

RESEARCH ARTICLE

Biodynamics of climbing: effects of substrate orientation on the locomotion of a highly arboreal lizard (*Chamaeleo calyptrotus*)

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SUMMARY

Arboreal substrates differ not only in diameter, but also in continuity and orientation. To gain more insight into the dynamics of small-branch locomotion in tetrapods we studied the veiled chameleon walking on inclined and declined perches of up to 60 deg slope. We found that forelimbs and hindlimbs contribute equally to the body's progression along inclines and declines. The higher-positioned limb's vertical impulses decreased with slope. And although vertical impulses in the lower-positioned limb increased with substrate slope, peak vertical forces decreased. The decrease in peak vertical forces in the lower-positioned limb can be explained by a considerable increase of tensile forces in the higher-positioned limb as the slope increases. In addition, limbs were more crouched on slopes whereas no changes in forward and backward reach were observed. Mediolateral impulses were the smallest amongst the force components, and lateral impulses (medially directed limb forces) exceeded medial impulses (laterally directed limb forces). On inclines and declines, limb placement was more variable than on level substrates. The tail never contacted the substrate during level locomotion; however, on inclines and declines, the tail was held closer to the substrate, with short substrate contacts in one-third of the analyzed trials. Regardless of substrate orientation the tail was always held straight above the branch; therefore, rotational moments induced by the tail were minimized.

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INTRODUCTION

During locomotion on branches that are smaller in diameter than the body's trunk, mediolateral movements of the center of mass (CoM) cause rolling moments (Cartmill, 1985; Preuschoft, 2002). Tetrapods that possess grasping extremities are assumed to stabilize the body by exerting torques around the substrates (Preuschoft, 2002). In addition, tetrapods moving on small branches reduce the distance of the body to the substrate to minimize the vertical oscillation of the CoM that causes rolling moments (e.g. Schmitt and Lemelin, 2002; Higham and Jayne, 2004a; Schmidt and Fischer, 2010). Other adjustments include the reduction of speed, stride frequency and peak forces (e.g. Schmitt and Lemelin, 2002; Lammers and Biknevicius, 2004; Schmitt and Hanna, 2004; Carlson and Demes, 2010; Lemelin and Cartmill, 2010).

Arboreal substrates differ not only in diameter, but also in continuity and orientation. The influences of substrate orientation on small-branch locomotion are not as well known, as studies of horizontal branch locomotion (e.g. Schmitt, 2010) and almost all studies focusing on climbing to date do not include kinetics (mammals: Nakano, 2002; Nyakatura et al., 2008; Schmidt and Fischer, 2010; Shapiro and Young, 2010; Stevens et al., 2011; lizards: Higham and Jayne, 2004a; Higham and Jayne, 2004b; Foster and Higham, 2012). Besides the studies of vertical climbing in Old and New World monkeys (Hirasaki et al., 1993; Hirasaki et al., 2000; Nakano, 2002; Hanna and Schmitt, 2011), only one study to date has investigated the kinetics of small-branch locomotion on shallow

inclines and declines [in *Monodelphis domestica* (Lammers, 2007)]. In lizards, there exists a fair number of studies investigating the effect of sloped surfaces on running performance as well as one kinetic analysis of geckos running vertically (Huey and Hertz, 1982; Irschick and Jayne, 1998; Jayne and Irschick, 1999; Autumn et al., 2006). However, kinetic data of lizards moving on inclined and declined perches are lacking. Despite theoretical assumptions, such as differences in force distribution between the forelimbs and hindlimbs (for details, see Lammers, 2007), deviations from these assumptions are likely to occur due to behavioral as well as morphological adaptations that differ between species (Nakano, 2002; Preuschoft, 2002). For example, peak vertical forces of the forelimbs and hindlimbs differ depending on the degree of climbing specialization in primate species moving on vertical inclines [*Ateles geoffroy*, *Macaca fuscata* (Hirasaki et al., 1993); *Macaca fascicularis* (Hanna and Schmitt, 2011)].

Chameleons are highly specialized among lizards and share the following traits with other arboreal tetrapods, such as mammals, that are linked to small-branch locomotion: (1) high mobility in the shoulder girdle, (2) a clear functional regionalization of the trunk, (3) the use of a compliant gait, (4) a functional parasagittalization of forelimbs and hindlimbs, at least for parts of the stride, (5) diagonal coupling of footfall events and (6) prehensile extremities/tail (with primates and some marsupials) (Peterson, 1984; Losos et al., 1993; Fischer et al., 2010). To gain more insight into the dynamics of small-branch locomotion in tetrapods, we

studied the veiled chameleon (*Chamaeleo calytratus*, Chamaeleonidae, Squamata; Dumeril and Bibron 1851) moving on small perches differing in slope from horizontal (0 deg) to shallow and steep inclines (30 deg/60 deg) and declines (−30 deg/−60 deg). We wanted to test whether the kinetic patterns found for different inclines and declines can be solely explained by changes in weight distribution between the forelimbs and hindlimbs or whether behavioral and/or morphological adaptations result in deviations from theoretical assumptions in the veiled chameleon.

The first hypothesis addresses changes in the vertical force component. On inclines and declines the line of gravity passing through the CoM moves progressively closer to the lower-positioned limb as the slope becomes steeper (Preuschoft, 2002). Thus, the lower-positioned limb has to carry more of the body weight as the incline or decline increases, while the amount of body weight carried by the higher-positioned limb decreases. Therefore, with an increase in incline and decline we expect an increase of vertical impulses in the lower-positioned limb. However, chameleons are expected to reduce peak vertical forces in order to minimize oscillations of the CoM and therefore maximize stability during walking on inclined/declined substrates. A reduction of peak vertical forces could be obtained by lowering the pivot height and/or by increasing the contact time of the forelimbs and hindlimbs, as seen in several mammals and marsupials moving on small branches (Lammers, 2007; Schmidt and Fischer, 2010; Stevens et al., 2011).

As soon as the line of gravity passing through the CoM falls behind the lower-positioned limb on sloped substrates, pitching moments about the lower-positioned limb will occur that must be counterbalanced by tensile (negative dorsoventral) forces exerted by the higher-positioned limb (Preuschoft, 2002). If chameleons avoid pitching backwards on inclines or forwards on declines, we should observe either an increase in backward reach of the hindlimbs during upwards locomotion or an increase in forward reach of the forelimbs during downward locomotion, as seen in several primates and marsupials (Lammers, 2007; Stevens et al., 2011).

With an increase in incline, we expect an increase in propulsive (anterior) forces in hindlimbs in order to overcome shear forces induced by the line of gravity that acts against the direction of movement. On declines we expect an increase braking (posterior) forces in forelimbs because the line of gravity acts with the direction of movement and passes the forelimbs closer than the hindlimbs (Preuschoft, 2002).

