

***In vivo* muscle activity in the hindlimb of the arboreal lizard, *Chamaeleo calypttratus*: general patterns and the effects of incline**

Timothy E. Higham* and Bruce C. Jayne

Department of Biological Sciences, University of Cincinnati, PO Box 210006, Cincinnati, OH 45221-0006, USA

*Corresponding author at present address: Section of Evolution and Ecology, University of California, One Shields Avenue, Davis, CA 95616, USA (e-mail: tehigham@ucdavis.edu)

Accepted 6 October 2003

Summary

Arboreal animals often move on surfaces with variable and steep inclines, but the changes in hindlimb muscle activity in response to incline are poorly understood. Thus, we studied the hindlimb muscle activity in the arboreal specialist, *Chamaeleo calypttratus*, moving up and down 45° inclines and on a horizontal surface. We quantified electromyograms (EMGs) from nine hindlimb muscles, and correlated EMGs with three-dimensional hindlimb kinematics. Kinematics changed little with incline, but the EMGs changed substantially. Most of the changes in EMGs were for amplitude rather than timing, and the EMGs of the hip and thigh muscles had more conspicuous changes with incline than those of the lower limb muscles. Unlike most other vertebrates, chameleons flexed the knee substantially during the first half of stance while the foot was anchored to the perch, and the amplitude of two large knee flexors increased when moving uphill compared to level and downhill. Thus, knee flexion in early stance probably contributes significantly

to propulsion in *C. calypttratus*. During stance, the caudofemoralis EMGs of *C. calypttratus* correlated well with femur retraction, knee flexion and posterior femur rotation, and their amplitudes were higher on uphill and level surfaces than on the downhill surface. During the second half of stance, iliotibialis EMGs correlated well with knee extension, and their amplitude was highest on the uphill surface and lowest on the downhill surface. Many of the muscles in the hindlimb of *C. calypttratus* changed activity with incline in a manner similar to the propulsive limb muscles in mammals. Although muscle strain often increases when animals need more power to move uphill, the minimal changes in the hindlimb kinematics of *C. calypttratus* with incline imply little change in muscle strain.

Key words: locomotion, lizard, *C. calypttratus*, arboreal, kinematics, incline, slope, hindlimb, electromyography, muscle.

Introduction

Most animals live in environments that require traversing inclines of variable steepness, and lizards are one of the most diverse lineages of vertebrates that are adept at moving on steep surfaces. However, most studies of animal locomotion examine movement on level (horizontal) surfaces. For diverse lineages of animals, the limited data indicate that inclines usually affect both limb movements (Schmitt, 1998; Jayne and Irschick, 1999; Zaaf et al., 2001) and muscle activity (Pierotti et al., 1989; Roy et al., 1991; Roberts et al., 1997; Carlson-Kuhta et al., 1998; Smith et al., 1998; Gillis and Biewener, 2002; Daley and Biewener, 2003). For example, some hindlimb muscles of the rat have relative amplitudes of electromyograms (EMGs) that are greatest on an uphill surface and lowest on a downhill surface (Gillis and Biewener, 2002).

When an animal moves uphill, the opposing forces exerted by gravity increase the cost of transport (Farley and Emshwiller, 1996; Wickler et al., 2000) and the mechanical power required by propulsive-phase muscles (Farley, 1997; Roberts et al., 1997; Swanson and Caldwell, 2000; Gabaldon

et al., 2001). In contrast, during downhill locomotion increased activity of some muscles may be required to slow the animal and retard passive flexion of the joints rather than enhance the speed of forward movement and actively flex the joints (Smith et al., 1998; Gabaldon et al., 2001). Whether motor patterns are altered similarly on inclines between animals that inhabit structurally different habitats or between animals from evolutionarily diverse lineages is not fully understood.

A key question in functional morphology is whether muscle activity is conserved during major evolutionary changes in morphology and environment (Peters and Goslow, 1983; Lauder and Shaffer, 1988; Wainwright et al., 1989; Lauder, 1994; Smith, 1994; Ashley-Ross, 1995; Biewener, 2002). Many animals living in arboreal habitats have morphological, behavioral or ecological specializations that allow them to move effectively in this habitat. For example, primates and chameleons have prehensile feet (Fig. 1; Cartmill, 1974; Peterson, 1984; Schmitt, 1998) and geckoes and anoles have toe pads (Irschick et al., 1996; Russell, 2002), all of which

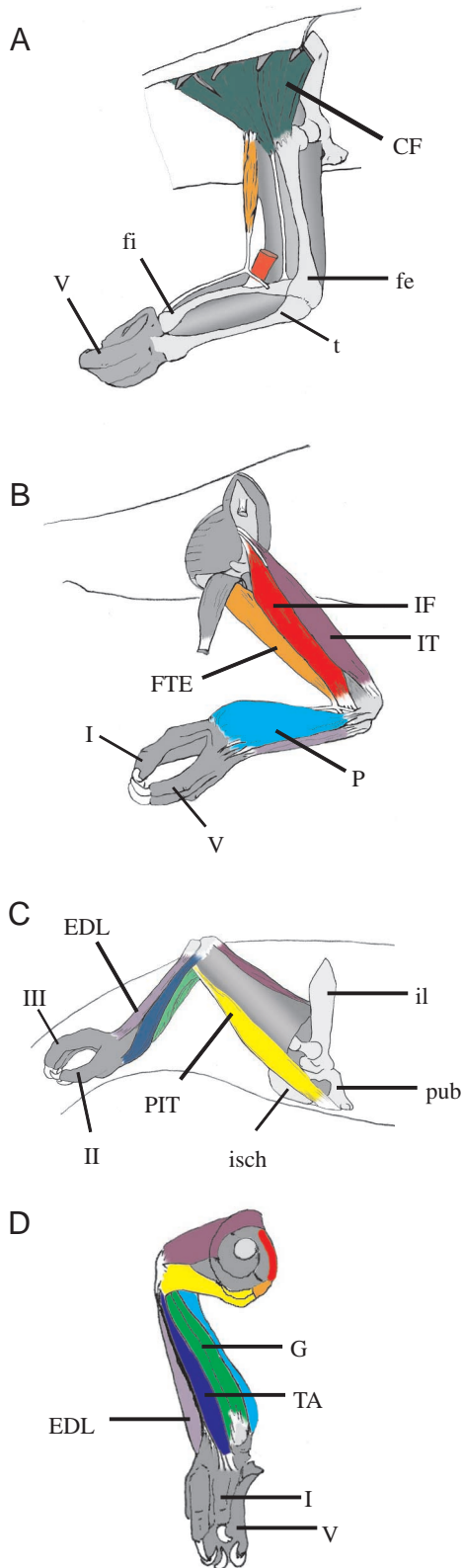


Fig. 1. Right hindlimb muscles of *C. calypttratus* represented by a lateral view of the deeper musculature (A), a lateral view of a protracted and depressed limb (B), a fully retracted and abducted limb (C), and an anterior view of a fully retracted and disarticulated limb (D). Bones are in light gray and connective tissue is in white. Abbreviations: I–V, digit numbers; CF, caudofemoralis; EDL, extensor digitorum longus; fe, femur; fi, fibula; FTE, flexor tibialis externus; G, gastrocnemius; IF, iliofibularis; il, ilium; IT, iliotibialis; isch, ischium; P, peroneus; PIT, puboischiotibialis; pub, pubis; t, tibia; TA, tibialis anterior.

and behavior, and include both terrestrial and arboreal forms. Furthermore, many lizards are adept at moving on steep inclines in their natural habitats. Inclines often affect the maximal speeds (performance) and limb movements (kinematics) of lizards in both laboratory and field experiments (Huey and Hertz, 1982; Irschick and Jayne, 1998, 1999a; Jayne and Ellis, 1998; Jayne and Irschick, 1999; Zaaf et al., 2001). However, previous EMG experiments with lizards have neither examined the effects of incline nor studied an arboreal species (Jayne et al., 1990a; Reilly, 1995; Nelson and Jayne, 2001). Thus, we examined the effects of incline on the *in vivo* activity of hindlimb muscles in the arboreal specialist *C. calypttratus* (veiled chameleon) and correlated muscle activity to the three-dimensional movements of the hindlimb.

