular systems. For example, greater aerobic capacity increases the ability of an animal to migrate, defend territories, and travel long distances to find mates and food (Dickinson *et al*. 2000; Garland *et al*. 1990; Irschick and Garland 2001; Nathan *et al*. 2008). Aerobic capacities are an emergent property of heart size, spleen mass, lung size and other sub-organismal systems (Hammond *et al*. 2000) In many animals including humans, “the race goes to the swift” (Miles 2004) in attaining higher social status (Impellisseri *et al*. 2008) finding mates, acquiring food, and escaping predators under the duress of the biophysical constraints of the environment (Husak, 2006a; Husak, 2006b; Husak *et al*., 2006). In other words, faster animals are more likely to escape predators and produce offspring. ‘Realizing’ (sensu Bauwens *et al*. 1995; Irschick 2003) fast sprint speeds results from dynamic combinations of behavioral and morphological components. On one hand, faster muscle contractions hasten the step cycle frequency (Aerts 1990, Biewener 2003). On the other hand, stride length can be increased statically by having longer limbs or increased dynamically via increased joint extension during the stance phase of a step (Biknevicius and Reilly 2006; Biewener 1991; Biewener 2003; Garland and Losos 1994).

The evolution of fast maximal sprint speed has evolved several times in lizards (Garland, 1984; Garland et al., 1990; Garland and Losos, 1994). However, the underlying mechanisms by which lizards coordinate high speed still await discovery in many evolutionary trajectories (McElroy and Reilly 2009). One perspective is to study stride length and stride frequency (Bonine and Garland 1999; Foster *et al.* 2015; Olberding *et al.* 2015) as predictors of sprint speed. While this allows researchers to understand if evolution chiefly acts on leg length or muscle contraction properties in general, is akin to studying overall aerobic capacity, but not the underlying organ systems or aerobic pathways. To truly understand the physiological and biomechanical properties that determine the sprint speed, a more fine-scale approach must be taken.

The detailed analysis of kinematics, or the observable movements of morphological components, can illuminate mechanisms that determine locomotor performance. For example, Snyder (1954) proposed that stride length is chiefly accomplished through femur retraction, associated with the caudofemoralis muscle. Others, including Reilly & Delancey (1997) and Felder & Jayne (1998) argued that some propulsion takes place by extension of the ankle joint in cursorial lizards, but that thrust chiefly occurs through contractions of the caudofemoralis. Conversely, McElroy *et al.* 2012 found that the gastrocnemius, powering ankle extension, advances sprint speed in the cursorial Florida Scrub Lizard, *Sceloporus woodi.* Two important missing pieces of information are 1) how these joint extensions, rotations, and retractions work together to directly and indirectly power movement and 2) how these coordinated contributions work under natural conditions. This is critical because none of these suborganismal traits operate in isolation and lizards rarely run in uniform, controlled environments. Given the contentious nature of the literature, the coordination of lizard sprint speed is a gravid system for examining how integrated suites of traits allow animals to perform in nature (Wainwright, 1994; Wainwright *et al.*, 2005)

Ascending arboreal habitats is often viewed as the initial selective pressure for the adhesive toe pads of geckos (Gamble *et al.* 2012; Russell *et al.* 2015). The adhesive system enables many geckos to exploit vertical and overhanging structures (Irschick et al., 1996; Russell, 1979). However, some Gekkotan species are secondarily terrestrial (Bauer et al., 1996; Bauer, 1999; Gamble *et al.* 2012; Johnson and Russell 2009), utilizing a variety of substrata. Selective pressures in non-arboreal habits are different. For example, the Namib Day Gecko, *Rhoptropus afer* (Peters 1869; Figure 2.1) escapes on diverse surfaces varying in heterogeneity and incline severity but predator evasion on relatively flat, but variable, surfaces is associated with smaller adhesive toe pads (Collins *et al.* 2015). This species sprints up to tens of meters away from simulated predators (Collins *et al.* 2015). Relative to other geckos, *R. afer* accommodates a cursorial lifestyle via a derived morphological pattern including reduced toe pads, longer limbs, and extended distal elements (FitzSimons 1943; Higham & Russell 2010; Odendaal 1979). I investigate locomotor kinematics in *R. afer* to determine how this species resolves the physical challenges faced during predator evasion based on the information obtained from Collins *et al.* (2015). Following the framework of Renous and Gasc (1977) as extended in Russell and Bels (2001), I grouped joint extensions and limb segment rotations/retractions into functional assemblages. Throughout this paper, these assemblages will be referred to by their action (*e.g.* peroneus and gastrocnemius = ankle extension).

Engaging and disengaging adhesive toe pads is rapid (~ 20 ms), but this process can occupy approximately 12.7% of stance time (Autumn et al. 2006). While adhesion was once thought to directly relate to microscopic topographical variation (Irschick *et al.* 1996; Mahendra, 1938), it is incline, not substrate texture, that triggers adhesion (Russell and Higham 2009). In some species, adhesion is triggered at approximately 10º incline, and the percentage of strides where adhesion is used increases to approximately 100% by 30º (Russell and Higham 2009). Furthermore, fore-aft and lateral forces decrease to zero when the subdigital toe pads are being unfurled (Autumn *et al.* 2006). Hence, this phase of the stride likely limits the animals' ability to propel itself forward throughout a stride.

I test the hypothesis that both proximal and distal hindlimb elements work together to determine sprint speed in *R. afer* on level and uphill surfaces (Figs. 2.3 – 2.10). Previous accounts of gecko kinematics suggest that geckos detach their toe pads from the substrate before lifting the foot, and therefore propulsion through ankle extension would not occur (Russell 1975; Zaaf *et al.* 1999). These authors contend that more proximal musculature would be especially important in geckos because they evolved to climb. Yet, *R. afer* is secondarily terrestrial, highly cursorial, and exhibits morphology suited to high sprint speed (Collins *et al.* 2015; Russell & Johnson 2009; Higham *et al.* 2015). I predict that kinematic coordination in *R. afer* will correspond with its derived morphology and habitat use. In other words, I predict that locomotion in *R. afer* is achieved through ankle and knee extension more than femur retraction and femur rotation.

Methods

Individuals (n=33, 3.15 – 5.2 cm Snout-Vent Length, 2 – 3.5 grams mass) were collected during November 2012 and June 2013 in Dorob National Park and the Gobabeb Research and Training Centre. Individual geckos were temporally housed in cloth bags just after capture and before experiments began. The geckos were recorded from oblique dorsal and lateral views as it ran down a 1.5 m long x 0.15 m wide raceway at 0^{o} and 30^{o} incline composed of 60-grit sandpaper. The walls of the trackway facing the lateral view camera was clear Plexiglas and the back wall was covered with white copy paper to enhance the contrast of the lizard's outline relative to the background. All trials were conducted in a laboratory at the Namibia Ministry of Fisheries (http://www.mfmr.gov.na) office in Swakopmund, Namibia. I used two high-speed Phantom video cameras to obtain lateral and dorsal views (recording at 500 – 1000 fps) (Korff and McHenry 2010; Walker 1998). I calibrated the three-dimensional space using a custom calibration object composed of Legos placed into the field of view prior to experiments. I conducted three trials per individual per day over the period of seven days. Animals were handled humanely under UCR IACUC AUP# A20110038E.

Prior to the first running trail, the following were marked with non-toxic correcting fluid and a black fine-tip sharpie maker: three equally spaced markers were placed down the midline of the dorsum of the animal beginning at the center of pectoral girdle and ending at the pelvic girdle (center of hip); the articulation between the femur and the pelvic girdle (hip), the articulation between the femur and tibia (knee); and the articulation between the tibia and proximal tarsals (ankle) (Figure 2.2). Three-dimensional coordinates of these markers were digitized using DLTdv5 in Matlab 2012b (R2012b The Mathworks Inc, MA, USA; Hedrick 2008). Following digitization, angles, accelerations, and velocities, were calculated for the hip, knee, and ankle joints for the entirety of each stride (Clemente et al., 2013; Jayne and Irschick, 1999a; Spezzano and Jayne, 2004). I only analyzed trials in which an individual ran down the center of the raceway without stopping through field of view. Data were processed using a custom script written in Matlab (R2012b The Mathworks Inc, MA, USA). Videos were digitized and joint angles and angular velocities for the hind limb were calculated for 2-5 strides per individual on each treatment.

I focused My attention on the stance phase of each stride in order to better understand how propulsion is determined. The following kinematic variables were calculated using joint angle and angular velocities derived from the high-speed video: total ankle extension and ankle extension speed, total knee extension and knee extension speed, total femur rotation and femur rotation speed, and total femur retraction and femur retraction speed (Table 2.1).

Data analysis:

Values for each individual were averaged. Then, averages for individuals on each treatment were log10 transformed in Microsoft Excel to ameliorate any non-normal and skewed data. After transforming data, all traits were normal and coefficients of skew were below 1.0. Spearman's (ϱ) rank correlation coefficient and Pearson's correlation coefficient were used to initially describe general relationships between all measured kinematics as well as the relationship between all measured kinematics and speed (Table 2.2). I computed principal components analysis (PCA) in JMP® (Version 10 for Mac. SAS Institute Inc., Cary, NC, USA, 1989–2007) to distill each joint movement and the speed of each joint movement to a component representing each joint (Table 3). These components ameliorate the problem of variance inflation due to multicolinearity (Petraitis *et al.* 1996) and to increase statistical power. These components were used in further analysis (Fig 2.3).

Structural Equation Modeling, Path Analysis, and Locomotion

Path models, initially developed by Sewell Wright (1943), are graphical depictions of equations that represent a hypothesis. They are a framework for testing the direct and indirect effects of independent variables on one or multiple dependent variables. Path analyses often are tested using a series of multiple regressions as originally proposed by Sewell Wright (1934). Path analysis simultaneously estimates the *a-priori* relationships of all variables to evaluate the effect or multiple predictors on one or more dependent variables (Shipley 1999; Tabachnick and Fidell 2001). One advantage of path models over regression models is that variables can simultaneously be dependent variables and predictors (Shipley 1999). Therefore, path models allow evaluation over a cascade of a hypothesized construction.

I specifically focus on the muscle groups that would power locomotion during the stance phase of a stride. The following variables were used in my path analysis: Femur Retraction, Femur Rotation, Knee Extension, and Ankle Extension (Table 2.1)(Fig 2.3). I concentrated on these joints and segments during the stance phase of a stride based on previous studies of lizard locomotion (*e.g.* Synder 1952; Nelson & Jayne 2001; Zaaf *et al.* 1999; Reilly 1995; McElroy *et al.* 2006). If ankle and knee extension, considered distal elements (farther away from the center of mass), exert the most influence on the model, then *R. afer* coordinates locomotion similar to the cursorial *S. woodi*. If femur rotation and retraction, considered proximal, exert the most influence on the model, then *R. afer* move forward in a manner similar to other geckos. Whereas previous studies made significant strides to determine how some kinematics or muscle groups more strongly correlate with speed relative to others, my analysis improves upon them by articulating and measuring how muscle groups operate together to move animals forward. I used maximum likelihood to model path coefficients in Ωnyx (von Oertzen *et al* 2016).

The integrated, non-independent nature of kinematics prevents researchers from non-invasively isolating joint and limb segment movements to experimentally determine their relative contribution to locomotion. One merit of using path analysis is the ability to statistically quantify the relative contributions of muscle groups to forward locomotion by via model nesting. Model nesting fixes one or more free parameters, yielding a model that is restricted relative to another (Tabachnick and Fidell 2001, Shipley 1999). Nesting models in this manner statistically 'fixes' joint and limb segment movements, allowing researchers to interpret their effect on the model in different treatments. Significance of these statistical "fixes" and their associated pathways are determined by model fit indices (did the model improve, stay the same, or degrade). I proceeded to test relative contributions to sprint speed by setting each path, one at a time, to zero and then measuring model fit. For ease of interpretation in figures, path coefficients were z-transformed in Ωnyx, setting the variance of all independent variables to one, displaying relative weights among paths. However, all goodness-of-fit and significance testing occurred on non-transformed scores. Single-headed arrows imply that one variable is causal relative to another. Double-headed arrows indicate a predicted relationship, but the nature of that relationship is unknown.

The path models in this study represent my theoretical model of causal relationships among variables. In other words, I present my theory of how kinematics, the observed motions powered by muscle contractions, determine forward locomotion on a level (0º) and incline (30º) treatment in *R. afer*.

Results

Univariate summary, principal components, and model fit

Sprint speed decreased between level (0º) (1.5 m/s) and inclined (30º) (1.19 m/s) treatments (p=0.0012) but no other measured variables significantly changed between treatments. Complete Correlation and Variance-Covariance Matrices are available in supplementary file one (S1).

I computed principal components for each joint and limb segment and saved the first component to represent respective combination of extension, rotation, or retraction and speed at which these movements occurred (Table 2.3). The first principal component summarized 70 – 89% of the variation in each joint and limb segment. Subsequently, these components were used to represent each joint and segment and their motions.

I tested the hypothesis that my theory about how locomotion is powered in *R. afer* fits the data for a level and inclined surface. Goodness of fit indices are used to determine how well my theory of locomotion matched the observed variance-covariance matrix produced by my data (Tabachnick and Fidel 2006; Shipley 2003). On the level (0º) treatment, AICc was 452.725, x^2 was 0.093, the Log Likelihood score equaled -203.862, and RMSEA equaled 0.0 (Table 2.1). On the inclined (30º) treatment, AICc equaled 362.47, x^2 was 6.231, the Log Likelihood score equaled -158.674, and RMSEA equaled 0.257.

What determines sprint speed?

Modeling joint and segment movements as direct contributors to sprint speed allowed us to determine which joints and limb segments determine sprint speed and how these joints and segment work in concert (Fig 2.3). Distal elements, especially the ankle (standardized path coefficient 0.76) and to a lesser degree the knee (standardized path coefficient -0.27), are the most important factors in determining forward speed on a level substrate (Table 2.1). The story is slightly different running uphill - proximal elements increase in their relative contributions to speed, more evenly distributing how locomotion is powered. Despite this increase, distal elements still exert the most control (Tables 2.1 & 2.2).

Evaluating the effect of statistically controlling for a joint or segment movement requires that each path associated with a particular joint or segment is set equal to zero. Then, fit indices are evaluated to determine if, this joint or segment significantly affected the model and the magnitude of this effect. For example, when statistically controlling for femur rotation on the level treatment, AICc decreased to 444.485, x^2 increased to 6.854, Log Likelihood decreased to -207.243, and RMSEA increased from 0.01 to 0.108 (Table 2.1). While statistically controlling for femur rotation on the level treatment was non-statistically significant according to a Log Likelihood ratio test ($p=0.08$, Table 2.1), decreases in goodness of fit indices indicate was biological significance. Statistically controlling for femur rotation on the incline treatment was significantly significant according to a Log Likelihood ration test ($p=0.03$, Table 2.1), AICc decreased to 354.104, x^2 increased to 12.987, and Log Likelihood decreased to -162.052 (Table 2.1). The effects of all joint and segment movements on locomotion in both treatments are summarized in Table 1.

Using path analysis, I was able to ask whether distal or proximal elements determine sprint speed. I tested the hypothesis that distal, not proximal elements determine sprint speed by simultaneously controlling for ankle and knee as distal elements and then femur retraction and rotation as proximal elements. Statistically controlling for distal elements by setting all ankle and knee paths equal to zero yielded a significant decrease in fit on the level (Log Likelihood Ratio test < 0.0001) and incline (Log Likelihood Ratio test < 0.0001). Proximal elements significantly affected My model on an incline (Log Likelihood Ratio test = 0.04) but not the level treatment (Log Likelihood Ratio test = 0.1).

Discussion

Is locomotor coordination associated with habitat use and morphology?

Rhoptropus afer achieve sprint speed with an integrated but behaviorally flexible suite of muscle group contributions (Wainwright *et al*. 2008). Distal elements, especially ankle extensor muscles, exert the most control over locomotion in the level treatment. I suggest adaptive simplification of the adhesive toe pad is associated with a reorganization of locomotion in *R. afer* (Higham *et al.* 2015). This is logical given that *R. afer* sprint away from predators similar to non-Gekkonid cursorial lizards such as *S. woodi* (McElroy *et al.* 2012, McElroy and McBrayer 2010). Thus, while geckos may have evolved musculature and coordination to climb, the diminution of the importance of climbing may be associated with the re-evolution of cursorial locomotion control.

The organization of locomotion is flexible - *R. afer* recruit the ankle and knee extensor muscles but femur retraction and rotation increase in their relative contributions. For secondarily terrestrial geckos such as *R. afer*, moving uphill is especially interesting because deployment of the adhesive system is hypothetically triggered at inclines of about 10°, but it is not recruited on horizontal surfaces (Russell and Higham 2009). In contrast, a detailed evaluation of the video attained for this dissertation revealed that the adhesive toe pad of *R. afer* is recruited on flat surfaces approximately 57% of the time. It is thought that sprint speed becomes limited on inclined surfaces above 10^{o} because of the time taken to insert the attachment and detachment phases of adhesion into the step cycle. This response is reflexive and apparently controlled by feedback from the vestibular system (Russell and Higham 2009). *Rhoptropus afer* are not hyperextending the toe pad before foot lift-off. Instead, they are using the toe pad as non-Gekkonids use their claw during the final propulsive phase of the stance. This likely allows them to increase velocity, but more work is necessary to elucidate the role of the toe pad in this secondarily terrestrial, cursorial lizard.

Path analysis

Path analysis is a powerful method to analyze the direct and indirect contributions of multiple integrated traits to an outcome (Mitchell 1992; Wootton 1994; Sinervo and DeNardo 1996). Path models are relatively ubiquitous in ecological, evolutionary, and organismal biological research (*e.g.* Arnold 1972, Garland and Losos 1994, Lesku *et al.* 2006) yet they are rare in the field of biomechanics. Locomotion, a central theme in biomechanics, is an emergent property controlled and constructed by underlying and integrated suites of traits. Therefore, I used path analysis to assess how locomotion is controlled in *R. afer*.