Chameleons move their laterally flattened body (and thus the CoM) above the substrate throughout the entire stride (Fischer et al., 2010). Thus, rotational moments are expected to play a minor role in stabilization on small branches in chameleons for this experimental setup. However, shear forces are expected to increase the more the limb is placed laterally and/or the more the limb is loaded (Lammers, 2007).

Another important aspect concerning the locomotor stability during small-branch locomotion is the use of the tail [auxiliary balancing mechanism in primates (Larson and Stern, 2006)]. A study of grasping forces in chameleons revealed that the prehensile tail can hold the body and, therefore, is an important safety device when moving in an arboreal habitat (Herrel et al., 2012; Losos et al., 1993). So far no studies exist that address the use of the tail during chameleon locomotion. Therefore, we are interested in the following questions: does the tail contact the substrate during locomotion on sloped or level small branches; if the tail contacts the substrate, are the peak forces generated by the tail comparable to those generated by the limbs; and is there a difference in tail posture between locomotion on level *versus* sloped substrates?

MATERIALS AND METHODS

Animals and experimental protocol

We studied the kinematic parameters of two male individuals of the veiled chameleon, *Chamaeleo calytratus*. The Committee for Animal Protection of the State of Thuringia, Germany, registered and allowed all experiments. Our specimens were 19.5 and 21 cm in snout–vent length and 105 and 170 g in mass, respectively. Before and after each trial animals were allowed to heat up under an incandescent light bulb so that body temperature was maintained between 29 and 31°C. The chameleons were filmed during walking on a wooden pole coated with nonskid cork. We studied level locomotion (0 deg) as well as upward and downward locomotion (30, 60, −30 and −60 deg). The speed of each trial was determined by plotting a digitized distinct mark on the pigmentation pattern on the side of the torso against time. Only strides in which the animals visually moved at a steady speed were included in the analysis. We analyzed five representative trials per individual and a total of 10 trials per slope.

Kinetic analysis

Three-dimensional substrate reaction forces of the fore- and hindlimbs were measured using a force-sensitive element of 5 cm length integrated into the middle of the pole. Only walks in which only one limb was placed completely on the force-sensitive element were used in the analysis. The diameter of the pole was 2 cm with an overall length of 2 m. The force-sensitive element was attached to the surface of one Kistler force plate and separated from non-instrumented segments by 2 mm gaps. Dynamic control experiments identified a maximal measurement error of 3.6% for forces between 0.1 and 1 N (Witte et al., 2002). Substrate reaction forces (SRFs) are composed of anteroposterior (a-p), mediolateral (m-l) and dorsoventral (d-v, equivalent to vertical forces on level substrates) components and are equal and opposite to the forces exerted by the animal (e.g. Schmitt, 2003b). These forces are directed orthogonal to each other. To determine the actual load carried by forelimbs and hindlimbs at different orientations, we summed the vertical components of d-v and a-p forces and impulses to calculate the vertical forces and impulses, respectively (Lammers, 2007). SRFs were collected at 500 Hz. Analog force data were amplified (8-Channel Charge Amplifier, Type 9865, Kistler, Winterthur, Zurich, Switzerland), converted into a digital format (NI USB-6229, National Instruments, Austin, TX, USA) and recorded with LabView 8.2 (National Instruments). All data were imported and filtered in Microsoft Excel using a moving average algorithm with a window of 10 frames at a sampling rate of 500 Hz. The resulting SRFs were calculated and normalized to the animal's body weight and are presented as body weight units (BW). Kinetic data included peak d-v, vertical, a-p and m-l forces and associated impulses (BW s). Anterior forces indicate propulsive forces and posterior forces indicate braking forces. Mediolateral forces exerted by the limbs were positive for medially directed (lateral SRF) and negative for laterally directed (medial SRF) forces, regardless of which limbs generated them. SRF recordings were synchronized with standard light recordings using a post-trigger.

Metric and kinematic analysis

To determine single limb events, two high-speed cameras (SpeedCam Visario g2, Weinberger, Nuremberg, Germany) recorded post-triggered sequences from each side of the animal at a frequency of 500 Hz. Using the standard light video recorded from the ipsilateral side of the limb that grasped the force-sensitive element, we digitized the following landmarks: shoulder joint, wrist, forelimb cleft, hip joint, hind limb cleft, upper and lower pole (diameter) and, for the

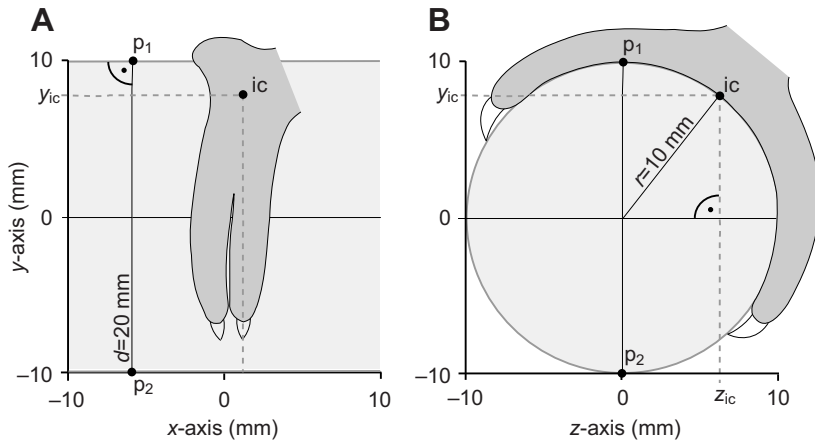


Fig. 1. Lateral view (A) and anterior view (B) of the forelimb grasping the pole to illustrate the calculation of the *y*- and *z*-coordinates of the initial contact (ic) based on 2D data obtained from the lateral view. The distance from the upper (*p*₁) and lower pole (*p*₂) reflects the diameter (*d*=20 mm) of the pole. The *y*-coordinate of the initial contact was determined by setting the null-axis to the middle of the pole using *p*₁ and *p*₂ as reference landmarks. The *z*-coordinate was estimated using the determined *y*-coordinate of the initial contact and the radius of the pole (*r*=10 mm). The *x*-axis corresponds to the direction of movement along the pole. The dimensions of the forefoot illustrated scale in size and are similar to the dimensions of the hind foot.

determination of speed, a distinct mark on the pigmentation pattern on the side of the torso. The digitization of the landmarks was conducted using the Open Source video analysis and modeling tool Tracker Version 4.62 for Windows (www.opensourcephysics.org). We estimated the digitization error by a 10× repetition of digitization of all landmarks of one randomly chosen frame of a sequence. The maximum digitization error was between 0.6 and 1 mm for the *x*- and *y*-coordinates of the shoulder and hip joint. All other landmarks were identified with a higher accuracy. For all trials regardless of substrate orientation, the *x*-axis corresponds to the direction of movement, the *y*-axis corresponds to the dorsoventral axis and the *z*-axis corresponds to the mediolateral axis. Metric parameters for both forelimbs and hindlimbs included stance phase and swing phase duration, stride duration and duty factor, as well as index duty factor (average hind limb duty factor in relation to average forelimb duty factor).

