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Context-dependent changes in motor control and kinematics during locomotion: modulation and decoupling

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Successful locomotion through complex, heterogeneous environments requires the muscles that power locomotion to function effectively under a wide variety of conditions. Although considerable data exist on how animals modulate both kinematics and motor pattern when confronted with orientation (i.e. incline) demands, little is known about the modulation of muscle function in response to changes in structural demands like substrate diameter, compliance and texture. Here, we used high-speed videography and electromyography to examine how substrate incline and perch diameter affected the kinematics and muscle function of both the forelimb and hindlimb in the green anole (*Anolis carolinensis*). Surprisingly, we found a decoupling of the modulation of kinematics and motor activity, with kinematics being more affected by perch diameter than by incline, and muscle function being more affected by incline than by perch diameter. Also, muscle activity was most stereotyped on the broad, vertical condition, suggesting that, despite being classified as a trunk-crown ecomorph, this species may prefer trunks. These data emphasize the complex interactions between the processes that underlie animal movement and the importance of examining muscle function when considering both the evolution of locomotion and the impacts of ecology on function.

1. Introduction

Animals necessarily interact with their environment when performing activities necessary for survival. Perhaps the most important examples involve locomotion, which is almost always important for capturing prey, evading predators and interacting with conspecifics. However, the environment through which animals move is often highly heterogeneous. Therefore, in order to be successful, species must be able to effectively perform locomotor behaviours under a variety of conditions. Furthermore, as muscles are the contractile units that generate movement, they, too, must be able to function effectively to power diverse behaviours under variable conditions.

Although morphological properties of muscle, such as physiological cross-sectional area [1–4], fibre length [3–6] and moment arm [6–8], can impact overall muscle function, studies of the *in vivo* function of muscle are necessary to determine the actual role of muscles in generating observed movements. Although there is extensive support for a link between locomotor kinematics and motor control patterns in a variety of species [9–12], this relationship may not always hold, despite the fact that muscles often power locomotion. Changes in the activity of specific muscles may not always result in changes in the kinematics of the corresponding joint or limb segment if the muscle activity changes are used to counteract changes in external forces acting on the animal or changes in the activity of other antagonistic muscle groups. For example, despite ample evidence that increases in incline require significant increases in muscle work (either through increased muscle recruitment or length change; e.g. [13–15]), extensive changes in kinematics are not always observed [16]. Similarly, changes in kinematics that are necessitated by changes in the external environment, such as habitat structure, may not alter the

demand placed on the muscles. Thus, despite their dependent relationship, motor control patterns may not be affected when locomotor kinematics are determined by extrinsic rather than intrinsic factors. The additional complexity of this potential decoupling between kinematics and motor control patterns, as well as the potential influence of substrate structure on this decoupling, emphasizes the importance of simultaneous measurement of *in vivo* muscle activity and locomotor movements, especially in the context of varying environmental demand.

Most locomotor challenges that animals face as they move through heterogeneous habitats can be divided into two types, orientational and structural demand. The orientation of a substrate determines the relative impact of gravity on stability and forward locomotion and thus can profoundly impact the cost of locomotion and overall locomotor performance [17–20]. Whereas the timing of muscle activity is fairly consistent with changes in incline, muscle recruitment tends to increase with increasing incline [13–15]. However, there are many other kinds of demands in terrestrial habitats (e.g. perch diameter, substrate rugosity and texture, compliance, three-dimensional clutter), all of which can be placed into the broad category of structural demands. Although kinematics and kinetics have been shown to change in response to at least some of these structural demands [16,21,22], how these types of challenges impact motor patterns is poorly understood.

Anolis, containing nearly 400 species, is among the best studied of lizard genera and has become a model system for a number of facets of biology (reviewed in [23,24]). Despite extensive research into differences in locomotor performance, morphology, and behaviour in the different *Anolis* ecomorphs [25–27], we know nothing about how variation in habitat structure influences the muscles that power locomotion in these species. We examined the *in vivo* muscle activity patterns and relevant limb kinematics of the green anole, *Anolis carolinensis*, running on two different inclines (0° and 90°) and perch diameters (1 cm and flat). This species is a trunk-crown ecomorph that regularly uses a wide range of substrate diameters and inclines, in proportion to what is available in its habitat [28]. We determined the function of the focal muscles based on hypotheses from the literature [29], and we tested the hypothesis that, as is the case with kinematics, muscle function will be modulated in response to changes in demand, resulting in a coupling of physiology and kinematics. Specifically, we expected that anoles would increase the intensity of motor unit recruitment in response to steeper inclines. On narrow perches, we expected increased recruitment in all of the muscles examined in this study given that they are associated with moving in the more crouched posture that is associated with narrow perches [16,30].

2. Material and methods

(a) Subjects

Seven adult male *A. carolinensis* Voigt 1832 (mass = 5.9 ± 0.4 g; snout–vent length (SVL) = 6.1 ± 0.2 cm) were obtained from commercial suppliers. Anoles were not fed within the 12 h prior to surgery to minimize the effect of undigested food on anaesthetized subjects.

Based on previous kinematic data [16] and hypothesized muscle function from the literature (electronic supplementary material,

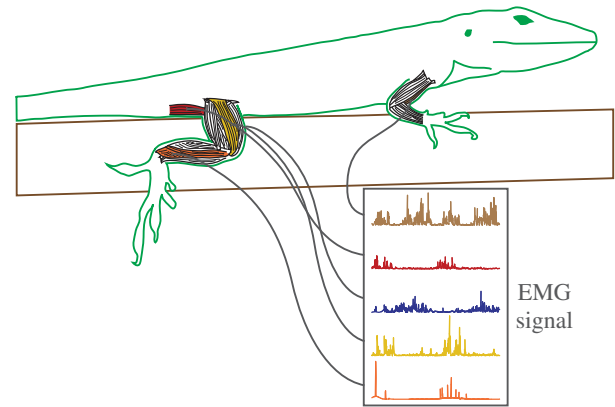


Figure 1. Schematic showing location of five of the six muscles implanted with EMG electrodes. EMG signals from top to bottom: biceps (brown), CF (red), PIT (located on ventral surface of proximal hindlimb; blue), ambiens (yellow), PB/PL (orange). (Online version in colour.)

