RESEARCH ARTICLE

Lizard tricks: overcoming conflicting requirements of speed *versus* climbing ability by altering biomechanics of the lizard stride

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SUMMARY

Adaptations promoting greater performance in one habitat are thought to reduce performance in others. However, there are many examples of animals in which, despite habitat differences, such predicted differences in performance do not occur. One such example is the relationship between locomotory performance to habitat for varanid lizards. To explain the lack of difference in locomotor performance we examined detailed observations of the kinematics of each lizard's stride. Differences in kinematics were greatest between climbing and non-climbing species. For terrestrial lizards, the kinematics indicated that increased femur adduction, femur rotation and ankle angle all contributed positively to changes in stride length, but they were constrained for climbing species, probably because of biomechanical restrictions on the centre of mass height (to increase stability on vertical surfaces). Despite climbing species having restricted stride length, no differences have been previously reported in sprint speed between climbing and non-climbing varanids. This is best explained by climbing varanids using an alternative speed modulation strategy of varying stride frequency to avoid the potential trade-off of speed *versus* stability on vertical surfaces. Thus, by measuring the relevant biomechanics for lizard strides, we have shown how kinematic differences among species can mask performance differences typically associated with habitat variation.

INTRODUCTION

The concept of niche partitioning is based on the understanding that a species can occupy a particular ecological microhabitat by having a morphology, physiology and behaviour adapted to suit that environment (Hutchinson, 1959; MacArthur and Levins, 1967). However, specialisation for one microhabitat is generally assumed to occur at the cost of reduced fitness in another habitat type, meaning an organism must often 'trade off' one environment for success in another. Understanding this selection process can lead to a deeper understanding of the probable origins for variation in morphological designs, but it is not always clear at what level of organisation selection for a trait in a certain environment acts on an organism.

Previous studies have suggested that natural selection does not act directly on morphology, but instead may act most directly on intermediate traits such as measures of locomotory performance (Arnold, 1983; Irschick and Garland, 2001). For many animals, locomotion is an important and ecologically relevant performance variable as organisms need to move to catch prey, escape from predators, find mates or defend territories (Garland, 1994; Garland and Losos, 1994). Some studies have shown such a direct relationship between locomotor performance and habitat. For example, *Anolis* lizards that occupy wide perches are long-limbed, quick and agile (Losos, 1990a; Losos, 1990b; Irschick and Losos, 1998), but have reduced speed and agility on narrow perches (Losos

and Sinervo, 1989; Macrini and Irschick, 1998). For 15 other various species of lizards, percentage of time moving in the field was correlated with treadmill endurance capability (Garland, 1999). Further, numerous studies support a link for species that frequently make use of open microhabitats with high sprint speed (Melville and Swain, 2000; Vanhooydonck and Van Damme, 2003; Gifford et al., 2008; Clemente et al., 2009b).

Other studies show a weaker relationship between ecology and locomotor performance. Among nine species of phrynosomatids, variation in morphology and performance was not related to the different substrates these species occupy (Miles, 1994). Habitat heterogeneity, availability of cover, and prey and predator abundance had only a weak association with stamina for 57 species of lizards (Garland, 1994). There was no evidence of a trade-off between running and climbing for 13 lacertilian species (Vanhooydonck et al., 2001), and no trade-off between sprinting, climbing and clinging ability for 18 species of lygosomine skinks (Goodman et al., 2007). One of the broadest locomotor studies (Van Damme and Vanhooydonck, 2001) analysed sprint speed in relation to foraging mode, activity, microhabitat use, mass, speed and ecology for 129 species of lizards. Activity, microhabitat and climate all had effects on sprint speed, but no difference was found between sit-and-wait and actively foraging species. Moreover, the effects of activity, microhabitat and climate were no longer significant when analysed in a

phylogenetic context. Thus the relationship for some locomotor performance traits with microhabitat can be surprisingly subtle.