Recording the limb movements and motor patterns of locomotion on different inclines can provide fundamental insights into whether some biologically realistic and important sources of environmental variation may require fundamental changes in limb function or motor output. Thus, we addressed the following major questions regarding the effects of incline on the muscle activity in the hindlimb of *C. calypttratus*. (1) Are the muscle activity patterns in the hindlimbs of chameleons affected by the incline of the locomotor surface? We hypothesized that most muscle activity in chameleons would be affected by incline as in other vertebrate taxa, such that the amount of activity of propulsive muscles would be greatest on the uphill and least on the downhill surface. Furthermore, the activity of muscles that prevent the collapsing of joints would be greatest on the downhill surface. Based on a study of chameleon kinematics (Higham and Jayne, 2004), we hypothesized that chameleons would rely more on actively pulling themselves forward on level and uphill surfaces. Thus, the muscles that flex the knee and retract the limb during the early part of stance may have disproportionately more activity. (2) Are the patterns of muscle activity in the hindlimbs of chameleons similar to those of terrestrial generalized lizards, despite substantial morphological differences? Although the kinematics of chameleons differ from those of generalized lizards (Higham and Jayne, 2004), we test the null hypothesis that motor pattern is conserved.

Materials and methods

Experimental subjects

We studied the veiled chameleon *Chamaeleo calypttratus* Dumeril and Bibron because it is arboreal and morphologically

facilitate grasping perches in arboreal habitats. Morphological specializations for arboreal habitats or the structure of the habitat itself could result in the alteration of the ancestral motor pattern of limb muscles during locomotion.

Lizard taxa vary considerably in their morphology, ecology

specialized compared to a generalized terrestrial lizard (Peterson, 1984). This particular species also has relatively large hindlimbs that are suitable for implanting electromyographic (EMG) electrodes. We obtained all lizards from a commercial dealer, and they were transported to the University of Cincinnati for experiments. Lizards were caged alone, fed crickets (supplemented with vitamins) three times a week, and provided water *ad libitum*. Incandescent light bulbs above the cages provided a light and heat source for 12 h per day and allowed the lizards to thermoregulate behaviorally and attain their preferred active temperature range of 29–31°C. We chose four lizards of similar size, as indicated by the following values (mean \pm S.E.M. and range): mass 90.0 \pm 3.4 g, 83.0–98.0 g; snout–vent length SVL 18.4 \pm 0.4 cm, 17.3–19.5 cm.

We determined the gross anatomy of the limb musculature by performing dissections of preserved specimens and using the work of Mivart (1870) and Snyder (1954). Our terminology follows that of Snyder (1954). Nine large muscles were well suited for percutaneously implanting EMG electrodes, and Fig. 1 illustrates the gross anatomy of these muscles.

Experimental protocol

We used Halothane to anesthetize all animals before implanting EMG electrodes. Animals recovered from anesthesia for approximately 2 h before the experiments began. *C. calytratus* moved along a 2.4 cm diameter wooden dowel, covered with cloth adhesive tape, with inclines of -45° (downhill), 0° (level), and 45° (uphill) after their body temperature reached 29–31°C, which is within the range of the active body temperatures that we observed in the laboratory for this species (Higham and Jayne, 2004). Each individual was tested within a single day and the order of inclines was randomized to minimize confounding effects of time. Following each experiment, the lizard was euthanized, and *post-mortem* dissections confirmed the position of EMG electrodes.

We obtained simultaneous dorsal and lateral views of the chameleons moving on the perch using a two-camera NAC HSV-500 (Tokyo, Japan) high-speed video system operating at 250 images s^{-1} . Video recordings were synchronized with EMG recordings using a 100 Hz square-wave generator that provided output to both the video system and the EMG tape recordings. To provide fixed points of reference, we drew lines at 10 cm intervals along the length of the surfaces. To facilitate digitizing the location of points on the lizards, we painted landmarks on the pelvis, knee, ankle, metatarsal and toe tip, as in Higham and Jayne (2004). Prior to each trial, we measured the body temperature of the chameleon using a thermocouple and a Tegam (Geneva, OH, USA) model 871A digital thermometer. From the video footage, we selected 3–4 strides per individual per incline that had similar speeds and duty factors among and within individuals. The grand means \pm S.E.M. for the speeds and duty factors for all of the 44 strides were 11.2 \pm 0.5 cm s^{-1} and 58.7 \pm 0.7%, respectively. Similar duty factors commonly indicate equivalent gaits, and an analysis of variance (ANOVA) confirmed that duty factors did

not vary significantly with incline ($F=2.8$, $P=0.07$, d.f.=2,32) or among individuals ($F=2.6$, $P=0.07$, d.f.=3,32). All of the locomotion of *C. calytratus* was a relatively slow walk.

For the chameleons, we analyzed non-consecutive strides from different trials, and the stationary surfaces used to obtain locomotion required a large number of trials to obtain reasonably steady-speed locomotion of similar speeds. The strides selected for analysis were in the middle of a bout of locomotion with three or more strides that subjectively appeared to be of similar speed. Hence, we selected strides with little if any acceleration.

Electromyography

We used fine-wire bipolar electrodes to record the *in vivo* activity of the muscle, and the general methods for constructing the electrodes followed those of Jayne (1988). We used 0.051 mm diameter polycoated stainless-steel wire (California Fine Wire Co., USA) with approximately 0.7 mm of insulation removed from the recording end. A ground wire was also implanted in the epaxial musculature of the proximal portion of the tail. We implanted each electrode through the skin using a 26-gauge hypodermic needle with the wires inserted through the tip. Following implantation, we used cyanoacrylate glue to attach electrode wires to the skin of the lizard's tail and plastic model cement to glue the two wires of each electrode together and all electrodes together into a single cable. The lengths of the wires from the animals to the probes of the recorder were approximately 2 m, which was sufficiently long to allow the lizards to move freely. We implanted a total of nine EMG electrodes into nine muscles of the right hindlimb (Fig. 1).

Electromyograms (EMGs) were amplified 5000 \times using Grass P511k series amplifiers (West Warwick, RI, USA) with high- and low-bandpass filters of 100 Hz and 10 KHz, respectively, and a 60 Hz notch filter. The analog EMG signals were recorded at 9.5 cm s^{-1} using a TEAC XR-7000 FM data recorder (Tokyo, Japan). We converted the analog signals to digital data using a Keithley 500A (Cleveland, OH, USA) analog-to-digital converter with an effective sampling rate of 8 kHz (Jayne et al., 1990b). Digital EMGs were filtered using a high-pass finite-impulse response filter to reduce the amplitude of the signal below 100 Hz to less than 10% of the original amplitude.

We used customized computer software (written by Garr Updegraff, San Clemente, CA, USA; garru@uci.edu) to subdivide each stride into bins for three separate analyses. First, we subdivided the stance and swing phase of each stride into fifteenths and tenths, respectively. This was done to facilitate pooling data from different strides so that the number of bins during stance divided by the total number of bins per stride equalled 60%, which approximated the average duty factor for all of the strides. Consequently, each bin often covered approximately 4% of the total stride, but the absolute duration of bins within a stride or among strides varied slightly as a result of strides with variation in duty factor or total duration. The average rectified amplitude (RA) of each bin (rectified integrated area divided by bin duration) for each

muscle was then converted to relative amplitude by dividing by the maximum value ever observed within a bin for a given individual and muscle. These data were used to generate a profile of mean relative amplitude pooled across all individuals for each incline with a 60% duty factor (the approximate mean value). Second, we subdivided each stride into 25 equal duration bins. The total rectified integrated area (RIA) for a given muscle was calculated per stride and the RIA for each bin was converted to relative area based on its percentage of the total RIA for the stride. For pairs of muscles within each stride we calculated the product moment correlation coefficients (r) between pairs of values of percent activity per bin per stride, using values from bins with the same elapsed time. We used these cross-correlations to clarify the extent to which the activity of different muscles overlapped. We expected synergistic and antagonistic pairs of muscles to have positive and negative correlations, respectively. Lastly, we subdivided each stride into 30 ms bins. We chose 30 ms (see Kinematics, below) so that at least one bin was available between successive video images that were digitized. We then summed the activity of all bins during a kinematic event and divided by the total per stride. Thus, for each muscle we could determine the percentage of activity that was during stance *versus* swing, joint extension *versus* flexion, etc.