table S1; [29]), six muscles were chosen for electromyography (EMG) implantation: biceps, caudofemoralis (CF), puboischiotibialis (PIT), ambiens pars dorsalis (AMB), peroneus longus (PL) and peroneus brevis (PB) (figure 1). These last two muscles were chosen instead of the synergistic gastrocnemius [29] because their position on the lateral, posterior side of the crus facilitated successful implantation and reduced the likelihood that electrodes would be pulled out during experimentation. Four points on the forelimb (shoulder, elbow, wrist and centre of the pectoral girdle) and five points on the hindlimb (hip, knee, ankle, base of the third metatarsal and centre of the pelvic girdle) were used to visualize the joints for subsequent kinematic analysis.

(b) Surgery and experimental protocol

These procedures follow those of Higham & Jayne [15]. Prior to surgery anoles were anaesthetized with an intramuscular injection of ketamine (100 mg kg^{-1}). Bipolar EMG electrodes, constructed from 0.051 mm diameter polycoated stainless-steel wire (California Fine Wire Co., Grover Beach, CA, USA), were implanted through the skin into the mid-bellies of each muscle using a 26-gauge hypodermic needle. To reduce the possibility of electrodes pulling out during the course of the experiment, EMG wires were individually sutured to the skin, immediately proximal to the implantation point and again on the dorsal surface of the lizard's back, using 5-0 coated vicryl suture (Ethicon, Inc., Somerville, NJ, USA). After surgery, lizards were placed in clean 10-gallon aquaria and allowed to recover from anaesthesia for 6–12 h.

Running trials took place on an apparatus identical to that described previously [16]. Lizards ran on 1 m long trackways at two perch diameters, a 9 cm wide flat perch, representing the large diameter treatment [16,30], and a narrow, 1.3 cm diameter perch. Perches were covered with cork shelf liner to enhance traction and were inclined at 0° and 90°. To allow calculation of three-dimensional kinematics, perches were mounted below a mirror inclined at 45°. EMG wires were suspended below the perch and were long enough to ensure unobstructed forward locomotion.

Dorsal and lateral videos of the running anoles were obtained simultaneously with two high-speed Photron APX-RS cameras (Photron USA, San Diego, CA, USA) at $500 \text{ frames s}^{-1}$. EMG signals were amplified 10 000 times using GRASS QP511 quad and P55 AC amplifiers (Natus Neurology Inc., Warwick, RI, USA) with a 60 Hz notch filter and low- and high-bandpass filters of 0.1 Hz and 3000 Hz, respectively. Signals were recorded at $5000 \text{ samples s}^{-1}$ using a BIOPAC MP150 data acquisition system with the UIM100C module and ACQKNOWLEDGE (v. 4.0) software (BIOPAC Systems, Inc., Goleta, CA, USA). An external

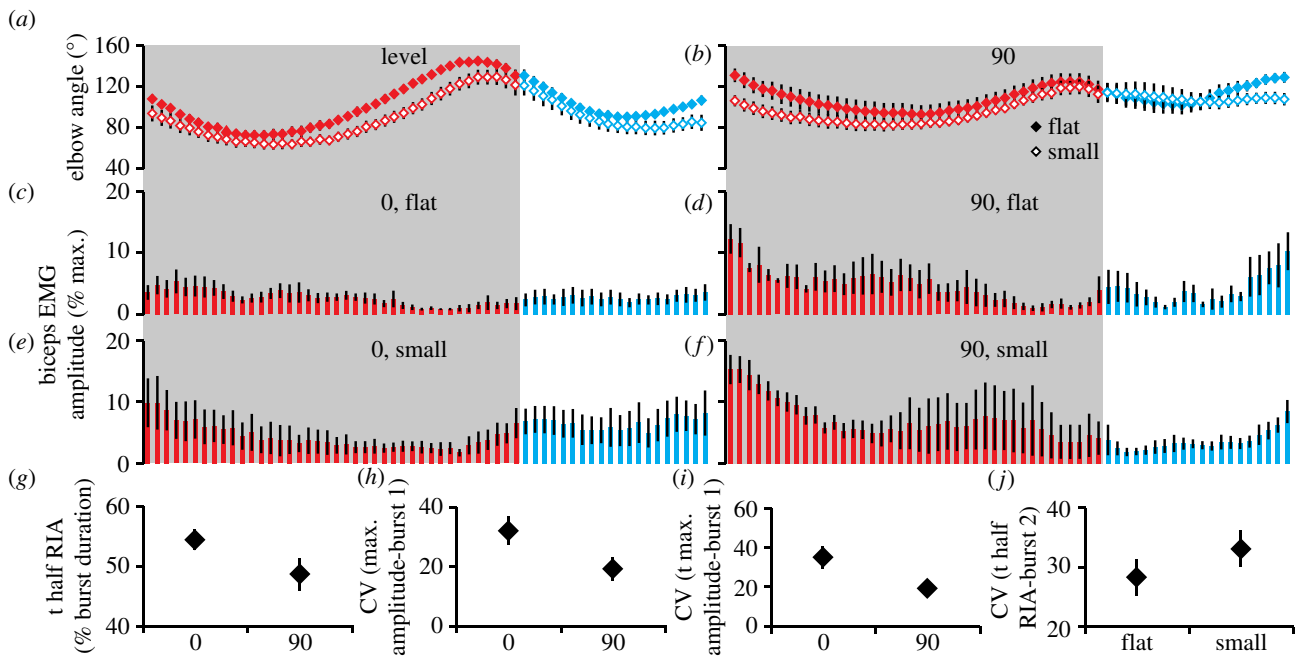


Figure 2. Binned joint angle (*a,b*), binned EMG amplitude (*c–f*) and significant relationships (*g–j*) for the biceps on the various conditions. (*a,b*) Closed, flat perch; open, small diameter perch. (*a–f*) Shaded region (red data), stance phase; unshaded region (blue data), swing phase. CV, coefficient of variation; t, time; RIA, rectified integrated area; max., maximum. Values are mean + s.e. (Online version in colour.)

trigger was used to sync EMG and video data. Trials were considered for analysis if anoles ran steadily through the field of view, on top of the perch.