The often poor relationship between locomotor performance traits and ecology may be due to two reasons. Firstly, whole-animal performance traits can be difficult to measure accurately. Differences in behaviour may affect results (e.g. certain species become more aggressive during experiments) and there might be differences between field and laboratory measurements (Djawdan and Garland, 1988). Secondly, differences in some aspects of whole-body performance may be masked by different solutions to biomechanical problems, and so relationships between a particular measure of performance and microhabitat can become blurred. For example, higher speeds can be achieved by taking longer strides, or by taking strides more frequently. If only one of these biomechanical aspects is constrained within an environment, then high speeds may still be achieved by changes in another aspect, and trade-offs among whole-body performance traits may be difficult to detect.

One solution to this problem may be to examine species variation in locomotor performance at a level below whole-body performance by measuring the relevant kinematics of the lizard's stride, because the kinematics of the stride can be considered as a collective result of the intrinsic properties of the locomotor system (Aerts et al., 2000; Van Damme et al., 1998; Vanhooydonck et al., 2002; Zaaf et al., 2001). An analysis of spatio-temporal gait characteristics for two species of gecko, which differed in climbing ability, showed that the ground-dwelling species achieved high speeds by changing stride length, while the climbing species modified stride frequency (Zaaf et al., 2001). Further, in a phylogenetically diverse sample of lizards, there were patterns of covariation between foraging mode and locomotor mechanics, showing that widely foraging species used a slower vaulting gait, while sit-and-wait species used a faster bouncing gait (McElroy et al., 2008). By measuring differences in the kinematic movement of the hindlimb it is possible to infer and detect differences in whole-body performance variables that are difficult to measure directly (e.g. sure-footedness, manoeuvrability or efficiency).

In this study we examine the hindlimb and pelvic kinematics for 11 species of Australian monitor (*Varanus*) lizards. Varanids have radiated into most environments within Australia, yet despite this, the body shape of the Varanidae family does not exhibit substantial morphological differentiation (Pianka, 1995; Thompson and Withers, 1997a). Habitat characteristics, however, are well documented for this group, making them ideal models in which to compare variation in kinematics with ecological characteristics. First we assess the extent to which habitat influences speed modulation *via* increased stride length *versus* stride frequency, and secondly we measure angular kinematic associations with habitat for species running at equivalent speeds. Based on previous studies, we hypothesise that variation in stride kinematics will be associated with changes in habitat, specifically differences in climbing habitat and foraging mode.

MATERIALS AND METHODS
Animals

We collected a total of 33 adult lizards from 11 species of Australian varanid for this study. All specimens used in the study were wild caught. Lizards were captured using a variety of techniques including pit trapping and hand foraging. Individuals that appeared sick, injured or obviously malnourished were not included. Owing to uncertainty in determining sex, males and females were not differentiated in the analysis. We made morphological measurements of snout-to-vent (SVL) length and hindlimb length for each
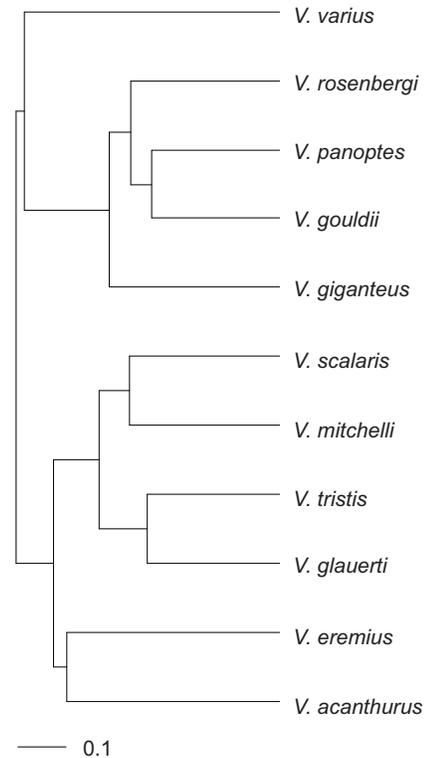

Fig. 1. Phylogeny for the 11 species of *Varanus* in the present study (based on Thompson et al., 2009). Scale bar units are in substitutions per site.

individual. Hindlimb length was further subdivided by measuring the upper hindlimb length from the hip joint to the knee, the lower hindlimb length from the knee joint to the ankle, and the hindfoot length from the ankle joint to the tip of the longest toe, excluding the claw.