For many muscles during downhill locomotion, large time intervals (>0.1 s) often elapsed between successive spikes of EMGs (e.g. Fig. 2, caudofemoralis, -45°), or the amplitudes of EMG spikes were often small. Thus, determining the onset and offset of EMGs for all experimental conditions was often not practical.

From all four individuals in our study, we obtained EMGs from one hip muscle, the caudofemoralis (CF), four thigh muscles including the iliofibularis (IF), iliotibialis (IT), flexor tibialis externus (FTE) and puboischiotibialis (PIT), and four lower leg muscles including the gastrocnemius (G), tibialis anterior (TA), extensor digitorum longus (EDL) and the peroneus (P) (Fig. 1).

Kinematics

We used customized video analysis software (Measurement TV, written by Garr Undergraff) to reconstruct the three-dimensional coordinates of each landmark. The x -axis of each video image was parallel to the overall direction of travel along the long axis of the locomotor surface. The y -axis was perpendicular to the surface and within a vertical plane passing through the long axis of each walkway, and $y=0$ for the top of the walkway along its entire length. The z -axis was perpendicular to the x - y plane.

For each chameleon on each incline, we digitized 27–32 images per stride, which provided images 30–150 ms apart. Three-dimensional coordinates were digitized for the right hip, knee, ankle, base and tip of the fourth toe (excluding claw). From a dorsal perspective, two-dimensional coordinates (x , z) were digitized for the right and left hips.

Three-dimensional angles described angles of joints within the hindlimb at footfall and at the end of stance. A detailed

explanation of the angular measurements can be found in Jayne and Irschick (1999). Smaller values of knee angle between 0° and 180° indicate greater flexion of the joint. For the ankle angle, decreasing values between 0° and 180° indicate greater dorsiflexion of the foot. The orientation of the pelvis was determined by a two-dimensional angle obtained from a dorsal (x , z) perspective, and the amount of pelvic rotation equalled the differences between maximum and minimum values.

For three angles that describe the orientation of the femur, we determined the maximum and minimum values for each complete stride cycle. Femur retraction was the two-dimensional angle between a line connecting the left and right hips and the long axis of the femur projected onto the x - z (horizontal) plane. Femur retraction values of 0° indicate when the femur is perpendicular to the longitudinal axis of the pelvis, and positive and negative values indicate measurements of femur retraction and protraction, respectively. Long-axis rotation of the femur was a three-dimensional angle between a vertical reference plane passing through the femur and the plane containing the femur and tibia. Greater clockwise long-axis rotation of the right femur, as seen in right lateral view, is indicated by greater positive values, and a value of zero indicates that the plane containing the femur and tibia is within the vertical reference plane passing through the femur. Femur depression was the three-dimensional angle between the long axis of the femur and a horizontal reference plane. Positive and negative values of femur depression indicate that the distal femur was either ventral or dorsal to the hip, respectively.

Statistical analyses

We used SAS version 8.0 for all statistical analyses, and $P < 0.05$ was the criterion for statistical significance. In the tabular summaries of the statistics, we provide degrees of freedom and F -values to clarify the potential effects of making multiple comparisons. To analyze the extent to which muscle activity overlapped with other muscles, we constructed correlation matrices and used the resulting r -values in two-way analyses of variance (ANOVAs) with individual as a random crossed factor and incline as a fixed crossed factor. The denominator for the F -test on the incline effect was the two-way interaction terms of the fixed effect and the individual factor (Zar, 1996). To determine the effects of incline on the angular variables, we performed ANOVAs on each angular variable with incline and individual as the independent variables. Finally, to determine whether incline significantly affected the percentage of activity of a certain muscle during a particular kinematic event, we performed ANOVAs on each percentage with incline and individual as the independent variables.

Results are presented as means \pm S.E.M., unless stated otherwise.

Results

Muscle anatomy

The caudofemoralis (CF) is a robust muscle originating from the transverse processes of the four most proximal caudal

vertebrae and inserting to both the greater trochanter of the femur and the proximal portion of the fibula *via* an auxiliary tendon (Fig. 1A). The iliofibularis (IF) originates *via* a tendon from the posterior and lateral margin of the ilium and inserts on the fibula distal to the insertion of the caudofemoralis auxiliary tendon (Fig. 1B). The iliotibialis (IT) originates *via* a tendon from the posterior portion of the ilium just dorsal to the origin of the iliofibularis and inserts to the proximal tibia *via* the connective tissue on the anterior face of the knee (Fig. 1B). The flexor tibialis externus (FTE) originates from the ilio-ischiadic tendinous arch, runs along the posterior and ventral portion of the thigh and sends a long tendon, running along the posterior edge of the lower leg, to the plantar ossicle (Fig. 1A,B). Additionally, the flexor tibialis externus sends a shorter tendon that crosses the iliofibularis and inserts on the fibula just proximal to the insertion of the iliofibularis (Fig. 1A,B). The puboischiotibialis (PIT) is on the ventral surface of the thigh and originates from the pubo-ischiatic symphysis (mid-ventral line) and inserts on the proximal portion of the tibia (Fig. 1C).

The gastrocnemius (G) originates from both the distal part of the femur and the posterior aspect of the tibia and runs along the posterior edge of the lower leg where it inserts on the plantar ossicle (Fig. 1D). The extensor digitorum longus (EDL) originates from the distal portion of the femur and from the posterior portion of the fibula and inserts onto both the fourth and fifth digit (Fig. 1C,D). The peroneus (P) originates from the proximal portion of the anterior face of the fibula and from the proximal portion of the posterior tibia and inserts on the proximal and dorsal portion of the fifth metatarsal (Fig. 1B). The tibialis anterior (TA) originates from the proximal portion of the tibia and inserts onto the proximal portion of the first metatarsal (Fig. 1C,D). Table 1 summarizes the hypothesized actions of the muscles based on their gross anatomy.

General description of limb movements and the effects of incline

At footfall, the knee of *C. calypttratus* extended well beyond 90° and the femur was protracted (Fig. 2A), which positioned the foot anterior to the hip. The angles of both the knee and ankle at footfall usually exceeded 120° and 140°, respectively (Fig. 2A). Maximal pelvic rotation was approximately coincident with footfall. Femur retraction of *C. calypttratus* began slowly at footfall, was fairly rapid at midstance, and continued to a maximum slightly before the end of stance (Fig. 2A). After footfall, the knee flexed maximally to an acute angle near midstance, whereupon the knee extended to a maximum at the end of stance (Fig. 2A). Clockwise long-axis femur rotation (as seen in right lateral view) began at footfall and continued throughout stance (Fig. 2A). Femur depression remained fairly constant throughout stance (Fig. 2A).

At the beginning of swing, the knee began to flex until just before mid-swing and then extended until footfall (Fig. 2A). The amount of knee flexion during swing was always less than the amount of flexion during stance. Femur protraction began at the beginning of swing and was rapid until mid-swing,

Table 1. *The muscles used in this study and their proposed actions*

Muscle	Proposed action based on anatomy
Caudofemoralis (CF)	Knee flexion; femur retraction and rotation
Ilio-fibularis (IF)	Knee flexion; limb elevation
Iliotibialis (IT)	Knee extension; femur retraction; limb elevation
Flexor tibialis externus (FTE)	Knee flexion; plantar flexion; femur retraction
Puboischiotibialis (PIT)	Knee flexion; femur depression
Gastrocnemius (G)	Plantar flexion
Tibialis anterior (TA)	Plantar flexion; digit opener
Extensor digitorum longus (EDL)	Dorsiflexion; digit opener
Peroneus (P)	Plantar flexion; digit opener

whereupon the femur was protracted slowly until footfall (Fig. 2A). Counterclockwise long-axis femur rotation of *C. calypttratus* also began near the beginning of swing, was rapid until mid-swing, and continued rotating slowly until footfall (Fig. 2A). Values of femur depression decreased sufficiently during early swing such that the knee was often more dorsal than the hip. From mid-swing until footfall, femur depression remained relatively constant (Fig. 2A).