After experimentation was complete, anoles were euthanized with an overdose of sodium pentobarbital (300 mg kg^{-1} intraperitoneal injection). Post-mortem dissections were performed to verify electrode placement and all the muscles of the hindlimb and proximal forelimb were removed for mass and fascicle length measurements (electronic supplementary material, table S2).

(c) Electromyography

Prior to all analyses, EMG signals were bandpass filtered (2500 Hz and 70 Hz high- and low-bandpass filtered, respectively) and rectified. Seven variables were calculated from these signals: onset and offset time, burst duration, magnitude and timing of peak burst amplitude, total rectified integrated area (RIA) during the stance phase, and the time during each burst at which half of the burst RIA was achieved. All calculations were performed using custom code written for MATLAB (written by K.L.F.).

Burst onset and offset times were calculated following the method described by Roberts & Galdón [31]. A signal envelope was obtained through additional smoothing using a lowpass filter (300 Hz). The boundaries of the burst were defined as occurring when signal envelope exceeded a cut-off value of twice the standard deviation of an inactive section. Burst duration was the time between burst onset and offset. Both burst onset time and duration were standardized by stride duration and onset time was expressed relative to footfall prior to statistical analyses.

Before doing any calculations using EMG amplitude data, signal noise was subtracted from the rectified EMG signal. The maximum activity observed in each burst was identified and the time at which that peak occurred was expressed relative to footfall and standardized by stride duration. The total RIA during stance phase was the product of EMG amplitude and time and reflected the relative proportion of the muscle that was active during the period of time for which it was calculated. Both amplitude variables (i.e. peak burst amplitude and total stance RIA) were expressed relative to the maximum amplitude ever observed for that muscle per individual. To approximate the shape of the EMG burst, we

calculated the time at which half of the total burst RIA was achieved (*sensu* [32]) and expressed this value relative to burst duration.

To confirm the hypothesized function of the muscles (electronic supplementary material, table S1), EMG signals from strides of different length were averaged. To facilitate this, signal amplitudes during stance and swing phases were divided into 40 and 20 equal-duration bins, respectively, to be consistent with the average duty factor of 66%. These binned amplitudes were expressed relative to the maximum amplitude observed for each individual and muscle to allow data to be pooled across individuals. The resulting trace for each muscle was then compared to the binned kinematic data for the joint at which each muscle was expected to act (e.g. figures 2–4).

(d) Kinematics

We obtained x , y and z coordinates for each point (see above) using DLT DV 5 custom software [33] for MATLAB (v. R2010a, The MathWorks, Natick, MA, USA). The x -axis represented fore-aft movement, the y -axis described dorsoventral movement, and the z -axis represented medio-lateral movement perpendicular to the x - y plane. These points were used to calculate body speed, femur depression, retraction and rotation angles, and elbow, knee and ankle angles as previously described in detail [16,30]. Briefly, body speed was calculated separately for each limb, dividing the distance travelled by the point at the centre of the pectoral/pelvic girdles during the stride by the duration of the stride. Although body size did not correlate significantly with speed, speed was standardized (divided by SVL) to facilitate comparisons with other individuals and species in past and future studies.

Although these kinematic variables have already been measured for this species on similar substrates [16], the data reported here are from separate experiments in which the animals were implanted with EMG electrodes. Thus, a comparison can be made between unimplanted individuals [16] and implanted individuals (this paper) to ensure that behaviour was not significantly altered by surgical procedures.

To facilitate comparisons with muscle activity data, angular data were binned as described for the EMG analysis. Minimum, maximum and excursion of the angular data were obtained for the entire stride for input into statistical analyses.