For the interpretation of m-l forces we estimated the position of the cleft of the manus and pes (initial contact of the limb) on the pole (Fig. 1). The landmarks *p*₁ and *p*₂ reflect the diameter of the pole and were used to transform the *y*-coordinate of the initial contact so that the null-axis was located in the middle of the pole. We then estimated the *z*-coordinate assuming that the distance from the middle of the pole to the initial contact (ic) was constant [10 mm, equal to the radius (*r*) of the pole]:

$$z_{ic} = \sqrt{(r^2 + y_{ic}^2)} \quad (1)$$

Thus, positive *y*- and *z*-coordinates reflect a contact position on the upper quadrant on the lateral side of the pole, while positive *y*- and

negative *z*-coordinates reflect a contact position on the lower quadrant on the medial side of the pole.

To test the influence of substrate orientation on the overall positioning of the distal limbs we calculated the forward and backward reach of the limbs. The forward reach of a limb is defined by the distance of the distal limb at touchdown to a line drawn vertically through the proximal pivot of that limb on the pole. Deductively, the distance of the distal limb shortly before lift off and a line drawn vertically through the proximal pivot of that limb on the pole defines the backward reach.

Further, we calculated the pivot height of the forelimbs and hindlimbs at the time of peak resultant force. Peak resultant force is the maximum resultant force of the d-v, a-p and m-l force components. Pivot height is defined by the height of the proximal pivots of the forelimbs (shoulder joint) and the hindlimbs (hip joint) relative to the substrate.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 for Windows, Prism Version 4.0c for Macintosh. The level of statistical significance was *P*<0.05. All parameters but the d-v impulse were normally distributed and so we conducted linear regression analyses to test for speed effects. The effect of speed on the d-v impulse was tested with a rank correlation analysis. For the analyses of speed effects, data from all trials were pooled as both individuals moved at similar speeds. Parameters that were affected by speed for both the forelimbs and the hindlimbs are stance phase and swing phase durations as well as the braking impulse. Because there were no differences in speed

Table 1. Mean ± s.e.m. peak forces and time of peak forces of forelimbs (FL) and hindlimbs (HL)

Variable	Limb	Substrate orientation (deg)				
		-60	-30	0	30	60
Peak propulsive force (BW)	FL	0	0	0.15±0.01	0.38±0.02	0.60±0.03
	HL	0	0	0.07±0.01	0.35±0.03	0.50±0.04
Peak braking force (BW)	FL	-0.47±0.02	-0.33±0.02	-0.12±0.01	-0.05±0.01	-0.02±0.01
	HL	-0.38±0.03	-0.28±0.03	-0.09±0.01	-0.06±0.01	-0.03±0.01
Peak vertical force (BW)	FL	0.65±0.02	0.79±0.02	0.85±0.03	0.62±0.02	0.44±0.05
	HL	0.45±0.02	0.51±0.04	0.71±0.01	0.64±0.04	0.61±0.03
Time of peak propulsive force (% of stance)	FL	0	0	80±0.03	68±0.057	24±0.01
	HL	0	0	44±0.02	33±0.030	35±0.02
Time of peak braking force (% of stance)	FL	52±0.02	55±0.06	43±0.10	8±0.01	3±0.09
	HL	67±0.04	44±0.07	79±0.08	5±0.12	7±0.01
Time of peak vertical force (% of stance)	FL	52±0.02	72±0.02	75±0.02	74±0.02	72±0.08
	HL	69±0.03	32±0.04	27±0.02	32±0.02	31±0.04

BW, body weight units.

between the different substrate orientations and limbs we conducted three-way mixed-model ANOVAs for each kinetic, metric and kinematic parameter with individual as a random factor and substrate orientation and limb as fixed factors. Individual showed no interaction with slope and/or limbs and therefore data are not presented. Although we used repetitive measurements in this study, we would like to point out that for some parameters the sample size of two individuals may have resulted in the absence of significant main effects of limb despite high *F*-values. For those variables that showed a significant two-way interaction of slope and limbs, we also compared the forelimb and hindlimb means for each slope using independent-sample *t*-tests. Because we already tested for random effects caused by individuals in the three-way mixed-model ANOVA, we could exclude possible effects in the independent-sample *t*-tests. *P*-values for those pair-wise

comparisons were adjusted by conducting the table-wide sequential Bonferroni test (Rice, 1989).

RESULTS

Substrate reaction forces and impulses

Level locomotion

During locomotion on the horizontal perch, a-p force traces of the forelimbs are characterized by two braking impulses (0–25% and 38–65% of stance duration), separated by a brief propulsive impulse and then one longer propulsive impulse at the end of the stance phase (65–100% of stance duration; Fig. 2). Anteroposterior forces of the hindlimbs are characterized by a braking impulse at the beginning and the end of the stance phase (0–20% and 65–100% of stance duration) with a propulsive impulse in the middle of stance

