RESEARCH ARTICLE

How forelimb and hindlimb function changes with incline and perch diameter in the green anole, *Anolis carolinensis*

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SUMMARY

The range of inclines and perch diameters in arboreal habitats poses a number of functional challenges for locomotion. To effectively overcome these challenges, arboreal lizards execute complex locomotor behaviors involving both the forelimbs and the hindlimbs. However, few studies have examined the role of forelimbs in lizard locomotion. To characterize how the forelimbs and hindlimbs differentially respond to changes in substrate diameter and incline, we obtained three-dimensional high-speed video of green anoles (*Anolis carolinensis*) running on flat (9 cm wide) and narrow (1.3 cm) perches inclined at 0, 45 and 90 deg. Changes in perch diameter had a greater effect on kinematics than changes in incline, and proximal limb variables were primarily responsible for these kinematic changes. In addition, a number of joint angles exhibited greater excursions on the 45 deg incline compared with the other inclines. *Anolis carolinensis* adopted strategies to maintain stability similar to those of other arboreal vertebrates, increasing limb flexion, stride frequency and duty factor. However, the humerus and femur exhibited several opposite kinematic trends with changes in perch diameter. Further, the humerus exhibited a greater range of motion than the femur. A combination of anatomy and behavior resulted in differential kinematics between the forelimb and the hindlimb, and also a potential shift in the propulsive mechanism with changes in external demand. This suggests that a better understanding of single limb function comes from an assessment of both forelimbs and hindlimbs. Characterizing forelimb and hindlimb movements may reveal interesting functional differences between *Anolis* ecomorphs. Investigations into the physiological mechanisms underlying the functional differences between the forelimb and the hindlimb are needed to fully understand how arboreal animals move in complex habitats.

Key words: locomotion, arboreal, kinematics, lizard, running, climbing.

INTRODUCTION

Locomotion is essential to an animal's survival and success, and is integral to activities such as evading predators and finding suitable mates, prey and habitat (Garland and Losos, 1994). To successfully perform these tasks, animals must deal with their environment and overcome obstacles and challenges that govern or constrain movement. Terrestrial animals often move over a range of speeds, up or down sloped surfaces, on compliant or smooth surfaces, or may be forced to negotiate a discontinuous environment (e.g. Irschick and Jayne, 1999; Kohlsdorf and Biewener, 2006; Olberding et al., 2012). Compared with terrestrial animals, arboreal animals often face a wider range of challenges, including steep inclines and declines, substrate diameters from flat to extremely narrow (e.g. twigs), and obstacles in the forms of leaves or other branches that force the animal to jump or turn (e.g. Higham et al., 2001; Mattingly and Jayne, 2004). Although behavioral and/or morphological adaptations are commonly associated with this complex habitat structure, little is known about the detailed three-dimensional limb movements during arboreal locomotion.

Changes in incline and perch diameter are among the greatest challenges that arboreal animals encounter. Moving up an incline increases the cost of locomotion by requiring a greater proportion of the locomotor effort to offset the increased influence of gravity (Taylor et al., 1972; Cartmill, 1985; Farley and Emshwiller, 1996;

Roberts et al., 1997; Preuschoft, 2002; Daley and Biewener, 2003; Autumn et al., 2006). This increase in muscle work and overall energetic cost of locomotion often leads to decreased locomotor performance (Huey and Hertz, 1982; Irschick and Jayne, 1998; Zaaf et al., 2001; Schmidt and Fischer, 2011). Based on several studies that have examined the effects of incline on kinematics (Vilensky et al., 1994; Irschick and Jayne, 1998; Jayne and Irschick, 1999; Zaaf et al., 2001; Nakano, 2002; Higham and Jayne, 2004a; Spezzano and Jayne, 2004; Schmidt and Fischer, 2011), kinetics (Autumn et al., 2006; Lammers et al., 2006; Lammers, 2007) and motor patterns (Fowler et al., 1993; Carlson-Kuhta et al., 1998; Gabaldón et al., 2001; Gillis and Biewener, 2002; Higham and Jayne, 2004b; Higham and Nelson, 2008), it is clear that incline has variable effects depending on the species. However, a decrease in the height of the center of mass (CoM) and stride length, more posterior hindlimb placement, and increased stride frequency and muscle activity are common responses to incline in both terrestrial generalists and arboreal specialists.

Narrower perches increase the chance of falling by constraining foot placement to a narrower base of support (Cartmill, 1985; Preuschoft, 2002), and often result in decreased performance (Losos and Sinervo, 1989; Losos and Irschick, 1996; Vanhooydonck et al., 2006a; but see Schmidt and Fischer, 2010). This is likely a result of kinematic changes necessary for increased stability; lowering the

CoM by adopting a crouched/sprawled posture through greater limb flexion (Peterson, 1984; Schmitt, 1994; Higham and Jayne, 2004a; Franz et al., 2005; Schmidt and Fischer, 2010) and increasing duty factor (Lammers and Biknevicius, 2004; Franz et al., 2005; Lammers, 2007; Gálvez-López et al., 2011) reduce peak vertical forces and are common strategies for dealing with narrow substrates in a range of vertebrate taxa.

Although the hindlimbs are the key propulsors in terrestrial vertebrates, with the forelimbs absorbing collisional energy and acting primarily as brakes (Lammers and Biknevicius, 2004; Autumn et al., 2006; Lee, 2010; Deban et al., 2012), the coordinated function of the forelimbs and the hindlimbs is poorly understood in arboreal vertebrates. Structural differences between the forelimbs and the hindlimbs have been well documented among vertebrates; the pectoral girdle is generally more mobile than the pelvic girdle and structural differences between the glenoid cavity and the acetabulum lead to the potential for a greater range of motion in the forelimb than in the hindlimb (Haines, 1952; Snyder, 1954; Peterson, 1971; Peterson, 1973; Peterson, 1974; Jenkins and Goslow, 1983; Peterson, 1984; Reynolds, 1985; Schmitt, 1994; Larson et al., 2001; Lammers, 2007; Zihlman et al., 2011). In most primates, the shallow glenoid cavity with reduced coracoid and acromion processes in the pectoral girdle compared with the deeper acetabulum of the pelvic girdle are likely related, in part, to the greater weight-bearing function of the hindlimbs relative to the mobile forelimbs (Reynolds, 1985; Zihlman et al., 2011). These structural differences between forelimbs and hindlimbs tend to be most exaggerated in arboreal species, including primates (Larson et al., 2000), with some of the most extreme examples found in lizards. Antero-posterior translation of pectoral girdle and greater pectoral girdle rotation permits greater long-axis humerus rotational excursion in chameleons (Peterson, 1971; Peterson, 1973; Peterson, 1984; Fischer et al., 2010), anoles (Peterson, 1971; Peterson, 1974) and varanids (Haines, 1952; Jenkins and Goslow, 1983). Comparative forelimb and hindlimb function has been related to incline and perch diameter in a variety of arboreal and terrestrial mammals, with the stabilizing limb taking a more lateral position than the propelling limb, although the limb that dominates a particular function depends on the species (Cartmill, 1985; Nakano, 2002; Lammers and Biknevicius, 2004; Lammers et al., 2006; Lammers, 2007; Schmidt and Fischer, 2010; Schmidt and Fischer, 2011). Although environmental variables affecting hindlimb kinematics in lizards have been studied extensively, especially in terrestrial species (reviewed in Russell and Bels, 2001a), only two studies have investigated forelimb functional changes with incline in lizards (both with geckos), finding more lateral placement and greater duty factor (Zaaf et al., 2001) and a greater propulsive role of the forelimbs (Autumn et al., 2006).

Lizards are among the most proficient of vertebrate climbers and offer some of the most spectacular examples of arboreal adaptations. In particular, the evolution, morphology, and locomotor behavior and performance of *Anolis* ecomorphs, in relation to habitat structure, have been studied extensively (Pounds, 1988; Losos and Sinervo, 1989; Losos, 1990a; Losos, 1990b; Losos, 1994; Losos and Irschick, 1996; Irschick and Losos, 1999; Higham et al., 2001; Perry et al., 2004; Toro et al., 2004; Vanhooydonck et al., 2006a). However, only a single study has examined three-dimensional kinematics of the hindlimb in response to these challenges (Spezzano and Jayne, 2004). In that study, *Anolis sagrei* decreased hip height and increased knee flexion, femur retraction, depression and long-axis rotation to increase stability on narrower and/or steeper surfaces, although perch diameter had a greater overall effect than incline on

kinematics. However, that study looked at only a single species and did not examine the forelimb (Spezzano and Jayne, 2004). As morphological characteristics of muscle, including mass and moment arms, vary greatly between anole species and ecomorphs (Vanhooydonck et al., 2006b; Herrel et al., 2008) and between other lizards with divergent ecologies (Zaaf et al., 1999; Aerts et al., 2000), we would expect variation in behavioral and functional changes in response to arboreal challenges. Therefore, investigation of the effects of these variables on forelimb kinematics as well as on hindlimb kinematics in other ecomorphs is needed.