Lizards were collected under Department of Environment and Conservation permit SF003972, and experiments were performed under the University of Western Australia animal ethics permit RA/3/100/235.

Phylogeny

Phylogenetic history and environment can both affect species variation (Harvey and Purvis, 1991). Therefore, we used phylogentically informed statistics where possible in our analysis. For our phylogeny we used branch lengths and patterns based on a maximum likelihood tree from 1038 bp of the NADH-2 gene (Fig. 1) (Thompson et al., 2009). The branch tips were set to unity using the chronopl function and the tree was pruned using the drop.tip function, both from the Ape package (Paradis et al., 2004) in R (R Foundation for Statistical Computing, Vienna, Austria).

Kinematics

To measure the three-dimensional (3D) kinematics of a lizard's stride, individuals were filmed while running using either the Peak Motus analysis system (Peak Performance Technologies, Oxford Metric Group, Oxford, UK) for small lizards or the Vicon 612 motion analysis system (Vicon Motion Systems, Oxford Metric Group) for large lizards.

In the Peak Motus system, two high-speed cameras (Peak HSC-200PM), operating at $200\,\text{frames}\,\text{s}^{-1}$, captured simultaneous dorsal and lateral views of a lizard running on a treadmill, at $0.3\,\text{m}\,\text{s}^{-1}$.
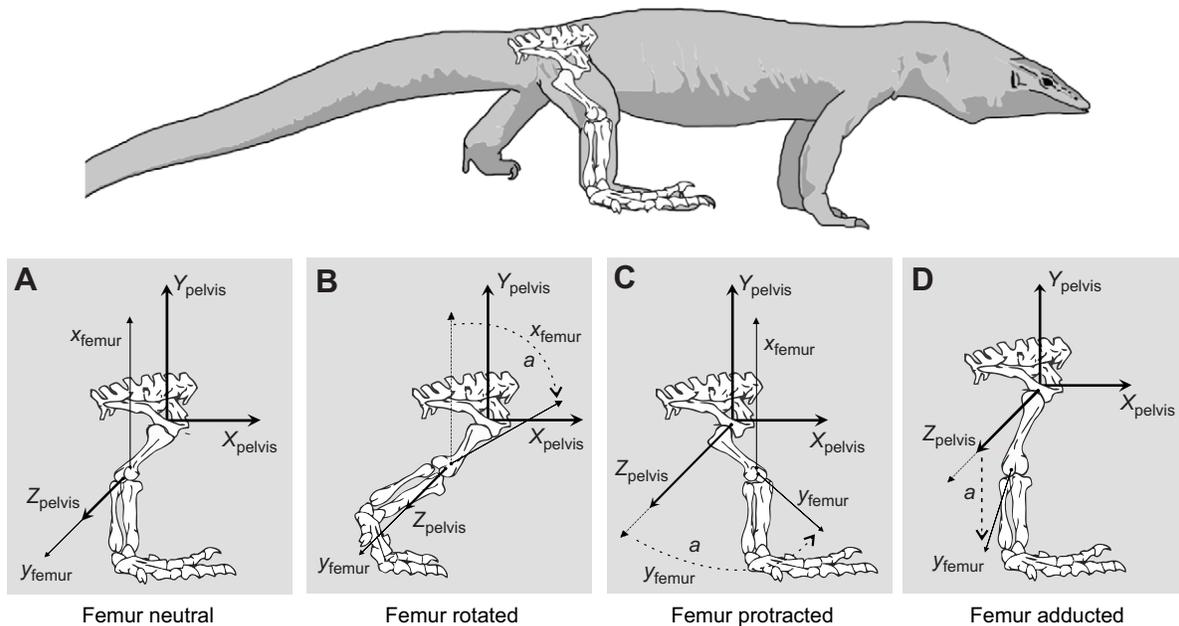
Fig. 2. Kinematic analysis of movement of the femur for the hindlimb of varanids. (A) Femur showing 0 deg of femur rotation, 0 deg of femur protraction/retraction and 0 deg of femur adduction. (B) Femur showing a deg of femur rotation, 0 deg of femur protraction/retraction and 0 deg of femur adduction. (C) Femur showing 0 deg of femur rotation, a deg of femur protraction and 0 deg of femur adduction. (D) Femur showing 0 deg of femur rotation, 0 deg of femur protraction/retraction and a deg of femur adduction.