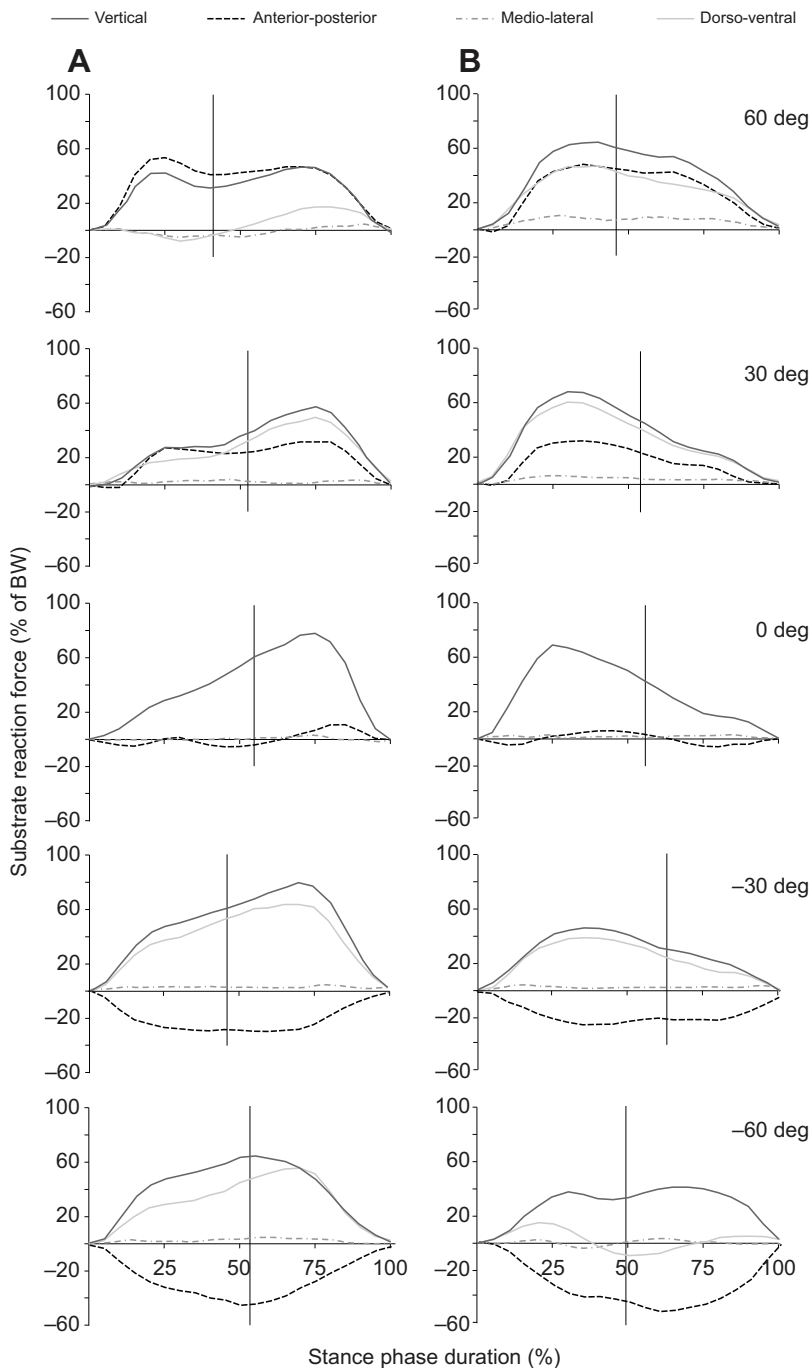


Fig. 2. Mean three-dimensional substrate reaction forces of the forelimbs (A) and hindlimbs (B) during stance phase. Vertical line indicates the time of stance phase when proximal pivot (shoulder/ hip joint) is in vertical line with the wrist/ankle joint. Dorsoventral forces on level substrates correspond to vertical forces.

Table 2. *F*-ratios from three-way ANOVAs performed separately on each kinetic, metric and kinematic parameter

Dependent variable	ANOVA effect		
	Slope × limb (4,4)	Slope (4,4)	Limb (1,1)
Peak braking force	18.30***	209.73***	159.35*
Peak vertical force	72.19***	196.32***	502.78*
Time of peak propulsive force	141.04***	322.36***	135.58
Pivot height at peak force	29.49***	28.50***	23.93
Time of peak vertical force	14.03**	2.02	48,616.27***
Vertical impulse	31.91***	0.75	21.93
Peak propulsive force	0.65	252.73***	4.07
Propulsive impulse	2.21	404.72***	2.44
Braking impulse	4.44	133.35***	0.27
Time of peak braking force	2.70	17.10***	4.45
z-coordinate of substrate contact	5.92	1.37	6745.04***
y-coordinate of substrate contact	2.38	1.64	488.63*
Swing duration	1.24	0.17	28.52
Net medio-lateral impulse	2.43	1.79	0.05
Speed	0.42	1.89	4.04
Duty factor	1.39	0.60	0.93
Stance duration	0.11	0.76	1.01
Forward reach	4.10	2.46	8.51
Backward reach	3.06	2.10	3.55

P*<0.05, *P*<0.025, ****P*<0.001.

Degrees of freedom are given in parentheses.

phase. Propulsive impulses did not differ between the forelimbs and hindlimbs and were similar in magnitude to braking impulses (supplementary material Table S1). Peak vertical forces in the forelimbs occurred at approximately 75±0.02% of stance phase and reached magnitudes of 0.85±0.03 BW. Peak vertical forces in the hindlimbs occurred earlier in stance phase, at approximately 27±0.02% of the stance phase, and were smaller than those of the forelimbs (Table 1). Vertical impulses did not differ between the forelimbs and hindlimbs (Fig. 3, supplementary material Tables S1, S2).

Net mediolateral impulses were positive albeit small and did not differ between the forelimbs and hindlimbs (Table 1, supplementary material Table S1). Lateral impulses (medially directed limb forces) outweighed medial impulses in both limbs.

Inclines

With an increase in incline, propulsive impulses in both forelimbs and hindlimbs increased whereas braking impulses decreased to trivial levels (Fig. 3). Incline force profiles are characterized by two peaks of propulsive forces in both limbs but are less distinct in the hindlimbs (Fig. 2). Propulsive impulses did not differ between the forelimbs and hindlimbs (Fig. 3, Table 2, supplementary material Table S1). Peak propulsive forces increased from level to steep inclined substrates and did not differ between the forelimbs and hindlimbs (Table 1, supplementary material Table S1). Peak propulsive forces in the forelimbs occurred in the last fourth of stance phase during level and slightly inclined locomotion (Table 1). On steep inclines the peak propulsive force of the forelimbs occurred early in stance phase

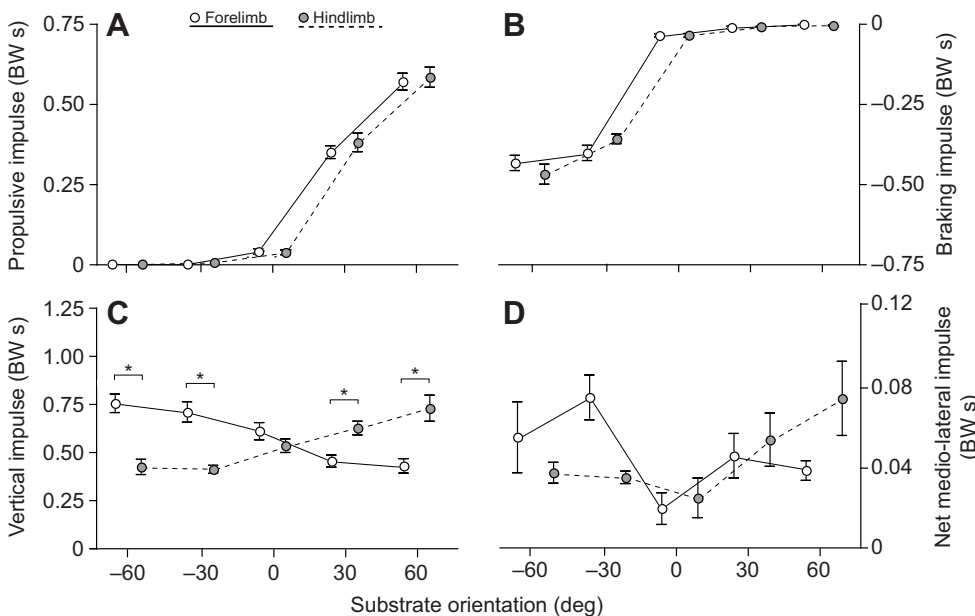


Fig. 3. Mean (±s.e.m.) propulsive (A), braking (B), vertical (C) and net medio-lateral impulses (D) of the forelimbs (white circles) and hindlimbs (grey circles) for all substrate orientations. Asterisks represent statistical differences between forelimbs and hindlimbs (supplementary material Table S2).