I tested a causal model of locomotion using maximum likelihood SEM. Previous researchers typically analyze kinematics as independent traits including ANOVA with post-hoc comparisons and multiple regression to test the correlation between traits and speed on different treatments. Others improve upon this and use principal components or discriminant analysis to reduce / decompose multiple traits into representative components, which then are used in ANOVA – family analyses. Yet, locomotor kinematics work together and are inherently linked via muscles, tendons, and bones. Where previous researchers either violated the assumption of independence of data or relied on correlations in decomposed data (Tabachnick and Fidell 2001) to make causal claims about locomotion, SEM permitted me to holistically investigate the determinants of speed in *R. afer*.

In a meta-analysis, Petraitis *et al.* (1996) found "most published path analyses in ecology should be viewed with extreme caution" mainly due to problems of variance inflation caused by multicollinearity. Careful planning of path analysis can alleviate some problems of multicollinearity (Graham 2003, Petraitis *et al.* 1996). Theoretically sound, real models are developed *a-priori* with potentially collinear relationships expressed by double headed arrows. Therefore, the inherently collinear nature is not only accounted for, but also measured and modeled as part of the theory of the system itself. For example, I used path analysis precisely because the anatomy and mechanics of locomotion are inherently linked and codependent. This codependence was modeled as a part of the hypothesis itself rather covariates that detract from the realism and interpretation of the analysis, thereby representing "shared" contributions of variables. Furthermore, I used PCA to decompose the biggest offenders of multicolinearity in my data other than step length and step frequency. When using components to represent multiple, and collinear, kinematics in a simplified path model, the effects were congruent with the larger, more detailed model.

One way of examining locomotor performance is by characterizing differences in stride length and stride frequency. I were interested in finer-scale details of locomotion – especially how limb segments and the muscles and tendons that extend and flex joints evolved to power sprint speed in *R. afer*. Therefore, I did not include stride frequency and stride length in my analysis. Exploratory analysis revealed that the problem of multicollinearity would be exacerbated by including stride length and stride frequency. The kinematics that construct the sub-organismal emergent properties of step length and step frequency are redundant, therefore drastically increase variance inflation (supplementary table (S2) of Variance Inflation Factors generated by JMP®

(Version 10 for Mac. SAS Institute Inc., Cary, NC, USA, 1989–2007). Dropping these two emergent properties increased model fit and reduced multicollinearity. While previous researchers (e.g. Petraitis *et al*. 1996) argued against dropping certain variables to ameliorate the problem of multicollinearity, it is theoretically sound to do so here because step length and step frequency are redundant with their underlying predictors. The kinematic variables of interest actually create stride length and stride frequency.

Future directions

Future workers should incorporate more individuals, but also more information about the individuals in the sample population. If multiple trials per individual are included in a study, then power can be gained by measuring within individual variation (Tabachnick and Fidell 2001). This is the next step in making SEM accessible to biomechanists, who often employ low sample sizes but have repeated measures within treatments.

While I did not directly test for “integration” (Young *et al*. 2009; Kane and Higham 2016; Wainwright *et al*. 2008), My path models show evidence that kinematic integration varies depending on the context. This type of analysis could be accomplished using path analysis in a manner similar to how I tested relative contributions to locomotion. Linkages, or double-headed arrows, between kinematics are deleted or held constant at zero while all other parameters of interest are estimated.

In the future, I recommend measuring whether the highly repeatable individual variation within populations are due to repeated “paths” or “structures” of locomotion (Garland & Losos 1994). For example, studies involving “many to one” mapping in fish indicate different ways to do the same thing such as capturing prey (Wainwright *et al*. 2005). Second, many animals employ high-speed locomotion during predator evasion. Using SEM in a comparative context would reveal species-level modifications of structural linkages to successfully evade predators (Garland and Losos 1994; Higham et al. 2015).

Alternative path models

my path model represents the kinematics that propel the animal during the stance phase of locomotion while running straight on a level (0°) and inclined (30°) incline. What remains to be seen is how animals navigate uneven, unpredictable terrain, how they maneuver through turns, accelerate, and how they enter refuges (Clemente and Wilson 2015; Wilson *et al*. 2015, Wynn *et al*. 2015). A more holistic picture of locomotion must be quantified in order to understand the role of biomechanics in evolution and ecology. Using path analysis or other SEM analyses would clarify subtle but crucial biological information in inherently non-independent model systems. For example, increasing sprint speed in lizards was thought to be as simple as increasing stride length, stride frequency, or both (McElroy *et al*. 2012). However, the underlying factors responsible for these emergent properties may change under different conditions. Furthermore, increasing speed could result from small changes across multiple joints or from one large contribution from one joint. Using an SEM approach will illuminate which muscle groups are labile, co-dependent, or under evolutionary constraints. This is well understood in fish locomotion and feeding (Kane and Higham 2015; Wainwright 1994; Wainwright *et al*. 2005) but under-appreciated in terrestrial locomotion.

References

Aerts, P. 1990. Mathematcial biomechanics and the "what?", "how?" and "why?" in functional morphology. *Netherlands Journal of Zoology* **40**, 153-172.

Arnold, S.J., 1972. Species densities of predators and their prey. *American Naturalist*, pp.220-236.

Arnold, S.J., 1983. Morphology, performance and fitness. *American Zoologist*, 23(2), pp.347-361.

Ashley-Ross, M. 1994a. Hindlimb kinematics during terrestrial locomotion in a salamander (*Dicamptodon tenebrosus*). *Journal of Experimental Biology* **193,** (1), pp.255-283.

Ashley-Ross, M. 1994b. Metamorphic and speed effects on hindlimb kinematics during terrestrial locomotion in the salamander *Dicamptodon tenebrosus*. *Journal of Experimental Biology* **193,** 1, pp.285-305.

Autumn, K., Hsieh, S., Dudek, D., Chen, J., Chitaphan, C. and Full, R. 2006. Dynamics of geckos running vertically. *The Journal of Experimental Biology* **209**, 260-272.

Badyaev, A.V., Foresman, K.R. and Young, R.L. 2005. Evolution of morphological integration: developmental accommodation of stress-induced variation. *The American Naturalist*, *166* **3**, pp.382-395.

Bauer, A., Russell, A. and Powell, G. 1996. The evolution of locomotor morphology in *Rhoptropus* (Squamata: Gekkonidae): functional and phylogenetic considerations. *African Journal of Herpetology* **45**, 8-30.

Bauer, A. M. (1999). Evolutionary scenarios in the Pachydactylus group geckos of southern Africa: new hypotheses. *African Journal of Herpetology* **48**, 53-62.

Bauwens, D., Garland Jr, T., Castilla, A.M. and Van Damme, R., 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioral covariation. Evolution, pp. 848-863.

Biknevicius, A.R. and Reilly, S.M., 2006. Correlation of symmetrical gaits and whole body mechanics: debunking myths in locomotor biodynamics. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, **305**, 11 pp.923-934.

Biewener, A. A. 1990. Biomechanics of mammalian terrestrial locomotion. *Science*. **250**, 1097-1103.

Biewener, A. A. 1991. Musculoskeletal design in relation to body size. *Journal of Biomechanics*. **24**, 19-31.

Biewener, A. A. 2003. Animal Locomotion. New York: Oxford University Press.

Birn-Jeffery, A.V. and Higham, T.E., 2014. The scaling of uphill and downhill locomotion in legged animals. *Integrative and comparative biology*, p.icu015.

Blums, P., Clark, R.G. and Mednis, A., 2002. Patterns of reproductive effort and success in birds: path analyses of long-term data from European ducks. *Journal of Animal Ecology*, **71,** 2, pp.280-295.

Bonine, K.E. and Garland Jr, T. 1999. Sprint performance of phrynosomatid lizards, measured on a high-speed treadmill, correlates with hindlimb length. *Journal of Zoology*, 248(02), pp.255-265.

Clemente, C.J. and Wilson, R.S., 2015. Balancing Biomechanical Constraints: Optimal Escape Speeds When There Is a Trade-off between Speed and Maneuverability. *Integrative and comparative biology*, p.icv103.

Clemente, C. J., Withers, P. C., Thompson, G. G. and Lloyd, D. 2013. Lizard tricks: overcoming conflicting requirements of speed versus climbing ability by altering biomechanics of the lizard stride. *The Journal of Experimental Biology* **216**, 3854-3862.

Collins, C.E., Russell, A.P. and Higham, T.E., 2015. Subdigital adhesive pad morphology varies in relation to structural habitat use in the Namib Day Gecko. *Functional Ecology* **29**, 1, pp.66-77.

Daley, M.A. and Biewener, A.A., 2003. Muscle force-length dynamics during level versus incline locomotion: a comparison of in vivo performance of two guinea fowl ankle extensors. *Journal of Experimental Biology* **206,** 17, pp.2941-2958.

Darwin, C.M.A. 1859. On the Origin of Species by Means of Natural Selection: A Facsimile of the First Edition. Harvard Paperbacks. Cambridge, MA.

Dickinson, M.H., Farley, C.T., Full, R.J., Koehl, M.A.R., Kram, R. and Lehman, S. 2000. How animals move: an integrative view. *Science* **288**, 5463, pp.100-106.

Farley, C. T. and Emshwiller, M. 1996. Efficiency of uphill locomotion in nocturnal and diurnal lizards. *Journal of Experimental Biology* **199**, 587-592.

Fieler, C.L. and Jayne, B.C., 1998. Effects of speed on the hindlimb kinematics of the lizard Dipsosaurus dorsalis. *Journal of Experimental Biology* **201,** 4, pp.609-622.

FitzSimons, V. F. M. 1943. The lizards of South Africa. Pretoria, South Africa: Transvaal Museum.

Foster, K.L., Collins, C.E., Higham, T.E. and Garland Jr, T., 2015. 11 Determinants of lizard escape performance: decision, motivation, ability, and opportunity. *Escaping From Predators: An Integrative View of Escape Decisions*, p.287.

Gamble, T., Greenbaum, E., Jackman, T. R., Russell, A. P. and Bauer, A. M. 2012. Repeated origin and loss of adhesive toepads in geckos. *PloS one* **7**, e39429.

Garland, T. 1984. Physiological Correlates of Locomotory Performance in a Lizard - an Allometric Approach. *American Journal of Physiology* **247**, R806-R815.

Garland, T. J. and Losos, J. B. 1994. Ecological morphology of locomotor performance in squamate reptiles. In *Ecological Morphology: Integrative Organismal Biology*, eds. P. C. Wainwright and S. M. Reilly), pp. 240-302. Chicago: University of Chicago Press.

Garland, T., Bennett, A. F. and Daniels, C. B. 1990. Heritability of Locomotor Performance and its Correlates in a Natural Population *Experientia* **46**, 530-533.

Garland Jr, T., Hankins, E. and Huey, R.B. 1990. Locomotor capacity and social dominance in male lizards. *Functional Ecology*, pp.243-250.

Graham, M.H., 2003. Confronting multicollinearity in ecological multiple regression. *Ecology* **84,** 11, pp.2809-2815.

Hammond, K.A., Chappell, M.A., Cardullo, R.A., Lin, R.S. and Johnsen, T.S.,2000. The mechanistic basis of aerobic performance variation in red junglefowl. *Journal of Experimental Biology* **203**(13), pp.2053-2064.

Hedrick, T.L. 2008. Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration & Biomimetics*. **3**, 034001.

Higham, T.E., Birn-Jeffery, A.V., Collins, C.E., Hulsey, C.D. and Russell, A.P. 2015. Adaptive simplification and the evolution of gecko locomotion: Morphological and biomechanical consequences of losing adhesion. *Proceedings of the National Academy of Sciences* **112,** 3, pp.809-814.

Higham, T. E. and Russell, A. P. 2010. Divergence in locomotor performance, ecology, and morphology between two sympatric sister species of desert-dwelling gecko. *Biological Journal of the Linnean Society* **101**, 860-869

Holzman, R., Day, S.W. and Wainwright, P.C. 2007. Timing is everything: coordination of strike kinematics affects the force exerted by suction feeding fish on attached prey. *Journal of Experimental Biology* **210,** 19, pp.3328-3336.

Huey, R. B. and Hertz, P. E. 1984. Effects of body size and slope on acceleration of a lizard (*Stellio stellio*). *The Journal of Experimental Biology* **110**, 113-123.

Husak, J.F. 2006a. Does speed help you survive? A test with collared lizards of different ages. *Functional Ecology* **20,** 1, pp.174-179.

Husak, J.F., 2006b. Does survival depend on how fast you can run or how fast you do run? *Functional Ecology* **20**, 6, pp.1080-1086.

Husak, J.F., Fox, S.F., Lovern, M.B. and Bussche, R.A. 2006. Faster lizards sire more offspring: sexual selection on whole-animal performance. *Evolution* ***60*** 10, pp.2122-2130.

Impellizzeri, F.M., Rampinini, E., Castagna, C., Bishop, D., Bravo, D.F., Tibaudi, A. and Wisloff, U. 2008. Validity of a repeated-sprint test for football. *International journal of sports medicine*, 29(11), pp.899-905.

Irschick, D.J., 2003. Measuring performance in nature: implications for studies of fitness within populations. *Integrative and Comparative Biology* **43**, 3, pp.396-407.

Irschick, D. J., Austin, C. C., Petren, K., Fisher, R. N., Losos, J. B. and Ellers, O. 1996. A comparative analysis of clinging ability among pad-bearing lizards. *Biological Journal of the Linnean Society* **59**, 21-35.

Irschick, D.J. and Garland Jr, T. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annual Review of Ecology and Systematics*, pp.367-396.

Jayne, B. C. and Irschick, D. J. 1999. Effects of incline and speed on the three-dimensional hindlimb kinematics of a generalized iguanian lizard (*Dipsosaurus dorsalis*). *The Journal of Experimental Biology* **202**, 143-159.

Johnson, M. K. and Russell, A. P. 2009. Configuration of the setal fields of *Rhoptropus* (Gekkota: Gekkonidae): functional, evolutionary, ecological, and phylogenetic implications of observed pattern. *Journal of Anatomy* **214**, 937-955.

Kane, E.A. and Higham, T.E. 2015. Complex systems are more than the sum of their parts: using integration to understand performance, biomechanics, and diversity. *Integrative and comparative biology*, p.icv033.

Kohlsdorf, T., Garland Jr, T. and Navas, C.A. 2001. Limb and tail lengths in relation to substrate usage in *Tropidurus* lizards. *Journal of Morphology* **248** 2, pp.151-164.

Korff, W. L. and McHenry, M. J. 2010. Environmental differences in substrate mechanics do not affect sprinting performance in sand lizards (*Uma scoparia* and *Callisaurus draconoides*). *The Journal of Experimental Biology* **214**, 122-130.

Lesku, J.A., Roth II, T.C., Amlaner, C.J. and Lima, S.L. 2006. A phylogenetic analysis of sleep architecture in mammals: the integration of anatomy, physiology, and ecology. *The American Naturalist* **168,** 4, pp.441-453.

Losos, J.B., 2010. Adaptive radiation, ecological opportunity, and evolutionary determinism. *The American Naturalist* **175,** 6 623-639.

Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D. and Smouse, P.E. 2008. A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences* **105***,* 49, pp.19052-19059.

Mahendra, B. C. 1938. Contributions to the bionomics, anatomy, reproduction and development of the Indian house-gecko, *Hemidactylus flaviviridis* Rüppel. *Proceedings of the Indian Academy of Sciences-Section B* **4**, 288-306.

McElroy, E. J., Archambeau, K. L. and McBrayer, L. D. 2012. The correlation between locomotor performance and hindlimb kinematics during burst locomotion in the Florida scrub lizard, *Sceloporus woodi*. *The Journal of Experimental Biology* **215**, 442-453.

McElroy, E. J. and McBrayer, L. D. 2010. Getting Up to Speed: Acceleration Strategies in the Florida Scrub Lizard, *Sceloporus woodi*. *Physiological and Biochemical Zoology* **83**, 643-653.

McElroy, E. J. and Reilly, S. M. 2009. The relationship between limb morphology, kinematics, and force during running: the evolution of locomotor dynamics in lizards. *Biological Journal of the Linnean Society* **97**, 634-651

Miles, D.B., 2004. The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. *Evolutionary Ecology Research* **6** 1, pp.63-75.

Mitchell, R.J., 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modeling. *Functional Ecology* pp.123-129.

Nava, S.S., Lindsay, C.R., Henderson, R.W. and Powell, R. 2001. Microhabitat, activity, and density of a dwarf gecko (*Sphaerodactylus parvus*) on Anguilla, West Indies. *Amphibia-Reptilia*, **22** 4, pp.455-464.

Odenaal, F. J. 1979. Notes on the adaptive ecology and behavior of four species of *Rhoptropus* (Gekkonidae) from the Namib Desert with special reference to a thermoregulatory mechanism employed by *Rhoptropus afer*. *Madoqua* **11**, 255-260.

Olberding, J.P., Herrel, A., Higham, T.E. and Garland, T., 2015. Limb segment contributions to the evolution of hind limb length in phrynosomatid lizards. *Biological Journal of the Linnean Society*.

Petraitis, P.S., Dunham, A.E. and Niewiarowski, P.H., 1996. Inferring multiple causality: the limitations of path analysis. *Functional Ecology*, pp.421-431.

Preuschoft, H. 1990. Gravity in primates and its relatin to body shape and locomotion. *Human Evolution* **5**, 559-578.

Ricklefs, R.E. and Miles, D.B. 1994. Ecological and evolutionary inferences from morphology: an ecological perspective. *Ecological morphology: Integrative rganismal Biology*. University of Chicago Press, Chicago, 101, pp.13-41.

Reilly, S. and Delancey, M. 1997. Sprawling locomotion in the lizard *Sceloporus clarkii*: quantitative kinematics of a walking trot. *Journal of Experimental Biology* **200**, 4, pp.753-765.