Illumination was provided by a 2000 W light. Prior to recording, eight landmarks were painted on each lizard, using Liquid Paper (Newell Rubbermaid, Atlanta, GA, USA), to mark the pelvis and the hindlimb joints of each lizard and facilitate digitising the video images. Markers were placed above the lumbar vertebra and the caudal vertebra to mark the pelvis. Hindlimb markings were placed on the hip, knee and ankle joints, the metatarsals at the base of the toe, and the tip of the toe excluding the claw. The joint of the head and neck was also used as a marker. A length of matchstick was glued upright at the midpoint between the lumbar vertebrae and the caudal vertebrae along the pelvis, to allow the degree of pelvic roll to be measured directly. The dorsal tip of the matchstick was painted with Liquid Paper and used as a marker. Peak Motus software (Peak Motus 2000 version 9.0) was used to digitize points and convert them to 3D coordinates. Both lateral and dorsal views were then manually digitized by clicking on the centre of each marker on the hindlimb and pelvis. The Vicon Motion Analysis system used 12 infrared cameras mounted on tripods or the wall around a runway in the gait laboratory. The infrared cameras operated at 250 frames s^{-1} and captured only the 3D position of retro-reflective spherical markers 5 mm in diameter, placed on the same eight landmarks used above. Lizards were then run down the centre of the room along a carpeted substrate. Comparisons between systems, using the same animals run in both, suggested there were no significant differences in kinematics resulting from the motion capture system (Clemente, 2006).

The 3D coordinates of the markers from both systems were then imported into BodyBuilder software (Vicon, Oxford Metric Group). A BodyBuilder model was written to calculate kinematic characteristics for the lizards relative to a 'right-handed' global coordinate system, such that the origin (0,0,0) was located at the proximal, bottom right-hand corner of the track on which they were captured. Where the treadmill was used, a point along the belt was also digitized to create a moving reference frame. The position and movement of each lizard was described by three axes: x, y and z. Positive values of x, y and z indicated greater-anterior, right-lateral and dorsal positions relative to a global coordinate system superimposed on the racetrack.

Digitized points were examined within the stride. Each stride analysis began at footfall of the right hindlimb, and ended at the next footfall of the same limb. The measured variables and the terminology used to describe these variables were similar to those used by previous studies (Fieler and Jayne, 1998; Irschick and Jayne, 1999; Jayne and Irschick, 1999; Irschick and Jayne, 2000). Each stride was described by two phases: the stance phase, the portion of the stride that the right hindfoot was in contact with the substrate; and the swing phase, the portion of the stride that the foot was not in contact with the substrate. Average forward velocity (m s^{-1}) was taken as distance moved along the x–y plane by the lumbar vertebra (m) divided by stride duration (s). Stride length (L_S) was the distance travelled by the lumbar marker along the x–y plane between successive footfalls.

Three angles described the movement of the femur relative to the hip (see Fig. 2). Femur rotation is the angle between the plane containing the femur and the tibia (assuming dorsiflexion of the knee), with a vertical reference plane passing through the hip and knee such that greater positive values indicate greater clockwise rotation of the knee joint (Fig. 2B). Femur retraction was the angle between the femur and a line perpendicular to the long axis of the pelvis, passing through the hip, such that values of 0 deg indicated the femur was perpendicular to the long axis of the pelvis and positive and negative values indicated greater amounts of retraction and protraction, respectively (Fig. 2C). Femur adduction described the angle between the femur and a horizontal plane passing though the hip, with positive and negative values indicating the knee was below or above the hip, respectively (Fig. 2D).