Table 3. Mean \pm s.e.m. speed, stance and swing duration, and duty factor for forelimbs (FL) and hindlimbs (HL) of the chameleon *Chamaeleo calytratus*

Variable	Limb	Substrate orientation (deg)				
		-60	-30	0	30	60
Speed (cm s^{-1})	FL	7.1 \pm 0.6	6.6 \pm 0.7	7.86 \pm 0.7	6.8 \pm 0.9	5.7 \pm 0.4
	HL	7.7 \pm 1.1	7.1 \pm 0.8	7.68 \pm 0.9	6.2 \pm 0.9	6.9 \pm 0.7
Stance duration (s)	FL	1.8 \pm 0.2	2.3 \pm 0.3	2.39 \pm 0.4	2.4 \pm 0.3	2.3 \pm 0.3
	HL	2.0 \pm 0.2	2.6 \pm 0.4	3.01 \pm 0.8	2.5 \pm 0.2	3.2 \pm 0.8
Swing duration (s)	FL	1.0 \pm 0.1	0.8 \pm 0.1	0.84 \pm 0.1	0.8 \pm 0.1	1.2 \pm 0.1
	HL	1.0 \pm 0.1	1.2 \pm 0.1	1.2 \pm 0.2	1.1 \pm 0.1	1.0 \pm 0.1
Duty factor (%)	FL	65 \pm 0.01	73 \pm 0.02	72 \pm 0.03	73 \pm 0.02	66 \pm 0.02
	HL	66 \pm 0.02	67 \pm 0.03	66 \pm 0.02	68 \pm 0.02	70 \pm 0.04

(Table 1). Peak propulsive forces in the hindlimbs occurred earlier on inclined substrates compared with level locomotion (Table 1). On inclines, transient braking forces occurred only at the beginning of the stance phase (Table 1).

Vertical impulses in the forelimbs decreased the more the slope increased, while vertical impulses in the hindlimbs increased with increasing slope (Fig. 3, Table 2). On inclines, vertical impulses in the hindlimbs exceeded those in the forelimbs, while peak vertical forces did not differ (Fig. 3, Table 1, supplementary material Table S2). The maximum amount of body weight carried by the forelimbs and hindlimbs decreased continuously from level to steep inclined substrates (Table 1). The timing of peak vertical forces was not affected by incline, with peak vertical forces occurring later in forelimbs than in hindlimbs (Table 1).

The mediolateral pattern parallels that found on the level: mediolateral impulses represented the smallest force components, and the lateral impulses exceeded medial impulses in both limbs. The slight increase in laterally oriented forces (net m-l impulse) in the hindlimbs was not significant and m-l impulses did not differ between the forelimbs and hindlimbs (Fig. 3, Table 2, supplementary material Table S1).

Declines

On declines the a-p forces are characterized solely by a braking impulse that increased with increase in decline and did not differ between the forelimbs and hindlimbs (Fig. 3, Table 2, supplementary material Table S2). Peak braking forces in both limbs increased from level to steeply declined substrates, with forelimb peak braking forces exceeding those of the hindlimbs (Tables 1, 2). In forelimbs the timing of peak braking forces was not affected by decline and occurred at approximately 50% of stance phase (Fig. 2). Peak braking forces in the hindlimbs occurred shortly after mid-stance on level and steep declines, while on shallow declines peak braking forces occurred before mid-stance (Fig. 2, Table 1).

Vertical impulses in the forelimbs increased the more the slope decreased, while vertical impulses in the hindlimbs decreased with decreasing slope (Fig. 3, Table 2, supplementary material Table S1). Both vertical impulses and peak vertical forces were greater in the forelimbs than in the hindlimbs (supplementary material Table S2). Peak vertical forces in both limbs decreased continuously from level substrates to steep declines (Table 1). The timing of peak vertical forces did not differ between level and shallow declines (Table 1). On steep declines peak vertical forces in forelimbs occurred earlier, as in hindlimbs (Tables 1, 2, supplementary material Table S2).

Mediolateral impulses continued to be the minor force component, with lateral impulses exceeding medial impulses in both limbs. Again, the slight increase in lateral-oriented forces (net m-l impulse) in the hindlimbs was not significant and m-l impulses did not differ

between the forelimbs and hindlimbs (Fig. 3, Table 2, supplementary material Table S1).

Across slopes

Substrate reaction forces and impulses

While on inclines propulsive forces dominated, solely braking forces were measured during declined locomotion (Fig. 2). Vertical forces were the greatest forces when walking on level and shallow inclined or declined substrates. Vertical forces remained the maximum forces in the lower-positioned limb, but larger a-p forces were measured for the higher-positioned limb (propulsive force on inclines and braking force on declines) on steep inclines and declines. Peak vertical forces in the forelimbs occurred in the last third of the stance phase at all substrate orientations except steep declines, where peak vertical forces in the forelimbs occurred at mid-support (time during stance phase when the hand is in vertical line with the shoulder joint; Fig. 2, Table 1). By contrast, peak vertical forces in the hindlimbs occurred earlier in the first third of the stance phase, with one exception again on steep declines, where peak vertical forces occurred in the last third of stance phase (Fig. 2, Table 1).

Metrics

Speeds ranged from 3.0 to 12.8 cm s^{-1} for both animals, regardless of the orientation of the substrate. The mean speed was 6.9 \pm 0.7 cm s^{-1} and did not differ between the different substrate orientations and individuals (Tables 2, 3). Stance and swing phase durations decreased with speed (Table 3). Neither stance nor swing duration was affected by substrate orientation (Table 2). Whereas the duty factor of the forelimbs and hindlimbs was not affected by substrate orientation, comparisons of duty factor between forelimbs and hindlimbs revealed differences between the steepest inclines and declines and level orientation (Fig. 4): forelimb duty factors on level and shallow inclined and declined substrates were larger than hindlimb duty factors, whereas we found the reverse pattern on steep inclines and declines.