Russell, A. P. 1975. A contribution to the functional analysis of the foot of the Tokay, *Gekko gecko* (Reptilia: Gekkonidae). *Journal of Zoology* **176**, 437-476.

Russell, A. P. (1979). Parallelism and Integrated Design in the Foot Structure of Gekkonine and Diplodactyline Geckos. *Copeia* **1979**, 1-21.

Russell, A.P., Baskerville, J., Gamble, T. and Higham, T.E. 2015. The evolution of digit form in Gonatodes (Gekkota: Sphaerodactylidae) and its bearing on the transition from frictional to adhesive contact in gekkotans. *Journal of Morphology* **276** 11, pp.1311-1332.

Russell, A. P. and Bels, V. 2001. Biomechanics and kinematics of limb-based locomotion in lizards: review, synthesis, and prospectus. *Comparative Biochemistry and Physiology- Part A* **131**, 89-112.

Russell, A. P. and Higham, T. E. 2009. A new angle on clinging in geckos: incline, not substrate, triggers the deployment of the adhesive system. *Proceedings of the Royal Society B: Biological Sciences* **276**, 3705-3709.

Shipley, B. 1999. Testing causal explanations in organismal biology: causation, correlation and structural equation modeling. *Oikos*, pp.374-382.

Sinervo, B. and DeNardo, D.F. 1996. Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. *Evolution*, pp.1299-1313.

Snyder, R. C. 1954. The anatomy and function of the pelvic girdle and hindlim in lizard locomotion. *The Ameican Journal of Anatomy* **95**, 1-45.

Spezzano, L. C. and Jayne, B. C. 2004. The effects of surface diameter and incline on the hindlimb kinematics of an arboreal lizard (*Anolis sagrei*). *The Journal of Experimental Biology* **207**, 2115-2131.

Tabachnick, Barbara G. and Linda S. Fidell. 2001. *Using Multivariate Statistics*.

Taylor, C. R., Rowntree, V. J. and Caldwell, S. L. (1972). Running up and down hills - some consequences of size. *Science* **178**, 1096-&.

von Oertzen, T., Brandmaier, A.M. and Tsang, S., 2015. Structural equation modeling with Ωnyx. *Structural Equation Modeling: A Multidisciplinary Journal* **22,** 1, pp.148-161.

Walker, J. A. 1998. Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerical differentiation algorithms. *The Journal of Experimental Biology* **201**, 981-995.

Wainwright, P. C. 1994. Functional morphology as a tool in ecological research. In *Ecological Morphology: Integrative Organismal Biology*., eds. P. C. Wainwright and S. M. Reilly), pp. 42-59. Chicago: University of Chicago Press.

Wainwright, P. C., Alfaro, M. E., Bolnick, D. I. and Hulsey, C. D. 2005. Many-to-one mapping of form to function: A general principle in organismal design? *Integrative and Comparative Biology* **45**, 256-262.

Wainwright, P. C., Mehta, R. S. and Higham, T. E. 2008. Stereotypy, flexibility and coordination: key concepts in behavioral functional morphology. *The Journal of Experimental Biology* **211**, 3523-3528.

Wilson, R.S., Husak, J.F., Halsey, L.G. and Clemente, C.J. 2015. Predicting the movement speeds of animals in natural environments. *Integrative and Comparative Biology* **55** 6, pp.1125-1141.

Wynn, M.L., Clemente, C., Nasir, A.F.A.A. and Wilson, R.S., 2015. Running faster causes disaster: trade-offs between speed, manoeuvrability and motor control when running around corners in northern quolls (*Dasyurus hallucatus*). *Journal of Experimental Biology* **218,** 3, pp.433-439.

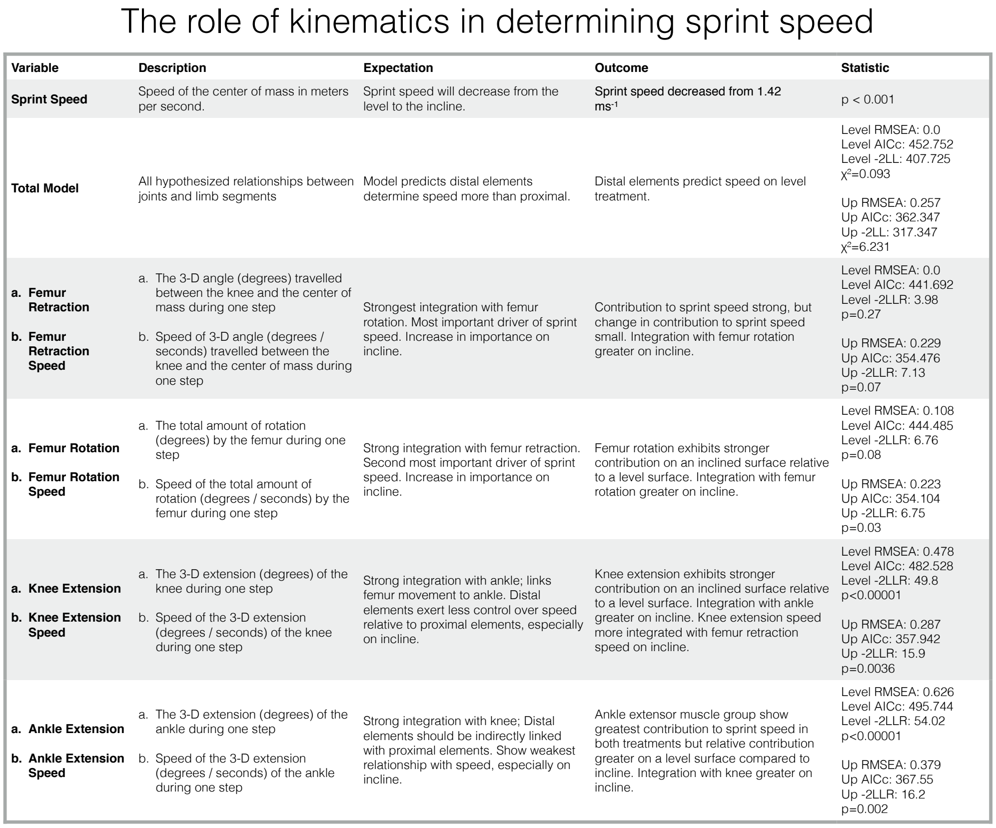
Wootton, J.T., 1994. Predicting direct and indirect effects: an integrated approach using experiments and path analysis. *Ecology 75,* 1, pp.151-165.

Wright, S., 1934. The method of path coefficients. *The Annals of Mathematical Statistics*. **5,** 3, pp.161-215.

Young, N.M., Hallgrímsson, B. and Garland Jr, T. 2009. Epigenetic effects on integration of limb lengths in a mouse model: selective breeding for high voluntary locomotor activity. *Evolutionary Biology* **36**, 1, pp.88-99.

Zaaf, A., Van Damme, R., Herrel, A. and Aerts, P. (2001). Spatio-temporal gait characteristics of level and vertical locomotion in a ground-dwelling and a climbing gecko. *The Journal of Experimental Biology* **204**, 1233-1246.

Table 1: Sprint speed and the hypothesized kinematic traits exerting control over forward speed in this study, their descriptions, expectations, and outcomes. In this table, the amount and speed of movement are grouped in rows for ease of explanation and interpretation. Low p-values (<0.05), derived from a Log-Likelihood Ratio Test, reject a more constrained model in favor of the more complete model that includes the joint movement in question. For example, constraining the ankle joint significantly degrades the model fit on level surfaces and incline surfaces. P-values should be considered along with model fit indices including RMSEA, which increase as model fit degrades, and AICc.



The role of kinematics in determining sprint speed

Variable	**Description**	**Expectation**	**Outcome**	**Statistic**
Sprint Speed	Speed of the center of mass in meters per second.	Sprint speed will decrease from the level to the incline.	Sprint speed decreased from 1.42 ms^{-1}	$p < 0.001$
Total Model	All hypothesized relationships between joints and limb segments	Model predicts distal elements determine speed more than proximal.	Distal elements predict speed on level treatment.	Level RMSEA: 0.0 Level AICc: 452.752 Level -2LL: 407.725 $\chi^2=0.093$ Up RMSEA: 0.257 Up AICc: 362.347 Up -2LL: 317.347 $\chi^2=6.231$
a. Femur Retraction **b. Femur Retraction Speed**	a. The 3-D angle (degrees) travelled between the knee and the center of mass during one step b. Speed of 3-D angle (degrees / seconds) travelled between the knee and the center of mass during one step	Strongest integration with femur rotation. Most important driver of sprint speed. Increase in importance on incline.	Contribution to sprint speed strong, but change in contribution to sprint speed small. Integration with femur rotation greater on incline.	Level RMSEA: 0.0 Level AICc: 441.692 Level -2LLR: 3.98 p=0.27 Up RMSEA: 0.229 Up AICc: 354.476 Up -2LLR: 7.13 p=0.07
a. Femur Rotation **b. Femur Rotation Speed**	a. The total amount of rotation (degrees) by the femur during one step b. Speed of the total amount of rotation (degrees / seconds) by the femur during one step	Strong integration with femur retraction. Second most important driver of sprint speed. Increase in importance on incline.	Femur rotation exhibits stronger contribution on an inclined surface relative to a level surface. Integration with femur rotation greater on incline.	Level RMSEA: 0.108 Level AICc: 444.485 Level -2LLR: 6.76 p=0.08 Up RMSEA: 0.223 Up AICc: 354.104 Up -2LLR: 6.75 p=0.03
a. Knee Extension **b. Knee Extension Speed**	a. The 3-D extension (degrees) of the knee during one step b. Speed of the 3-D extension (degrees / seconds) of the knee during one step	Strong integration with ankle; links femur movement to ankle. Distal elements exert less control over speed relative to proximal elements, especially on incline.	Knee extension exhibits stronger contribution on an inclined surface relative to a level surface. Integration with ankle greater on incline. Knee extension speed more integrated with femur retraction speed on incline.	Level RMSEA: 0.478 Level AICc: 482.528 Level -2LLR: 49.8 p<0.00001 Up RMSEA: 0.287 Up AICc: 357.942 Up -2LLR: 15.9 p=0.0036
a. Ankle Extension **b. Ankle Extension Speed**	a. The 3-D extension (degrees) of the ankle during one step b. Speed of the 3-D extension (degrees / seconds) of the ankle during one step	Strong integration with knee; Distal elements should be indirectly linked with proximal elements. Show weakest relationship with speed, especially on incline.	Ankle extensor muscle group show greatest contribution to sprint speed in both treatments but relative contribution greater on a level surface compared to incline. Integration with knee greater on incline.	Level RMSEA: 0.626 Level AICc: 495.744 Level -2LLR: 54.02 p<0.00001 Up RMSEA: 0.379 Up AICc: 367.55 Up -2LLR: 16.2 p=0.002

Table 2: Pairwise correlations among all variables measured in this study.

Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
StrideLength	Speed	0.4445	33	0.1194	0.6835	0.0096
StepFrequency	Speed	0.6648	33	0.4165	0.8208	0.0001
StepFrequency	StrideLength	-0.3523	33	-0.6206	-0.0103	0.0443
FemurRotationSpeed	Speed	0.2013	33	-0.1526	0.5094	0.2613
FemurRotationSpeed	StrideLength	0.1468	33	-0.2069	0.4666	0.4149
FemurRotationSpeed	StepFrequency	0.0885	33	-0.2628	0.4191	0.6243
KneeSpeed	Speed	0.6772	33	0.435	0.828	0.0001
KneeSpeed	StrideLength	0.1206	33	-0.2323	0.4455	0.5037
KneeSpeed	StepFrequency	0.6181	33	0.3488	0.7931	0.0001
KneeSpeed	FemurRotationSpeed	0.2532	33	-0.0987	0.5488	0.1551
FemurRetraction	Speed	-0.0861	33	-0.4171	0.265	0.6337
FemurRetraction	StrideLength	0.3218	33	-0.0242	0.599	0.0678
FemurRetraction	StepFrequency	-0.3131	33	-0.5927	0.0339	0.0761
FemurRetraction	FemurRotationSpeed	0.0116	33	-0.333	0.3535	0.9489
FemurRetraction	KneeSpeed	-0.272	33	-0.5628	0.0786	0.1256
FemurRetractionSpeed	Speed	0.406	33	0.0728	0.6576	0.0191
FemurRetractionSpeed	StrideLength	0.1032	33	-0.2489	0.4312	0.5676
FemurRetractionSpeed	StepFrequency	0.3632	33	0.0227	0.6282	0.0378
FemurRetractionSpeed	FemurRotationSpeed	0.0875	33	-0.2638	0.4182	0.6284
FemurRetractionSpeed	KneeSpeed	0.0637	33	-0.2858	0.3983	0.7246
FemurRetractionSpeed	FemurRetraction	0.3919	33	0.0561	0.648	0.0241
AnkleSpeed	Speed	0.6614	33	0.4115	0.8188	0.0001
AnkleSpeed	StrideLength	0.1963	33	-0.1577	0.5055	0.2737
AnkleSpeed	StepFrequency	0.5494	33	0.2541	0.7511	0.0009
AnkleSpeed	FemurRotationSpeed	0.1003	33	-0.2517	0.4288	0.5787
AnkleSpeed	KneeSpeed	0.653	33	0.3992	0.8139	0.0001
AnkleSpeed	FemurRetraction	0.0473	33	-0.3009	0.3843	0.7939
AnkleSpeed	FemurRetractionSpeed	0.0496	33	-0.2988	0.3863	0.7839
AnkleExtension	Speed	0.3719	33	0.0328	0.6342	0.0331
AnkleExtension	StrideLength	0.4504	33	0.1267	0.6874	0.0085
AnkleExtension	StepFrequency	0.0292	33	-0.3173	0.3688	0.8718
AnkleExtension	FemurRotationSpeed	0.0587	33	-0.2905	0.3941	0.7456
AnkleExtension	KneeSpeed	0.4006	33	0.0664	0.654	0.0209
AnkleExtension	FemurRetraction	0.2462	33	-0.106	0.5436	0.1672
AnkleExtension	FemurRetractionSpeed	-0.1532	33	-0.4717	0.2007	0.3948
AnkleExtension	AnkleSpeed	0.8485	33	0.7129	0.923	0.0001
KneeExtension	Speed	0.3185	33	-0.0278	0.5966	0.0708
KneeExtension	StrideLength	0.4439	33	0.1187	0.6831	0.0097
KneeExtension	StepFrequency	-0.0272	33	-0.3671	0.3191	0.8805
KneeExtension	FemurRotationSpeed	0.242	33	-0.1105	0.5404	0.1748
KneeExtension	KneeSpeed	0.7633	33	0.5692	0.8769	0.0001
KneeExtension	FemurRetraction	-0.029	33	-0.3687	0.3174	0.8726
KneeExtension	FemurRetractionSpeed	-0.2109	33	-0.5168	0.1427	0.2387
KneeExtension	AnkleSpeed	0.4042	33	0.0708	0.6565	0.0196
KneeExtension	AnkleExtension	0.5078	33	0.1992	0.7248	0.0026
FemurRotation	Speed	0.028	33	-0.3184	0.3678	0.877
FemurRotation	StrideLength	0.3886	33	0.0523	0.6458	0.0254
FemurRotation	StepFrequency	-0.2386	33	-0.5378	0.1141	0.1812
FemurRotation	FemurRotationSpeed	0.3944	33	0.0591	0.6497	0.0231
FemurRotation	KneeSpeed	0.0266	33	-0.3196	0.3666	0.8832
FemurRotation	FemurRetraction	0.3141	33	-0.0327	0.5934	0.075
FemurRotation	FemurRetractionSpeed	0.0644	33	-0.2852	0.3989	0.7217
FemurRotation	AnkleSpeed	-0.1359	33	-0.4578	0.2176	0.4509
FemurRotation	AnkleExtension	-0.027	33	-0.3669	0.3193	0.8816
FemurRotation	KneeExtension	0.2535	33	-0.0984	0.549	0.1546

Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|
StrideLength	Speed	0.374	0.032
StepFrequency	Speed	0.674	0.0001
StepFrequency	StrideLength	-0.3558	0.0421
FemurRotationSpeed	Speed	0.2797	0.1149
FemurRotationSpeed	StrideLength	0.234	0.19
FemurRotationSpeed	StepFrequency	0.1029	0.5686
KneeSpeed	Speed	0.6959	0.0001
KneeSpeed	StrideLength	0.0535	0.7676
KneeSpeed	StepFrequency	0.6688	0.0001
KneeSpeed	FemurRotationSpeed	0.2463	0.167
FemurRetraction	Speed	-0.1217	0.5
FemurRetraction	StrideLength	0.3168	0.0724
FemurRetraction	StepFrequency	-0.2489	0.1626
FemurRetraction	FemurRotationSpeed	0.107	0.5536
FemurRetraction	KneeSpeed	-0.1915	0.2857
FemurRetractionSpeed	Speed	0.2951	0.0954
FemurRetractionSpeed	StrideLength	0.0886	0.624
FemurRetractionSpeed	StepFrequency	0.3744	0.0318
FemurRetractionSpeed	FemurRotationSpeed	-0.0638	0.7241
FemurRetractionSpeed	KneeSpeed	0.1441	0.4238
FemurRetractionSpeed	FemurRetractoin	0.3379	0.0544
AnkleSpeed	Speed	0.7851	0.0001
AnkleSpeed	StrideLength	0.1598	0.3745
AnkleSpeed	StepFrequency	0.6495	0.0001
AnkleSpeed	FemurRotationSpeed	0.0836	0.6439
AnkleSpeed	KneeSpeed	0.6347	0.0001
AnkleSpeed	FemurRetraction	0.0608	0.7367
AnkleSpeed	FemurRetractionSpeed	0.237	0.1843
AnkleExtension	Speed	0.4763	0.0051
AnkleExtension	StrideLength	0.5281	0.0016
AnkleExtension	StepFrequency	0.0297	0.8695
AnkleExtension	FemurRotationSpeed	0.0505	0.7803
AnkleExtension	KneeSpeed	0.2864	0.1061
AnkleExtension	FemurRetraction	0.3189	0.0705
AnkleExtension	FemurRetractionSpeed	0.0391	0.8289
AnkleExtension	AnkleSpeed	0.741	0.0001
KneeExtension	Speed	0.2239	0.2103
KneeExtension	StrideLength	0.5053	0.0027
KneeExtension	StepFrequency	-0.1126	0.5325
KneeExtension	FemurRotationSpeed	0.2754	0.1208
KneeExtension	KneeSpeed	0.5709	0.0005
KneeExtension	FemurRetraction	0.1481	0.4109
KneeExtension	FemurRetractionSpeed	-0.1434	0.426
KneeExtension	AnkleSpeed	0.2012	0.2615
KneeExtension	AnkleExtension	0.3643	0.0371
FemurRotation	Speed	-0.0281	0.8768
FemurRotation	StrideLength	0.3623	0.0383
FemurRotation	StepFrequency	-0.2119	0.2364
FemurRotation	FemurRotationSpeed	0.618	0.0001
FemurRotation	KneeSpeed	-0.0595	0.7423
FemurRotation	FemurRetraction	0.2741	0.1227
FemurRotation	FemurRetractionSpeed	0.016	0.9294
FemurRotation	AnkleSpeed	-0.1504	0.4035
FemurRotation	AnkleExtension	-0.0244	0.8928
FemurRotation	KneeExtension	0.3018	0.0878

Table 2 (continued): Pairwise correlations among all variables measured in this study.

Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
StrideLength	Speed	0.1669	33	-0.1871	0.4826	0.3532
StepFrequency	Speed	0.5842	33	0.3014	0.7726	0.0004
StepFrequency	StrideLength	-0.696	33	-0.8388	-0.4634	0.0001
FemurRotationSpeed	Speed	0.2821	33	-0.0677	0.5702	0.1117
FemurRotationSpeed	StrideLength	-0.0485	33	-0.3854	0.2998	0.7888
FemurRotationSpeed	StepFrequency	0.2454	33	-0.1069	0.543	0.1686
KneeSpeed	Speed	0.1992	33	-0.1546	0.5078	0.2663
KneeSpeed	StrideLength	-0.4829	33	-0.7087	-0.1673	0.0044
KneeSpeed	StepFrequency	0.5391	33	0.2403	0.7446	0.0012
KneeSpeed	FemurRotationSpeed	0.1648	33	-0.1892	0.4809	0.3595
FemurRetraction	Speed	-0.1919	33	-0.5021	0.1621	0.2847
FemurRetraction	StrideLength	0.2276	33	-0.1256	0.5295	0.2028
FemurRetraction	StepFrequency	-0.3065	33	-0.5879	0.0411	0.0828
FemurRetraction	FemurRotationSpeed	-0.4887	33	-0.7124	-0.1746	0.0039
FemurRetraction	KneeSpeed	-0.3875	33	-0.645	-0.0509	0.0259
FemurRetractionSpeed	Speed	0.2126	33	-0.141	0.5181	0.235
FemurRetractionSpeed	StrideLength	0.0309	33	-0.3158	0.3702	0.8646
FemurRetractionSpeed	StepFrequency	0.1697	33	-0.1843	0.4848	0.345
FemurRetractionSpeed	FemurRotationSpeed	-0.0281	33	-0.3678	0.3183	0.8768
FemurRetractionSpeed	KneeSpeed	-0.1651	33	-0.4811	0.189	0.3586
FemurRetractionSpeed	FemurRetraction	0.2461	33	-0.1061	0.5435	0.1673
AnkleSpeed	Speed	0.5539	33	0.2601	0.7539	0.0008
AnkleSpeed	StrideLength	-0.4155	33	-0.6641	-0.0842	0.0162
AnkleSpeed	StepFrequency	0.7123	33	0.4885	0.8482	0.0001
AnkleSpeed	FemurRotationSpeed	0.2035	33	-0.1503	0.5111	0.256
AnkleSpeed	KneeSpeed	0.4519	33	0.1285	0.6884	0.0083
AnkleSpeed	FemurRetraction	-0.3357	33	-0.6088	0.0086	0.0562
AnkleSpeed	FemurRetractionSpeed	-0.2483	33	-0.5452	0.1038	0.1635
AnkleExtension	Speed	0.1933	33	-0.1607	0.5032	0.2812
AnkleExtension	StrideLength	0.2019	33	-0.1519	0.5099	0.2598
AnkleExtension	StepFrequency	-0.0664	33	-0.4006	0.2834	0.7135
AnkleExtension	FemurRotationSpeed	0.0341	33	-0.3129	0.373	0.8506
AnkleExtension	KneeSpeed	0.0879	33	-0.2633	0.4186	0.6265
AnkleExtension	FemurRetraction	-0.1205	33	-0.4454	0.2324	0.5041
AnkleExtension	FemurRetractionSpeed	-0.5018	33	-0.7209	-0.1915	0.0029
AnkleExtension	AnkleSpeed	0.6412	33	0.382	0.8069	0.0001
KneeExtension	Speed	-0.1467	33	-0.4665	0.2071	0.4154
KneeExtension	StrideLength	-0.0875	33	-0.4183	0.2637	0.6281
KneeExtension	StepFrequency	-0.0467	33	-0.3839	0.3014	0.7964
KneeExtension	FemurRotationSpeed	0.061	33	-0.2884	0.396	0.736
KneeExtension	KneeSpeed	0.8054	33	0.6388	0.8999	0.0001
KneeExtension	FemurRetraction	-0.2642	33	-0.557	0.087	0.1373
KneeExtension	FemurRetractionSpeed	-0.3273	33	-0.6028	0.0181	0.063
KneeExtension	AnkleSpeed	0.0403	33	-0.3073	0.3784	0.8237
KneeExtension	AnkleExtension	0.1341	33	-0.2193	0.4564	0.457
FemurRotation	Speed	0.2994	33	-0.049	0.5828	0.0906
FemurRotation	StrideLength	0.166	33	-0.188	0.4819	0.3557
FemurRotation	StepFrequency	0.1067	33	-0.2456	0.4341	0.5546
FemurRotation	FemurRotationSpeed	0.3376	33	-0.0064	0.6102	0.0547
FemurRotation	KneeSpeed	0.0687	33	-0.2813	0.4025	0.7042
FemurRotation	FemurRetraction	0.1883	33	-0.1657	0.4993	0.294
FemurRotation	FemurRetractionSpeed	-0.1475	33	-0.4672	0.2062	0.4127
FemurRotation	AnkleSpeed	0.0613	33	-0.288	0.3963	0.7345
FemurRotation	AnkleExtension	0.0096	33	-0.3348	0.3518	0.9575
FemurRotation	KneeExtension	0.022	33	-0.3237	0.3626	0.9031

Spearman's ρ

Variable	by Variable	Spearman ρ	Prob>|ρ|
StrideLength	Speed	0.0946	0.6006
StepFrequency	Speed	0.5766	0.0004
StepFrequency	StrideLength	-0.7129	0.0001
FemurRotationSpeed	Speed	0.3372	0.055
FemurRotationSpeed	StrideLength	0.0244	0.8928
FemurRotationSpeed	StepFrequency	0.2639	0.1378
KneeSpeed	Speed	0.2517	0.1577
KneeSpeed	StrideLength	-0.4823	0.0045
KneeSpeed	StepFrequency	0.5544	0.0008
KneeSpeed	FemurRotationSpeed	0.1524	0.3972
FemurRetraction	Speed	-0.1678	0.3507
FemurRetraction	StrideLength	0.2042	0.2543
FemurRetraction	StepFrequency	-0.297	0.0932
FemurRetraction	FemurRotationSpeed	-0.383	0.0278
FemurRetraction	KneeSpeed	-0.3854	0.0268
FemurRetractionSpeed	Speed	0.264	0.1376
FemurRetractionSpeed	StrideLength	0.0124	0.9455
FemurRetractionSpeed	StepFrequency	0.1531	0.395
FemurRetractionSpeed	FemurRotationSpeed	-0.0946	0.6006
FemurRetractionSpeed	KneeSpeed	-0.0963	0.5941
FemurRetractionSpeed	FemurRetraction	0.2423	0.1743
AnkleSpeed	Speed	0.5558	0.0008
AnkleSpeed	StrideLength	-0.4288	0.0128
AnkleSpeed	StepFrequency	0.6879	0.0001
AnkleSpeed	FemurRotationSpeed	0.2376	0.183
AnkleSpeed	KneeSpeed	0.502	0.0029
AnkleSpeed	FemurRetraction	-0.3486	0.0468
AnkleSpeed	FemurRetractionSpeed	-0.2644	0.1371
AnkleExtension	Speed	0.2246	0.2089
AnkleExtension	StrideLength	0.1594	0.3755
AnkleExtension	StepFrequency	-0.0466	0.7966
AnkleExtension	FemurRotationSpeed	0.0408	0.8217
AnkleExtension	KneeSpeed	0.0919	0.611
AnkleExtension	FemurRetraction	-0.0414	0.8189
AnkleExtension	FemurRetractionSpeed	-0.4706	0.0057
AnkleExtension	AnkleSpeed	0.6477	0.0001
KneeExtension	Speed	-0.1086	0.5474
KneeExtension	StrideLength	-0.0912	0.6136
KneeExtension	StepFrequency	-0.0212	0.9067
KneeExtension	FemurRotationSpeed	0.0411	0.8203
KneeExtension	KneeSpeed	0.763	0.0001
KneeExtension	FemurRetraction	-0.257	0.1488
KneeExtension	FemurRetractionSpeed	-0.2767	0.119
KneeExtension	AnkleSpeed	0.1297	0.472
KneeExtension	AnkleExtension	0.1347	0.4549
FemurRotation	Speed	0.2433	0.1724
FemurRotation	StrideLength	0.2303	0.1973
FemurRotation	StepFrequency	0.0809	0.6545
FemurRotation	FemurRotationSpeed	0.3516	0.0448
FemurRotation	KneeSpeed	-0.002	0.9912
FemurRotation	FemurRetraction	0.266	0.1345
FemurRotation	FemurRetractionSpeed	-0.13	0.4708
FemurRotation	AnkleSpeed	0.017	0.925
FemurRotation	AnkleExtension	0.0598	0.7409
FemurRotation	KneeExtension	0.0037	0.9838

Table 3.3: Each joint and segment movement and movement speed are represented by the first principal component when each total movement and speed were transformed using Principal Components Analysis.

```
Femur Rotation
Eigenvalues
Number              Eigenvalue  Percent    Cum Percent
                  1      1.359   67.948         67.948
                  2      0.641   32.052            100
Eigenvectors
                    Prin1       Prin2
FemurRotationSpeed     0.70711   -0.70711
FemurRotation          0.70711    0.70711


Femur Retraction
Eigenvalues
Number              Eigenvalue  Percent    Cum Percent
                  1     1.3241   66.203         66.203
                  2     0.6759   33.797            100
Eigenvectors
                    Prin1       Prin2
FemurRetraction        0.70711   -0.70711
FemurRetractionSpeed   0.70711    0.70711

Knee Extension
Eigenvalues
Number              Eigenvalue  Percent    Cum Percent
                  1      1.786   89.301         89.301
                  2      0.214   10.699            100
Eigenvectors
                    Prin1       Prin2
KneeSpeed              0.70711    0.70711
KneeExtension          0.70711   -0.70711

Ankle Extension
Eigenvalues
Number              Eigenvalue  Percent    Cum Percent
                  1     1.7935   89.675         89.675
                  2     0.2065   10.325            100
Eigenvectors
                    Prin1       Prin2
AnkleSpeed             0.70711    0.70711
AnkleExtension         0.70711   -0.70711
```

Figure 2.1: *Rhoptropus afer* near Swakopmund, Namibia. Photo credit: Tim Higham.

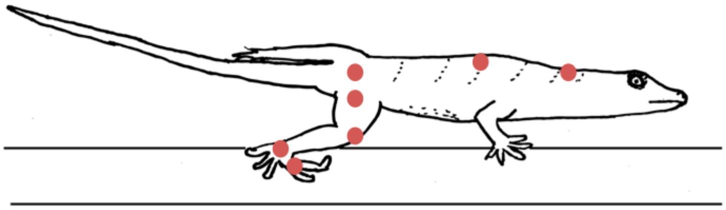


Figure 2.2: Red dots in this image represent points on each individual used to calculate 3-D angles, rotations, and retractions during each trial.

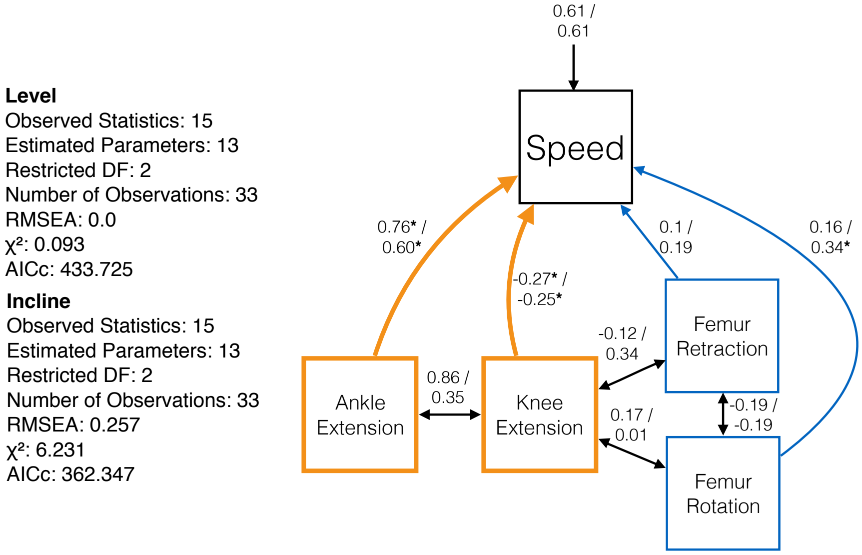
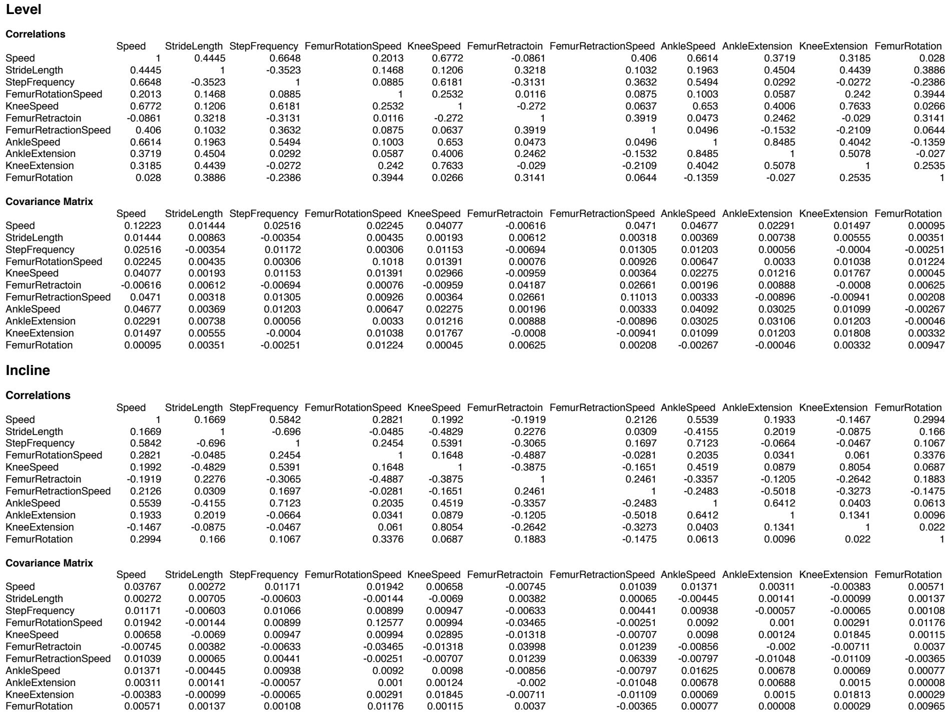


Figure 2.3: Numbers represent relative relationships among traits. Single-headed arrows imply that one variable is causal relative to another. Double-headed arrows (black) indicate a predicted relationship, but the nature of that relationship is unknown. Asterisks indicate a significant predictor of sprint speed according a Log Likelihood ratio test ($p<0.05$). The numbers on the top half of each path represent contributions on a level surface while the numbers on the bottom half of each path represent contributions on an incline surface. I tested the hypothesis that sprint speed is principally modulated via proximal elements (femur retraction and femur rotation, highlighted in blue) rather than distal elements (knee and ankle extension, highlighted in orange). I compared models when proximal elements were constrained to zero vs. when distal elements were constrained to zero. By evaluating RMSEA, AICc, and calculating significance from Log Likelihood ratios, I determined if data fit the model significantly better or was significantly degraded when each joint was constrained.

Supplementary information 1: Correlation and Variance-Covariance matrices for the level and incline treatments.