Incline significantly affected only one of the 19 angular variables measured, which is similar to the minimal effect of incline on kinematics of *C. calypttratus* found by Higham and Jayne (2004). The ankle angle at the end of stance was significantly affected by incline such that the greatest values were on the downhill and lowest on uphill surface (Fig. 2A). The speeds of locomotion for the kinematic data in Higham and Jayne (2004) and this study were very slow (grand means=17 and 11 cm s⁻¹, respectively) and the duty factors were similar (grand means=63% and 59%, respectively). Thus, we had no evidence that the electrodes substantially affected the locomotion of the chameleons in this study compared to the previous kinematic study.

Muscle activity on a level perch

The average duty factor was approximately 60%, thus a muscle with constant amplitude and continuous activity would have 60% activity during stance and 40% during swing. More than two-thirds of the rectified integrated area (RIA) occurred during stance for the caudofemoralis, flexor tibialis externus, puboischiotibialis, extensor digitorum longus, and peroneus regardless of incline (Table 2).

The onset of caudofemoralis activity began immediately following footfall and usually continued throughout the stance phase of the stride (Fig. 2B). The activity of the caudofemoralis usually ceased just after the beginning of the swing phase (Fig. 2B). The location of burst activity of the iliofibularis during a stride varied considerably between individuals. Occasionally a small burst of iliofibularis activity occurred during early stance, but most commonly the burst of

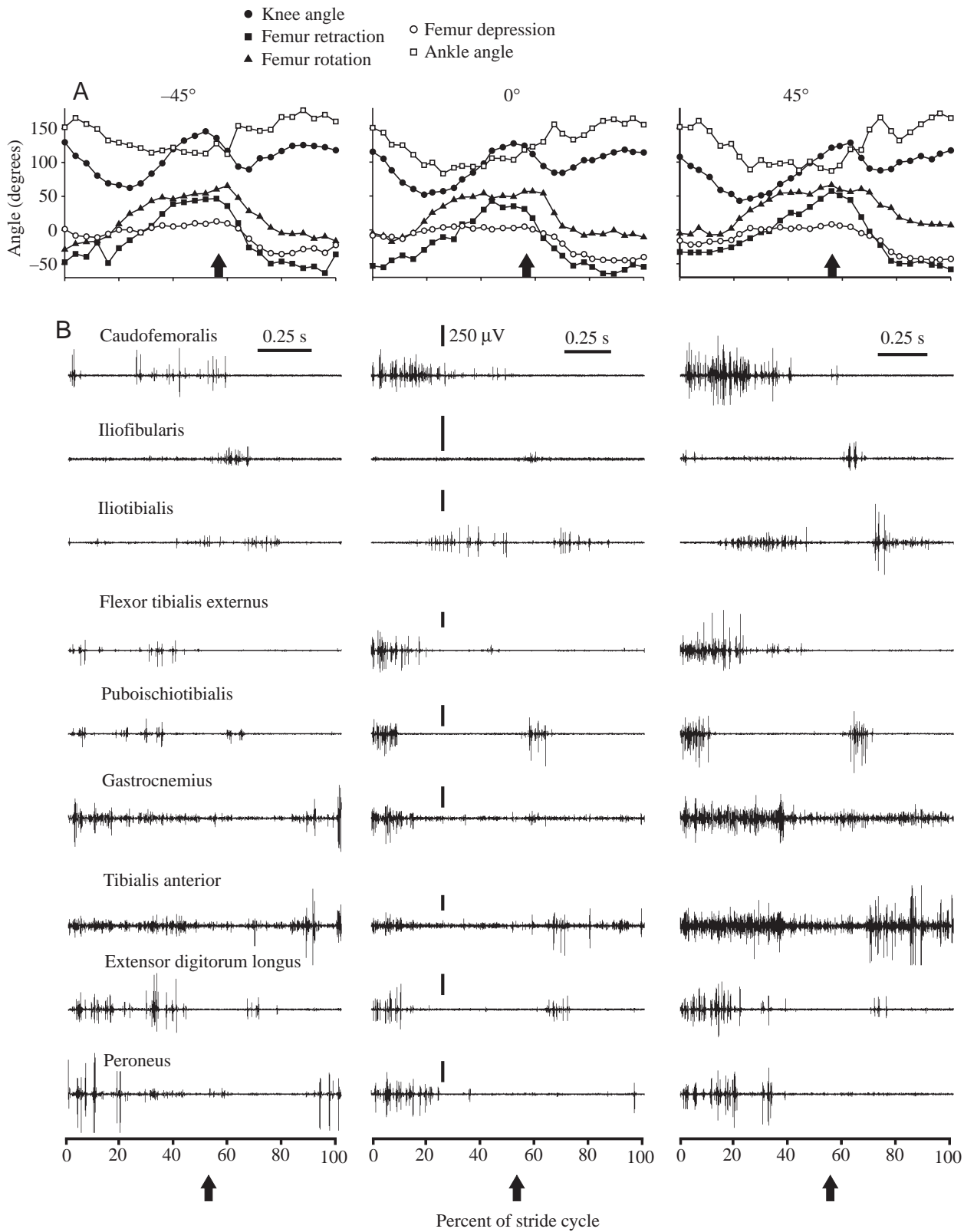


Fig. 2. (A) Kinematics and (B) EMGs versus time (as a percentage of stride cycle) for one stride from a single individual of *C. calypttratus* on inclines of -45° (left), 0° (middle), and 45° (right). The arrows indicate the end of stance, and footfall occurs at time 0%. The vertical bars indicate the voltage scales ($250 \mu\text{V}$), which are constant between all panels within a row.

iliofibularis activity was at the beginning of swing (Fig. 2B). The iliotibialis usually had one burst of activity beginning at midstance and continuing until the end of stance and another burst near mid-swing (Fig. 2B). The activity of the flexor tibialis externus began immediately following footfall and usually ended near midstance (Fig. 2B). The puboischiotibialis had an initial burst of activity immediately following footfall and lasting for approximately one-fourth of stance, and a second burst of activity occurred at the beginning of swing and lasted for approximately one-fourth of the swing phase (Fig. 2B).

The gastrocnemius was active throughout stance and had lower amounts of activity throughout swing (Fig. 2B). The

Table 2. Mean values of the percentages of rectified integrated area during different phases of the stride cycle and F-values for the main effect of incline from two-way ANOVAs performed separately on each variable

Variable ^a	Percent of activity per stride at each incline			F-value Incline (d.f. 2,6)
	-45°	0°	45°	
CF stance	67	93	86	7.5*
CF stance flex	15	50	51	27.0**
CF swing flex	25	2	1	11.4*
CF retraction	72	93	84	6.5*
CF post rotation	76	95	87	5.1
IF stance	51	38	57	3.7
IF stance flex	32	20	24	2.6
IF swing flex	28	39	18	2.4
IF retraction	51	39	55	2.2
IF elevation	38	53	32	2.5
IT stance	43	49	65	6.9*
IT stance ext	20	40	52	18.9**
IT swing	39	37	29	1.8
IT retraction	44	50	63	5.6*
IT elevation	42	43	29	4.2
FTE stance	79	90	95	2.1
FTE stance flex	33	68	80	11.7*
FTE swing flex	10	4	1	1.7
FTE retraction	82	90	94	1.6
PIT stance	72	73	82	1.5
PIT stance flex	37	62	71	5.3*
PIT swing flex	20	23	14	1.7
G stance	59	69	61	0.7
TA stance	48	54	49	0.2
EDL stance	75	83	88	0.6
P stance	70	84	84	3.5

* $P < 0.05$, ** $P < 0.01$.

Muscle abbreviations are listed in Table 1.

^aEach variable is a combination of a muscle and a phase of the stride. The description following the muscle abbreviation (e.g. stance flex) indicates the percentage of the total rectified integrated area of those muscles during those kinematic events.

Flex, flexion of the knee; ext, extension; retraction, femur retraction; post rotation, clockwise long-axis femur rotation as seen in right lateral view; elevation, femur elevation.

tibialis anterior had variable activity that often occurred throughout the stride (Fig. 2B). The extensor digitorum longus had a strong burst of activity immediately following footfall and lasted for up to one-half of stance (Fig. 2B). A second burst of activity of the extensor digitorum longus with lower amplitude and relatively short duration occurred just before mid-swing (Fig. 2B). The peroneus became active at footfall or just prior to the end of swing and was active for approximately half of stance (Fig. 2B).