Kinematics

Limb placement

While the animals always grasped the level substrate on the middle of the upper quadrant of the pole, the touchdown position of the forelimbs and hindlimbs was much more variable on inclines and declines. And while we did not observe differences within a limb, hindlimbs were placed more laterally than forelimbs (Fig. 5, Table 2).

Limb forward and backward reach

Forelimb forward (5.6 \pm 0.9 cm) and backward (5.4 \pm 0.8 cm) reach were not affected by substrate orientation (Fig. 6A,B). The forward reach of the hindlimbs decreased from level (0 deg: 5.9 \pm 0.3 cm) to

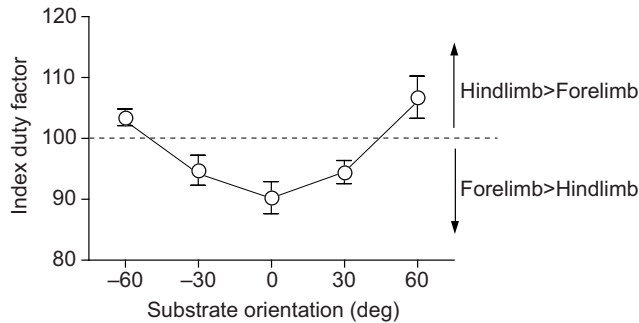


Fig. 4. Mean (\pm s.e.m.) index duty factor for all substrate orientations. Values above 100 reflect higher hindlimb duty factors than for the forelimbs. Values lower than 100 reflect higher forelimb duty factors than for the hindlimbs.

declined substrates (-30 deg: 4.6 ± 0.7 cm; -60 deg: 4.5 ± 0.7 cm) as well as to steeply inclined substrates (60 deg: 4.4 ± 1.0 cm; Fig. 6A,B). The backward reach of the hindlimbs was not affected by substrate orientation (Table 2). Neither forward nor backward reach differed between the forelimbs and hindlimbs (Table 2).

Pivot height

Forelimb pivot height at peak force decreased from 3.1 ± 0.1 cm on level and shallow inclined substrates to 2.3 ± 0.1 cm on steep inclined substrates as well as all declined substrates (Fig. 6C, Table 2). Hind limb pivot height was approximately 2.3 ± 0.1 cm and did not differ between the different substrate orientations (Fig. 6C, Table 2). Pivot heights at peak force were equal for forelimbs and hindlimbs on declines and steep inclines, whereas on level and shallow inclined substrates forelimbs were more extended than hindlimbs (Fig. 6C).

Tail use

During level small-branch locomotion chameleons never touched the substrate with their tail. On sloped substrates (inclines and declines) we observed a tail–substrate contact of the distal part of the tail in only 25 to 30% of the trials and only for very brief periods of the stride. The contact forces fell below the sensitivity of the force plates and, therefore, were significantly below the forces exerted by the limbs. On level substrates the tail was always held straight, either parallel to the perch or slightly dorsally elevated. On inclines and declines the tail posture changed: the tail was held ventrally depressed and commonly displayed a curvature in its distal end. No tail rotation was observed on either level or sloped substrates.

DISCUSSION

This is the first comprehensive study investigating the effects of incline and decline on the kinetics and kinematics of small-branch locomotion in chameleons. Our data support previous kinematic data recorded for hindlimbs (Higham and Jayne, 2004a) and provide further insight into the biomechanics of climbing in tetrapods. We verified our first hypothesis that vertical impulse of the lower-positioned limb increased on inclines (30 , 60 deg) and shallow declines (-30 deg). This pattern was predicted by Preuschoft (Preuschoft, 2002); as the line of gravity passing through the CoM aligns more closely with the lower-positioned limb, this limb takes on a greater role in body weight support. On steep declines, however, we observed no further increase in vertical impulses compared with shallow declines. This stagnation in forelimb vertical impulse coincided with an increased vertical impulse in the hindlimbs during

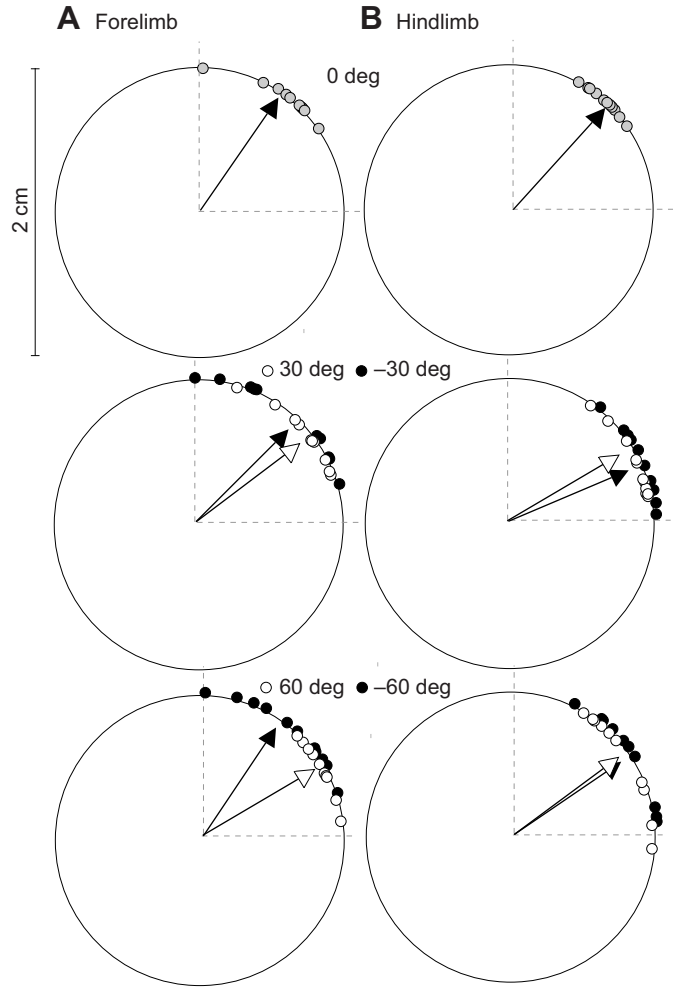


Fig. 5. Location of substrate contact of hands (A) and feet (B) at touchdown for level (grey circles), incline (open circles) and decline (black circles) branch locomotion. Circles represent data from both animals. Mean values are indicated with arrows.

the second half of stance phase. This suggests that the grasping hind feet of chameleons hold on to the steeply declined substrate during the latter half of stance phase, thereby retaining an appreciable degree of body weight support by the hindlimbs and limiting further loading of the forelimbs.