We investigated forelimb and hindlimb kinematics of the green anole, *Anolis carolinensis*, which is a trunk-crown ecomorph, encountering a wide range of inclines (0 to 90 deg) and substrate diameters [1 cm to flat (Mattingly and Jayne, 2004)]. It uses its arboreal habitat opportunistically, occupying most arboreal and terrestrial substrates in the absence of other species, but moves higher in the trees when living sympatrically with other species. This is exemplified by the competitive interaction between *A. carolinensis* and *A. sagrei*, a trunk-ground species that prefers slightly larger diameters, in Florida (Collette, 1961; Mattingly and Jayne, 2004; Losos, 2009). Its flexibility and competition with *A. sagrei* makes *A. carolinensis* an ideal subject for understanding the kinematic basis of performance changes in arboreal habitats in both forelimbs and hindlimbs. Although some kinematic changes associated with lowering CoM and increasing stability on the more challenging surfaces (e.g. increased elbow/knee flexion and/or humerus/femur elevation) are expected based on previous mammalian and lacertilian literature, we expect *A. carolinensis* to modulate forelimb and hindlimb kinematics differently because of anatomical differences between the limbs. However, we expect *A. carolinensis* to modulate hindlimb kinematics similarly to *A. sagrei*, rotating, retracting and depressing the femur more, and decreasing stride and step lengths on narrower perches and steeper inclines.

MATERIALS AND METHODS
Subjects
Four adult male *Anolis carolinensis* Voigt 1832 [mass=6.1±0.7 g; snout–vent length (SVL)=6.0±0.2 cm] were obtained from commercial suppliers and housed individually in 10-gallon aquaria. The aquaria were heated with 100 W lights for 12 h per day, and perches in the aquaria allowed the lizards to behaviorally thermoregulate to their preferred active temperature (28–36°C) (Licht, 1968). Additional lights providing a source of UVB were also placed above the aquaria. Lizards were fed vitamin-enriched crickets every other day and were given water *ad libitum*.

Although our sample size was limited to four individuals, statistical significance can still be determined with confidence if the amount of variation within treatments is less than the variation between treatments (Harmon and Losos, 2005). We calculated the standard deviation within treatments for each variable and divided that by the total standard deviation across treatments for each variable and always found less within-treatment variation than between-treatment variation (within-treatment variation ranged from 14.2 to 88.2% of between-treatment variation). Therefore, our sample size was sufficient to avoid type I errors.

Prior to running trials, several joints were marked with white nail polish to enhance visualization in the video. Points included the shoulder/hip, the elbow/knee, the wrist/ankle, and the base and tip of the third metacarpal/fourth metatarsal. The opposite shoulder/hip joint was also marked as was the midpoint between the shoulders and hips, to aid in determining pectoral/pelvic girdle rotation (Fig. 1).

Research was conducted under Clemson University Animal Care and Use protocol no. AUP2010-50.

Experimental setup

Lizards ran on 1 m long flat (9 cm wide) and small (1.3 cm diameter) perches that were attached by their ends to a 1×1.2 m plywood sheet, which could be rotated on the wall to any angle. Because large diameter surfaces, such as tree trunks or large branches, result in hindlimb kinematics similar to those with flat surfaces in *A. sagrei* (Spezzano and Jayne, 2004), the flat perch in our study represented a large diameter treatment. The perches were mounted 0.5 m from the plywood to discourage the lizards from jumping off the perch, and were suspended 1.1 m above the ground. Both perches were covered in cork shelf liner to simulate a natural surface and to enhance traction. A mirror was mounted to the plywood above the perches at a 45 deg angle.

Two high speed Photron APX-RS cameras (Photron USA, San Diego, CA, USA) were used simultaneously to obtain dorsal (using the mirror) and lateral video of the lizards running on the perches. Cameras recorded at 500 frames s^{-1} with a shutter speed of 1/2000 s. We obtained two to five strides of steady locomotion for both the hindlimb and the forelimb of each individual running on both perches at 0, 45 and 90 deg. We selected trials in which: (1) both the forelimb and the hindlimb were visible, (2) the lizard remained on the top of the perch, and (3) the lizard ran steadily through the field of view.

Kinematics

We digitized the sequences using DLT DV 3 custom software (Hedrick, 2008) for MATLAB (version R2009a, The MathWorks, Natick, MA, USA) in order to obtain *x*, *y* and *z* coordinates. The *x*-axis described antero-posterior movement, parallel to the direction of travel, the *y*-axis described dorso-ventral movement, perpendicular to the perch, and the *z*-axis described medio-lateral movement perpendicular to the *x*–*y* plane. Three-dimensional coordinates were obtained for the following points: tip of the nose, right and left shoulder/hip, mid-pectoral/pelvic girdle, elbow/knee, wrist/ankle, base and tip of the third metacarpal, and base and tip of the fourth metatarsal (Fig. 1). The forelimb and the hindlimb were

analyzed separately and, in each case, the coordinates were transformed to place the shoulder/hip at the origin (0,0,0) to facilitate visualization of the limb such that positive *x*, *y* and *z* indicated anterior, dorsal and lateral positions relative to the hip. All calculations based on these coordinates were performed in Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA).

Body speed was calculated for each stride by digitizing a point along the midline of the body (the tip of the nose or the middle of the pectoral or pelvic girdles) that showed minimal medio-lateral excursion. The distance traveled by that point between consecutive frames was divided by the duration between frames to calculate instantaneous speed. Speed was then standardized by dividing by SVL.

Stride length was calculated as the two-dimensional (*x*–*y*) distance traveled during a complete stride cycle whereas step length was the distance traveled during stance. Both of these variables were standardized to the SVL of the individual. Duty factor was the percentage of the total stride during which the limb of interest was in contact with the ground, and stride frequency was the number of complete strides per second (Hz).

Hip height was calculated as the two-dimensional distance between the *y*-coordinate of the right hip and the perch (set to *y*=0). From this, minimum and maximum hip height ($Y_{hip,min}$, $Y_{hip,max}$), hip height at footfall ($Y_{hip,FF}$), change in hip height through stance ($Y_{hip,FF}$–$Y_{hip,min}$) and total vertical excursion of the hip ($Y_{hip,EX}$) were determined. Variables for shoulder height were calculated similarly.

Three-dimensional angles were calculated for the elbow/knee, wrist/ankle and metacarpophalangeal (MCP)/metatarsophalangeal (MTP) joints as described previously (Jayne and Irschick, 1999; Spezzano and Jayne, 2004). Elbow/knee angle was calculated as the three-dimensional angle created by the humerus/femur and ulna/tibia with smaller angles between 0 and 180 deg indicating greater flexion at each joint. Wrist/ankle angle was calculated as the three-dimensional angle created by the ulna/tibia and the third metacarpal/fourth metatarsal, with smaller angles less than 180 deg indicating greater dorsiflexion of the fore-feet and hind-feet. MCP/MTP angle was calculated as the three-dimensional angle
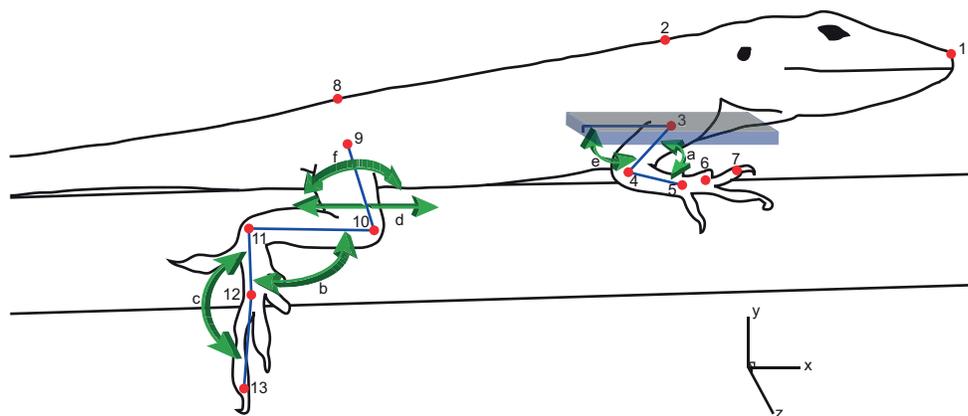

Fig. 1. Diagram of *Anolis carolinensis*, indicating anatomical landmarks digitized (red dots) and angular variables measured (green arrows) from video analysis. a, elbow/knee, the angle between the humerus/femur and the ulna/crus; b, wrist/ankle, the angle between the ulna/crus and the third metacarpal/fourth metatarsal; c, metacarpophalangeal/metatarsophalangeal joints, the angle between the third metacarpal/fourth metatarsal and the corresponding phalanges; d, humerus/femur retraction, the two-dimensional angle, in the horizontal (*x*–*z* plane), between the humerus/femur and the line connecting the right and center of the shoulders/hips; e, humerus/femur depression, the three-dimensional angle between the humerus/femur and a horizontal plane through the shoulder/hip; f, humerus/femur rotation, the three-dimensional angle between a vertical plane through the humerus/femur and a plane containing the upper and lower limbs. 1, nose; 2, center of shoulder; 3, right shoulder; 4, elbow; 5, wrist; 6, third metacarpophalangeal joint; 7, tip of third forelimb phalanx; 8, center of hip; 9, right hip; 10, knee; 11, ankle; 12, fourth metatarsophalangeal joint; 13, tip of fourth hindlimb phalanx.

created by the third metacarpal/fourth metatarsal and toe tip, with angles greater than 180 deg indicating plantar flexion of the fore-toe and the hind-toe. Minimum, maximum, excursion and angle at footfall (FF) and end of stance (ES) were calculated for each of these angular variables (Fig. 1).