Level

Correlations

	Speed	StrideLength	StepFrequency	FemurRotationSpeed	KneeSpeed	FemurRetractoin	FemurRetractionSpeed	AnkleSpeed	AnkleExtension	KneeExtension	FemurRotation
Speed	1	0.4445	0.6648	0.2013	0.6772	-0.0861	0.406	0.6614	0.3719	0.3185	0.028
StrideLength	0.4445	1	-0.3523	0.1468	0.1206	0.3218	0.1032	0.1963	0.4504	0.4439	0.3886
StepFrequency	0.6648	-0.3523	1	0.0885	0.6181	-0.3131	0.3632	0.5494	0.0292	-0.0272	-0.2386
FemurRotationSpeed	0.2013	0.1468	0.0885	1	0.2532	0.0116	0.0875	0.1003	0.0587	0.242	0.3944
KneeSpeed	0.6772	0.1206	0.6181	0.2532	1	-0.272	0.0637	0.653	0.4006	0.7633	0.0266
FemurRetractoin	-0.0861	0.3218	-0.3131	0.0116	-0.272	1	0.3919	0.0473	0.2462	-0.029	0.3141
FemurRetractionSpeed	0.406	0.1032	0.3632	0.0875	0.0637	0.3919	1	0.0496	-0.1532	-0.2109	0.0644
AnkleSpeed	0.6614	0.1963	0.5494	0.1003	0.653	0.0473	0.0496	1	0.8485	0.4042	-0.1359
AnkleExtension	0.3719	0.4504	0.0292	0.0587	0.4006	0.2462	-0.1532	0.8485	1	0.5078	-0.027
KneeExtension	0.3185	0.4439	-0.0272	0.242	0.7633	-0.029	-0.2109	0.4042	0.5078	1	0.2535
FemurRotation	0.028	0.3886	-0.2386	0.3944	0.0266	0.3141	0.0644	-0.1359	-0.027	0.2535	1

Covariance Matrix

	Speed	StrideLength	StepFrequency	FemurRotationSpeed	KneeSpeed	FemurRetractoin	FemurRetractionSpeed	AnkleSpeed	AnkleExtension	KneeExtension	FemurRotation
Speed	0.12223	0.01444	0.02516	0.02245	0.04077	-0.00616	0.0471	0.04677	0.02291	0.01497	0.00095
StrideLength	0.01444	0.00863	-0.00354	0.00435	0.00193	0.00612	0.00318	0.00369	0.00738	0.00555	0.00351
StepFrequency	0.02516	-0.00354	0.01172	0.00306	0.01153	-0.00694	0.01305	0.01203	0.00056	-0.0004	-0.00251
FemurRotationSpeed	0.02245	0.00435	0.00306	0.1018	0.01391	0.00076	0.00926	0.00647	0.0033	0.01038	0.01224
KneeSpeed	0.04077	0.00193	0.01153	0.01391	0.02966	-0.00959	0.00364	0.02275	0.01216	0.01767	0.00045
FemurRetractoin	-0.00616	0.00612	-0.00694	0.00076	-0.00959	0.04187	0.02661	0.00196	0.00888	-0.0008	0.00625
FemurRetractionSpeed	0.0471	0.00318	0.01305	0.00926	0.00364	0.02661	0.11013	0.00333	-0.00896	-0.00941	0.00208
AnkleSpeed	0.04677	0.00369	0.01203	0.00647	0.02275	0.00196	0.00333	0.04092	0.03025	0.01099	-0.00267
AnkleExtension	0.02291	0.00738	0.00056	0.0033	0.01216	0.00888	-0.00896	0.03025	0.03106	0.01203	-0.00046
KneeExtension	0.01497	0.00555	-0.0004	0.01038	0.01767	-0.0008	-0.00941	0.01099	0.01203	0.01808	0.00332
FemurRotation	0.00095	0.00351	-0.00251	0.01224	0.00045	0.00625	0.00208	-0.00267	-0.00046	0.00332	0.00947

Incline

Correlations

	Speed	StrideLength	StepFrequency	FemurRotationSpeed	KneeSpeed	FemurRetractoin	FemurRetractionSpeed	AnkleSpeed	AnkleExtension	KneeExtension	FemurRotation
Speed	1	0.1669	0.5842	0.2821	0.1992	-0.1919	0.2126	0.5539	0.1933	-0.1467	0.2994
StrideLength	0.1669	1	-0.696	-0.0485	-0.4829	0.2276	0.0309	-0.4155	0.2019	-0.0875	0.166
StepFrequency	0.5842	-0.696	1	0.2454	0.5391	-0.3065	0.1697	0.7123	-0.0664	-0.0467	0.1067
FemurRotationSpeed	0.2821	-0.0485	0.2454	1	0.1648	-0.4887	-0.0281	0.2035	0.0341	0.061	0.3376
KneeSpeed	0.1992	-0.4829	0.5391	0.1648	1	-0.3875	-0.1651	0.4519	0.0879	0.8054	0.0687
FemurRetractoin	-0.1919	0.2276	-0.3065	-0.4887	-0.3875	1	0.2461	-0.3357	-0.1205	-0.2642	0.1883
FemurRetractionSpeed	0.2126	0.0309	0.1697	-0.0281	-0.1651	0.2461	1	-0.2483	-0.5018	-0.3273	-0.1475
AnkleSpeed	0.5539	-0.4155	0.7123	0.2035	0.4519	-0.3357	-0.2483	1	0.6412	0.0403	0.0613
AnkleExtension	0.1933	0.2019	-0.0664	0.0341	0.0879	-0.1205	-0.5018	0.6412	1	0.1341	0.0096
KneeExtension	-0.1467	-0.0875	-0.0467	0.061	0.8054	-0.2642	-0.3273	0.0403	0.1341	1	0.022
FemurRotation	0.2994	0.166	0.1067	0.3376	0.0687	0.1883	-0.1475	0.0613	0.0096	0.022	1

Covariance Matrix

	Speed	StrideLength	StepFrequency	FemurRotationSpeed	KneeSpeed	FemurRetractoin	FemurRetractionSpeed	AnkleSpeed	AnkleExtension	KneeExtension	FemurRotation
Speed	0.03767	0.00272	0.01171	0.01942	0.00658	-0.00745	0.01039	0.01371	0.00311	-0.00383	0.00571
StrideLength	0.00272	0.00705	-0.00603	-0.00144	-0.0069	0.00382	0.00065	-0.00445	0.00141	-0.00099	0.00137
StepFrequency	0.01171	-0.00603	0.01066	0.00899	0.00947	-0.00633	0.00441	0.00938	-0.00057	-0.00065	0.00108
FemurRotationSpeed	0.01942	-0.00144	0.00899	0.12577	0.00994	-0.03465	-0.00251	0.0092	0.001	0.00291	0.01176
KneeSpeed	0.00658	-0.0069	0.00947	0.00994	0.02895	-0.01318	-0.00707	0.0098	0.00124	0.01845	0.00115
FemurRetractoin	-0.00745	0.00382	-0.00633	-0.03465	-0.01318	0.03998	0.01239	-0.00856	-0.002	-0.00711	0.0037
FemurRetractionSpeed	0.01039	0.00065	0.00441	-0.00251	-0.00707	0.01239	0.06339	-0.00797	-0.01048	-0.01109	-0.00365
AnkleSpeed	0.01371	-0.00445	0.00938	0.0092	0.0098	-0.00856	-0.00797	0.01625	0.00678	0.00069	0.00077
AnkleExtension	0.00311	0.00141	-0.00057	0.001	0.00124	-0.002	-0.01048	0.00678	0.00688	0.0015	0.00008
KneeExtension	-0.00383	-0.00099	-0.00065	0.00291	0.01845	-0.00711	-0.01109	0.00069	0.0015	0.01813	0.00029
FemurRotation	0.00571	0.00137	0.00108	0.01176	0.00115	0.0037	-0.00365	0.00077	0.00008	0.00029	0.00965

Supplementary information 2: Variance inflation factors for all kinematics (raw scores) and step length and step frequency, which were not included in subsequent analyses.

Variance Inflation Factors

Level

	Speed	StrideLength	StepFrequency	FemurRotationSpeed	KneeSpeed	FemurRetractoin	FemurRetractionSpeed	AnkleSpeed	AnkleExtension	KneeExtension	FemurRotation
Speed	37.6287	-30.8275	-41.8019	-1.1171	-3.0974	0.5099	0.881	16.0054	-12.4142	2.6129	2.4375
StrideLength	-30.8275	28.0362	42.2585	1.0753	0.8188	0.4421	-2.4566	-22.9844	16.9127	-1.488	-2.6669
StepFrequency	-41.8019	42.2585	248.2936	3.9752	-141.358	-4.1187	-6.8696	-217.608	176.2804	106.1001	-3.8012
FemurRotationSpeed	-1.1171	1.0753	3.9752	1.3647	-2.7581	0.0265	-0.1929	-2.4653	1.9011	1.923	-0.6702
KneeSpeed	-3.0974	0.8188	-141.358	-2.7581	209.2653	15.7941	-7.7122	35.2718	-31.21	-162.442	2.2359
FemurRetractoin	0.5099	0.4421	-4.1187	0.0265	15.7941	4.105	-2.8494	-7.4497	4.4122	-12.1105	-0.5281
FemurRetractionSpeed	0.881	-2.4566	-6.8696	-0.1929	-7.7122	-2.8494	3.9302	15.4071	-11.0249	6.587	0.3404
AnkleSpeed	16.0054	-22.9844	-217.608	-2.4653	35.2718	-7.4497	15.4071	321.0321	-260.445	-21.6379	0.0342
AnkleExtension	-12.4142	16.9127	176.2804	1.9011	-31.21	4.4122	-11.0249	-260.445	213.9022	18.8604	0.8331
KneeExtension	2.6129	-1.488	106.1001	1.923	-162.442	-12.1105	6.587	-21.6379	18.8604	127.9943	-2.1216
FemurRotation	2.4375	-2.6669	-3.8012	-0.6702	2.2359	-0.5281	0.3404	0.0342	0.8331	-2.1216	1.9751

Incline

	Speed	StrideLength	StepFrequency	FemurRotationSpeed	KneeSpeed	FemurRetractoin	FemurRetractionSpeed	AnkleSpeed	AnkleExtension	KneeExtension	FemurRotation
Speed	104.4754	-118.767	-143.137	-0.2033	43.212	-1.0364	2.4474	-35.7098	19.4528	-37.2848	4.1983
StrideLength	-118.767	138.555	160.8315	0.6384	-45.7659	2.0756	-4.1659	44.4147	-25.9741	40.054	-6.046
StepFrequency	-143.137	160.8315	279.6528	-1.6079	-109.164	0.389	-5.7186	-22.7558	23.1358	90.3793	-7.3885
FemurRotationSpeed	-0.2033	0.6384	-1.6079	2.0317	2.602	1.396	-0.6376	1.0682	-1.0543	-1.9858	-1.1067
KneeSpeed	43.212	-45.7659	-109.164	2.602	84.7317	1.2244	-1.1555	3.4935	-7.1361	-70.4245	0.6193
FemurRetractoin	-1.0364	2.0756	0.389	1.396	1.2244	2.5136	-1.0742	2.2521	-1.9469	-0.5104	-1.3711
FemurRetractionSpeed	2.4474	-4.1659	-5.7186	-0.6376	-1.1555	-1.0742	2.6568	1.8267	-0.0271	1.1833	1.32
AnkleSpeed	-35.7098	44.4147	-22.7558	1.0682	3.4935	2.2521	1.8267	96.226	-64.526	0.6803	-0.308
AnkleExtension	19.4528	-25.9741	23.1358	-1.0543	-7.1361	-1.9469	-0.0271	-64.526	45.3438	3.4557	0.6747
KneeExtension	-37.2848	40.054	90.3793	-1.9858	-70.4245	-0.5104	1.1833	0.6803	3.4557	59.8811	-0.75
FemurRotation	4.1983	-6.046	-7.3885	-1.1067	0.6193	-1.3711	1.32	-0.308	0.6747	-0.75	2.3483

Chapter 3: How does the subdigital adhesive toe pad alter the control of locomotion and enhances sprint performance in the Namib Day Gecko, *Rhoptropus afer?*

<u>Summary</u>:

Novel morphological structures theoretically alter the trajectory of evolution by expanding the niche space available to an organism and increasing ecologically relevant performances in this new space. This chapter tests the hypothesis that adhesion, a morphological novelty, alters the ecology and mechanics of sprint speed in *R. afer*. I found that individuals vary in the use of the morphologically novel toe pad, and that these individuals diverge in their mechanics of locomotion. Second, in individuals that do not always use the toe pad, the deployment or hyperextension of the toe pad rearranges locomotor coordination.

<u>Introduction:</u>

Novel morphological structures and biomechanics

Novel morphological structures may profoundly influence functional diversification by promoting the exploitation of new niches (Darwin 1859; Dornburg et al., 2011; Hunter, 1998). These evolutionarily novel structures increase performance of an ecologically relevant task in a descendent compared to its ancestor (Dornburg et al., 2011; Vermeij, 2006). Famous examples include cleidoic eggs in amniotes (Packard and Packard, 1980), the evolution of limbs in tetrapods (Shubin et al., 2006), and the suspended jaw of fish (Wilga et al., 2001). These novel structures, often called key innovations, are an important concept in macroevolution because they are thought to promote adaptive radiation.

The converse to morphological novelty is adaptive simplification (Fong *et al.* 1995; Lande 1978). Simplification such as digit reduction in artiodactyls (Clifford 2010), bipedal rodents (Moore *et al*. 2016), and limb loss in snakes (Gans 1975; Wiens and Slingluff 2001) may be more rampant and as important as novel structures (Fong *et al*. 1995). While morphological novelty is often associated with a selective advantage (Schluter, 2000), less is known about the causes and consequences of their secondary reduction or loss. Character simplification occurs in conjunction with the relaxation of selection on the trait in question or when the trait is selected against (Fong *et al*. 1995). Yet, simplified states of novelties may themselves have advantages in new environments (Gans 1975; Skinner *et al*. 2008). Quantifying the functional basis for adaptive simplification could clarify the mechanistic basis for the reduction of morphological novelties. For example, a recent study by Higham *et al*. (2016) indicates that independent reductions & losses of adhesion in a clade of geckos, Pachydactylus, leads to elevated rates of morphological and biomechanical evolution due to relaxed morphological constraints.

Gecko adhesion and locomotion

Gecko adhesion evolved independently at least eleven times and has been lost nine times (Gamble et al., 2012). Toe pads are considered a novel morphological structure, permitting the exploitation of inclined and inverted surfaces and leading to increased rates of diversification (Gamble et al., 2012; Russell, 1979). Adhesion occurs through a finely tuned, hierarchically arranged locomotor system culminating in adhesive toe pads (Autumn and Peattie, 2002; Russell, 1975). Toe pads are characterized by subdigital scansors that carry highly organized (Johnson and Russell, 2009) fields of microfibrillar setae (10 – 100+ μm in length). Each setae bare branched tips terminating in spatulae (0.2 – 0.4 μm wide) that create reversible bonds with the substratum. Adhesion is achieved by a combination of van der Waals forces and frictional loading (Autumn and Peattie, 2002; Autumn et al., 2002; Johnson and Russell, 2009; Tian et al., 2006).

During locomotion, the setae are engaged with the substratum through the unfurling of hyperextended digits subsequent to the beginning of stance at the heel strike (Russell, 1975;

Russell, 2002; Zaaf et al., 2001). The digits tips that contain the adhesive toe pad are rolled down from proximal to distal. Through a complex arrangement of connective tissue and digital muscles, the setae are pulled towards the center of the mass (COM) and the setae are thus engaged (Russell, 1975; Russell, 2002). The adhesive force of the setae is then transmitted through the dermis to the plantar aponeurosis, and then to the COM via lateral digital tendons (Russell, 1975; Russell, 2002). The adhesive bond is released through digital hyperextension - the digits are hyperextended from distal to proximal just before the swing phase of limb cycling (Russell, 1975; Russell, 2002; Russell and Higham, 2009; Zaaf et al., 2001) (Fig. 3). The digital muscles of geckos power hyperextension, which connect to the distal bony elements of the digits through elongate tendons (Russell, 1975; Russell, 2002).

Engaging and disengaging setae is rapid (~ 20 ms), but this process occupies approximately 12.7% of stance time (Autumn et al., 2006). While adhesion was once thought to directly relate to microscopic topographical variation (Irschick et al., 1996; Mahendra, 1938), it is incline, not substrate texture, that triggers adhesion (Russell and Higham, 2009). Gecko adhesion is triggered, with some variability, at approximately 10° incline, and the percentage of strides where adhesion is used increases to approximately 100% by 30° (Russell and Higham, 2009). Employment of the adhesive system incurs a lower limit on the stance phase of locomotion, meaning that when adhesion is used some COM velocity may be lost. Furthermore, fore-aft and lateral forces decrease to zero when the subdigital toe pads are being unfurled (Autumn et al., 2006). Hence, this phase of the stride likely limits the animals' ability to propel itself forward throughout a stride.

Animals move as a system of levers and pulleys

The limbs of terrestrial animals act as a series of levers and fulcrums - muscles produce force that is transmitted via tendons to bones (*i.e.* the lever), which rotate around a joint (*i.e.* the fulcrum). These rotations sum together and are applied to the ground to move the animal (Biewener, 1990; Biewener, 1991; Biewener, 2003; Borelli and Maquet, 1989; Smith and Savage, 1956). How fast or how forceful an animal moves is largely determined by the gear ratio of each joint. The distance from a joint to the application of force, in this case the ground, is known as the out lever (Fig. 1). Short out levers amplify the force produced by a muscle contraction and are favored where more force is vital for locomotion, such as digging or climbing. Conversely, long out levers reduce force but amplify muscle contraction velocity and are favored for faster speeds (Biewener, 2003; Borelli and Maquet, 1989; Smith and Savage, 1956; Snyder 1954). When geckos lift the most distal portion of their digits from the substrate before full power is reached, they effectively shorten the out lever (Autumn et al., 2006; Russell and Bels, 2001; Russell and Higham, 2009; Snyder, 1954), thereby reducing their potential for speed (Figs. 2 & 3). Because some secondarily terrestrial geckos sprint on relatively level surfaces evade predators, it is logical that natural selection would favor a mechanism to overcome the potential velocity disadvantage associated with a shortened out lever.