Secondly, we expected a decrease in peak vertical forces on sloped substrates in order to minimize oscillations of the CoM, as seen in *Monodelphis domestica* (Lammers and Biknevicius, 2004; Lammers, 2007). Although data on pivot height on sloped perches are not published yet for *M. domestica*, data on sloped terrestrial trackways showed both a lowering of the pivot height in the higher-positioned limbs on sloped substrates and a decrease in speed, the latter corresponding to an increase in contact time. Both changes found in *M. domestica* are linked to the reduction in peak vertical forces on sloped substrates (Lammers et al., 2006). In chameleons, we found a similar decrease of peak vertical forces in the lower-positioned limb as the slope increased (albeit this was not significant for shallow inclines). However, we did not find a decrease in speed on either inclines or declines, and only on declined substrates did we observe a decrease in pivot height for the lower-positioned limb (forelimb). But we found a concomitant increase in tensile forces exerted by the contralateral, higher-positioned limb as the slope

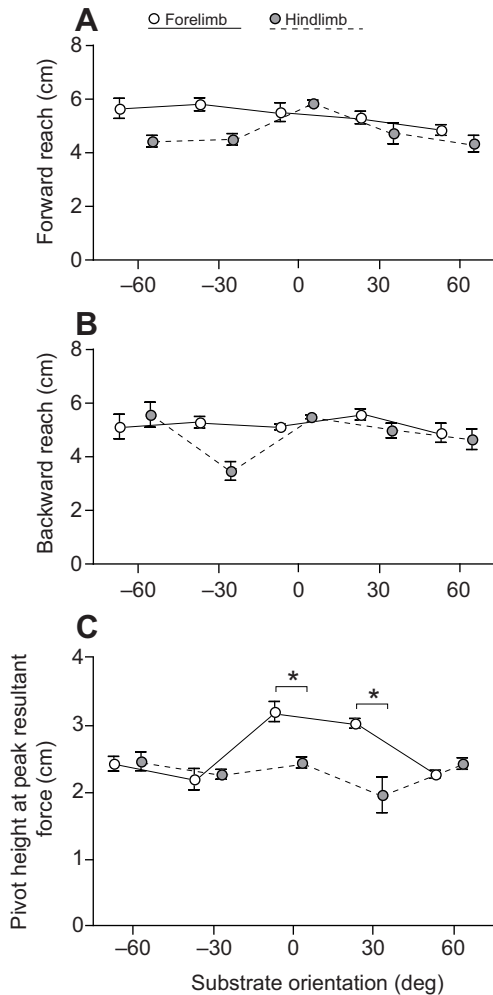


Fig. 6. Mean (\pm s.e.m.) values of (A) forward reach, (B) backward reach and (C) pivot height at peak resultant force for forelimbs (white circles) and hindlimbs (grey circles) at all substrate orientations. Asterisks represent statistical differences between forelimbs and hindlimbs (supplementary material Table S2).

increased. This increase in tensile forces results in a reduction of the load acting on the lower-positioned limb and therefore could explain the reduction of peak vertical forces in the lower-positioned limb.

Pitching moments occur when the line of gravity passing through the animal's CoM falls behind or in front of the lower-positioned limb. Several studies of mammals walking on inclined and declined substrates have revealed changes in forward and backward reach in order to shift the line of gravity between the points of contact to avoid pitching moments (Nyakatura et al., 2008; Schmidt and Fischer, 2011; Stevens et al., 2011). In chameleons, these moments were not incurred at the shallow slopes due to the overall more crouched posture of chameleons on sloped substrates. But besides this change in limb configuration, chameleons did not adjust their limb kinematics on non-level substrates. One explanation could be that chameleons already use the greatest forward and backward reach in forelimbs and hindlimbs in order to maximize the contact time on the substrate; this is supported by previous kinematic data of horizontal branch locomotion in chameleons (Fischer et al., 2010). In addition, contrary to rats, squirrels and cotton-top tamarins, chameleons have strong tong-like grasping extremities that secure

the substrate contact even when limbs exert tensile forces to the substrate, as seen in the higher-positioned limb on steep inclines and declines.

While tensile forces in the dorsoventral direction can easily be recognized in the force profiles because they are measured as negative SRFs, the identification of tensile forces ('pulling') exerted in the direction of movement (anteroposterior) is interpretable by pairing force profiles with limb kinematics. Positive propulsive forces exerted on an inclined substrate before mid-support (hand/feet in vertical line with proximal joint) reflect pulling forces whereas positive propulsive forces exerted after mid-support reflect forces exerted by pushing the body up an incline (and *vice versa* for declines: pushing before mid-support and pulling after mid-support). Distinguishing between pushing and pulling forces is important for the interpretation of the results found for anteroposterior forces on sloped substrates. With an increase in incline, we expected larger propulsive (anterior) forces in the hindlimbs in order to overcome shear forces induced by the line of gravity that acts against the direction of movement. On declines we expected larger braking (posterior) forces in forelimbs because the line of gravity acts with the direction of movement and passes the forelimbs closer than the hindlimbs (Preuschoft, 2002). Contrary to these expectations we found that the forelimbs and hindlimbs contributed equally to body propulsion on inclines as well as braking on declines. If we now differentiate between pushing and pulling, then the two peaks seen in propulsive force profiles on inclines would reflect maximum pulling (first peak) and maximum pushing forces (second peak). With an increase in incline, both forces increased in forelimbs and hindlimbs, although forelimbs mainly pushed the body forward, while hindlimbs mainly exerted pulling forces to contribute to the body's progression. On steep inclines pushing and pulling forces became equally high in both forelimbs and hindlimbs, thus indicating that maximum forces are exerted to overcome gravity. For hindlimbs this is supported by electromyography data that showed a burst activity of three of the main knee flexors (flexor tibialis externus, puboischiotibialis and caudofemoralis) during early stance phase as well as a burst activity of a knee extensor (iliotibialis) later in stance phase (Higham and Jayne, 2004b). For at least one of the knee flexors (caudofemoralis) and the knee extensor the amplitude increased on inclined substrates (Higham and Jayne, 2004b), which supports our findings that impulses increased as well. In addition, changes in muscle activity were mainly related to changes in amplitude rather than timing (Higham and Jayne, 2004b), which coincides with the only small changes observed for kinematic parameters (Higham and Jayne, 2004a; present study). The converse is true for the declines. On shallow declines, pushing and pulling forces are equally high in forelimbs and hindlimbs, and then there is a shift in hindlimb function on steep declines in that the braking forces are increased in the second half of stance phase, as limbs hold on to the substrate while the body continues to move forward.