Three angles described the orientation and movement of the right humerus/femur. Humerus/femur retraction was calculated as the two-dimensional angle (in the *x*–*z* plane) between a line connecting the left and right shoulder/hip and the humerus/femur, where positive angles indicate retraction and negative angles indicate protraction; 0 deg indicates the humerus/femur is perpendicular to the antero-posterior axis of the body. Humerus/femur depression was calculated as the three-dimensional angle between the humerus/femur and a horizontal plane containing the right shoulder/hip such that positive angles indicate depression and negative angles indicate elevation of the elbow/knee relative to the shoulder/hip. Humerus/femur long-axis rotation was calculated as the three-dimensional angle between a vertical plane containing the humerus/femur and the plane containing the upper and lower limbs, where positive angles indicate clockwise rotation and negative values indicate counter-clockwise rotation. Angles at FF, ES and overall angular excursion were calculated for all three of these variables and minimum and maximum angles were recorded for depression and long-axis rotation (Fig. 1).

Pectoral/pelvic rotation was calculated as the two-dimensional (*x*–*z*) angle between the antero-posterior axis of the body (containing the nose and midpoint of the pectoral/pelvic girdles) and a line connecting the left and right shoulders/hips. Positive angles indicate clockwise rotation where the right shoulder/hip is posterior to the left shoulder/hip, whereas negative angles indicate the right shoulder/hip is anterior to the left shoulder/hip (counter-clockwise rotation) (Fig. 1).

Movement of the skin and/or soft tissue independent of the underlying skeletal structures may affect the accuracy of the above-mentioned angular variables (e.g. Filipe et al., 2006). Although other techniques, such as three-dimensional X-ray reconstruction of moving morphology (XROMM) (Brainerd et al., 2010), would potentially help eliminate some of this error by allowing the skeletal structures to be tracked directly, the extent to which the skin and skeletal elements are decoupled during locomotion in lizards is unclear.

Average angular velocities were calculated during extension (for the elbow/knee, wrist/ankle and metacarpal/metatarsal joints) and flexion (for knee/elbow joints) during stance, with greater positive values indicating faster extension and greater negative values for the elbow/knee indicating faster flexion. Greater positive and negative values of humerus/femur depression indicate faster depression and elevation, respectively, of the upper limbs. Greater positive and negative values of humerus/femur retraction indicate faster retraction and protraction, respectively, of the upper limbs. Greater positive and negative values of humerus/femur long-axis rotation indicate faster clockwise and counter-clockwise rotation, respectively, of the upper limbs. Angular velocities were calculated in deg s^{-1}.

Lastly, the linear velocity of the distal tip of the metacarpal/metatarsal during swing phase was calculated and standardized to SVL s^{-1} such that greater positive values indicates faster swing in the anterior direction.

Minimum, maximum and excursions for all variables were determined from the entire stride, except for the velocity variables, which were determined only from the stance portion of the stride. All values are reported as means ± s.e.m.

Statistical analyses

All statistical analyses were performed using JMP (version 9.0, SAS Institute Inc., Cary, NC, USA). Because the lizards ran at different speeds depending on treatment (ranging from 2.11 to 21.24 SVL s^{-1} overall, and averaging 11.89±0.96, 8.42±0.54 and 4.57±0.25 SVL s^{-1} for 0, 45 and 90 deg, respectively), the effects of speed were removed by regressing all the variables individually against body speed (SVL s^{-1}). The residuals of all variables that exhibited significant relationships ($\alpha \leq 0.1$) with speed were saved and used for future analyses, whereas all other variables were kept in their original form. All variables were averaged across strides for each individual prior to further analyses.

Variables associated with time (i.e. velocities, stride frequency and duty factor) were isolated from the remaining angular and linear distance variables in order to run analyses separately on each set of variables. For the angular set, a principal components analysis (PCA) was used to reduce dimensionality and isolate the 15 variables most important for describing the variation in the data. The selected variables were those with the highest loadings chosen from both the first and second principal components (PC1 and PC2), but the number of variables chosen from the PC1 was proportional to the percentage of variation explained by that axis, with the remaining variables chosen from PC2. For example, if 80% of the variation in the data was explained by PC1, the top 12 variables, by loading, were chosen from that axis, whereas the remaining three had the highest loading on PC2. These 15 variables were then used in a discriminant function analysis (DFA) in an effort to see whether they could predict, and therefore explain, the six treatments tested. Variables that loaded heavily (greater than 0.3) on each axis of the DFA were considered most important in describing the kinematic changes that occurred across treatments (see Tables 1–6). Because fewer variables were included in the velocity and timing variable set, a PCA was not needed to reduce dimensionality and variables were loaded directly into the DFA. Finally, because the DFA does not allow us to determine which treatments separate significantly from each other, we performed a one-way ANOVA on each DFA axis using treatments as the grouping variable and DFA scores as the dependent variable (see Tables 7, 8).

When variables from both limbs were combined into a single analysis, variation caused by differences between the limbs overwhelmed the majority of the variation within each limb. Therefore, to increase resolution of variables causing within-limb variation, forelimb and hindlimb variables were analyzed separately. For the combined analysis, to better visualize the changes that occurred with changes in perch diameter, values obtained on the small diameter perches were subtracted from the values on the flat perches for each individual. Therefore, in the combined analyses, variables for which values were greater than zero represented variables that had greater values on flat diameters, whereas those with values less than zero were variables that had greater values on small diameters.

RESULTS
General description and overall differences in forelimb and hindlimb kinematics

At FF, the femur was protracted 30 deg or more, depressed between 2 and 16 deg, and rotated clockwise by at least 20 deg (Fig. 2). The knee was generally anterior and ventral to the hip and was extended more than 90 deg on flat perches, but was flexed to 50–60 deg on small diameters. Ankle angle was, on average, obtuse on flat perches and acute on small diameters. The fourth toe was always extended more than 120 deg at FF (Fig. 2).

Table 1. Loadings from a discriminant function (DF) analysis (F=1.34, P=0.21) of the difference in joint angles between flat and small diameter treatments in the forelimb and hindlimb of *Anolis carolinensis*

Variable	DF1 (52.4%)	DF2 (25.6%)
Min. pectoral/pelvic rotation	**–0.30**	–0.07
Ex. pectoral/pelvic rotation	0.20	–0.16
Humerus/femur retraction angle (ES)	**0.58**	**0.43**
Ex. humerus/femur retraction angle	0.14	–0.08
Min. humerus/femur depression angle	**0.50**	0.18
Max. humerus/femur depression angle	**0.33**	–0.01
Humerus/femur depression angle (FF)	0.00	**–0.34**
Humerus/femur depression angle (ES)	**0.81**	**0.36**
Ex. humerus/femur depression angle	0.19	–0.11
Min. humerus/femur rotation angle	**–0.71**	**–0.58**
Max. humerus/femur rotation angle	**–0.55**	**–0.57**
Max. elbow/knee angle	**0.67**	–0.05
Elbow/knee angle (ES)	**0.72**	0.16
Wrist/ankle angle (FF)	**–0.75**	0.10
Ex. wrist/ankle angle	–0.01	0.14

Loadings with a magnitude ≥0.3 are in bold.
Positive loadings indicate angles that are greater on flat perches than on small diameter perches, whereas negative loadings indicate angles that are greater on small diameter perches than on flat perches.
Percentages of variation explained by each DF axis are indicated in parentheses.
FF, footfall; ES, end of stance; Min., minimum; Max., maximum; Ex., excursion (maximum – minimum).

Table 3. Loadings from a discriminant function (DF) analysis (F=1.52, P=0.13) of joint angles in the forelimb of *Anolis carolinensis* in response to flat and small diameter perches at 0, 45 and 90 deg

Variable	DF1 (60.7%)	DF2 (20.6%)
Min. shoulder height	**0.37**	–0.07
Max. shoulder height	**0.45**	–0.08
Humerus retraction angle (ES)	–0.07	–0.18
Min. humerus depression angle	**–0.39**	–0.29
Max. humerus depression angle	**–0.45**	**–0.43**
Humerus depression angle (FF)	**–0.43**	**–0.37**
Ex. humerus depression angle	**–0.44**	**–0.44**
Min. humerus rotation angle	**–0.69**	–0.29
Max. humerus rotation angle	**–0.47**	**–0.37**
Humerus rotation angle (FF)	**–0.42**	**–0.32**
Humerus rotation angle (ES)	**–0.36**	**–0.39**
Min. elbow angle	**0.73**	0.03
Min. wrist angle	**0.56**	**0.44**
Ex. wrist angle	**–0.54**	**–0.41**
Toe angle (FF)	–0.28	**–0.35**

Loadings with a magnitude ≥0.3 are in bold.
Percentages of variation explained by each DF axis are indicated in parentheses.
FF, footfall; ES, end of stance; Min., minimum; Max., maximum; Ex., excursion (maximum – minimum).