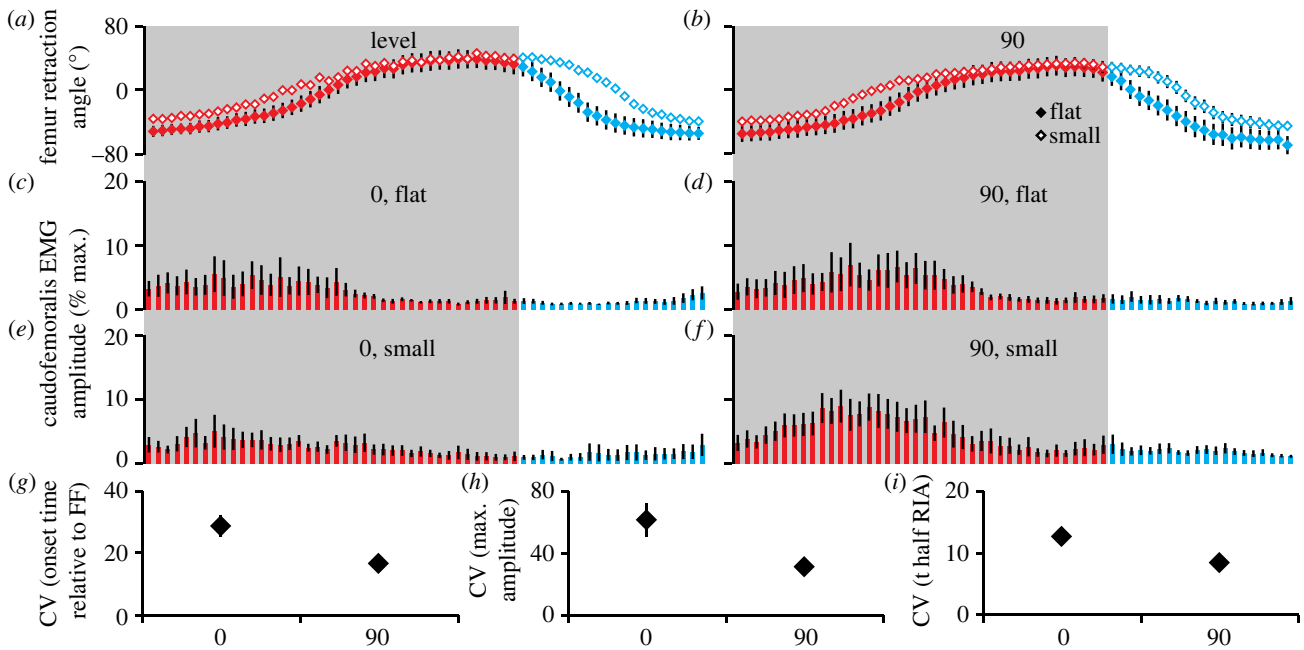


Figure 3. Binned joint angle (*a,b*), binned EMG amplitude (*c–f*) and significant relationships (*g–i*) for the CF on the various conditions. (*a,b*) Closed, flat perch; open, small diameter perch. (*a–f*) Shaded region (red data), stance phase; unshaded region (blue data), swing phase. Abbreviations are the same as given in figure legend 2. Values are mean + s.e. (Online version in colour.)

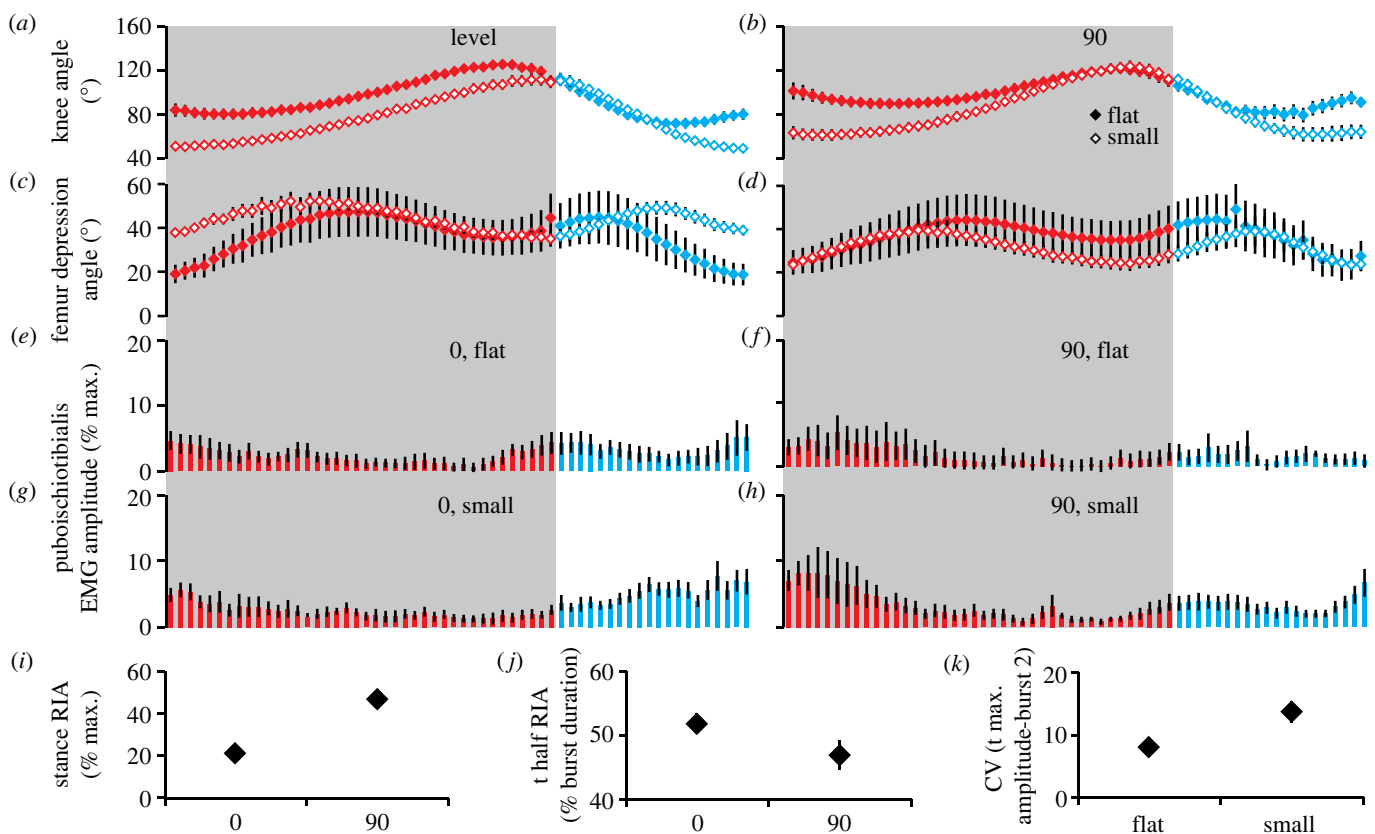


Figure 4. Binned joint angle (*a–d*), binned EMG amplitude (*e–h*) and significant relationships (*i–k*) for the PIT on the various conditions. (*a–d*) Closed, flat perch; open, small diameter perch. (*a–h*) Shaded region (red data), stance phase; unshaded region (blue data), swing phase. Abbreviations are the same as given in figure legend 2. Values are mean + s.e. (Online version in colour.)

(e) Statistical analyses

JMP (version 9.0, SAS Institute Inc., Cary, NC, USA) was used to perform all statistical analyses. The effect of speed (SVL s^{-1}) was removed prior to all analyses by regressing all kinematic and EMG variables individually against speed and saving residuals of the variables that showed a significant ($\alpha \pm 0.1$) relationship with speed.

The kinematic analyses performed were similar to those performed on previously published data [16] in order to confirm that EMG implantation did not interfere with normal movement. Briefly, temporal (angular velocities, stride frequency and duty factor) and angular variables (minimum, maximum and excursion of joint angles) were separated and input into separate discriminant function analyses (DFA). The variables that

loaded heavily (greater than 0.3) on each of the first two DF axes were considered important for explaining the differences in kinematics between treatments. A similar DFA was performed on temporal data. For a more detailed explanation of these statistical methods, see [16]. As the kinematic changes between treatments were similar in these data as in individuals that were not implanted with EMG electrodes, the surgery did not affect our results.