Table 1. Habitat characteristics for 11 species of varanid lizard (genus *Varanus*) in this study

Species	Microhabitat (Thompson and Withers, 1997a)	Retreat type (Thompson et al., 2009)	Foraging strategy	Climbing ability	Openness
V. acanthurus	Sedentary terrestrial	Rocks and trees	SW	Non-climbing	Closed
V. eremius	WF terrestrial	Burrows	WF	Non-climbing	Open
V. giganteus	WF terrestrial	Burrows	WF	Non-climbing	Open
V. glauerti	Arboreal/rock	Oblique rock crevices	WF	Climbing	Semi-open
V. gouldii	WF terrestrial	Burrows	WF	Non-climbing	Open
V. mitchelli	Aquatic	Rocks and trees	SW	Climbing	Semi-open
V. panoptes	WF terrestrial	Burrows	WF	Non-climbing	Open
V. rosenbergi	WF terrestrial	Burrows	WF	Non-climbing	Semi-open
V. scalaris	Arboreal/rock	Rocks and trees	WF	Climbing	Semi-open
V. tristis	Arboreal/rock	Rocks and trees	SW	Climbing	Semi-open
V. varius	Arboreal/rock	Rocks and trees	WF	Climbing	Semi-open

SW, sit and wait predator; WF, widely foraging predator.

Two joint angles described the movement of the hindlimb segments relative to each other. The knee joint described the posterior facing angle between the femur and tibia, such that a value of 90 deg indicated that the tibia was perpendicular to the femur, and smaller values between 0 and 180 deg indicated greater flexion of the joint. The ankle joint was between the dorsal aspect of the foot and the tibia, so that a value of 90 deg indicated that the foot was perpendicular to the tibia, and a value of 180 deg indicated that the foot was aligned with the tibia.

Placing the matchstick perpendicular to the dorsal surface of the pelvis allowed a coordinate system to be attached to the pelvis, measuring roll and yaw. Each angle was calculated relative to the global coordinate system. To correct for lizards running at an angle to the global coordinate system, the average angle of forward movement of the pelvis (on the *x*–*y* plane) was calculated throughout the stride and aligned with the *x*-axis. Pelvic roll was measured as rotation of the pelvis along the *x*-axis, and pelvic yaw was measured as the side-to-side movement of the pelvis within the *x*–*y* plane.

Analysis

We performed the analysis in two parts, assessing firstly the extent to which habitat influences speed modulation *via* increased stride length *versus* stride frequency, and secondly angular kinematic associations with habitat for species running at equivalent speeds.

To determine whether species use predominately stride length or stride frequency to modulate speed, we determined the linear regression of slope for stride length (S_{SL}) and stride frequency (S_{SF}) against speed for each individual lizard (*N*=26 individuals, mean trials per individual=9). We excluded individuals for which neither stride length nor stride frequency showed a significant relationship ($P<0.05$) with speed. We then averaged these slopes for the remaining individuals within each species (species average S_{SL} and S_{SF}; *N*=10 species). These slopes were found to be weakly related to size (R^2=0.35, *P*=0.072), with smaller lizards tending to modulate speed *via* stride length, and larger lizards *via* stride frequency. We corrected for size using phyl.resid function from the phytools package (Revell, 2012) in R, accounting for phylogenetic non-independence of data points to determine size-corrected slopes for stride length and frequency with speed. We used SVL as our measure of size because it was correlated with body mass and was less susceptible to variation from diet and body condition. We then examined size-corrected slopes using the phylANOVA function from the phytools package (Revell, 2012) in R (R core development team 2012) to perform a phylogenetically informed analysis of variance for slopes between different habitat groups.

Species were grouped using five ecological characteristics used previously (Table 1). Microhabitat characteristics (Thompson and Withers, 1997a) and retreat site (Thompson et al., 2009) have previously been used. Further, we used three other ecological characteristics – openness of habitat, foraging mode and climbing ability – based on an extensive literature search and used elsewhere (Clemente, 2006; Clemente et al., 2009a; Clemente et al., 2009b).