The only significant negative cross-correlation of relative EMG amplitude between pairs of muscles for locomotion on the level perch was for the iliotibialis and the puboischiotibialis (Table 3), and the near absence of overlapping activity of these two muscles (Figs 2, 3) agrees well with their antagonistic functions as a knee extensor and flexor, respectively. Several of the lower limb muscles had substantial overlap of major activity (Figs 2, 3), and hence significant positive cross correlations between the relative amplitudes of activity (Table 3).

Effects of incline on muscle activity

The effects of incline were more conspicuous for hip and thigh muscles than for lower leg muscles (Figs 2, 3). Furthermore, changes in motor pattern in response to incline were predominantly due to changes in amplitude rather than changes in timing (Figs 2, 3). Effectively all of the muscles studied had less intense activity for downhill locomotion than for both level and uphill locomotion.

The percentage of muscle activity during stance changed significantly with incline for the caudofemoralis, which had much more of its activity during stance on level (93%) and uphill (86%) than on the downhill (67%) (Table 2). The peak relative amplitude of the caudofemoralis within an entire stride increased from downhill to level and from level to uphill (Fig. 3). Large amplitude activity of the caudofemoralis was conspicuously absent during early stance as the knee flexed on the downhill compared to the level and uphill surfaces (Fig. 3; Table 2). Effectively no caudofemoralis activity occurred in early swing as the knee flexed during level and uphill locomotion, whereas a significantly greater amount of activity occurred during this time interval for downhill strides (Fig. 3; Table 2).

Activity of the iliotibialis was nearly absent during early stance on the level and uphill surfaces, and the peak relative amplitude per stride increased from downhill to level and level to uphill (Fig. 3). On the uphill surface, more than half the iliotibialis activity occurred as the knee extended during stance and there was significantly less activity during this period for strides on the level and downhill surfaces (Table 2).

The peak activity of the flexor tibialis externus during the entire stride on the downhill surface was markedly less than the values for both the level and uphill surfaces (Fig. 3). Additionally, the amount of flexor tibialis activity during knee flexion during stance on the downhill was less than half that of the uphill and level surfaces (Table 2).

The peak activity per stride of the puboischiotibialis

Table 3. Mean *r*-values from cross-correlations between pairs of muscles for each incline

	CF	IT	FTE	PIT	G	TA	EDL	P	IF
Downhill									
CF									
IT	-0.13								
FTE	0.30	-0.16							
PIT	-0.02	-0.29	0.25						
G	0.31	0.06	0.25	-0.04					
TA	0.19	0.18	0.22	-0.08	0.77**				
EDL	0.05	0.00	0.25	0.30	0.07	0.07			
P	0.27	-0.04	0.17	0.25	0.10	-0.02	0.43*		
IF	-0.08	0.14	0.02	0.18	0.20	0.22	0.13	0.00	
Level									
CF									
IT	-0.06								
FTE	0.42*	-0.33							
PIT	0.18	-0.43*	0.63**						
G	0.36	-0.10	0.38	0.27					
TA	0.16	0.04	0.23	0.09	0.81**				
EDL	0.23	-0.21	0.50**	0.59**	0.20	0.16			
P	0.37	-0.19	0.43*	0.39*	0.13	-0.02	0.66**		
IF	0.04	-0.14	0.05	0.27	0.11	0.08	0.11	0.01	
Uphill									
CF									
IT	0.19								
FTE	0.50**	-0.24							
PIT	0.21	-0.30	0.58**						
G	0.40*	0.02	0.34	0.19					
TA	0.16	0.11	0.10	0.02	0.57**				
EDL	0.52**	-0.21	0.78**	0.64**	0.29	0.17			
P	0.60**	-0.04	0.70**	0.40*	0.38	0.09	0.73**		
IF	0.26	0.19	0.06	0.20	0.22	0.06	0.07	0.08	

Asterisks indicate significant correlation coefficients; * $P < 0.05$, ** $P < 0.01$.

Correlations that were affected significantly by incline are in boldface.

See Table 1 for muscle abbreviations.

increased from downhill to level and from level to uphill (Fig. 3). For the puboischiotibialis a clear maximum in relative amplitude occurred shortly after footfall on the uphill and level surfaces (Fig. 3), and the percentage of activity during knee flexion within stance for uphill and level strides was approximately twice that of downhill strides (Table 2).

Most cross-correlations of muscle activity were not affected greatly by incline (Table 3). Only three of the 36 combinations of muscles were affected significantly by incline and each of these three pairs had higher cross correlations going from downhill to level and from level to uphill (Table 3). Although the cross correlations between activity of the iliotibialis and flexor tibialis externus muscles were not significantly affected by incline as indicated in the ANOVA, this negative cross correlation was significant for the level surface but not for either the downhill or uphill data. Thus, the overlap in activity of these two muscles was least on the level surface. The highly significant positive cross correlations for the level and uphill surfaces emphasize the similarity of the timing of peak activity

of the flexor tibialis externus and the puboischiotibialis muscles near the beginning of stance, whereas the absence of a significant cross correlation for surface suggests these muscle do not retain fundamentally similar timing relationships on the downhill compared to the other surfaces.

Discussion

Muscle activity and kinematics of chameleons

Our study is the first to determine the *in vivo* muscle activity of the hindlimb during locomotion of a specialized arboreal lizard. The most conspicuous changes in amplitude with incline were for hip and thigh muscles rather than the lower limb muscles.

At footfall, the femur of *C. calytratus* is maximally protracted, and unlike most lizards, the knee of *C. calytratus* is extended well beyond 90° (Higham and Jayne, 2004). Thus, knee flexion during early stance seems likely to pull the body forward. Consequently, muscles that flex the knee during early