Model system

Rhoptropus afer (Peters 1869) escapes on surfaces varying in substrate heterogeneity and incline severity (Collins *et al.* 2015). This species sprints up to tens of meters away from simulated predators (Collins *et al.* 2015; Odendaal 1979). Relative to other geckos, *R. afer* accommodates a cursorial lifestyle via a derived morphological pattern including longer limbs, extended distal elements, and reduced toe pads (Higham & Russell 2010; FitzSimons 1943). Therefore, *R. afer* is an ideal system to investigate the functional and mechanistic ramifications of a secondarily simplified novel morphological structure. During careful video analysis for Chapter 2, I observed that *R. afer* hyperextends its toe pad on a level surface in approximately 43% of strides but rolls over its toe pad in others. In this chapter, I test two hypotheses: 1) Coordination of sprint speed is altered when the toe pad is used and 2) Using the adhesive toe pad during level locomotion enhances sprint speed.

Methods

Data collection:

Individuals (n=33) were recorded from oblique dorsal and lateral views as they ran down a 1.5 m long x 0.15 m wide trackway at 0° and 30° incline, surfaced with 60-grit sandpaper. The walls of the trackway facing the lateral view camera were clear Plexiglas, and the back wall was covered with white copy paper to enhance the contrast of the lizard's outline relative to the background. I used two high-speed Phantom video cameras to obtain lateral and dorsal views (at 500 – 1000 fps) (Korff and McHenry, 2010; Walker, 1998). All running trials were conducted in a laboratory at the Namibia Ministry of Fisheries (http://www.mfmr.gov.na) in Swakopmund, Namibia.

Prior to the first running trial, the following joints were marked with non-toxic correcting fluid and a black fine-tip sharpie maker: three equally spaced markers were placed down the midline of the dorsum of the animal beginning at the pectoral girdle (center of shoulders) and ending at the pelvic girdle (center of hip); the articulation between the femur and the pelvic girdle (hip), the articulation between the femur and tibia (knee); the articulation between the tibia and proximal tarsals (ankle). Three-dimensional coordinates of these markers were digitized using DLTdv5 in Matlab 2012b (R2012b The Mathworks Inc, MA, USA; Hedrick 2008). Following digitization, angles, accelerations, and velocities were calculated for the hip, knee, and ankle joints for the entirety of each stride, with 2-5 strides per individual on each treatment. (Clemente et al., 2013; Jayne and Irschick, 1999a; Spezzano and Jayne, 2004). Data were processed using a custom script written in Matlab (R2012b The Mathworks Inc, MA, USA). Use of the adhesive toe pad was determined by carefully examining the tip of the longest digit (digit 3) in each video during stance. If they toe pad was hyperextended throughout the entire stance, then I coded the trial as “non-adhering”. If the toe pad was pressed to the trackway and remained on the trackway throughout the stance, then the trial was coded as “adherer”.

Data analysis:

Data were log10 transformed to ameliorate the non-normal and skewed nature of the data. After transforming data, all traits were normal and coefficients of skew were below 1.0. Common Factor Analysis (diagonal = 1) using “Principal Components” (PCA) with Oblimin rotation (weight 1) was used to distill the average kinematic values (Table 1A & B) in JMP® 12.1.0. I used maximum likelihood estimation (ML) to model these factors as path coefficients in subsequent analyses in Onyx (Shipley 1999; Tabachnick and Fidell 2001; von Oertzen *et al* 2016). Using PCA to decompose the data conferred power to test multiple, nested path models (Shipley 1999; Tabachnick and Fidell 2001). Within the level treatment, I subsequently used PCA (diagonal = 1) using “Principal Components” with Oblimin rotation (weight 1) to decompose the average kinematic values of only those individuals that varied in their use of adhesion (n=18) in JMP® 12.1.0. I used Kruskall-Wallis pair-wise comparisons in JMP® 12.1.0 to test the hypothesis that toe pads enhance sprint speed on the level treatment.

Results

For all 33 individuals in this dataset, three factors explained 58.5% of kinematic variation. These three factors describe a distal – proximal gradient in joint kinematics (Table 1A & B). Factor 1 describes 21% of the data, loading strongly on Ankle Extension Speed and Total Ankle Extension. Factor 2, describing 20% of the data, loads strongly on Knee Extension Speed and Total Knee Extension. Factor 3, describing 18% of the data, loads strongly on Stride Length, Stride Frequency, Total Femur Retraction, and Femur Rotation. Thus, the factor loadings indicate modularity in the control of locomotion.

Ninety-three total strides were analyzed in this study. Twenty-seven out of 47 strides on a level surfaces employed adhesion on the level treatment. On the incline treatment, 34 out of 46 strides employed adhesion (Table 1C). Thirty-three individuals were used in this study. Of the sample population, 15 individuals always applied the adhesive toe pad to the surface of the trackway on the level and incline treatment (Table 1D). These individuals are called "adherers" and "chronic users" through the rest of this paper. Interestingly, 18 individuals varied in their use of the adhesive toe pad. These are referred to as "variants".

Adherers and variants coordinated locomotion differently (Fig. 4A-C; Table 2). Adherers relied more on ankle extensor muscles to propel itself forward on the level treatment (Fig. 1B) while variants relied more on knee extensor muscles (Fig. 4C; Table 2).

For the 18 variants, four factors explained 99% of the kinematic variation. These four factors describe a similar distal – proximal gradient (Table 3A & B). Factor 1 describes 29% of the data, loading strongly on Ankle Extension Speed and Total Ankle Extension. Factor 2, describing 26% of the variation, loads strongly on the knee joint. Factor 3, describing 21% of the variation, loads strongly on femur retraction. Finally, factor 4 describes 23% of the data and loads strongly on femur rotation (Table 3A & B). Within this sub-sample, adhesion was used in 19 of the 36 strides analyzed (Table 3C). I focused on the 15 of 18 variants that varied in their use of adhesion on the level treatment.

Within variant sub-sample, relative contributions to sprint speed are significantly different on the level treatment when these individuals employ their adhesive toe pad (Table 4; Fig. 5A & B). When the adhesive toe pad was employed, ankle and knee extension were the principal drivers of locomotion (Table 4; Figure 5A). When the adhesive toe pad was hyperextended on the level treatment, femur retraction and femur rotation have relatively stronger roles (Table 4; Figure 5B). Locomotor speed on the level treatment was faster in the 15 individuals tested when adhesion was used ($p<0.05$, Figure 6).

Discussion

Two important findings are presented in this study. First, adaptive simplification is accompanied by altered locomotor coordination in *R. afer* (Higham *et al.* 2016). This species uses habitat structures quite differently from its climbing congeners (Higham and Russell 2009; Johnson and Russell 2009). *Rhoptropus afer* escape predators by sprinting over variable but often flat terrain compared to many geckos. Altering coordination my release *R. afer* from biomechanical constraints allowing this species to be successful in a secondarily terrestrial lifestyle (Bauer *et al.* 1996; Collins *et al.* 2015; Higham and Russell 2009: Johnson and Russell 2009). Second, I found evidence that *R. afer* use their adhesive toe pad to increase their speed on a level surface (Figure 6). Selection against active hyperextension during the stance phase would increase the out lever, thereby amplifying sprint speed. This is an important finding because it is the first to suggest that the gecko subdigital adhesive to pad is used for a task other than climbing.

Intraspecific variation in the size, use, and coordination of a secondarily simplified novel trait

Rhoptropus afer vary across their range in habitat use during predator evasion, adhesive pad morphology, and the use and coordination of the adhesive toe pad (Collins *et al.* 2015). Biologists representing disparate fields have long recognized the role of individual variation in intraspecific competition, predator-prey interactions, adaptive syndromes, and speciation (Bolnick *et al.* 2003; Careau and Garland 2012; Darwin 1859; Irschick and Garland 2001; Cespedes and Lailvaux 2015; Schall and Pianka 1980; Sih *et al.* 2004). Natural selection acts directly on sprint speed, endurance, thermal performance curves, and behavioral syndromes (Arnold and Bennett 1988; Darwin 1859; Garland *et al.* 1990; Miles 2004; Sih *et al.* 2004). However, this problem is under-appreciated in terrestrial locomotor biomechanics. Given the striking individual and temporal variability in adhesive toe pad mechanics found in this study, more work is needed to construct a comprehensive framework for how natural selection shapes biomechanics (Higham *et al.* 2016; Wilson *et al.* 2015). For example, do individuals within a species vary in their approaches to predator evasion? Schall and Pianka (1960) suggested that individuals adopt dissimilar escape trajectories in order to be unpredictable to predators. It is possible that *R. afer* adopt dissimilar adhesion control strategies to vary their use of habitat structures. Whereas some geckos are restricted to specific habitat features (Bauer *et al.* 1996; Russell and Delaugerre 2016), *R. afer* has a multitude of substrata available (Collins *et al.* 2015). Furthermore, species may vary in the mechanisms by which they achieve high performance. Many-to-one mapping should be applied to studies of individuals within populations to determine if and how the underlying mechanisms of locomotion are evolutionarily constrained or labile (Wainwright *et al.* 2005).

References

Arnold, S.J. and Bennett, A.F., 1988. Behavioural variation in natural populations. V. Morphological correlates of locomotion in the garter snake (*Thamnophis radix*). *Biological Journal of the Linnean Society*, *34*(2), pp.175-190.

Autumn, K., Liang, Y. A., Hsieh, S. T., Zesch, W., Chan, W. P., Kenny, T. W., Fearing, R. and Full, R. J. (2000). Adhesive force of a single gecko foot-hair. *Nature* **405**, 681-685.

Autumn, K. and Peattie, A. M. (2002). Mechanisms of Adhesion in Geckos. *Integrative and Comparative Biology* 42, 1081-1090.

Bauer, A., Russell, A. and Powell, G. (1996). The evolution of locomotor morphology in Rhoptropus (Squamata: Gekkonidae): functional and phylogenetic considerations. *African Journal of Herpetology* **45**, 8-30.

Biewener, A. A. (1990). Biomechanics of mammalian terrestrial locomotion. *Science*. **250**, 1097-1103.

Biewener, A. A. (1991). Musculoskeletal design in relation to body size. *Journal of Biomechanics*. **24**, 19-31.

Biewener, A. A. (2003). Animal Locomotion. New York: Oxford University Press.

Bejder L, Hall BK (2002) Limbs in whales and limblessness in other vertebrates: Mechanisms of evolutionary and developmental transformation and loss. Evol Dev 4(6):445–458.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. and Forister, M.L., 2003. The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist*, *161*(1), pp.1-28.

Borelli, G. A. and Maquet, P. (1989). On the movement of animals: Springer-Verlag.

Careau, V. and Garland Jr, T., 2012. Performance, personality, and energetics: correlation, causation, and mechanism. Physiological and Biochemical Zoology, 85(6), pp.543-571.

Cespedes, A.M. and Lailvaux, S.P., 2015. An individual-based simulation approach to the evolution of locomotor performance. *Integrative and comparative biology*, *55*(6), pp.1176-1187.

Clemente, C. J., Withers, P. C., Thompson, G. G. and Lloyd, D. (2013). Lizard tricks: overcoming conflicting requirements of speed versus climbing ability by altering biomechanics of the lizard stride. *The Journal of Experimental Biology* **216**, 3854-3862.

Clifford, A.B., 2010. The evolution of the unguligrade manus in artiodactyls. *Journal of Vertebrate Paleontology*, *30*(6), pp.1827-1839.

Collins, C.E., Russell, A.P. and Higham, T.E., 2015. Subdigital adhesive pad morphology varies in relation to structural habitat use in the Namib Day Gecko. Functional Ecology, 29(1), pp.66-77.

Darwin, C.M.A. (1859). On the Origin of Species by Means of Natural Selection: A Facsimile of the First Edition. Harvard Paperbacks. Cambridge, MA.

Dornburg, A., Sidlauskas, B., Santini, F., Sorenson, L., Near, T. J. and Alfaro, M. E. (2011). The influence of an innovative locomotor strategy on the phenotypic diversification of triggerfish (Family: Balistidae). *Evolution* **65**, 1912-1926.

Fong DW, Kane TC, Culver DC. 1995. Vestigialization and loss of nonfunctional characters. Annu Rev Ecol Syst 26:249–268.

Gamble, T., Greenbaum, E., Jackman, T. R., Russell, A. P. and Bauer, A. M. (2012). Repeated origin and loss of adhesive toepads in geckos. *PloS one* **7**, e39429.

Gans C (1975) Tetrapod limblessness: Evolution and functional corollaries. Am Zool 15(2):455–467.

Garland, T., Bennett, A. F. and Daniels, C. B. (1990). Heritability of Locomotor Performance and its Correlates in a Natural Population *Experientia* **46**, 530-533.

Higham, T.E., Birn-Jeffery, A.V., Collins, C.E., Hulsey, C.D. and Russell, A.P., 2015. Adaptive simplification and the evolution of gecko locomotion: Morphological and biomechanical consequences of losing adhesion. *Proceedings of the National Academy of Sciences*, *112*(3), pp.809-814.

Higham, T.E., Rogers, S.M., Langerhans, R.B., Jamniczky, H.A., Lauder, G.V., Stewart, W.J., Martin, C.H. and Reznick, D.N., 2016, September. Speciation through the lens of biomechanics: locomotion, prey capture and reproductive isolation. In *Proc. R. Soc. B* (Vol. 283, No. 1838, p. 20161294). The Royal Society.

Higham, T. E. and Russell, A. P. (2010). Divergence in locomotor performance, ecology, and morphology between two sympatric sister species of desert-dwelling gecko. *Biological journal of the Linnean Society* **101**, 860-869.

Hildebrand, M. (1985). The adaptive significance of tetrapod gait selection. *American Zoologist* **20**, 255-267.

Hunter, J. P. (1998). Key innovations and the ecology of macroevolution. *Trends in ecology & evolution* **13**, 31-36.

Irschick, D. J., Austin, C. C., Petren, K., Fisher, R. N., Losos, J. B. and Ellers, O. (1996). A comparative analysis of clinging ability among pad-bearing lizards. *Biological journal of the Linnean Society* **59**, 21-35.

Irschick, D. J. and Garland, T. (2001). Integrating function and ecology in studies of adaptation: Investigations of locomotor capacity as a model system. Annual Review of Ecology and Systematics 32, 367-+.

Jayne, B. C. and Irschick, D. J. (1999b). Effects of incline and speed on the three-dimensional hindlimb kinematics of a generalized iguanian lizard (*Dipsosaurus dorsalis*). *The Journal of Experimental Biology* **202**, 143-159.

Johnson, M. K. and Russell, A. P. (2009). Configuration of the setal fields of *Rhoptropus* (Gekkota: Gekkonidae): functional, evolutionary, ecological, and phylogenetic implications of observed pattern. *Journal of Anatomy* **214**, 937-955.

Lande R (1978) Evolutionary mechanisms of limb loss in tetrapods. Evolution 32(1): 73–92.

Mahendra, B. C. (1938). Contributions to the bionomics, anatomy, reproduction and development of the Indian house-gecko, *Hemidactylus flaviviridis* Rüppel. *Proceedings of the Indian Academy of Sciences-Section B* **4**, 288-306.

Miles, D. B. (2004). The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. *Evolutionary Ecology Research* **6**, 63-75

Moore, T.Y., Organ, C.L., Edwards, S.V., Biewener, A.A., Tabin, C.J., Jenkins, F.A. and Cooper, K.L., 2015. Multiple phylogenetically distinct events shaped the evolution of limb skeletal morphologies associated with bipedalism in the jerboas. *Current Biology*, *25*(21), pp.2785-2794.

Russell, A. P. (1975). A contribution to the functional analysis of the foot of the Tokay, Gekko gecko (Reptilia: Gekkonidae). *Journal of Zoology* **176**, 437-476.

Russell, A. P. (1979). Parallelism and Integrated Design in the Foot Structure of Gekkonine and Diplodactyline Geckos. *Copeia* **1979**, 1-21.

Russell, A. P. (2002). Integrative functional morphology of the gekkotan adhesive system (Reptilia: Gekkota). *Integrative and Comparative Biology* **42**, 1154-1163.

Russell, A. P. and Bels, V. (2001). Biomechanics and kinematics of limb-based locomotion in lizards: review, synthesis, and prospectus. *Comparative Biochemistry and Physiology- Part A* **131**, 89-112.

Russell, A.P. and Delaugerre, M.J., 2016. Left in the dust: differential effectiveness of the two alternative adhesive pad configurations in geckos (Reptilia: Gekkota). *Journal of Zoology*.

Schall, J.S. and Pianka, E.R., 1980. Evolution of escape behavior diversity. *American Naturalist*, pp.551-566.

Schluter, D. (2000). The ecology of adaptive radiation: Oxford University Press.

Shipley, B. 1999. Testing causal explanations in organismal biology: causation, correlation and structural equation modelling. *Oikos*, pp.374-382.

Sih, A., Bell, A. and Johnson, J.C., 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in ecology & evolution*, *19*(7), pp.372-378.

Skinner A, Lee MSY, Hutchinson MN (2008) Rapid and repeated limb loss in a clade of scincid lizards. BMC Evol Biol 8:310.

Smith, J. M. and Savage, R. J. (1956). Some locomotory adaptations in mammals. *Journal of the Linnean Society of London, Zoology* **42**, 603-622.

Snyder, R. C. (1954). The anatomy and function of the pelvic girdle and hindlim in lizard locomotion. *The Ameican Journal of Anatomy* **95**, 1-45.

Spezzano, L. C. and Jayne, B. C. (2004). The effects of surface diameter and incline on the hindlimb kinematics of an arboreal lizard (*Anolis sagrei*). *The Journal of Experimental Biology* **207**, 2115-2131

Tabachnick, Barbara G. and Linda S. Fidell "Using multivariate statistics." (2001).

Tian, Y., Pesika, N., Zeng, H., Rosenberg, K., Zhao, B., McGuiggan, P., Autumn, K. and Israelachvili, J. (2006). Adhesion and friction in gecko toe attachment and detachment. *Proceedings of the National Academy of Sciences* **103**, 19320-19325.

Vermeij, G. J. (2006). Historical contingency and the purported uniqueness of evolutionary innovations. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 1804-1809.