We further tested for morphological association with ecological characteristics. To do this we calculated residuals for each segment of the hindlimb from SVL using the phyl.resid function in R to remove any effects of body size. Residuals were then analysed against habitat groups by ANOVA using the phylANOVA function from the phytools package (Revell, 2012) in R.

The second part of our analysis examined movement of the hindlimb and the pelvis in relation to ecology and stride dynamics. As speed modulation techniques were found to be related to climbing ability and retreat type, we focused on these ecological parameters to understand how and why lizards differentially modulate speed either by stride length or frequency. We included only strides for lizards running at near-equivalent speeds to remove any effects this may have on stride kinematics. To do this we calculated the percentage of maximum speed (%max) for each stride; the maximum speed of each individual was measured in a previous study (Clemente et al., 2009b). Strides were excluded if they were less than 30%max or greater than 50%max. We chose this range as it represents a medium paced trot, and includes the greatest proportion of strides.

For these strides we examined seven variables defined above, three describing movement of the femur, two joint angles described the movement of the hindlimb segments relative to each other, and two describing movement of the pelvis. We used the absolute angle value for each variable at midstance as well as the total change in each angle during the stance portion of the stride, to total 14 variables. We chose midstance because ground reaction forces are generally highest during this phase of the stride (Alexander, 1977; Christian, 1995; Blob and Biewener, 2001) and the total change in angles represents the compliance of the limb during the stance phase. Variables were corrected for size using phyl.resid function from the phytools package (Revell, 2012), and then standardised so that they all have a variance of 1 and a mean of 0 using the scale function (Becker et al., 1988) from the base package in R. Because we had only 11 species, and therefore more variables than cases, we reduced our parameter size using forward stepwise model selection with Wilk's lambda as the criterion for inclusion in the model. This was

Table 2. Relationship between habitat and relative lengths of limb segments in 11 species of varanid lizard

Habitat grouping	HFOOT	LHL	UHL
Microhabitat	$F_{3,7}=0.435$, $P=0.846$	$F_{3,7}=1.326$, $P=0.496$	$F_{3,7}=0.045$, $P=0.995$
Retreat type	$F_{2,8}=0.209$, $P=0.859$	$F_{2,8}=2.920$, $P=0.193$	$F_{2,8}=0.105$, $P=0.927$
Foraging strategy	$F_{1,9}=0.044$, $P=0.865$	$F_{1,9}=1.875$, $P=0.257$	$F_{1,9}=1.080$, $P=0.371$
Climbing ability	$F_{1,9}=0.636$, $P=0.607$	$F_{1,9}=1.792$, $P=0.341$	$F_{1,9}=0.157$, $P=0.765$
Openness	$F_{2,8}=0.295$, $P=0.787$	$F_{2,8}=1.791$, $P=0.310$	$F_{2,8}=0.019$, $P=0.984$

Lengths of the limb are represented by residual values from regression against snout-to-vent length. HFOOT, hind foot length; LHL, lower hind limb length; UHL, upper hind limb length.

implemented using the greedy.wilks function from the klaR package (Weihs et al., 2005) in R using either climbing ability or retreat as a grouping factor. The resulting formula was then used in a discriminate function analysis, using the function lda from the MASS package (Venables and Ripley, 2002) in R. This reduced our kinematic variables to a single function, which best described kinematic variation due to climbing ability or retreat site. We then used the first discriminate function (LD1) to test for a significant relationship with S_{SL} using independent contrasts function pic from the ape package (Paradis et al., 2004) in R, to confirm a correlative relationship between these variables. Standardized contrasts were positivised and regressed through the origin (Garland et al., 1992) using the lmorigin function from the Ape package (Paradis et al., 2004) in R.

RESULTS
Morphological associations with habitat
We examined whether morphological variation in the length of segments of the hindlimb were related to habitat. However, none of the habitat groupings showed a significant association with relative length of any segment of the hindlimb (Table 2).