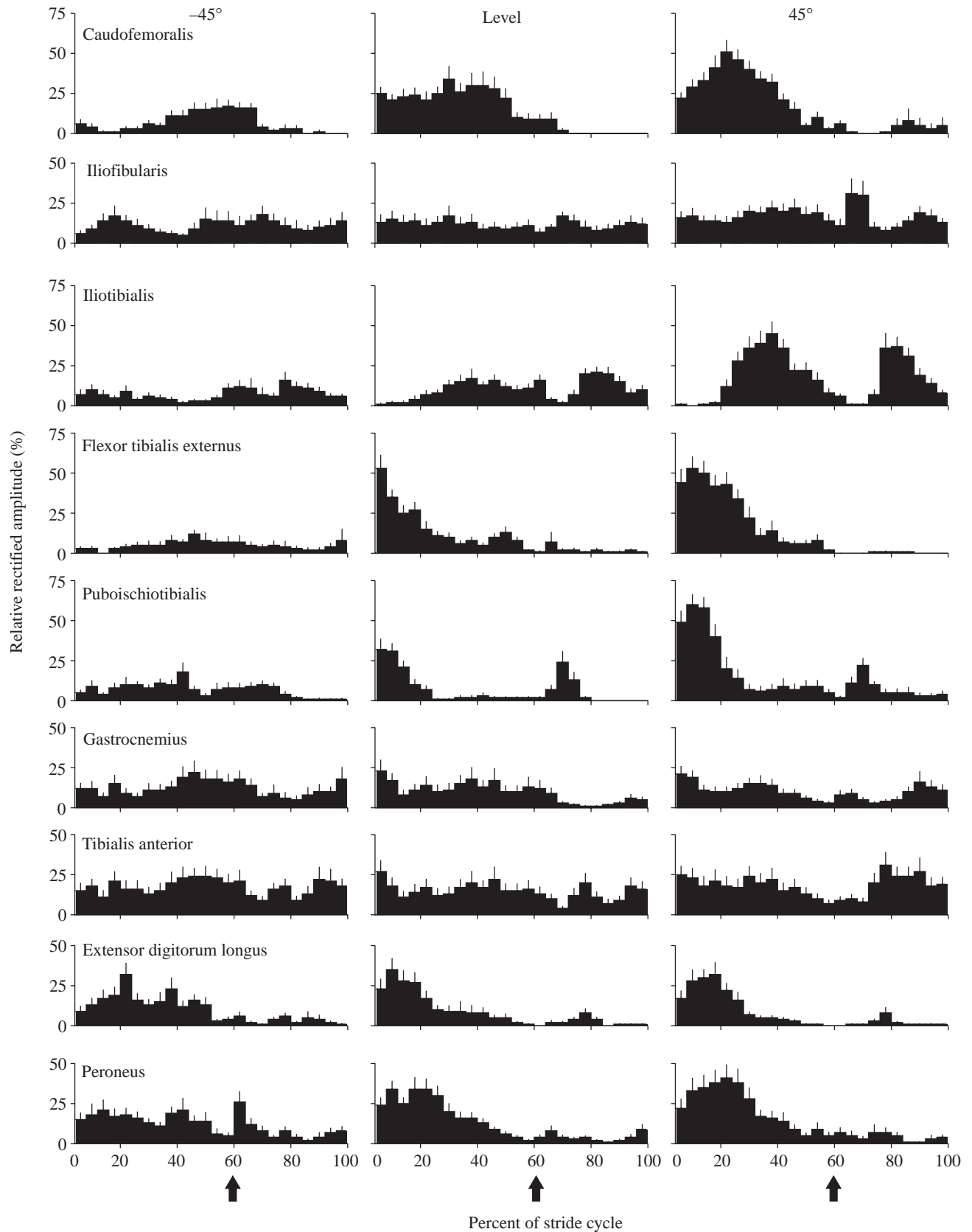


Fig. 3. The relative rectified amplitude (mean \pm s.e.m.) for each muscle from individuals of *C. calypttratus* on inclines of -45° (left), 0° (middle), and 45° (right). Each stride was divided into 15 equal duration bins during stance and 10 equal duration bins during swing. Each bin was scaled to the largest value ever observed for a bin from a given muscle over all three inclines. Time zero is footfall and the arrow indicates the end of stance.

stance are likely to be important for propelling the body forward in a fashion analogous to the salamander, *Dicamptodon tenebrosus* (Ashley-Ross, 1995). The muscles most likely to be responsible for knee flexion during the first half of stance in *C. calypttratus* are the flexor tibialis externus, puboischiotibialis and the caudofemoralis. These muscles all had bursts of activity during early stance and were all hypothesized to flex the knee, and the greatest amplitude occurred immediately following footfall. Thus, actively pulling the body forward is partly determined by the activity of these three muscles. During femur retraction, the caudofemoralis and flexor tibialis externus were active and thus could contribute to another mechanism of propulsion.

Plantar flexion and dorsiflexion could not be measured as easily in chameleons as in other lizards (Irschick and Jayne, 1999b; Jayne and Irschick, 1999). The highly specialized foot of *C. calypttratus* has two opposing groups of digits, each of which is enclosed in skin, rather than having five independent digits (Fig. 1). We measured ankle angle as the three-dimensional angle between the lower limb and the fourth metatarsal (within the lateral group of digits). Consequently, the ankle angle is affected both by closing and opening the foot (to grasp and release the perch) and posteriorly directed movements of the collective foot structures relative to the lower limb. The movements of the chameleon feet were also not on a flat substrate as they grasped the round perch. Thus, we assumed that the functional equivalent of plantar flexion occurred predominantly during stance and the reverse movement occurred during swing. The anatomy of the peroneus and gastrocnemius and the large portion of their activity during stance suggest that these muscles are involved in the posterior rotation of the foot in *C. calypttratus*.

The hyperextension of the toe at the end of stance and very early swing in many terrestrial lizards (Irschick and Jayne, 1999b; Jayne and Irschick, 1999) suggests that using the foot to actively push off from the flat substrate is important. In contrast, the change of nearly 90° in the orientation of the long axis of the foot of *C. calypttratus* largely precludes this mechanism of propulsion. Furthermore, the foot of *C. calypttratus* grasps the perch and does not hyperextend at the end of stance. The grasping foot of chameleons could thus facilitate a mechanism for propulsion that relies on pulling the body, but at the cost of a reduced ability to push during late stance by plantar flexion similar to that of other terrestrial lizards.

Comparisons with alligators and lizards

The caudofemoralis of both crocodylians and lepidosaur reptiles is a robust muscle originating from caudal vertebrae and inserting on the femur and proximal lower limb and is involved in femur retraction, posterior femur rotation and knee flexion (Gatesy, 1990, 1997; Russell and Bauer, 1992; Reilly, 1995; Nelson and Jayne, 2001). Previous EMG studies of lizards and crocodylians have found that the caudofemoralis usually becomes active late in swing, which could retard femur protraction, anterior femur rotation and knee extension (Reilly, 1995; Gatesy, 1997; Nelson and Jayne, 2001). The onset of

activity of the caudofemoralis of *C. calypttratus* was usually at footfall and only rarely during late swing. However, the slow locomotor speed of *C. calypttratus* may preclude the need to actively decelerate femur protraction with the caudofemoralis.

Nelson and Jayne (2001) found that passive changes in strain of the caudofemoralis muscle of the desert iguana, *Dipsosaurus dorsalis*, were only affected by knee extension when the femur was substantially anterior to the hip. Thus, although the caudofemoralis of *C. calypttratus* appears likely to flex the knee during early stance, the lack of activity and position of the femur suggest the caudofemoralis is not important for the second flexion of the knee during swing.

We found that the activity of the iliofibularis of *C. calypttratus* was variable but when a burst was evident, it was usually in early swing (Fig. 2). The activity of the iliofibularis of *Alligator mississippiensis* starts during late stance and continues into early swing (Gatesy, 1997), and the iliofibularis of the savannah monitor, *Varanus exanthematicus*, is active mainly during swing (Jayne et al., 1990a).

The puboischiotibialis of alligators has two distinct bursts, one from late swing to late stance, and another shorter burst at the stance/swing transition (Gatesy, 1997). In alligators, the puboischiotibialis probably helps to prevent limb collapse during stance by resisting femoral abduction (Gatesy, 1997). The activity of the pubotibialis (similar to our PIT) of *Sceloporus clarkii* begins after footfall and peaks when the knee angle is 90° (Reilly, 1995), thus contributing to knee flexion. A smaller burst of activity in *S. clarki* also occurs at mid-swing (Reilly, 1995). Similarly, the puboischiotibialis of *C. calypttratus* had two very distinct bursts, but one burst was in early to mid-swing and the other was in early stance. Thus, the timing of activity of the puboischiotibialis of *C. calypttratus* is different from that of the distantly related alligator, but relatively similar to *S. clarki*. The burst of activity in early stance could help maintain the horizontal orientation of the femur and thus prevent femur elevation.

The gastrocnemius and peroneus longus of *Sceloporus clarkii* are both plantar flexors that have peak bursts during the first half of stance (Reilly, 1995). Although the activity of the gastrocnemius in *C. calypttratus* was variable, the peroneus generally had a similar pattern of activity as that of *S. clarki*. However, the morphology of the foot and ankle of *C. calypttratus* differs substantially from a generalized lizard. Activation of the peroneus while the foot of *C. calypttratus* is anchored to the perch could move the knee away from the body and in an anterior direction, thus flexing the ankle during early stance (Fig. 2).

The appendicular anatomy of *C. calypttratus* differs considerably from that of *Varanus*, *Dipsosaurus*, and *Sceloporus*. However, the major features in the timing of muscles in common to these lizard taxa when moving on a level surface appear similar.

Effects of incline on chameleons

The hindlimb kinematics of *C. calypttratus* changed very little with changes in incline, whereas the amplitudes of muscle

activity changed substantially. Moving uphill requires an increase in propulsive forces compared to the level, because of the increased component of gravity opposing the direction of movement. One method that chameleons use to propel their body during the first half of stance is to flex their knee. The activity of both the flexor tibialis externus and the puboischiotibialis of *C. calytratus* increased substantially while moving uphill compared to level, and their peak amplitude occurred immediately following footfall. Thus, these two muscles are probably the most important for early knee flexion and for pulling the body forward. The activity of the caudofemoralis increased when moving uphill, and probably contributes to knee flexion, but the peak amplitude occurred after the knee began to flex at footfall and may be better correlated with the role in long axis rotation of the femur for propelling the chameleon uphill.