At FF, the humerus was generally protracted slightly, more depressed (between 12 and 43 deg, on average) than the femur, and rotated counter-clockwise by at least 45 deg. The elbow was generally anterior and ventral to the shoulder at FF and the elbow, wrist and third toe were all extended beyond 90 deg at FF (Fig. 2).

The wrist and ankle were located in a position lateral to the elbow and knee, respectively, on flat perches, but the ankle was placed more medially under the knee on small diameters. The third metacarpal, fourth metatarsal and toes were consistently oriented laterally relative to the long axis of the perch throughout the stride on the level surfaces, regardless of perch diameter, but they became oriented more medially, in line with the wrist/ankle, on the small diameter at steeper inclines, especially at 90 deg.

The humerus and femur retracted during the majority of stance, usually achieving maximum retraction at ES, although the humerus often had an additional period of retraction immediately following toe-off. Both the humerus and the femur underwent clockwise long-axis rotation through the entire duration of stance but the humerus rotated over a greater range than the femur (83.96±3.18 and 31.23±1.64 deg, respectively). The femur usually achieved greatest rotation at ES, but the humerus achieved maximal rotation 5–10% of the stride before the ES. Humeral rotation and retraction were generally faster (0.29±0.02 and 0.75±0.12 deg s^{-1}, respectively) than for the femur (0.13±0.01 and 0.35±0.03 deg s^{-1}, respectively). The femur usually depressed during the first half of stance before being elevated almost back to the original position during the second half of stance. The

Table 2. Loadings from a discriminant function (DF) analysis (F=2.27, P=0.0032) of the difference in angular velocities between flat and small diameter treatments in the forelimb and hindlimb of *Anolis carolinensis*

Variable	DF1 (85.2%)	DF2 (7.2%)
Stride frequency	**0.53**	**0.50**
Duty factor	**0.69**	**–0.38**
Relative swing velocity	**0.80**	–0.28
Humerus/femur retraction velocity	–0.14	–0.07
Humerus/femur depression velocity	**–0.45**	0.26
Humerus/femur rotation velocity	–0.05	**0.43**
Elbow/knee angle flexion velocity	–0.14	**0.63**
Elbow/knee angle extension velocity	–0.18	**0.78**
Elbow/knkle angle extension velocity	0.11	**0.54**
Toe angle extension velocity	**–0.35**	–0.11

Loadings with a magnitude ≥0.3 are in bold.
Positive loadings indicate angles that are greater on flat perches than on small diameter perches, whereas negative loadings indicate angles that are greater on small diameter perches than on flat perches.
Percentages of variation explained by each DF axis are indicated in parentheses.

Table 4. Loadings from a discriminant function (DF) analysis (F=1.72, P=0.035) of angular velocities in the forelimb of *Anolis carolinensis* in response to flat and small diameter perches at 0, 45 and 90 deg

Variable	DF1 (70.4%)	DF2 (19.3%)
Stride frequency	**0.49**	**–0.32**
Duty factor	**0.56**	–0.03
Relative swing velocity	**0.91**	0.09
Humerus retraction velocity	0.18	**0.59**
Humerus depression velocity	**–0.44**	0.14
Humerus rotation velocity	**0.39**	–0.03
Elbow angle flexion velocity	0.04	0.28
Elbow angle extension velocity	**0.54**	0.04
Wrist angle extension velocity	0.26	0.09
Toe angle extension velocity	–0.05	0.05

Loadings with a magnitude ≥0.3 are in bold.
Percentages of variation explained by each DF axis are indicated in parentheses.

Table 5. Loadings from a discriminant function (DF) analysis (F=2.90, P=0.0026) of joint angles in the hindlimb of *Anolis carolinensis* in response to flat and small diameter perches at 0, 45 and 90 deg

Variable	DF1 (76.0%)	DF2 (14.6%)
Relative stride length (SVL)	**0.87**	−0.29
Relative step length (SVL)	**0.91**	−0.26
Femur retraction angle (ES)	**−0.37**	**0.74**
Min. femur depression angle	**−0.51**	**0.50**
Max. femur depression angle	**−0.43**	**0.64**
Femur depression angle (FF)	**−0.53**	**0.46**
Femur depression angle (ES)	**−0.46**	**0.67**
Ex. femur depression angle	**−0.35**	**0.68**
Min. femur rotation angle	0.28	**−0.73**
Max. femur rotation angle	**0.42**	**−0.63**
Femur rotation angle (FF)	0.23	**−0.66**
Femur rotation angle (ES)	**0.45**	**−0.67**
Max. knee angle	0.12	**0.35**
Knee angle (FF)	**0.67**	**−0.50**
Ankle angle (FF)	**0.43**	**−0.49**

Loadings with a magnitude ≥0.3 are in bold.

Percentages of variation explained by each DF axis are indicated in parentheses.

FF, footfall; ES, end of stance; Min., minimum; Max., maximum; Ex., excursion (maximum − minimum).

humerus, however, was elevated for most of stance, reaching a more elevated position at ES (12.46±1.24 deg below horizontal) compared with FF (29.57±3.21 deg below horizontal; Fig. 2).

The femur and the humerus protracted and rotated counter-clockwise through most of swing and the knee and elbow flexed and then extended in the first and second halves of swing. However, the ankle and the fourth hind-toe spent most of swing flexing with a brief extension at the end of swing, contrasting with the wrist and third toe of the forelimb, which generally maintained constant angles or extended during swing. The femur depressed during the first half of swing and elevated during the second half, causing the knee to describe an arc as the hindlimb came into position at the end of swing. The elbow, however, tended to trace a linear path dorsally and anteriorly as the humerus elevated through swing (Fig. 2). Swing phase velocity was slower in the forelimb (14.56±1.13 SVL s^{-1}) than in the hindlimb (18.36±1.01 SVL s^{-1}).

Table 6. Loadings from a discriminant function (DF) analysis (F=2.40, P=0.0018) of angular velocities in the hindlimb of *Anolis carolinensis* in response to flat and small diameter perches at 0, 45 and 90 deg

Variable	DF1 (47.2%)	DF2 (28.6%)
Stride frequency	−0.08	**0.61**
Duty factor	−0.02	**−0.54**
Relative swing velocity (limb length/s)	**0.75**	−0.19
Femur retraction velocity	**0.39**	**0.75**
Femur depression velocity	−0.18	**0.62**
Femur rotation velocity	0.18	**0.78**
Knee angle flexion velocity	**−0.64**	**0.47**
Knee angle extension velocity	−0.10	**0.85**
Ankle angle extension velocity	0.10	**0.63**
Toe angle extension velocity	**0.45**	**−0.40**

Loadings with a magnitude ≥0.3 are in bold.

Percentages of variation explained by each DF axis are indicated in parentheses.

Effects of surface diameter and incline

With the exception of the combined and forelimb DFAs of the angular variables (Fig. 3A,C), all DFAs were significant (Tables 1–6). However, as only one misclassification occurred (the hindlimb of one individual running at 90 deg was classified with the 0 deg treatment in the combined DFA), we have confidence in the ability of the chosen variables to define treatments.

Decreasing perch diameter significantly affected more variables than increasing incline (41 and 28 variables affected by diameter and incline, respectively; Fig. 3C–F, Tables 3–6). In addition, the kinematics of the proximal joints (shoulder/hip, humerus/femur) were more affected than distal joints (40/50 and 19/40 of proximal and distal variables, respectively; chi-squared goodness-of-fit test, Pearson χ^2=13.03, d.f.=1, P=0.0003) and, in many instances, there were opposite trends in kinematics between the limbs (see below).

Changes in forelimb kinematics with perch diameter and incline

Forelimb stride frequency increased on the small diameter perch at 0 deg (flat: 7.79±1.69 Hz, small: 9.44±0.77 Hz) and 45 deg (flat: 6.56±0.85 Hz, small: 7.53±0.21 Hz), but decreased at 90 deg (flat: 6.08±0.87 Hz, small: 5.63±0.80 Hz; Fig. 3B,D). Forelimb swing phase velocity was significantly faster on the small perch (17.09±1.85 SVL s^{-1}) than on the flat perch (12.04±0.87 SVL s^{-1}), resulting in increased duty factor (small: 0.68±0.02, flat: 0.54±0.05; Fig. 3B,D). The shoulders were significantly closer to the surface and rotated slightly more on the smaller diameter perch (min. height: 4.27±0.87 mm, min. rotation: −37.51±3.89 deg) than on the flat perch (min. height: 8.53±0.66 mm, min. rotation: −37.45±2.03 deg; Fig. 3A,C).