Wainwright, P. C., Alfaro, M. E., Bolnick, D. I. and Hulsey, C. D. (2005). Many-to-one mapping of form to function: A general principle in organismal design? *Integrative and Comparative Biology* **45**, 256-262.

Wake DB. 1991. Homoplasy: the result of natural selection, or evidence of design limitations. Am. Nat. 138:543-67

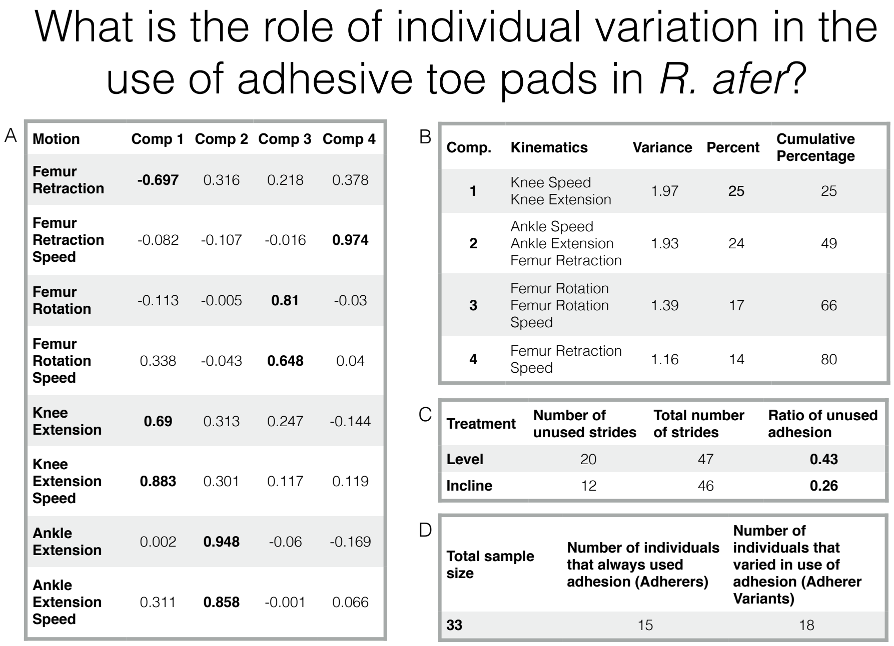
Wiens JJ, Slingluff JL (2001) How lizards turn into snakes: A phylogenetic analysis of body-form evolution in anguid lizards. Evolution 55(11):2303–2318.

Wilga, C. D., Hueter, R. E., Wainwright, P. C. and Motta, P. J. (2001). Evolution of upper jaw protrusion mechanisms in elasmobranchs. *American Zoologist* **41**, 1248-1257.

Wilson, R.S., Husak, J.F., Halsey, L.G. and Clemente, C.J., 2015. Predicting the movement speeds of animals in natural environments. *Integrative and comparative biology*, *55*(6), pp.1125-1141.

Zaaf, A., Van Damme, R., Herrel, A. and Aerts, P. (2001). Spatio-temporal gait characteristics of level and vertical locomotion in a ground-dwelling and a climbing gecko. *The Journal of Experimental Biology* **204**, 1233-1246.

Table 3.1: **A**: Three components were extracted using principal components analysis to summarize and reduce the number of variables. The cross-loading of factors was minimized using oblimin rotation. The number of factors retained was determined by examining a scree plot using the broken stick method. Bold font indicates loadings equal to or greater than 0.4 **B**: The first component, explaining 21% of the variance, described movement around the ankle. Component 2, explaining 20% of the variance, described movement around the knee. Component 3, explaining 18% of the variance, described movement around the hip. **C**. The variability of toe pad use facilitated comparisons of locomotion with and without adhesion. **D**. The number of individuals that always used adhesion was similar to the number of individuals that varied in the use of adhesion, allowing for comparisons among two "user" groups.



What is the role of individual variation in the use of adhesive toe pads in *R. afer*?

A

Motion	**Comp 1**	**Comp 2**	**Comp 3**	**Comp 4**
Femur Retraction	**-0.697**	0.316	0.218	0.378
Femur Retraction Speed	-0.082	-0.107	-0.016	**0.974**
Femur Rotation	-0.113	-0.005	**0.81**	-0.03
Femur Rotation Speed	0.338	-0.043	**0.648**	0.04
Knee Extension	**0.69**	0.313	0.247	-0.144
Knee Extension Speed	**0.883**	0.301	0.117	0.119
Ankle Extension	0.002	**0.948**	-0.06	-0.169
Ankle Extension Speed	0.311	**0.858**	-0.001	0.066

B

Comp.	**Kinematics**	**Variance**	**Percent**	**Cumulative Percentage**
1	Knee Speed Knee Extension	1.97	**25**	25
2	Ankle Speed Ankle Extension Femur Retraction	1.93	24	49
3	Femur Rotation Femur Rotation Speed	1.39	17	66
4	Femur Retraction Speed	1.16	14	80

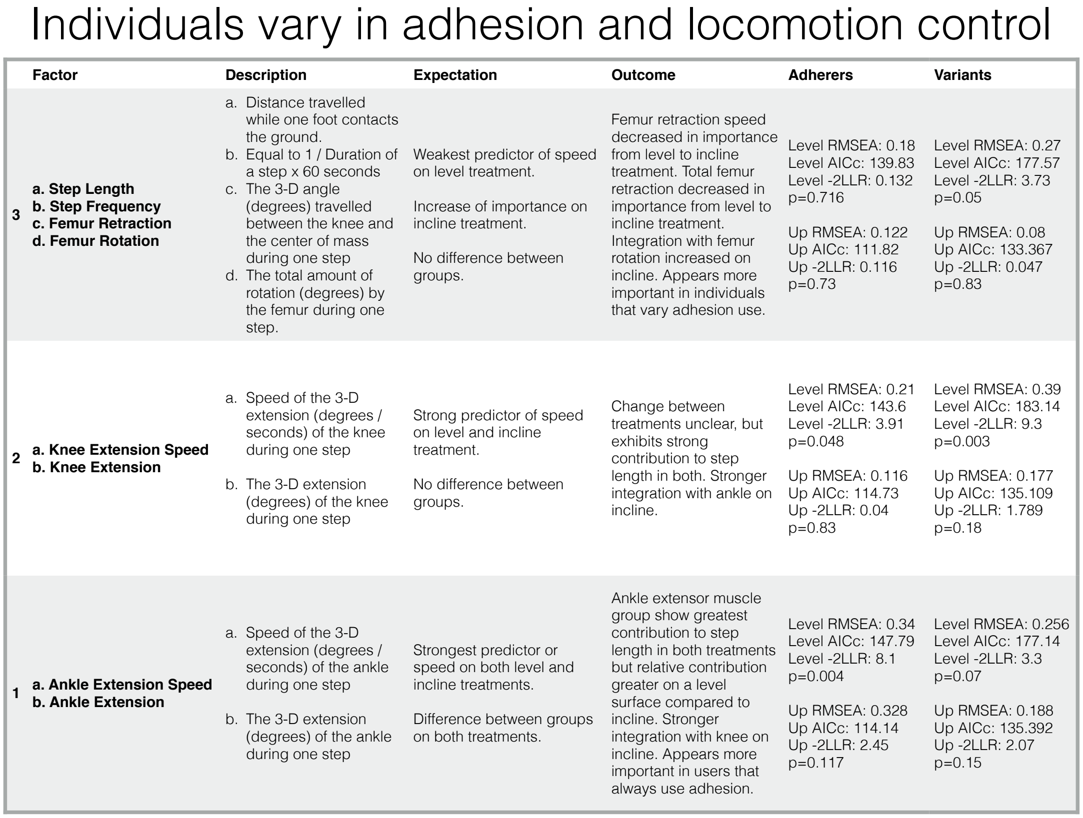
C

Treatment	**Number of unused strides**	**Total number of strides**	**Ratio of unused adhesion**
Level	20	47	**0.43**
Incline	12	46	**0.26**

D

Total sample size	**Number of individuals that always used adhesion (Adherers)**	**Number of individuals that varied in use of adhesion (Adherer Variants)**
33	15	18

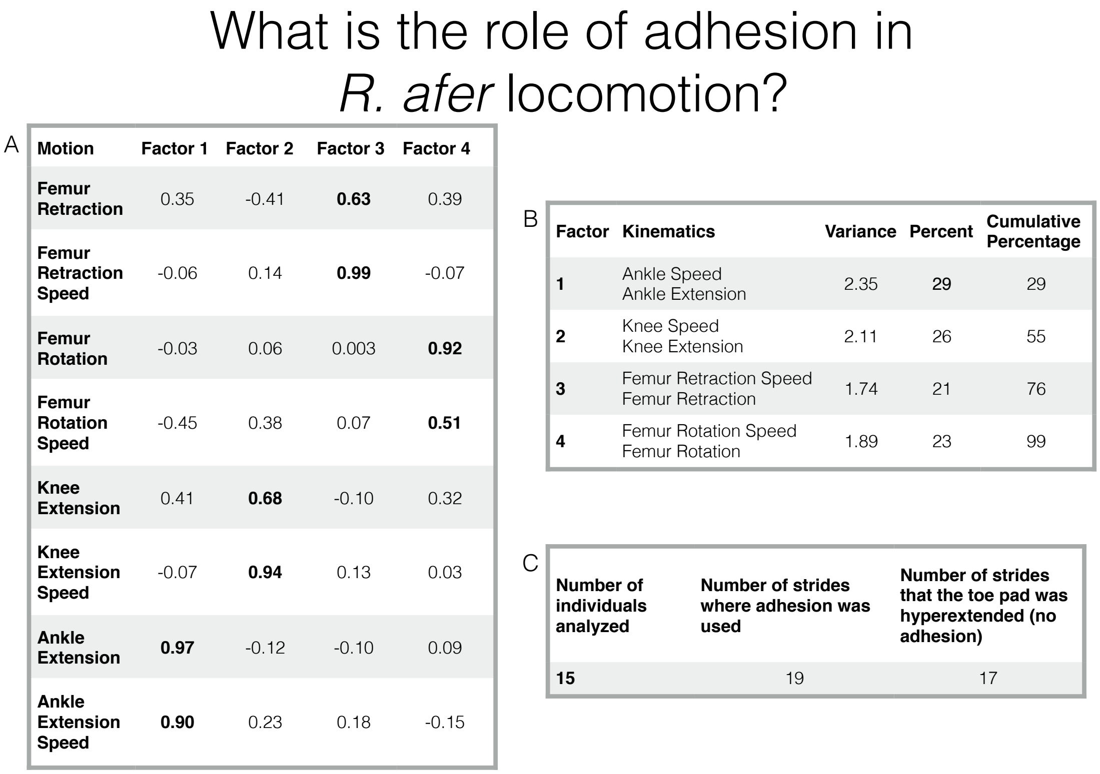
Table 3.2: Three components representing the suite of kinematic traits exerting control over forward speed in this study, their descriptions, expectations, and outcomes for individuals that always employ adhesion (Adherers) and individuals that vary in their use of adhesion (Variants). Low p-values (<0.05), derived from a Log-Likelihood Ratio Test, reject a more constrained model in favor of the more complete model that includes the principal factor in question. For example, constraining Component 1, representing the ankle joint, significantly degrades the model fit on a level surface in Adherers (p=0.004), but not Variants (p=0.07), indicating that the muscles extending the ankle are relatively more important in individuals that only use adhesion, or that they are more important when adhesion is used.



Individuals vary in adhesion and locomotion control

	Factor	Description	Expectation	Outcome	Adherers	Variants
3	**a. Step Length** **b. Step Frequency** **c. Femur Retraction** **d. Femur Rotation**	a. Distance travelled while one foot contacts the ground. b. Equal to 1 / Duration of a step x 60 seconds c. The 3-D angle (degrees) travelled between the knee and the center of mass during one step d. The total amount of rotation (degrees) by the femur during one step.	Weakest predictor of speed on level treatment. Increase of importance on incline treatment. No difference between groups.	Femur retraction speed decreased in importance from level to incline treatment. Total femur retraction decreased in importance from level to incline treatment. Integration with femur rotation increased on incline. Appears more important in individuals that vary adhesion use.	Level RMSEA: 0.18 Level AICc: 139.83 Level -2LLR: 0.132 p=0.716 Up RMSEA: 0.122 Up AICc: 111.82 Up -2LLR: 0.116 p=0.73	Level RMSEA: 0.27 Level AICc: 177.57 Level -2LLR: 3.73 p=0.05 Up RMSEA: 0.08 Up AICc: 133.367 Up -2LLR: 0.047 p=0.83
2	**a. Knee Extension Speed** **b. Knee Extension**	a. Speed of the 3-D extension (degrees / seconds) of the knee during one step b. The 3-D extension (degrees) of the knee during one step	Strong predictor of speed on level and incline treatment. No difference between groups.	Change between treatments unclear, but exhibits strong contribution to step length in both. Stronger integration with ankle on incline.	Level RMSEA: 0.21 Level AICc: 143.6 Level -2LLR: 3.91 p=0.048 Up RMSEA: 0.116 Up AICc: 114.73 Up -2LLR: 0.04 p=0.83	Level RMSEA: 0.39 Level AICc: 183.14 Level -2LLR: 9.3 p=0.003 Up RMSEA: 0.177 Up AICc: 135.109 Up -2LLR: 1.789 p=0.18
1	**a. Ankle Extension Speed** **b. Ankle Extension**	a. Speed of the 3-D extension (degrees / seconds) of the ankle during one step b. The 3-D extension (degrees) of the ankle during one step	Strongest predictor or speed on both level and incline treatments. Difference between groups on both treatments.	Ankle extensor muscle group show greatest contribution to step length in both treatments but relative contribution greater on a level surface compared to incline. Stronger integration with knee on incline. Appears more important in users that always use adhesion.	Level RMSEA: 0.34 Level AICc: 147.79 Level -2LLR: 8.1 p=0.004 Up RMSEA: 0.328 Up AICc: 114.14 Up -2LLR: 2.45 p=0.117	Level RMSEA: 0.256 Level AICc: 177.14 Level -2LLR: 3.3 p=0.07 Up RMSEA: 0.188 Up AICc: 135.392 Up -2LLR: 2.07 p=0.15

Table 3.3: **A**: Four factors were extracted using principal factors analysis. The cross-loading of factors was minimized using oblimin rotation. The number of factors retained was determined by examining a scree plot using the broken stick method. Bold font indicates loadings greater than 0.5 **B**: The first factor, explaining 29% of the variance, described movement around the ankle. Factor 2, explaining 26% of the variance, described movement around the knee. Factor 3, explaining 21% of the variance, described femur retraction or hip extension. Factor 4, explaining 23% of the variance, describes femur rotation. **C**. Fifteen individuals varied their use of the toe pad on a level surface.



What is the role of adhesion in *R. afer* locomotion?

A

Motion	Factor 1	Factor 2	Factor 3	Factor 4
Femur Retraction	0.35	-0.41	**0.63**	0.39
Femur Retraction Speed	-0.06	0.14	**0.99**	-0.07
Femur Rotation	-0.03	0.06	0.003	**0.92**
Femur Rotation Speed	-0.45	0.38	0.07	**0.51**
Knee Extension	0.41	**0.68**	-0.10	0.32
Knee Extension Speed	-0.07	**0.94**	0.13	0.03
Ankle Extension	**0.97**	-0.12	-0.10	0.09
Ankle Extension Speed	**0.90**	0.23	0.18	-0.15

B

Factor	Kinematics	Variance	Percent	Cumulative Percentage
1	Ankle Speed Ankle Extension	2.35	**29**	29
2	Knee Speed Knee Extension	2.11	26	55
3	Femur Retraction Speed Femur Retraction	1.74	21	76
4	Femur Rotation Speed Femur Rotation	1.89	23	99

C

Number of individuals analyzed	Number of strides where adhesion was used	Number of strides that the toe pad was hyperextended (no adhesion)
15	19	17

Table 3.4: Three factors representing the suite of kinematic traits exerting control over forward speed in this study, their descriptions, expectations, and outcomes for runs where adhesion was employed and when the toe pad was hyperextended. For ease of interpretation and due to their theoretical autocorrelation, kinematics related to femur retraction and rotation were combined in this table. Low p-values (<0.05), derived from a Log-Likelihood Ratio Test, reject a more constrained model in favor of the more complete model that includes the principal factor in question. No p-values fall below 0.05 in this analysis; however, examining RMSEA, AICc, and -2LLR indicates that the muscles powering ankle extension have the strongest contribution to forward speed when adhesion is employed while femur retraction and rotation have the strongest contribution when the toe pad is hyperextended.