Moving downhill requires less propulsive force (compared to level locomotion) because of the increased component of gravity contributing to forward movement, but some muscles must actively resist the passive flexion of joints in order to maintain posture and control speed. Early knee flexion during stance can occur passively, and in order to prevent the joint from collapsing, a knee extensor should be active. For the first 20% of the stride cycle, the relative amplitude of the iliotibialis was slightly greater on the downhill compared to both the level and the uphill surfaces in *C. calytratus* (Fig. 3), and it extends the knee. Thus, the iliotibialis may help prevent knee flexion when moving downhill. The iliotibialis also extends the knee during the second half of swing, and the relative amplitude of this burst of muscle activity increased markedly on uphill surfaces. Perhaps this increased amplitude of swing-phase activity reflects an increased force requirement for knee extension because of greater torque on the lower limb with the change in limb orientation.

The activity of the hindlimb muscles of *C. calytratus* changed markedly with incline, supporting the idea that animals modulate the activity of muscles as the demands in the environment change. However, the primary form of modulating muscle activity was changing amplitude rather than timing relationships. Furthermore, the most conspicuous changes in amplitude that we found were for propulsive muscles, which increased in activity on an uphill surface and decreased in activity on a downhill surface. The lack of significant changes within an incline for the cross-correlations between most pairs of muscles (Table 3) further supports the conclusion that, despite changes in the intensity of muscle activity, the shape of the bar graphs indicating the relative timing of peaks and periods of minimal or no activity changed very little with incline for most muscles. Consequently, the synergistic or antagonistic relationships between pairs of muscles were usually not affected by incline. The most notable exceptions to the overall preservation in the relative timing of peak activity within a muscle were for the flexor tibialis externus and the puboischiotibialis on the downhill incline compared to the other two surfaces (Fig. 3). In contrast to conspicuous peak of activity in early stance for these two

muscles on the level and uphill surfaces, such peaks were absent on the downhill incline. Thus, these two muscles were involved in the only cases where the cross-correlations between pairs of muscle activity changed significantly with incline.

Inclines and other vertebrates

All previous EMG studies on the effects of incline on vertebrate locomotion are for mammals and birds (Pierotti et al., 1989; Roy et al., 1991; Roberts et al., 1997; Carlson-Kuhta et al., 1998; Smith et al., 1998; Galbaldon et al., 2001; Gillis and Biewener, 2002; Daley and Biewener, 2003). During level locomotion in cats, the knee and ankle joints flex briefly after footfall and then extend throughout stance (Carlson-Kuhta et al., 1998). However, flexion of the knee and ankle is absent during the stance phase of cats on uphill grades. At paw liftoff on level and uphill surfaces, both the knee and ankle of cats flex substantially for more than half of swing and then extend prior to footfall (Carlson-Kuhta et al., 1998).

Hip extension in mammals serves a similar function to femur retraction and rotation in sprawling-limbed vertebrates, since all of these movements tend to advance the hip forward relative to the point of foot contact. In cats, the hip begins to extend immediately after footfall regardless of incline (Carlson-Kuhta et al., 1998; Smith et al., 1998), and the amplitude of activity of stance-phase muscles that extend the hip (propulsive muscles) increases when moving uphill compared to the level (Pierotti et al., 1989; Carlson-Kuhta et al., 1998). The propulsive muscles of *C. calytratus* are ones that flex the knee for the first half of stance, extend the knee in the second half of stance, and retract and rotate the femur throughout stance, and these muscles increased in activity when moving uphill as compared to level (Fig. 3). Thus, a common response among distantly related groups of vertebrates is to increase the recruitment intensity of propulsive-phase muscles when moving uphill. These results are further supported by a study of rats, where the activity of the biceps femoris (hip extensor and knee flexor) and vastus lateralis (knee extender) muscles were the highest moving uphill (Gillis and Biewener, 2002).

During downhill locomotion, the activity of stance-phase muscles of cats that extend the hip to propel the body decreases (Smith et al., 1998). The muscles involved in propulsion in *C. calytratus* also decrease in activity when moving downhill as compared to level locomotion (Fig. 3). The muscles that flex the hip, and thus prevent forward movement, have increased activity when the cats move downhill (Smith et al., 1998). During downhill locomotion, the rectus femoris muscle in cats, which extends the knee, also has increased activity (Smith et al., 1998). Additionally, the timing of the onset of rectus femoris activity occurs earlier in stance for downhill locomotion compared to level locomotion. Thus, cats prevent the collapse of the knee joint when moving downhill both by increasing the amplitude of activity of the rectus femoris and by shifting the timing of activity.

Not all increases in EMG amplitude are associated with the need to increase propulsion when moving uphill. For example,

during uphill locomotion the iliopsoas of cats flexes the hip and lifts the limb during swing, and this muscle has increased activity compared to level locomotion (Carlson-Kuhta et al., 1998). The iliotibialis of *C. calyptatus* is, in part, a swing-phase muscle that extends the knee, and it had increased activity when moving uphill during swing compared to the level. When moving uphill the limb is more nearly perpendicular to gravity, and this increases the torque, resulting in an increase in activity of some swing-phase muscles in order to extend the knee.

During uphill locomotion, the most anterior and posterior positions of the limb during the entire stride in cats shift posteriorly compared to level locomotion, resulting in a reduction of the anterior placement of the paw at footfall relative to the hip (Carlson-Kuhta et al., 1998). On both uphill and level surfaces, cats have asymmetric longitudinal excursions of the foot relative to the hip, in which the magnitude of posterior placement exceeds that of anterior placement (Carlson-Kuhta et al., 1998), but this asymmetry is greater on the uphill. Similarly, in *Dipsosaurus dorsalis*, the most anterior position of the foot is less than the most posterior position relative to the hip, and the most posterior position increases when moving uphill *versus* moving on a level surface (Jayne and Irschick, 1999). The most anterior (X_{\max}) and posterior (X_{\min}) positions of the ankle relative to the hip in *C. calyptatus* are unaffected by incline (Higham and Jayne, 2004). Furthermore, rather than having marked asymmetry in longitudinal positions relative to the hip, the maximum anterior distance of the ankle closely approximated the maximum posterior distance of the ankle in *C. calyptatus*, further supporting the idea that chameleons pulling their body forward in early stance contributes to propulsion.

For generalized terrestrial lizards, the hindlimb kinematics change with speed in a manner that increases the muscle strain of the caudofemoralis (Nelson and Jayne, 2001). Such increased muscle strain is commonly correlated with increased power requirements for diverse vertebrate musculoskeletal systems performing a variety of tasks. For example, muscle fascicles of running turkeys actively shorten on an uphill surface, whereas they have isometric activity on a level surface (Roberts et al., 1997). Furthermore, the strain of some muscles while they are active in rats is greater on the uphill than on the level surface (Gillis and Biewener, 2002). The hindlimb kinematics of cats, generalized lizards, and most vertebrates change considerably when moving on inclines (Carlson-Kuhta et al., 1998; Smith et al., 1998; Jayne and Irschick, 1999). In contrast, the kinematics of *C. calyptatus* change minimally with incline and may indicate that power output is modulated primarily by altering force rather than strain during muscle activity.

This research was supported by NSF grant IBN 9983003 to D. J. Irschick and B.C.J. The acquisition of a high-speed video system was supported by NSF grant BIR 9217409 to B.C.J. R. German, D. Gist, M. Maynard, and A. Horner provided valuable comments on early drafts of the

manuscript. M. Rucker, A. Horner, and L. Spezzano, Jr assisted with experiments. We also thank Kristin Lierl for the artwork of the limb musculature.