Long-axis humeral rotation, but not retraction, was significantly greater on the smaller diameter perch (max. rotation: 29.58±6.25 deg, ES retraction: 54.78±4.16 deg) than on the flat perch (max. rotation: −0.12±4.96 deg, ES retraction: 53.81±3.45 deg), especially at 45 deg (Fig. 3A,C). Depression and vertical excursion were greater on the small perch diameter (max: 51.05±5.57 deg, excursion: 44.78±4.22 deg) than on the flat surface (max: 25.89±1.56 deg, excursion: 24.97±1.32 deg), especially at 45 deg, and elevation generally increased with incline on flat surfaces (Fig. 3C). Retraction of the humerus was faster on the smaller perch (0.77±0.168 deg s^{-1}) than on the flat perch (0.72±0.18 deg s^{-1}), and at 45 deg (1.14±0.258 deg s^{-1}) than at the other two inclines (0 deg: 0.72±0.108 deg s^{-1}, 90 deg: 0.38±0.158 deg s^{-1}). The humerus elevated faster on the small diameter perch than on the flat perch at 0 deg (small: −0.28±0.058 deg s^{-1}, flat: −0.23±0.048 deg s^{-1}) and 45 deg (small: −0.21±0.048 deg s^{-1}, flat: −0.15±0.018 deg s^{-1}), but slower at 90 deg (small: −0.15±0.058 deg s^{-1}, flat: −0.15±0.038 deg s^{-1}; Fig. 3B,D).

Elbow flexion was greater on the small diameter perch than on the flat surface (Table 9). Similarly, wrist flexion and angular excursion were greater on the small diameter perch than on the flat surface and at 45 deg than at the other two inclines (Table 9). However, wrist extension at FF was greater on the small diameter perch than on the flat surface (Table 9). Lastly, MCP extension at FF was greater on the small diameter perch at 45 deg (166.14±1.28 deg) than in all other treatments and slower on the small diameter perch than on the flat surface at 0 deg (small: 1.55±0.318 deg s^{-1}, flat: 2.14±0.438 deg s^{-1}) and 45 deg (small: 0.97±0.088 deg s^{-1}, flat: 1.48±0.188 deg s^{-1}; Fig. 3B,D).

Changes in hindlimb kinematics with perch diameter and incline

Hindlimb stride frequency was greater on the small diameter perch compared with the flat surface at 0 deg (small: 9.44±0.94 Hz, flat:

Table 7. Results of one-way ANOVAs showing significant separation of treatments on each axis of forelimb and hindlimb DFAs

Treatment	Forelimb DFA joint angles		Forelimb DFA angular velocities		Hindlimb DFA joint angles		Hindlimb DFA angular velocities	
	$DF1_{76.45}$	$DF2_{25.93}$	$DF1_{38.5}$	$DF2_{10.58}$	$DF1_{277.68}$	$DF_{53.23}$	$DF1_{25.69}$	$DF2_{15.56}$
Flat; 0 deg	A	B	B	B,C	B	B	B,C	C
Flat; 45 deg	B	B	B	A	A	B	B,C	C
Flat; 90 deg	A	A,B	B	C	C	B	A	B,C
Small; 0 deg	D	A	A	C	E	B	C	A
Small; 45 deg	C	C	A	A,B	C	A	B	A
Small; 90 deg	B	B	B	B,C	D	B	A	A,B

Subscripts are *F*-values, d.f=5,23, *P*<0.0001 for all ANOVAs.
Treatments that do not share letters are significantly different.

8.94±0.83 Hz) and 45 deg (small: 7.44±0.26 Hz, flat: 6.40±0.81 Hz), but was generally lower on the small diameter perch at 90 deg (small: 5.48±0.95 Hz, flat: 6.08±0.90 Hz; Fig. 3B). Both stride and step lengths decreased on the small diameter perch (stride length: 0.96±0.03 SVL, step length: 0.59±0.01 SVL) compared with the flat surface (stride length: 1.28±0.09 SVL, step length: 0.80±0.04 SVL; Fig. 3E). Hindlimb swing phase velocity was slower on the small diameter perch (15.98±0.80 SVL s^{-1}) than on the flat surface (20.74±1.62 SVL s^{-1}) and duty factor was lower on the small diameter perch (small: 0.61±0.02, flat: 0.64±0.02; Fig. 3B,F). Also, pelvic girdle rotation was greater on the small diameter perch (min. rotation: –30.94±3.87 deg) than on the flat surface (min. rotation: –24.76±2.12 deg; Fig. 3A).

Femur retraction was greatest, and rotation was smallest, on the small diameter perch at 45 deg (ES retraction: 68.58±4.43 deg, min. rotation: 12.48±8.17 deg; Fig. 3A,E). Femur depression and vertical excursion were greater on the small diameter perch (max. depression: 42.10±4.71 deg, excursion: 30.12±3.07 deg) than on the flat surface (max. depression: 13.26±0.81 deg, excursion: 12.97±0.79 deg), especially at 45 deg (Fig. 3A,E). Femur elevation was generally greater with incline on flat surfaces (Fig. 3A). In addition, the femur rotated, retracted and depressed faster on the small diameter perch (rotation: 0.13±0.018 deg s^{-1}, retraction: 0.38±0.048 deg s^{-1}, depression: 0.20±0.038 deg s^{-1}) than on the flat surface (rotation: 0.12±0.028 deg s^{-1}, retraction: 0.32±0.038 deg s^{-1}, depression: 0.08±0.018 deg s^{-1}; Fig. 3B,F).

Knee extension was greater on the small diameter perch than on the flat surface (Table 9) and at ES was greatest on the small diameter perch at 45 deg (137.81±6.51 deg; Fig. 3A,E). However, knee flexion at FF was greater on the small diameter perch (Table 9; Fig. 3A,E). The knee extended faster, but flexed slower on the small diameter perch than on the flat surface and flexed slower at 90 deg than at

Table 8. Results of one-way ANOVAs showing significant separation of treatments on each axis of combined DFAs

Treatment	Combined DFA joint angles		Combined DFA angular velocities	
	$DF1_{54.49}$	$DF2_{26.62}$	$DF1_{85.86}$	$DF2_{7.24}$
Forelimb; 0 deg	A	B,C	C	A,B,C
Forelimb; 45 deg	B	A	C	A,B
Forelimb; 90 deg	B	A	A,B	A
Hindlimb; 0 deg	C	A	B	C
Hindlimb; 45 deg	C	C	B	B,C
Hindlimb; 90 deg	C	B	A	A,B

Subscripts are *F*-values, d.f.=5,23, *P*<0.001 for all ANOVAs.
Treatments that do not share letters are significantly different.

the other two inclines (Table 9; Fig. 3E). In addition, ankle flexion at FF was greater on the small diameter perch compared with the flat surface (Table 9; Fig. 3A,E) and was greatest at 45 deg (67.67±5.87 deg). Lastly, the MTP extension was slower on the small diameter perch than on the flat surface at 0 deg (small: 0.25±0.028 deg s^{-1}, flat: 0.74±0.208 deg s^{-1}) and 45 deg (small: 0.15±0.128 deg s^{-1}, flat: 0.54±0.148 deg s^{-1}), but not at 90 deg (small: 0.12±0.028 deg s^{-1}, flat: 0.12±0.028 deg s^{-1}; Fig. 3B,F).

DISCUSSION

Anolis carolinensis adopted strategies for dealing with changes in incline and perch diameter similar to those of other vertebrates, increasing stride frequency to increase dynamic stability, and increasing limb flexion to lower the CoM and reduce torque and the tendency to topple (Schmitt, 1994; Higham and Jayne, 2004a; Franz et al., 2005; Schmidt and Fischer, 2010; Gálvez-López et al., 2011; Lammers and Zurcher, 2011). However, kinematics generally differed between the forelimbs and the hindlimbs, and limb function was modulated differently in response to changes in habitat structure (Fig. 3). A key conclusion is that, when dealing with a small diameter perch, the forelimb and the hindlimb exhibited opposite kinematic trends (Fig. 3), suggesting that the propulsive mechanisms in anoles shift with external demand. Additionally, the humerus exhibited a greater range of motion than the femur in all treatments. This might allow the humerus to be more functionally plastic.

Of the functional demands that we manipulated in this study, perch diameter had the greatest impact on limb kinematics. Interestingly, of the variables affected by incline, the majority revealed a difference between the 45 deg treatment and the other two treatments (0 and 90 deg), which were similar. The proximal joints (shoulder/hip and humerus/femur) appeared to be primarily responsible for these changes in kinematics across treatments. Despite the added energetic cost associated with moving up inclined surfaces (Taylor et al., 1972; Farley and Emshwiller, 1996; Roberts et al., 1997), the greater impact of perch diameter on kinematics in our study may be explained by the constraints that narrow surfaces impose on foot placement.