Statistical analyses for each EMG variable were performed separately for each muscle. To test for significant differences between mean values of the treatments, mixed-model analyses of variance (ANOVAs) were performed in which individual was a random factor and incline and perch diameter were fixed factors. As there was never a significant interaction between incline and perch diameter, this interaction was removed from all analyses. The correct *F*-values and degrees of freedom for perch diameter and incline effects were obtained using the mean squares of the two-way interaction between fixed and individual factors as the denominator of each fixed factor [34].

In addition to testing for differences between mean EMG values, we tested for differences in the coefficient of variation (CV) of each variable between treatments using a two-way ANOVA in which incline and perch diameters were fixed effects. As above, the interaction between incline and perch diameter was never significant and so was removed from analyses.

3. Results

Overall, the experimental treatments affected kinematics much more strongly than they affected muscle activity patterns. Of the 32 kinematic variables examined, 63% were affected by the treatments, whereas only 10% of the 100 muscle activity variables were affected. However, although more of the kinematic variables were affected by perch diameter (47%) than by incline (25%), the opposite trend was seen in the muscle activity data (2% and 8% significant for perch diameter and incline, respectively). These relationships were not affected by the use of different statistical tests on the two datasets.

(a) Changes in kinematics with perch diameter and incline

Detailed descriptions of limb movements of this species on a variety of inclines and perch diameters have been published elsewhere using different individuals from this study [16]. We refer interested readers to those descriptions and here focus on highlighting the significant differences relevant to the focal muscles of this study.

Of the kinematic variables considered in this study, approximately twice as many variables were significantly affected by changes in perch diameter than by changes in incline (table 1). In general, individuals had a faster stride frequency and a lower duty factor on the narrow perch than on the flat perch (table 1). In the forelimb, the elbow had a smaller excursion and was generally held more extended on the vertical treatment than on the level treatment but flexed more on the small diameter perch than on the flat perch (table 1). In the hindlimb, the femur had a greater rotational excursion on the vertical than on the horizontal treatment but was more retracted and depressed on the small diameter perch than on the flat perch (table 1). The femur also rotated, retracted and depressed faster on the narrow perch than on the flat perch (table 1). Like the elbow, the knee was held in

a more extended posture at 90° than at 0° but it was generally more flexed and had a greater angular excursion on the small diameter than on the flat perch (table 1). However, it extended faster on the narrow perch than on the flat perch (table 1). Finally, the ankle extended less at end of stance, had less angular excursion, and extended slower on the small diameter perch than on the flat perch (table 1).

Overall, joint kinematics of the anoles in this study was similar to the kinematics of individuals that had not been surgically implanted with EMG electrodes [16]. Therefore, we conclude that electrode implantation did not alter normal movement.

(b) General description of muscle activity patterns

The overall timing of muscle activity appeared to coincide with hypothesized functions of the muscles (electronic supplementary material, table S1, figures 2–6). The biceps had two bursts of activity during the stride. The first burst occurred during the entire period of stance phase elbow flexion. It began immediately prior to footfall, although sometimes as early as half of the way through swing phase, and generally ended one-half to two-thirds of the way through stance phase, generally after the end of elbow flexion (figures 2*a–f* and 5). The beginning of the second, smaller burst approximately coincided with the end of elbow extension towards the end of stance phase and continued briefly during the first one-quarter of swing phase as the elbow was flexing during the first portion of limb recovery (figures 2*a–f* and 5).

The CF was generally characterized by a single prolonged burst of activity. Activity began shortly before footfall, often in the last three-fourths of swing phase, and generally ended one-half to two-thirds of the way through stance (figures 3*a–f* and 6). However, in some trials, CF activity continued through all of stance and ended shortly after the beginning of swing. The PIT generally had a double burst, including a high amplitude, short burst centred around footfall and coinciding with a small amount of femur depression at the beginning of stance phase, and a lower amplitude, longer burst centred around end of stance, coincident with the end of knee extension and the beginning of knee flexion as the limb is brought forward in recovery during the swing phase (figures 4*a–h* and 6). The AMB also had two bursts of activity. The first burst was generally larger in amplitude and occurred during knee extension, beginning shortly after footfall and ending around two-thirds of stance phase (figure 6). The second, smaller burst occurred during swing phase in the last one-half to one-third of knee flexion (figure 6). Both the PB and PL had double bursts, the first beginning in late swing phase and ending shortly after footfall, and the second burst generally occurring in the second half of stance during the major propulsive period of ankle extension (figure 6).

(c) Changes in muscle activity with perch diameter and incline

Overall, changes in incline had a stronger impact on muscle activity patterns than changes in perch diameter; a total of eight variables, from the biceps, CF and PIT, were significantly affected by incline, whereas only two variables, one each from the biceps and PIT, were affected by perch diameter.

Substrate diameter did not significantly affect the means of any of the variables considered here. However, the shape of the second burst of the biceps, as measured by the time,