References

- Ashley-Ross, M. A. (1995). Patterns of hind limb motor output during walking in the salamander *Dicamptodon tenebrosus*, with comparisons to other tetrapods. *J. Comp. Physiol. A* **177**, 273-285.
- Biewener, A. A. (2002). Future directions for the analysis of musculoskeletal design and locomotor performance. *J. Morphol.* **252**, 38-51.
- Carlson-Kuhta, P., Trank, T. V. and Smith, J. L. (1998). Forms of forward quadrupedal locomotion. II. A comparison of posture, hindlimb kinematics, and motor patterns for upslope and level walking. *J. Neurophysiol.* **79**, 1687-1701.
- Cartmill, M. (1974). Pads and claws in arboreal locomotion. In *Primate Locomotion* (ed. F. A. Jenkins), pp. 45-83. New York: Academic Press.
- Daley, M. A. and Biewener, A. A. (2003). Muscle force-length dynamics during level *versus* incline locomotion: a comparison of *in vivo* performance of two guinea fowl ankle extensors. *J. Exp. Biol.* **206**, 2941-2958.
- Farley, C. T. (1997). Maximum speeds and maximum power outputs in lizards. *J. Exp. Biol.* **200**, 2189-2195.
- Farley, C. T. and Emshwiller, M. (1996). Efficiency of uphill locomotion in nocturnal and diurnal lizards. *J. Exp. Biol.* **199**, 587-592.
- Gabaldon, A. M., Nelson, F. E. and Roberts, T. J. (2001). Gastrocnemius muscle mechanics in turkeys during uphill and downhill running. *Am. Zool.* **41**, 1448.
- Gatesy, S. M. (1990). Caudofemoral musculature and the evolution of theropod locomotion. *Paleobiol.* **16**, 170-186.
- Gatesy, S. M. (1997). An electromyographic analysis of hindlimb function in *Alligator* during terrestrial locomotion. *J. Morphol.* **234**, 197-212.
- Gillis, G. B. and Biewener, A. A. (2002). Effects of surface grade on proximal hindlimb muscle strain and activation during rat locomotion. *J. Appl. Physiol.* **93**, 1731-1743.
- Higham, T. E. and Jayne, B. C. (2004). Locomotion of lizards on inclines and perches: hindlimb kinematics of an arboreal specialist and a terrestrial generalist. *J. Exp. Biol.* **207**, 233-248.
- Huey, R. B. and Hertz, P. E. (1982). Effects of body size and slope on sprint speed of a lizard *Stellio (Agama) stellio*. *J. Exp. Biol.* **97**, 401-409.
- Irschick, D. J., Austin, C. C., Petren, K., Fisher, R. N., Losos, J. B. and Ellers, O. (1996). A comparative analysis of clinging ability among pad-bearing lizards. *Biol. J. Linn. Soc.* **59**, 21-35.
- Irschick, D. J. and Jayne, B. C. (1998). Effects of incline on speed, acceleration, body posture, and hindlimb kinematics in two species of lizard, *Callisaurus draconoides* and *Uma scoparia*. *J. Exp. Biol.* **201**, 273-287.
- Irschick, D. J. and Jayne, B. C. (1999a). A field study of effects of incline on the escape locomotion of a bipedal lizard, *Callisaurus draconoides*. *Physiol. Biochem. Zool.* **72**, 44-56.
- Irschick, D. J. and Jayne, B. C. (1999b). Comparative three-dimensional kinematics of the hindlimb for high-speed bipedal and quadrupedal locomotion of lizards. *J. Exp. Biol.* **202**, 1047-1065.
- Jayne, B. C. (1988). Muscular mechanisms of snake locomotion: an electromyographic study of the sidewinding and concertina modes of *Crotalus cerastes*, *Nerodia fasciata* and *Elaphe obsoleta*. *J. Exp. Biol.* **140**, 1-33.
- Jayne, B. C., Bennett, A. F. and Lauder, G. V. (1990a). Muscle recruitment during terrestrial locomotion: how speed and temperature affect fibre type use in a lizard. *J. Exp. Biol.* **152**, 101-128.
- Jayne, B. C., Lauder, G. V. and Reilly, S. M. (1990b). Short communication: the effect of sampling rate on the analysis of digital electromyograms from vertebrate muscle. *J. Exp. Biol.* **154**, 557-565.
- Jayne, B. C. and Ellis, R. V. (1998). How inclines affect the escape behaviour of a dune-dwelling lizard, *Uma scoparia*. *Anim. Behav.* **55**, 1115-1130.
- Jayne, B. C. and Irschick, D. J. (1999). Effects of incline and speed on the three-dimensional hindlimb kinematics of a generalized iguanian lizard (*Dipsosaurus dorsalis*). *J. Exp. Biol.* **202**, 143-159.
- Lauder, G. V. (1994). Homology, form, and function. In *Homology: The Hierarchical Basis of Comparative Biology* (ed. B. K. Hall), pp. 151-196. San Deigo: Academic Press.
- Lauder, G. V. and Shaffer, H. B. (1988). Ontogeny of functional design in tiger salamanders (*Ambystoma tigrinum*): are motor patterns conserved during major morphological transformations? *J. Morphol.* **197**, 249-268.
- Mivart, S. G. (1870). On the myology of *Chamaeleon parsonii*. *Proc. Zool. Soc. Lond.* **1870**, 850-889.

- Nelson, F. E. and Jayne, B. C.** (2001). The effects of speed on the *in vivo* activity and length of a limb muscle during the locomotion of the iguanian lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **204**, 3507-3522.
- Peters, S. E. and Goslow, G. E., Jr** (1983). From salamanders to mammals: continuity in musculoskeletal function during locomotion. *Brain Behav. Evol.* **22**, 191-197.
- Peterson, J. A.** (1984). The locomotion of *Chamaelea* (Reptilia: Sauria) with particular reference to the forelimb. *J. Zool., Lond.* **202**, 1-42.
- Pierotti, D. J., Roy, R. R., Gregor, R. J. and Edgerton, V. R.** (1989). Electromyographic activity of cat hindlimb flexors and extensors during locomotion at varying speeds and inclines. *Brain Res.* **481**, 57-66.
- Reilly, S. M.** (1995). Quantitative electromyography and muscle function of the hind limb during quadrupedal running in the lizard *Sceloporus clarkii*. *Zool. Anal. Complex Syst.* **98**, 263-277.
- Roberts, T. J., Marsh, R. L., Weyland, P. G. and Taylor, C. R.** (1997). Muscular force in running turkeys: The economy of minimizing work. *Science* **275**, 1113-1115.
- Roy, R. R., Hutchison, D. L., Pierotti, D. J., Hodgson, J. A. and Edgerton, V. R.** (1991). EMG patterns of rat ankle extensors and flexors. *J. Appl. Physiol.* **70**, 2522-2529.
- Russell, A. P.** (2002). Integrative functional morphology of the gekkotan adhesive system (Reptilia: Gekkota). *Integr. Comp. Biol.* **42**, 1154-1163.
- Russell, A. P. and Bauer, A. M.** (1992). The *m. caudifemoralis longus* and its relationship to caudal autotomy and locomotion in lizards (Reptilia: Sauria). *J. Zool., Lond.* **227**, 127-143.
- Schmitt, D.** (1998). Forelimb mechanics during arboreal and terrestrial quadrupedalism in Old World monkeys. In *Primate Locomotion: Recent Advances* (ed. E. Strasser, J. Fleagle, A. Rosenberger and H. McHenry), pp. 175-200. New York: Plenum Press.
- Smith, J. L., Carlson-Kuhta, P. and Trank, T. V.** (1998). Forms of forward quadrupedal locomotion. III. A comparison of posture, hindlimb kinematics, and motor patterns for downslope and level walking. *J. Neurophysiol.* **79**, 1702-1716.
- Smith, K. K.** (1994). Are neuromotor systems conserved in evolution? *Brain Behav. Evol.* **43**, 293-305.
- Snyder, R. C.** (1954). The anatomy and function of the pelvic girdle and hindlimb in lizard locomotion. *Amer. J. Anat.* **95**, 1-46.
- Swanson, S. C. and Caldwell, G. E.** (2000). An integrated biomechanical analysis of high speed incline and level treadmill running. *Med. Sci. Sports Exer.* **32**, 1146-1155.
- Wainwright, P. C., Sanford, C. P., Reilly, S. M. and Lauder, G. V.** (1989). Evolution of motor patterns: aquatic feeding in salamanders and ray-finned fishes. *Brain Behav. Evol.* **34**, 329-341.
- Wickler, S. J., Hoyt, D. F., Cogger, E. A. and Hirschbein, M. H.** (2000). Preferred speed and cost of transport: the effect of incline. *J. Exp. Biol.* **203**, 2195-2200.
- Zaaf, A., Van Damme, R., Herrel, A. and Aerts, P.** (2001). Spatio-temporal gait characteristics of level and vertical locomotion in a ground-dwelling and a climbing gecko. *J. Exp. Biol.* **204**, 1233-1246.
- Zar, J. H.** (1996). *Biostatistical Analysis*. Upper Saddle River, New Jersey: Prentice Hall.