Forelimbs and hindlimbs in vertebrate locomotion

Independent of treatment, the humerus of *A. carolinensis* in our study was protracted more and exhibited a greater range of rotation than the femur, which remained rotated in a more clockwise orientation than the humerus. In addition, the wrist was extended more than the ankle. These angular differences may be explained by anatomical differences between the limbs (Humphry, 1876; Russell and Bauer, 2008) and girdles (Haines, 1952; Snyder, 1954; Peterson, 1971; Peterson, 1973; Peterson, 1974; Jenkins and Goslow, 1983; Peterson,
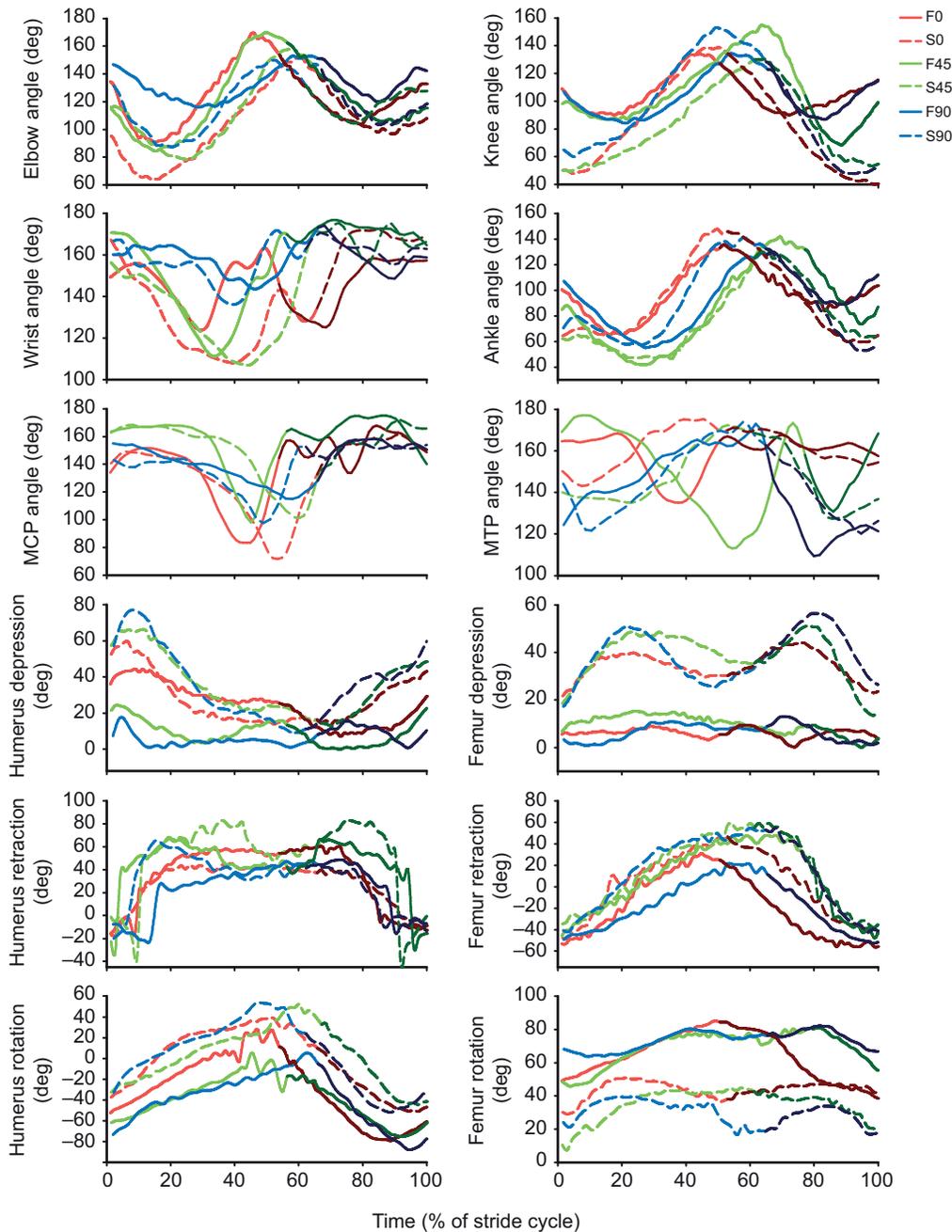
Fig. 2. Angles of forelimb (left) and hindlimb (right) joints *versus* time (as a percentage of stride cycle) for a representative stride in *Anolis carolinensis*. Red, 0 deg; green, 45 deg; blue, 90 deg; F/solid lines, flat perch; S/dashed lines, small diameter perch. Footfall begins at 0% and the transition from brighter to darker shades indicates the end of stance.

1984; Reynolds, 1985; Schmitt, 1994; Zihlman et al., 2011). The forelimb girdle of habitual climbers is adapted to allow a greater range of motion to meet the demands for flexibility in arboreal habitats (Reynolds, 1985; Zihlman et al., 2011). Anoles and chameleons lack or modify the attachment of the clavicle, which braces the anterior edge of the pectoral girdle in terrestrial species, and possess girdle musculature oriented to facilitate rotation and antero-posterior translation of the girdle (Peterson, 1971; Peterson, 1973; Peterson, 1974; Peterson, 1984). Rotation and translation of the scapulocoracoid, in addition to a sagitally oriented coracosternal orientation and modified glenoid cavity, allows a greater degree of humerus protraction/retraction and long-axis rotation than is possible in the femur (Jenkins and Goslow, 1983).

Terrestrial vertebrates generally exhibit a division in function between the forelimbs (braking) and the hindlimbs (propulsion)

(Deban et al., 2012). Kimura et al. (Kimura et al., 1979) argued that primates were hindlimb driven whereas non-primate mammals were forelimb driven, linking these differences in function with differences in placement of the CoM (posterior in primates and anterior in non-primate mammals). However, Demes et al. (Demes et al., 1994) found that differences in CoM position translated into differences in peak vertical forces, not propulsive forces. With few exceptions, forelimbs exert net braking forces whereas hindlimbs exert net propulsive forces in a variety of primates and non-primate mammals [e.g. horses, cats, dogs and chipmunks (Demes et al., 1994; Lammers and Biknevicius, 2004)]. Similarly, lizard hindlimbs are often the primary propulsors on terrestrial surfaces (e.g. Autumn et al., 2006), resulting in few studies examining forelimb function (Russell and Bels, 2001a). Despite the functional dichotomy between forelimbs and hindlimbs in terrestrial systems, the relative
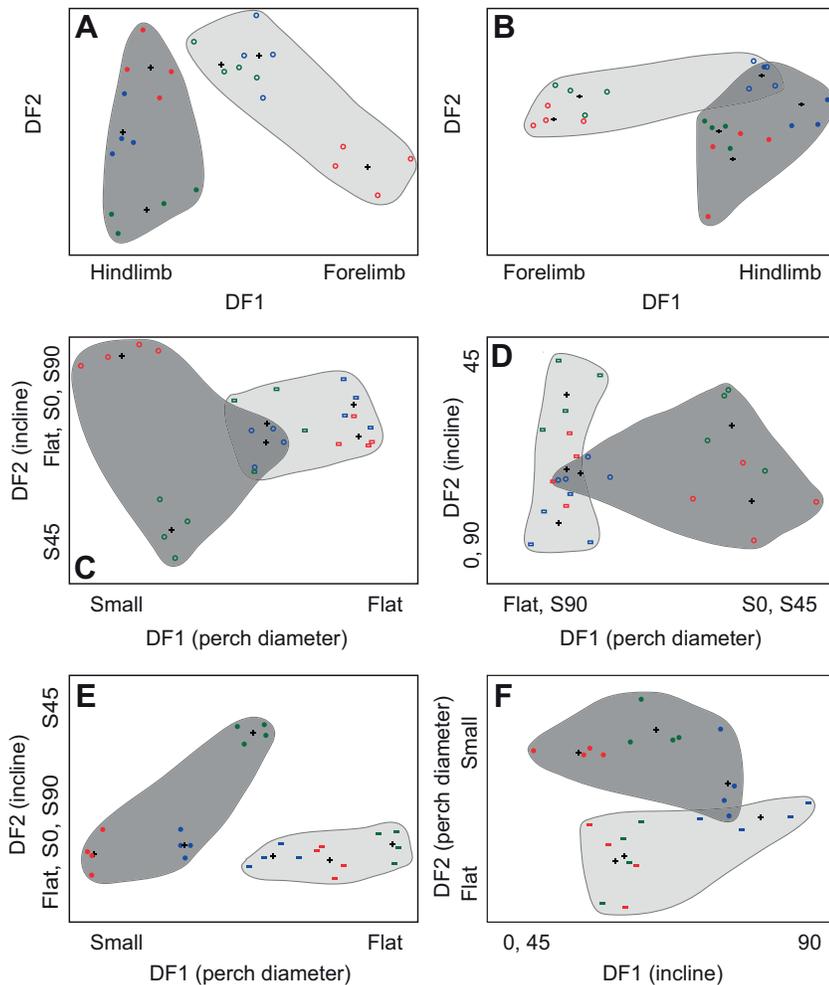
Fig. 3. First two axes of discriminant function (DF) analyses of combined forelimb and hindlimb joint angles (A) and angular velocities (B), forelimb joint angles (C) and angular velocities (D), and hindlimb joint angles (E) and angular velocities (F) of *Anolis carolinensis*. The mean of each treatment is indicated by a + symbol. For A and B, each point represents the difference between the flat and small perch diameter treatments, averaged across strides for each individual, at 0 deg (red), 45 deg (green) and 90 deg (blue) inclines. Variables loading heavily on the same side of the axis as the points on the DFA indicate inclines at which angles are greater on the flat perch than on the small diameter perch. Forelimb and hindlimb points are shaded in light and dark gray, respectively. Canonical loadings on each axis can be seen in Tables 1 and 2. Open circles, forelimb; closed circles, hindlimb. For C–F, points represent individuals (strides averaged within each treatment) on flat (rectangles) and small diameter (circles) perches at 0 deg (red), 45 deg (green) and 90 deg (blue) inclines. Flat and small diameter points are shaded in light and dark gray, respectively. Canonical loadings on each axis can be seen in Tables 3 and 4 (C,D) and Tables 5 and 6 (E,F).