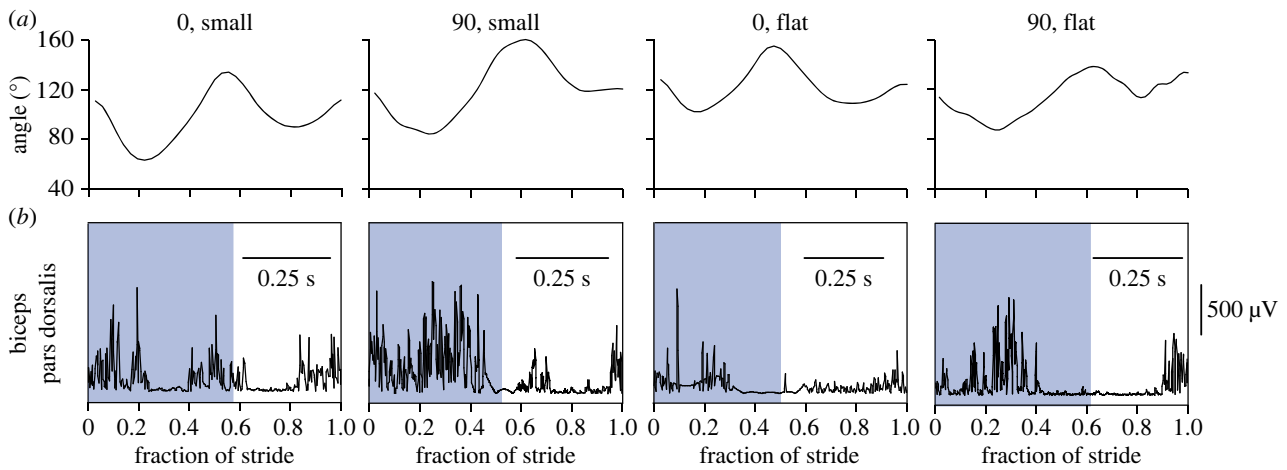


Figure 5. Elbow angle (a) and biceps EMG trace (b) for a representative stride in each condition. Shaded area represents stance phase. (Online version in colour.)

Table 1. Joint angle (Pillai's Trace $F = 2.75$, $p = 0.029$, describing 96.45% of total variation) and angular velocity (Pillai's Trace $F = 2.18$, $p = 0.011$, describing 93.43% of total variation) variables that loaded heavily (greater than 0.3) on the first two axes of discriminant function analyses. FF, footfall; ES, end of stance; Min., minimum; Max., maximum; Ex., excursion; V, velocity (deg s^{-1}); values are means \pm s.e.m.

	perch diameter		incline	
	small	flat	0°	90°
joint angle				
Max. elbow angle (FF)	106.53 \pm 5.75°	123.44 \pm 5.00°	103.73 \pm 5.46°	126.24 \pm 5.29°
Min. elbow angle			66.46 \pm 5.17°	80.97 \pm 6.31°
Ex. elbow angle			74.68 \pm 2.77°	47.49 \pm 6.60°
Ex. femur rotation			11.65 \pm 4.39°	14.04 \pm 2.25°
Min. femur retraction	-49.77 \pm 7.39°	-63.28 \pm 11.75°		
Max. femur retraction	49.46 \pm 7.91°	40.69 \pm 4.28°		
Min. femur depression	27.66 \pm 2.28°	19.18 \pm 5.77°		
Max. knee angle (FF)	73.67 \pm 7.34°	97.44 \pm 6.40°	79.09 \pm 6.36°	92.03 \pm 7.39°
Min. knee angle	54.71 \pm 4.09°	84.85 \pm 5.40°	63.96 \pm 3.88°	75.60 \pm 5.61°
Max. knee angle (ES)			122.09 \pm 3.86°	126.54 \pm 4.27°
Ex. knee angle	66.47 \pm 7.20°	42.68 \pm 6.19°		
Max. ankle angle	121.27 \pm 3.76°	132.43 \pm 5.22°		
Ex. ankle angle	59.10 \pm 6.25°	71.42 \pm 5.26°		
joint angular velocity				
stride frequency (strides s^{-1})	5.69 \pm 0.38	5.56 \pm 0.34		
duty factor	0.63 \pm 0.02	0.71 \pm 0.03		
V. femur rotation	2.76 \pm 1.35	2.01 \pm .89		
V. femur retraction	40.56 \pm 20.29	17.20 \pm 2.70		
V. femur depression	7.38 \pm 1.91	6.39 \pm 1.43		
V. knee flexion			-8.85 \pm 0.95	-8.38 \pm 2.10
V. knee extension	12.90 \pm 3.61	9.46 \pm 2.49		
V. ankle extension	13.95 \pm 3.36	19.39 \pm 4.47		

relative to the beginning of the burst, at which half RIA was achieved, was more variable on the small diameter perch (CV = 33.40 \pm 2.92) than on the flat perch (CV = 28.31 \pm 3.06; $F_{1,14} = 4.82$, $p = 0.046$; figure 2j). Similarly, the time,

relative to footfall, at which the maximum amplitude of the second burst of the PIT was reached was more variable on the narrower perch (CV = 13.56 \pm 1.68) than on the flat perch (CV = 7.83 \pm 1.32; $F_{1,19} = 4.82$, $p = 0.041$; figure 4k).