Toe pad use alters locomotion control

Factor	**Description**	**Expectation**	**Outcome**	**Adhesion Employed**	**Toe Pad Hyperextended**
a. Femur Retraction & **b. Femur Rotation**	a. The 3-D angle (degrees) travelled between the knee and the center of mass during one step b. The total amount of rotation (degrees) by the femur during one step.	The strongest predictor of speed when adhesion is employed.	Femur retraction and rotation increased in importance when the toe pad was hyperextended.	RMSEA: 0.01 AICc: 200.778 -2LLR: 0.05 p=0.97	RMSEA: 0.214 AICc: 175.458 -2LLR: 4.16 p=0.12
a. Knee Extension **b. Knee Extension Speed**	a. The 3-D extension (degrees) of the knee during one step b. Speed of the 3-D extension (degrees / seconds) of the knee during one step	Strongest contribution to speed when adhesive toe pad is hyperextended (not in use).	Knee extension increased in importance when the toe pad was hyperextended.	RMSEA: 0.01 AICc: 212.124 -2LLR: 0.14 p=0.24	RMSEA: 0.148 AICc: 181.462 -2LLR: 0.17 p=0.68
a. Ankle Extension **b. Ankle Extension Speed**	a. The 3-D extension (degrees) of the ankle during one step. b. The total amount of rotation (degrees) by the femur during one step	Weakest contribution when adhesion is employed and relatively stronger when adhesive toe pad is hyperextended (not in use).	Ankle extension was less important when the toe pad was hyperextended.	RMSEA: 0.21 AICc: 215.222 -2LLR: 4.5 p=0.07	RMSEA: 0.184 AICc: 182.661 -2LLR: 1.27 p=0.24

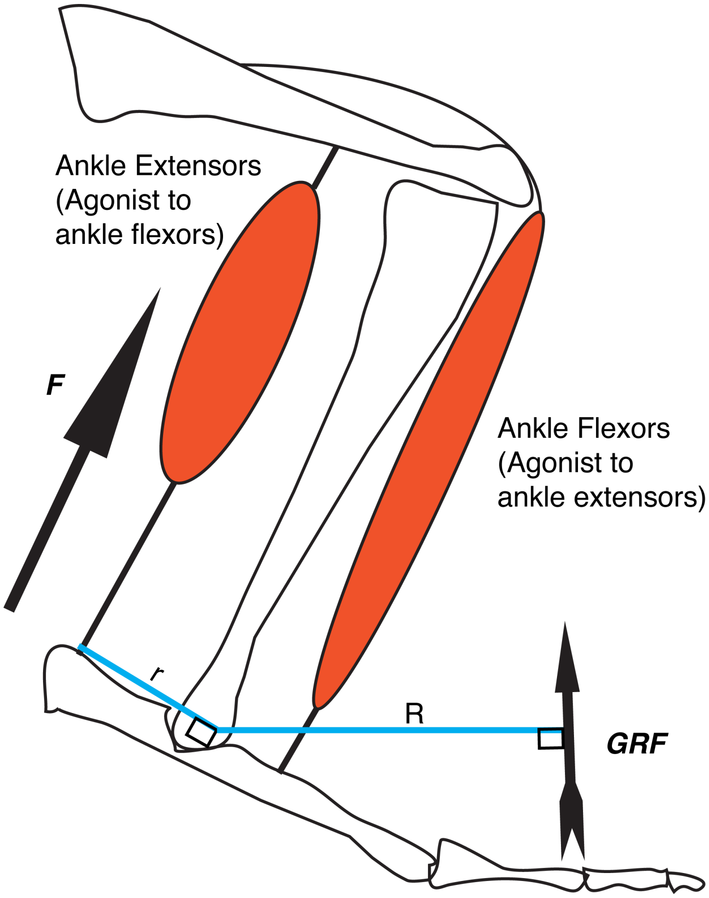


Figure 3.1: Agonist muscle groups of the ankle, (colored red), tensile force (*F)* produced by the ankle extensors, the ground reaction force (GRF) at the ankle, and the moment arms (muscle moment, r and GRF moment, R) of each force vector in a typical vertebrate limb during mid stance of a stride. Effective Mechanical Advantage (EMA) is equal to r / R and can be increased by increasing the length of the r (in-lever) or by decreasing R (out-lever). This can be accomplished by lengthening and shortening the bony elements associated with each moment arm. Conversely, a distance or velocity advantage can be increased by increasing the out-lever and decreasing the in-lever. Redrawn and modified from Biewener (2003).

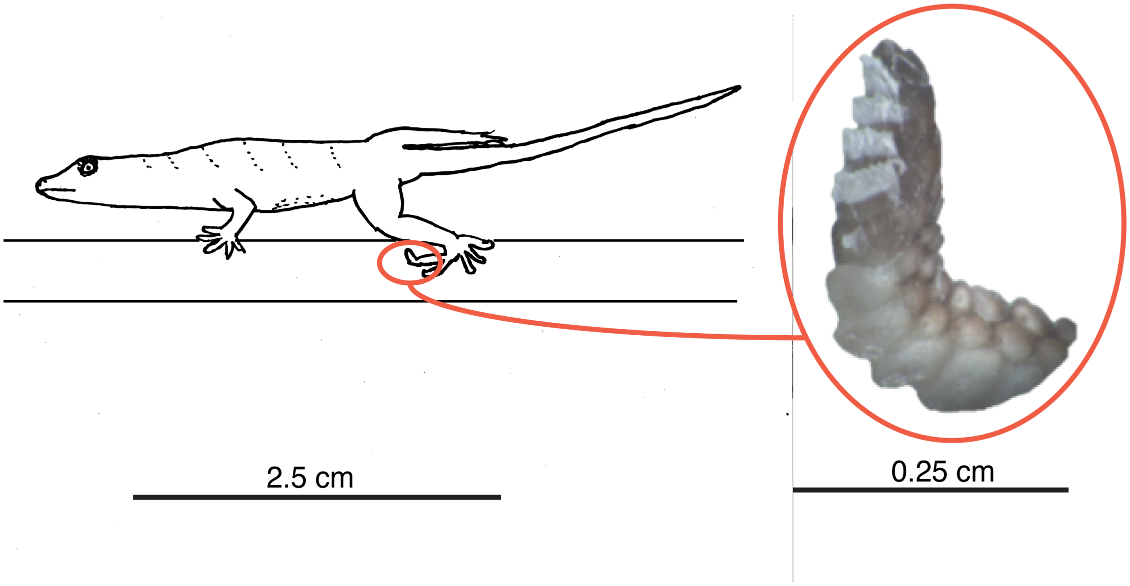


Figure 3.2: Hyperextension of the distal toe and subdigital adhesive toe pad during locomotion. Left: Generalized gecko running on a level trackway during mid-stance of the right pes. Right: The orientation of the subdigital adhesive system in *R. afer* during hyperextension.

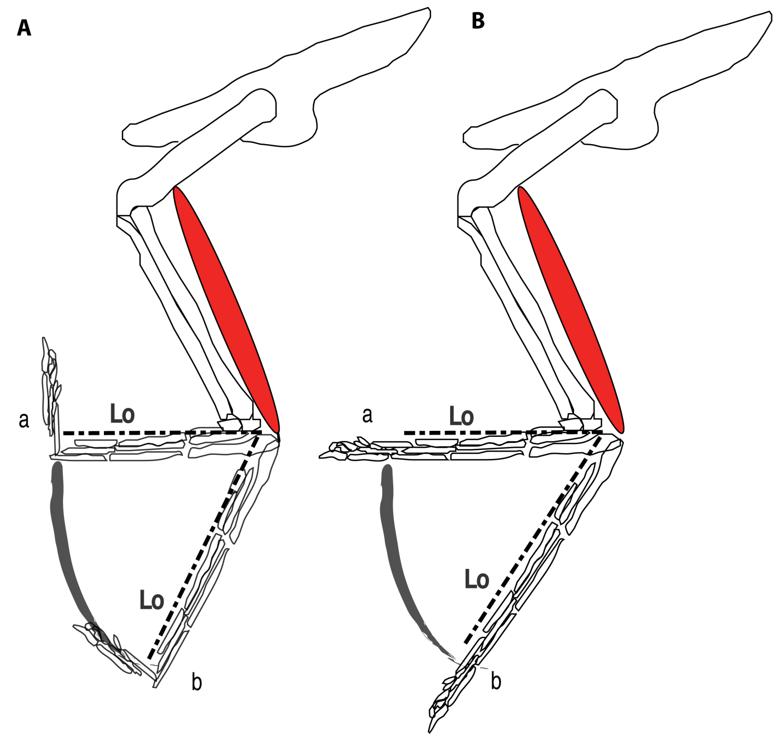


Figure 3.3: A consequence of hyperextension on the lever mechanics of locomotion in lizards illustrated in a lateral schematic of a parasagittal view of the left hind limb. In A, the distal elements of the hyperextended gecko foot travel from a to b (powered by the ankle extensor muscles, represented by the red oval) in the same amount of time that the distal elements of the non-gekkotan lizard also travel from a to b. The gecko foot exhibits increased mechanical advantage, but reduced velocity advantage. Redrawn and modified from Hildebrand *et al*. 1985, and Higham *et al*. 2011.

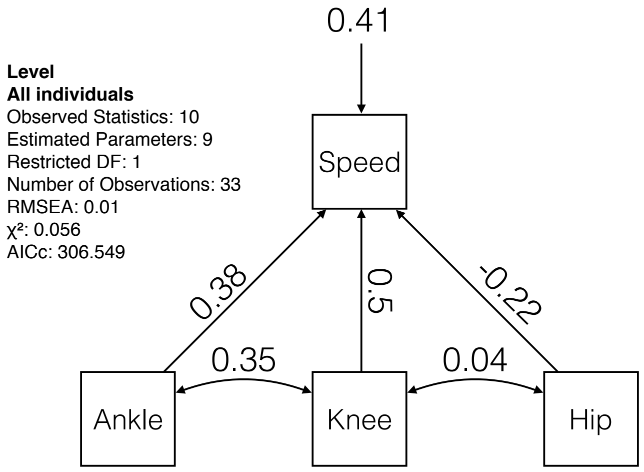


Figure 3.4a: Principal components were used in a series of structural equations models to examine how the control of locomotion changes in response to the use of the adhesive toe pad on different treatments. This path model is for all individuals on the level treatment.

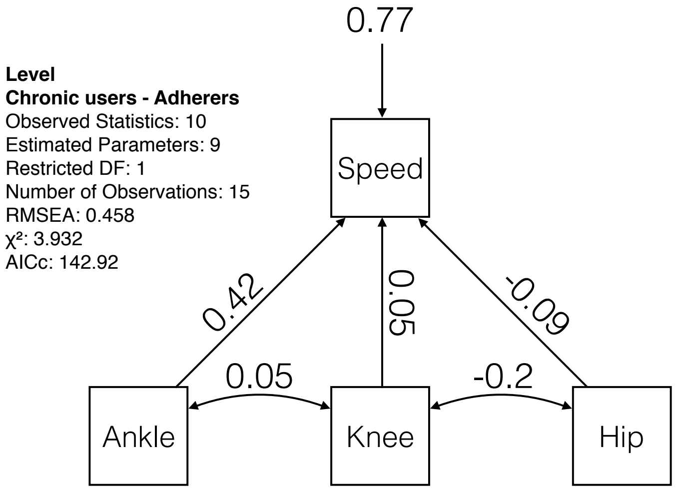


Figure 3.4b: Principal components were used in a series of structural equations models to examine how the control of locomotion changes in response to the use of the adhesive toe pad on different treatments. This path model is for individuals that always use adhesion (Adherers) on a level treatment.

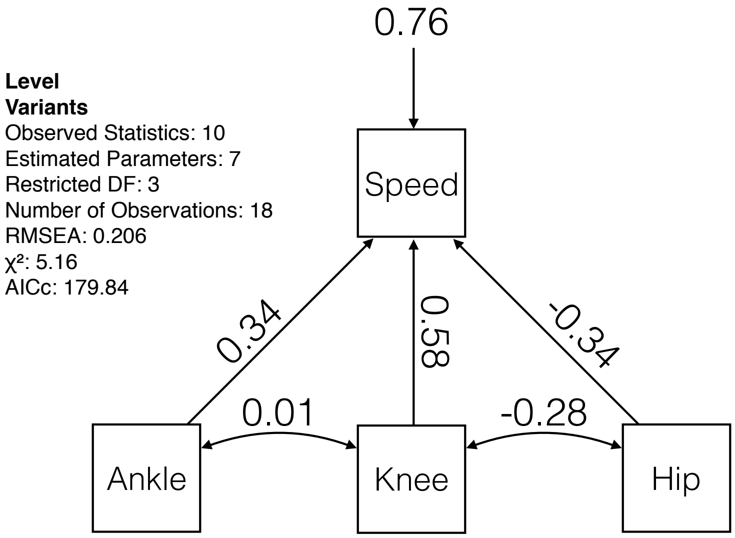


Figure 3.4c: Principal components were used in a series of structural equations models to examine how the control of locomotion changes in response to the use of the adhesive toe pad on different treatments. This path model is for individuals that vary in their use of adhesion (Variants) on a level treatment.

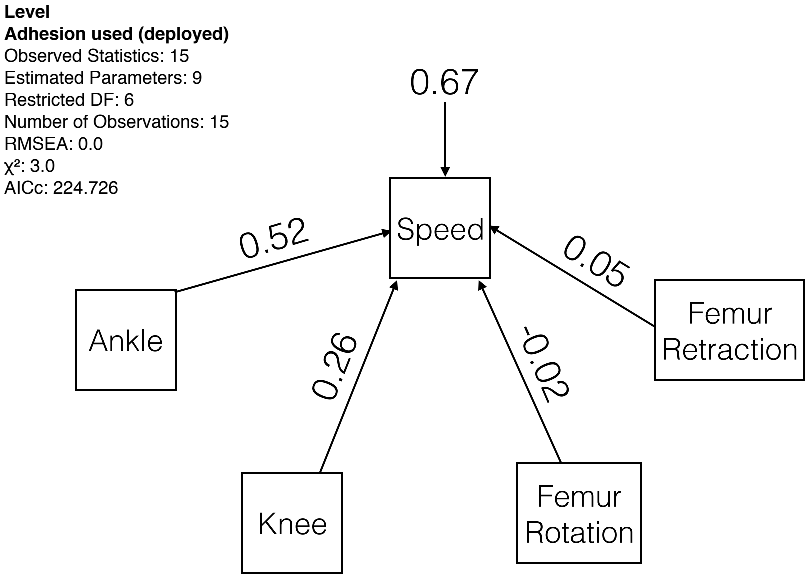


Figure 3.5: Principal components were used in a series of structural equations models to examine how the control of locomotion changes in response to the use of the adhesive toe pad on a level surface. Relative contributions to locomotion are significantly different when these individuals employ their adhesive toe pad. **A**. Path model for individuals when the toe pad is employed.

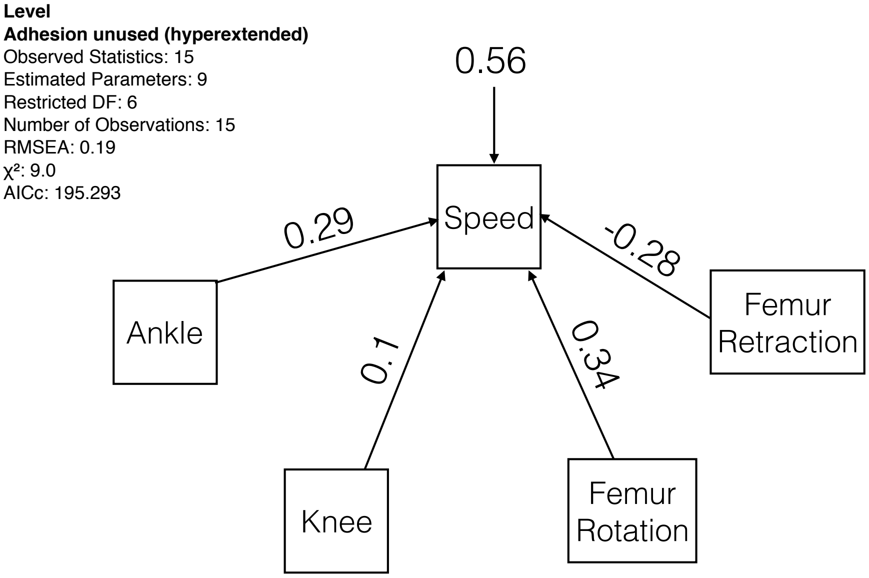
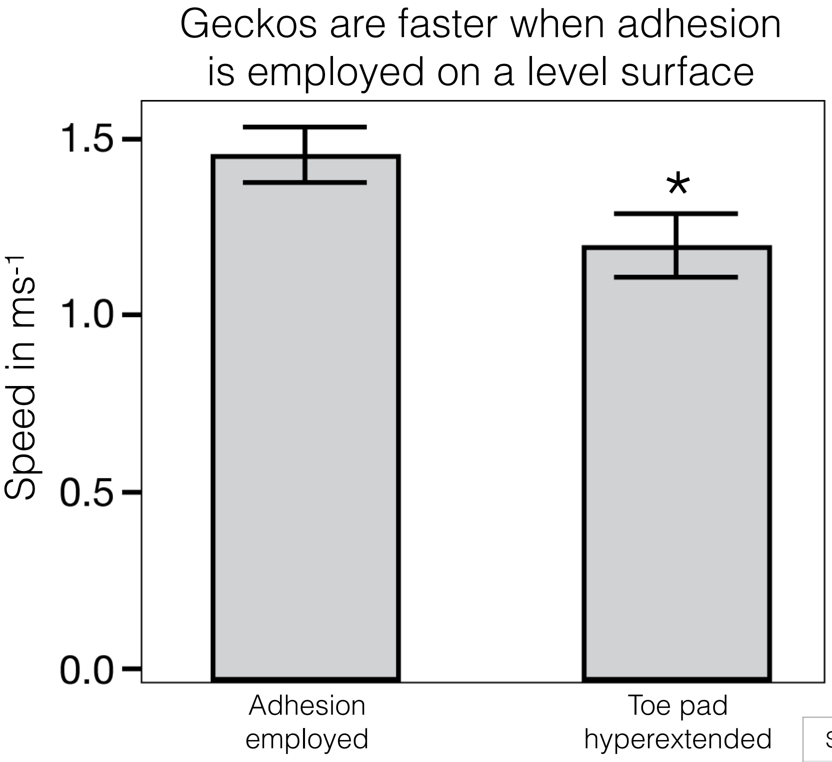


Figure 3.5: Principal components were used in a series of structural equations models to examine how the control of locomotion changes in response to the use of the adhesive toe pad on a level surface. Relative contributions to locomotion are significantly different when these individuals employ their adhesive toe pad. **B**. Path model for individuals when the toe pad is hyperextended.



S	Z	P>|Z|
201	-2.36	0.02

Figure 3.6: Locomotor speed on a level surface when adhesion was employed and when the toe pad was hyperextended. Error bars are +/- 1 S.E. *Rhoptorpus afer* ran faster when the toe pad was in contact with the surface (Wilcoxon / Kruskal-Wallis).