importance of forelimbs for propelling an animal commonly increases in arboreal situations to assist in overcoming the greater propulsive challenges of the system (Arnold, 1998; Zaaf et al., 1999; Autumn et al., 2006). The greater flexibility, anatomically and kinematically, of the forelimb of arboreal specialists, may make it a particularly effective structure for propulsion and stabilization in complex arboreal situations, where a greater range of motion is

beneficial (Reynolds, 1985; Zihlman et al., 2011). Because pulling the CoM would assist in keeping the animal against arboreal surfaces, propulsion from the forelimbs likely increases locomotor stability relative to the pushing motion seen in hindlimbs. Although it appears from our study that anoles exhibit greater kinematic flexibility of forelimb compared with the hindlimb, measurements of forces exerted by *A. carolinensis* running on a range of inclines

Table 9. Selected significant variables for distal joints in the forelimb and hindlimb of *Anolis carolinensis*

	Perch diameter		Incline		
	Small	Flat	0 deg	45 deg	90 deg
Forelimb					
Min. elbow angle	74.81±3.16	87.7±2.71			
Min. wrist angle	112.79±3.72	62.18±3.72	118.40±3.86	109.05±3.04	124.86±3.60
Ex. wrist angle	122.08±2.37	53.99±2.70	56.01±3.94	67.42±2.85	50.82±3.57
Wrist angle (FF)	163.54±1.61	157.10±6.99			
Max. knee angle	137.67±3.06	135.62±3.89			
Hindlimb					
Knee angle (FF)	52.74±2.26	96.35±4.78			
Knee extension velocity	0.37±0.04	0.29±0.03			
Knee flexion velocity	−0.13±0.01	−0.28±0.06	−0.30±0.09	−0.18±0.03	−0.15±0.02
Ankle angle (FF)	72.83±4.03	101.67±5.04			

Angles and angular velocities are given in deg and deg s^{-1}.
FF, footfall; Min., minimum; Max., maximum; Ex., excursion.
Values are means ± s.e.m.

and perch diameters are needed to confirm the shift in the propulsive roles of the forelimbs and hindlimbs.

Changes with incline and perch diameter

Climbing up steeper surfaces or narrower branches presents a number of functional challenges for arboreal species. Steeper inclines increase resistance to locomotion by increasing the proportion of gravity acting parallel to the surface and reducing the proportion holding the animal against the substrate (Cartmill, 1985; Preuschoft, 2002). In addition, the gravitational force acting on the animal is shifted downwards, towards the hindlimb when climbing and towards the forelimb when descending head-first (Preuschoft, 2002). As a result of this weight shift, individual leg function changes. On level surfaces, forelimbs of a wide range of animals exert net braking forces and posterior limbs push against the substrate towards the midline of the body (Full et al., 1991; Demes et al., 1994; Lammers and Biknevicius, 2004; Schmitt and Bonnono, 2009). On inclines, however, substrate reaction force data indicate that all four limbs pull the body upwards, towards the point of contact between the feet and the substrate (Autumn et al., 2006; Goldman et al., 2006; Schmitt and Bonnono, 2009). Thus, the relative propulsive contribution of the forelimb is dependent, at least partially, on the orientation of the animal, likely increasing in importance with increasing slope.

Perches of narrow diameter increase the likelihood of toppling because the sloped sides and the narrow base of support increase the proportion of the gravitational force acting tangentially to the perch, creating a toppling moment that increases with deflection of the CoM away from the perch (Preuschoft, 2002; Lammers and Biknevicius, 2004; Lammers and Gauntner, 2008). Although claws and adhesive structures of lizards help grip and maintain contact with the surface in the face of these challenges (Zani, 2000), both sets of limbs must undergo changes in posture and function to contribute to overcoming the greater challenges for propulsion and stability. One way arboreal animals can circumvent, to some extent, the negative impacts of a smaller diameter substrate involves placing the foot more laterally on the perch. This increases the angle of the arc subtended by the limbs, reducing the tangential component of the adduction force and increasing the normal component, aiding with grip maintenance (Cartmill, 1985; Schmidt and Fischer, 2010). However, lateral foot placement in small mammals correlates with a reduced propulsive component of force because a greater proportion of force is directed medially to maintain grip (Lammers and Biknevicius, 2004; Lammers, 2007; Schmidt and Fischer, 2010; Schmidt and Fischer, 2011). Forelimbs can assume a greater propulsive role than the hindlimbs on small diameter perches [opossums (Lammers and Biknevicius, 2004)] or on inclines [geckos (Autumn et al., 2006); opossums (Lammers, 2007)]. Our study supports the idea that forelimbs become increasingly important for

propulsion in arboreal circumstances; *A. carolinensis* placed the hindlimb laterally on narrow perches, maintaining a medial forelimb position, indicating that the forelimb may adopt a more propulsive role while the hindlimb assists in stabilization (Lammers and Biknevicius, 2004; Schmidt and Fischer, 2010). Although lateral foot placement indicates an increased role in stabilization in the hindlimb, a number of other variables that contribute to stability (e.g. swing phase velocity) did not change as expected (Lammers and Biknevicius, 2004; Franz et al., 2005; Lammers, 2007; Gálvez-López et al., 2011). However, whether the functional correlation between foot position and role in propulsion is similar in mammals and lizards remains to be investigated.

Anolis carolinensis modulated its kinematics to aid with stability in several ways. Forelimb stride frequency, duty factor and swing phase velocity were all greater on the small diameter perch compared with the flat surface (Fig. 3B,D). Combinations of these strategies have been observed in a number of other vertebrates moving on small diameter surfaces and are thought to increase dynamic stability and reduce peak vertical forces by applying force over a greater proportion of the stride (Lammers and Biknevicius, 2004; Franz et al., 2005; Lammers, 2007; Schmidt and Fischer, 2010; Gálvez-López et al., 2011; Lammers and Zurcher, 2011). In addition, *A. carolinensis* increased both elbow and knee flexion to decrease the height of the shoulder and hip above the surface (Fig. 3C,E). However, the decrease in hip height on small diameters was not significant, likely because femur depression also increased to assist in lateral placement of the hind feet on the perch (Fig. 3A). Limb flexion lowers the CoM, decreasing the gravitational component acting to destabilize or slow locomotion on both inclines and narrow perches (Cartmill, 1985; Arnold, 1998). This also allows a greater proportion of force to act parallel to the surface and aid in propulsion, and may reduce peak vertical forces by reducing vertical oscillations of the CoM, a factor that becomes especially important on compliant surfaces (Schmitt, 1994; Arnold, 1998; Lammers and Biknevicius, 2004; Gálvez-López et al., 2011). However, it is also possible that greater knee flexion has a negative impact on limb muscle function by shifting the operating lengths of the muscles. Future work examining how muscle function in *Anolis* lizards responds to changes in habitat structure would reveal any shifts in function.