Tetrapods with grasping abilities are capable of exerting torques around the substrate in order to stabilize the body during locomotion. Torques can counterbalance rotational moments that occur when the laterally flattened body is moved mediolaterally or when limbs are placed more laterally. Although mediolateral movements of the vertebral column are reduced in chameleons (Fischer et al., 2010), movements of the body around the substrate nevertheless occur, e.g. if an animal needs to hide from a potential predator in order to prevent detection (Heatwole, 1968). Although the direct measurement of torques was not possible within this study, we were able to use m-l and d-v SRFs to derive occurring shear forces, which

in part reflect occurring rotational moments (Lammers and Gauntner, 2008; Lammers, 2009). Medially directed peak forces exerted by the limbs (mean: $5.7 \pm 1.0\%$, max.: 16.5%) ranged within values observed for primates walking on small horizontal branches (Schmitt, 2003a). Although we observed the trend towards larger medially directed forces exerted by limbs that were more strongly loaded (lower-positioned) on inclines and declines, we could not verify our hypothesis due to the lack of statistical significance. Also, the correlation of larger medially directed limb forces with a more lateral limb placement on slopes revealed no statistical significance. The placement of contralateral limbs contacting the substrate in equal distance from the top to each side of the branch is interpreted to increase the stability during small-branch locomotion by reducing torque moments. Chameleons stabilize against occurring shear forces using tong-like grasping extremities that exert adduction forces and torques around the substrate (Cartmill, 1985) [also see 'opposable digits strategy' (Lammers and Gauntner, 2008)]. Future studies will test the hypothesis that adduction forces and torques increase on sloped substrates in order to counterbalance increasing shear forces. Indeed, existing hindlimb electromyography data strongly support that hypothesis: chameleons walking on level and sloped (45 , -45 deg) substrates showed an increase in muscle activity of plantar hindlimb flexors (gastrocnemius, tibialis anterior) from level to inclined substrates but not from level to declined substrates (Higham and Jayne, 2004b).

In addition, the overall slow speed of chameleons (level locomotion: 0.02 – 0.12 m s^{-1}) distinguishes them from other species with grasping extremities [level locomotion: 0.6 – 2.0 m s^{-1} (Schmitt and Lemelin, 2002; Schmitt, 2003a)]. And, although the stance duration does not increase on sloped substrates (even decreases on declines), the overall low speed resulted in longer contact times compared with other species studied. Thus, chameleons carry loads on their locomotor apparatus over a longer time (higher impulses) and transfer forces more continually (lower peak forces) and less dynamically than faster animals. In comparison to other grasping arboreal mammals walking on small horizontal branches [primates (Schmitt and Hanna, 2004); woolly opossum (Schmitt and Lemelin, 2002)], chameleons exerted higher forelimb than hindlimb vertical peak forces, a pattern more aligned with mammals with less grasping ability [cotton-top tamarin (Schmitt and Hanna, 2004); gray short-tailed opossum (Lammers and Biknevicius, 2004)]. Schmitt and Hanna suggested that possessing nails rather than claws on the digits may result in the different kinetic adaptations found between cotton-top tamarins and other arboreal primates (Schmitt and Hanna, 2004). Although chameleons share a higher forelimb-to-hindlimb vertical peak ratio than cotton-top tamarins walking on level small branches, chameleons exerted distinctly lower peak forces in both the forelimbs and hindlimbs, which we conclude is linked to the much slower speed of chameleons compared with cotton-top tamarins [0.61 m s^{-1} (Schmitt and Hanna, 2004)]. This conclusion is further supported by the findings of Lammers and Biknevicius (Lammers and Biknevicius, 2004), which show that the decrease in speed coincides with a reduction in peak vertical forces in *M. domestica* from peak vertical forces of 1.1 BW in fast terrestrial trials to 0.5 BW in slow terrestrial trials.

The results of the present study support the hypothesis of the prehensile tail as a safety device during climbing. Although the tail of the chameleons in this study did not grasp the substrate during locomotion, it was held closer to the substrate on slopes. This could enable a fast tail grip had the chameleon begun to fall or if there was any other disturbance during locomotion (e.g. branch movements). It could also act to draw the caudal torso

toward the substrate, minimizing the distance of the CoM to the substrate. The times when the tail contacted the substrate could serve as tactile assurance of the proximity of the tail to the substrate. In contrast to primates, the tail was not used to generate rotational moments as a means of stabilizing the body (Larson and Stern, 2006), but rather was held straight during the stride, suggesting that limb grasp adequately counters any rotational moments in chameleons.

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AUTHOR CONTRIBUTIONS

All authors contributed equally to the conception, design and execution of the study, the interpretation of the findings, as well as drafting and revising the article.

COMPETING INTERESTS

No competing interests declared.

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Table S1. Mean \pm s.e.m. force impulses of forelimbs (FL) and hindlimbs (HL) of the chameleon *Chamaeleo calyptratus*

Variable (BW·s)	Limb	Substrate orientation (deg)				
		-60	-30	0	30	60
Propulsive impulse	FL	0	0	0.04 \pm 0.01	0.35 \pm 0.02	0.57 \pm 0.03
	HL	0	0	0.04 \pm 0.01	0.38 \pm 0.03	0.58 \pm 0.03
Braking impulse	FL	-0.43 \pm 0.02	-0.40 \pm 0.03	-0.03 \pm 0.01	-0.01 \pm 0.00	0
	HL	-0.47 \pm 0.03	-0.36 \pm 0.06	-0.04 \pm 0.01	-0.01 \pm 0.00	0
Vertical impulse	FL	0.76 \pm 0.05	0.72 \pm 0.05	0.62 \pm 0.05	0.47 \pm 0.03	0.44 \pm 0.04
	HL	0.43 \pm 0.04	0.42 \pm 0.02	0.54 \pm 0.04	0.63 \pm 0.04	0.74 \pm 0.07
Net mediolateral impulse	FL	0.06 \pm 0.02	0.09 \pm 0.02	0.02 \pm 0.01	0.05 \pm 0.01	0.04 \pm 0.01
	HL	0.04 \pm 0.02	0.04 \pm 0.00	0.03 \pm 0.01	0.05 \pm 0.01	0.08 \pm 0.02

BW, body weight.

Table S2. Results of independent *t*-tests of selected kinetic, metric and kinematic parameters for forelimb *versus* hindlimb comparison for each slope

Parameter	Substrate orientation (deg)				
	-60	-30	0	30	60
Peak braking force	0.016	n.s.	0.026	n.s.	n.s.
Peak vertical force	0.001*	0.001*	0.002*	n.s.	n.s.
Time of peak propulsive force	n.s.	n.s.	0.001*	0.001*	0.001*
Pivot height at peak force	n.s.	n.s.	0.001*	0.003*	n.s.
Time of peak vertical force	0.001*	0.001*	0.001*	0.001*	0.001*
Vertical impulse	0.001*	0.001*	n.s.	0.002*	0.002*

*Significant *P*-values after table-wide sequential Bonferroni correction.