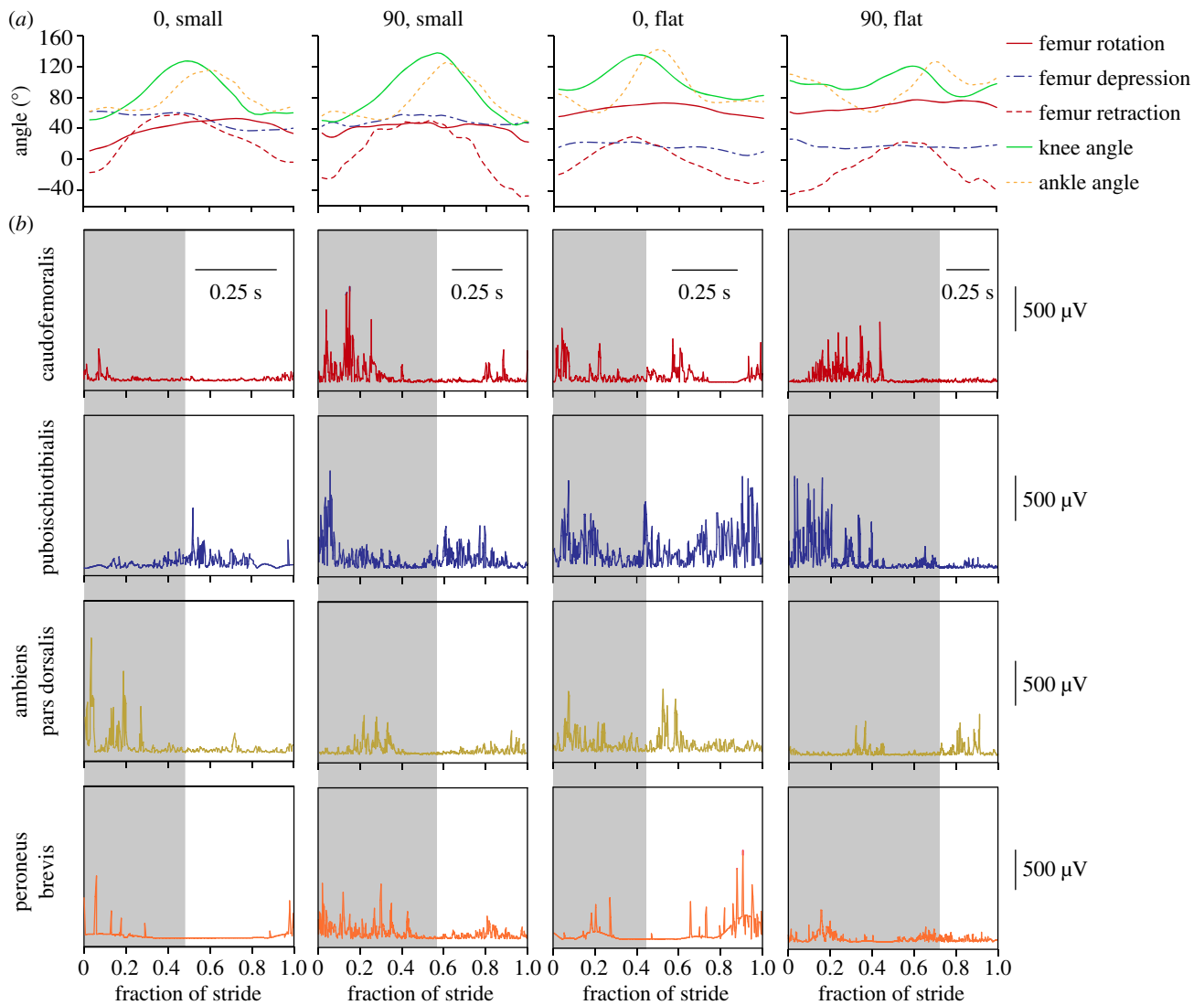


Figure 6. Joint angle (a) and EMG traces ((b) four rows) for the hindlimb muscles and corresponding joints. Shaded area represents stance phase. Femur retraction/rotation = CF; femur depression, knee flexion = PIT; knee extension = ambiens; ankle angle = PB. (Online version in colour.)

Mean differences in muscle activity patterns, in response to changes in incline, were only observed in the biceps and PIT. The first burst of the biceps ($F_{1,4} = 8.17$, $p = 0.046$; figure 2g) and the second burst of the PIT ($F_{1,4} = 12.26$, $p = 0.025$; figure 4j) had a more front-loaded shape on the vertical (time to half RIA, relative to FF, as % of burst duration; biceps = 48.77 ± 2.57 ; PIT = 46.98 ± 2.23) than on the horizontal treatment (biceps = 54.30 ± 1.60 ; PIT = 51.95 ± 1.44) and the PIT had a greater total recruitment during stance on 90° (RIA, as % max = 46.48 ± 4.40) than on 0° (RIA, as % max = 20.96 ± 2.29 ; $F_{1,4} = 23.52$, $p = 0.0083$; figure 4i). The remaining variables that were affected by incline all showed a significant increase in variability on the level treatment compared with the vertical treatment. These variables included the maximum amplitude (0° CV = 32.10 ± 4.64 , 90° CV = 19.19 ± 3.97 ; $F_{1,15} = 4.73$, $p = 0.046$; figure 2h) and the time, relative to FF, at which that max. amplitude was achieved (0° CV = 35.05 ± 5.70 , 90° CV = 19.34 ± 2.85 ; $F_{1,15} = 5.08$, $p = 0.04$; figure 2i) for the first burst of the biceps, and the onset time (0° CV = 28.69 ± 3.39 , 90° CV = 16.61 ± 2.37 ; $F_{1,20} = 7.80$, $p = 0.011$; figure 3g), maximum amplitude (0° CV = 61.61 ± 10.65 , 90° CV = 31.44 ± 5.39 ; $F_{1,20} = 6.69$, $p = 0.018$; figure 3h), and shape (0° CV = 12.76 ± 1.31 , 90°

CV = 8.47 ± 1.27 ; $F_{1,20} = 5.93$, $p = 0.024$; figure 3i) of the CF burst.

4. Discussion

We assessed simultaneous changes in three-dimensional limb kinematics and motor control of a lizard moving in a simulated arboreal environment with varying inclines and perch diameters. Limb kinematics were impacted more than muscle activity patterns in response to our treatments, and both were more strongly affected by different substrate characteristics; more kinematic variables were significantly affected by perch diameter than by incline (table 1; [16]), whereas more EMG variables were significantly affected by incline than by perch diameter (figures 2–4). This decoupling of kinematics and muscle activity suggests that muscles and limb movements do not respond to changes in substrate in the same way, and that a given pattern of neural control can result in different kinematic patterns (possibly impacted by external factors rather than internal control). Furthermore, different variables were found to be significantly different with changes in perch diameter than with

changes in incline, indicating that these substrate variables pose distinct functional challenges.