Increased limb flexion reduces effective limb length and thus has a negative impact on step length and stance duration; further kinematic adjustments occurring at the shoulder and hip joints may help mitigate this in *A. carolinensis*. We found significant increases in humerus rotation, but decreases in humerus retraction on small diameter perches (Fig. 3A,C, Fig. 4). In contrast, femur rotation decreased while retraction increased (Fig. 3A,E, Fig. 4). Increasing rotation and retraction are two mechanisms that can contribute to increasing step length in lizards [for a general discussion see Russell
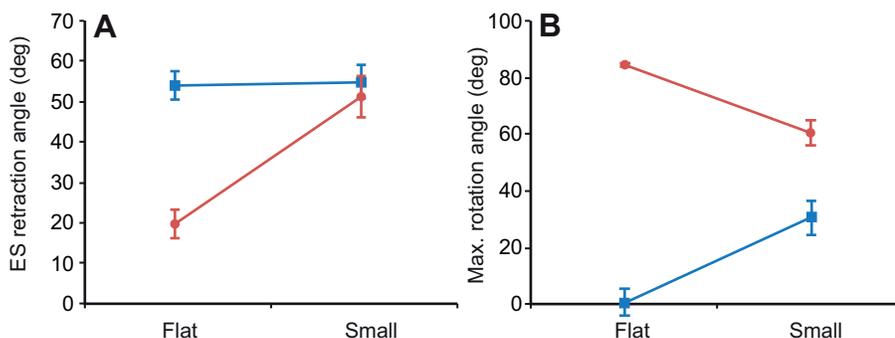

Fig. 4. Mean end of stance retraction angle (A) and maximum rotation angle (B) of the humerus (blue) and femur (red) of *Anolis carolinensis* on flat and small diameter perches. Variables chosen were identified as significant in discriminant function analyses (Fig. 3). ES, end of stance. Max., maximum. Values are means ± s.e.m.

and Bauer (Russell and Bauer, 2008)]. It is interesting, however, that the forelimbs and hindlimbs in *A. carolinensis* appear to use different mechanisms to overcome the reduction in effective limb length. The forelimb is capable of a greater range of motion, especially long-axis humerus rotation, because of anatomical specialization of the *Anolis* pectoral girdle that is not seen in the pelvic girdle (Peterson, 1971; Peterson, 1973; Peterson, 1974). In addition, the pectoral girdle rotated more on the small diameter perch compared with the flat surface, allowing a further increase in long-axis humerus rotation. This contrasts with the reduction in long-axis femur rotation on the small diameter perch despite the increase in pelvic rotation (Fig. 3A). Although increased pelvic rotation may contribute to instability by increasing lateral displacement of the CoM (Peterson, 1984; Preuschoft, 2002; Lammers and Gauntner, 2008), increasing pelvic rotation may be important to increase antero-posterior excursion of the femur (Peterson, 1984; Fischer et al., 2010). In contrast, rotation of the pectoral girdle is unlikely to have as great an effect on stability and therefore may allow a greater reliance on rotation as the primary forelimb propulsive mechanism. Further, excessive forelimb retraction may increase the possibility of interference between forelimb at ES and the ipsilateral hindlimb at FF because these events often occur at approximately the same time.

The variables impacted by the small diameter perch were exaggerated at 45 deg, resulting in a distinction between the 45 deg treatment and the 0 and 90 deg treatments. The humerus rotated most at 45 deg while the femur further reduced long-axis rotation, but both the humerus and femur depressed more on this incline (Fig. 3A,C,E). Greater depression of the humerus and femur may increase the range of antero-posterior as well as rotational movement by altering the orientation of these limb segments in the glenoid/acetabular cavities (Peterson, 1973). Thus, when combined with increases in wrist extension at FF, humerus rotation, knee extension at ES, and ankle flexion at FF, greater humerus and femur depression may indicate an effort to increase step length at 45 deg. Compared with the 45 deg treatment, the range of limb movement appears to be more restricted at 0 and 90 deg, suggesting possible behavioral and/or biomechanical constraints on step length on these two inclines. Therefore, 45 deg may be a preferable incline for effective locomotion in *A. carolinensis*, although this species appears to use inclines randomly in its environment, showing no particular preference for a specific incline (Mattingly and Jayne, 2004). Determining whether the 45 deg treatment results in optimal limb function requires further investigation.

Climbing in *Anolis* ecomorphs

Changes in perch diameter and incline resulted in similar changes in hindlimb kinematics in *A. carolinensis* and *A. sagrei* (Spezzano and Jayne, 2004). *Anolis sagrei* was affected more by perch diameter than by incline, the femur retracted and depressed more, the knee flexed more, contributing to a decrease in hip height, and stride and step lengths decreased on small diameters (Spezzano and Jayne, 2004). However, unlike *A. carolinensis*, *A. sagrei* increased femur rotation with decreasing perch diameter and increasing incline (Spezzano and Jayne, 2004). Further, pelvic rotation was not affected by perch diameter in *A. sagrei* (Spezzano and Jayne, 2004). Average minimum, maximum and excursion of femur retraction appeared to be similar or greater in *A. carolinensis* than *A. sagrei*, but there was considerably less long-axis femur rotation in *A. carolinensis*. Therefore, the reduction in long-axis femur rotation by *A. carolinensis* on small diameters may be the result of a preferential increase in femur retraction allowed by greater pelvic

rotation. That *A. sagrei* appears to maintain a similar pelvic rotation regardless of treatment may indicate a greater sensitivity to instability caused by lateral undulation on these small diameters. In addition, as limb length has been correlated with preference of perch diameter in *Anolis* (Losos, 1990a; Losos, 1990b; Mattingly and Jayne, 2004), it is unsurprising that the longer-legged *A. sagrei* [relative leg length ~84% of SVL (Spezzano and Jayne, 2004)], a trunk-ground species, appears to use greater perch diameters than are available on average in its environment (Mattingly and Jayne, 2004) and exhibits greater limb flexion on small diameters (Spezzano and Jayne, 2004) than *A. carolinensis* (relative leg length ~58% of SVL). However, *A. carolinensis*, a trunk-crown species, demonstrates an inconsistent preference for perch diameters, using substrates in proportion to what is available in its habitat in the Bahamas (Mattingly and Jayne, 2004), yet showing a preference for larger diameters in several habitats in Louisiana (Irschick et al., 2005a; Irschick et al., 2005b). Thus, *A. sagrei*'s preference for larger diameters may stem from longer relative leg lengths, which renders locomotion on narrower perches less stable, whereas the shorter relative leg lengths in *A. carolinensis* may facilitate the greater flexibility in habitat preference that is observed in some populations. As these considerations likely affect pelvic and hindlimb angles and excursions, locomotor kinematics may contribute to habitat use distinctions among *Anolis* ecomorphs.

Morphological differences between ecomorphs, especially in body size (ranging from 130 to 191 mm in crown giants and 33 to 51 mm in grass-bush species) and relative leg length (smallest in twig ecomorphs and longest in grass-bush and trunk-ground ecomorphs) have been related to sprinting and jumping performance, such that longer-legged species can sprint faster and jump further on larger diameters, but are more negatively affected by decreases in perch diameter than shorter-legged species (Losos and Sinervo, 1989; Losos, 1990a; Losos, 1990b; Irschick and Losos, 1999). Our data suggest possible kinematic explanations for these patterns. Similarly, shorter limbs keep the body closer to the substrate surface, which aids in stability (Schmitt, 1994; Higham and Jayne, 2004a; Franz et al., 2005; Schmidt and Fischer, 2010). Therefore, species with shorter limbs may be expected to exhibit fewer and less extreme changes in kinematics as diameter changes. This may explain, in part, the relatively consistent performance observed in species with shorter limbs (Losos and Sinervo, 1989).

Further experimentation on other ecomorphs is necessary to test these predictions and to clarify the underlying kinematic and biomechanical changes that explain the correlation between morphology and performance in this system. Furthermore, the forelimb has the potential to augment stabilization and/or propulsion during arboreal locomotion, potentially relieving functional restrictions in the hindlimb. Therefore, kinematic data of the forelimb of other ecomorphs is an essential component for understanding differences in arboreal locomotion and performance in *Anolis*.

Conclusions

Anolis carolinensis not only moves its forelimbs and hindlimbs differently under a specific condition, but also modulates forelimb and hindlimb function differently with changes in perch incline and diameter. Although the majority of these differences can be explained by anatomy, their functional consequences are less clear. We found that both forelimb and hindlimb angular velocities generally increased on the small diameter perch relative to the flat surface (the femur and humerus rotated and retracted faster, the elbow and knee extended faster, and the knee flexed faster;

Fig. 3B,D,F), but these faster angular velocities may have been caused by either increased muscle recruitment or passive collapse/extension of the joints caused by the shift in weight distribution as the body moved over the joints. To determine the relative contributions of the forelimbs and hindlimbs to propulsion, ground reaction force (GRF) data and patterns of *in vivo* muscle function are needed. GRF data from a variety of vertebrate taxa indicate that perch diameter and incline can affect whether the forelimb or the hindlimb adopts the primary propulsive role (Lammers and Biknevicius, 2004; Autumn et al., 2006; Lammers, 2007; Schmidt and Fischer, 2010). A single study has examined the neuromuscular responses to changes in arboreal structure, but it focused on a lizard (*Chamaeleo calyptratus*) with a highly specialized gripping mechanism (Higham and Jayne, 2004b). Higham and Jayne (Higham and Jayne, 2004b) found that the hindlimbs can be used to both pull (early stance) and push (latter half of stance) when moving up an incline. Whether this is common across lizards is not fully understood. In addition, different groups of lizards utilize very different morphological features during locomotion. For example, *Anolis* lizards employ an adhesive system, which is not actively modulated to the degree observed in other pad-bearing lizards (Russell and Bels, 2001b), and this might result in very different patterns of neuromuscular modulation. Understanding how this morphological diversity relates to the underlying physiological control of locomotion might reveal key functional axes of variation among groups of arboreal lizards. Therefore, further experimentation assessing both GRF patterns and *in vivo* muscle function is essential for understanding arboreal locomotion in lizards.

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