(a) Temporal heterogeneity of function within muscles

All muscles examined in this study, with the exception of the CF, were characterized by two bursts during a single stride cycle. Timing of these bursts relative to angular changes at the corresponding joints (figures 2–6) indicate that, contrary to what has been hypothesized based on anatomical position, these muscles probably have more than one function. For example, although the first, mid-stance burst in the AMB and the second, late-stance burst in both the PL and PB support the hypothesized functions of these muscles (electronic supplementary material, table S1), the remaining bursts of activity in these muscles seem to occur during knee and ankle flexion, respectively. As these muscles act to resist flexion when active, they likely are performing a stabilizing function in these instances. In contrast with this additional antagonistic function, the two bursts of the PIT appear to temporally segregate the dual function of this muscle, with femur depression occurring during the first, early-stance burst and knee flexion occurring during the second, late-stance burst. These patterns of muscle activity suggest an added complexity to muscle function that cannot be gleaned from morphological dissections alone. Furthermore, double bursts are not unheard-of in vertebrates [9,15,35], but it is worthwhile to consider that such activity patterns may be important for temporally separating multiple functions of a single muscle. This temporal segregation, in addition to the recent research that has identified spatial segregation of function within muscles during locomotion [10], highlights the complex function of muscles under dynamic conditions.

(b) Effect of substrate on muscle activity

As incline increases, an increasing component of gravity acts to resist forward locomotion [17,36], necessitating an increase in muscle work. Based on studies examining the effects of incline on vertebrate muscle activity [13–15,18,37], it is common for animals to increase intensity, rather than altering timing of muscle activity to increase muscle work on steeper inclines. In our study, the PIT was the only muscle with greater stance phase recruitment on steeper inclines, despite the fact that we examined multiple propulsive muscles. In addition, temporal aspects of the biceps and PIT muscles were altered, with greater front-loading of the muscle bursts on vertical than on level treatments. Although the onset time of these bursts did not differ significantly across treatments, this change in burst shape indicates that a greater number of motor units were being recruited earlier in the burst to facilitate a more rapid initiation of movement at the beginning of the stride on the steeper incline. Whether or not this also indicates changes in the recruitment timing of different fibre-types remains to be determined.

Interestingly, the majority of the EMG variables that were affected by substrate were significant for changes in CV rather than differences in mean values. Within-individual variability was always significantly greater on the level than on the vertical incline, and on the narrow perch diameter than on the broad surface. The degree of stereotypy of locomotion is a reflection of the degree to which an animal is specialized for that particular locomotor mode in its preferred habitat [38,39]. Therefore, although *A. carolinensis* is commonly

observed in all areas of the arboreal habitat, and on the ground [28], the decreased variability in muscle activity patterns observed on steep, broad substrates may indicate a functional preference for tree trunks. However, future work on more species with varying degrees of specialization will be needed to determine whether there is a link between substrate preference and stereotypy of muscle activity patterns.

(c) Decoupled kinematics and muscle activity in response to substrate

Surprisingly, kinematics and muscle activity exhibited a decoupled response to changes in substrate, particularly perch diameter. Despite considerable differences in limb posture and movement on the different substrate diameters (table 1; [16]), there were no changes in mean values for any of the EMG variables examined and only two changes in variability (figures 2 and 4). The significant changes in kinematics, namely increases in limb flexion, depression and retraction on the narrower substrate than on the broad surface, likely require a change in the length of the muscle–tendon units of most muscles investigated here (a possible exception is the bi-articular PL, which may have minimal net length change through simultaneous knee and ankle flexion). As *A. carolinensis* is very small and these muscles insert via short tendons (biceps, CF and PL/PB) or no tendon at all (PIT and AMB; [29]), these changes in limb posture probably result primarily in changes of the muscle length itself. If these operating lengths became suboptimal, muscle force generation would decrease, requiring a compensatory increase in fibre recruitment in order to maintain a similar force output [4,7]. The absence of any change in muscle intensity with substrate diameter may reflect the opportunistic nature of this species' habitat use; the expected changes may be more evident in ecomorphs with a more specialized habitat preference, in which optimal muscle lengths may have become adapted for the preferred substrate condition. However, this hypothesis remains to be tested. Interestingly, populations of green anoles differ in habitat use in LA, USA [40]. Populations that occupy habitat dominated by broad leaves use broader surfaces, on average, than populations living in habitat dominated by narrower substrates [40]. However, within each of these populations, anoles generally preferred to use broader surfaces than were available on average [40], potentially indicating the challenges of instability and its negative impact on locomotor performance on suboptimal, narrow perches. A comparison of kinematics and muscle activation in these populations may reveal the basis for this preference. Although it is possible that other muscles not examined may have been impacted, this is not likely given that we implanted most of the important propulsive muscles in the limbs. In addition, simultaneously recording from every muscle in the limbs is not feasible given the limited size of the animals.

In summary, despite considerable changes in limb kinematics with substrate, and perch diameter in particular [16], there were few significant changes in muscle activity patterns. This apparent decoupling of the response of muscle and limb movement to substrate highlights the complex nature of animal locomotion and how little we understand about muscle function in ecologically relevant contexts. It is evident that biomechanical and neuromechanical studies must use caution when interpreting observed changes in kinematics and motor control signals since altered

kinematics do not necessarily imply active modulation and muscle activity patterns cannot be used in isolation when attempting to infer movement. Furthermore, the possibility that different physiological and biomechanical variables can be decoupled in their contribution to locomotion may complicate our theories about the evolution of different locomotor modes or how locomotor adaptation for demanding substrates may have been achieved. The fact that kinematics can change significantly without corresponding changes in motor control is intriguing, and might be widespread among vertebrates that live in complex habitats. Examination of more specialized *Anolis* ecomorphs to see whether they exhibit a similar decoupling of movement and motor control may be a promising and fruitful next step in the investigation of the evolution of kinematic flexibility for a single motor pattern.

Habitat complexity stretches beyond consideration of incline and perch diameter in isolation. Arboreal habitats are characterized by numerous challenges that must be

dealt with or circumvented such as substrate compliance, rugosity, swaying caused by wind or animal movement, obstacles, sharp turns and gaps that must be bridged. These and other variables occur in various combinations and arboreal animals must deal with or circumvent each of them, often in quick succession. Given that the forelimbs are likely the first to contact a new surface first during a transition, much of the modulation might stem from feedback following this initial footfall. Examining the role of combinations of these challenges, as well as transitioning between combinations during a locomotor event, would be a highly interesting avenue of future research.

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Data accessibility. Kinematic/electromyography data and inventory of video files: Dryad doi:10.5061/dryad.c6500.

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