Speed modulation among habitat types
Speed modulation techniques varied among the species studied (Table 3). Some species tended to modulate speed primarily through changes in stride frequency, for example *V. varius*, where ~85% of the change speed is due to changes in frequency (Table 3). Other species, such as *V. eremius*, primarily used changes in stride length to alter speed, with 60% of the variation in speed being explained by changes in stride length (Table 3).

When comparing among habitat types, results were the same for stride length and stride frequency, so we present only results for the former below. There was no effect for slopes of stride length *versus* speed with microhabitat type ($F_{2,7}=6.82$, $P=0.065$), foraging mode ($F_{1,8}=1.55$, $P=0.275$) or habitat openness ($F_{2,7}=1.32$, $P=0.397$).

However, there was a significant effect of retreat type ($F_{2,7}=11.5$, $P=0.016$) and climbing habit ($F_{1,8}=9.46$, $P=0.049$). Results from these latter two analyses are consistent, indicating that species from arboreal habitats (that retreat to spaces in trees and rocks crevices) modulate speed primarily through changes in stride frequency whereas species from terrestrial habitats (retreat to burrows) modulate speed more through changes in stride length.

Kinematic modulation among habitat types
Variation in the kinematics of strides was visible among species (Fig. 3). Only climbing ability and retreat site showed any association with speed modulation *via* stride length (see paragraph above), thus we focus on these habitat classifiers below. Detailed examination of the movement of the hindlimb using climbing ability as a grouping factor in forward stepwise regression retained four kinematic variables: ankle angle at midstance, femur rotation at midstance, femur adduction at midstance and the change in femur rotation. Alternatively, when retreat site is used as the grouping variable, three variables were retained: the change in pelvic yaw, pelvic yaw at midstance, and the change in knee angle. Table 4 shows the loading for these variables when they are entered into a linear discriminant analysis.

Climbing ability returned only a single discriminant function as it was a binary classification. This function clearly separated climbing and non-climbing species, with climbing species being negatively loaded (Fig. 4A). Femur adduction at midstance showed the highest loading (Table 4), indicating that non-climbing species adduct the femur more than climbing species, and therefore adopt a more upright posture while walking (Fig. 3, compare left and right). The ankle angle at midstance also showed a strong positive loading, suggesting greater extension at the ankle joint for terrestrial species (i.e. digitigrade), but a more plantigrade foot posture for climbing species. Femur rotation at midstance was negatively weighted, indicating greater clockwise rotation of the femur for climbing

Table 3. Regression parameters showing the relationship between raw stride kinematics and speed for 10 species of varanid lizard

Species	N	SVL (mm)	Stride length			Stride frequency		
			Slope	Intercept	R^2	Slope	Intercept	R^2
V. acanthurus	3	143.89	0.398±0.039	−0.716±0.035	0.67	0.603±0.037	0.717±0.036	0.81
V. eremius	3	152.95	0.605±0.054	−0.819±0.023	0.87	0.396±0.054	0.818±0.023	0.70
V. giganteus	3	679.33	0.203±0.028	−0.234±0.031	0.87	0.797±0.028	0.234±0.031	0.98
V. glauerti	2	154.43	0.233±0.022	−0.700±0.021	0.51	0.769±0.023	0.701±0.021	0.91
V. gouldii	6	301.66	0.355±0.051	−0.500±0.025	0.57	0.645±0.051	0.500±0.025	0.83
V. panoptes	3	601.00	0.222±0.123	−0.277±0.073	0.47	0.779±0.122	0.277±0.072	0.94
V. rosenbergi	1	367.59	0.434	−0.493	0.56	0.565	0.493	0.69
V. scalaris	1	206.00	0.345	−0.837	0.92	0.658	0.836	0.98
V. tristis	1	238.00	0.221	−0.606	0.37	0.779	0.606	0.88
V. varius	2	655.00	0.148±0.127	−0.182±0.015	0.49	0.854±0.129	0.183±0.015	0.95

Values are means ± s.e.m. SVL, snout-to-vent length.

Table 4. Loadings for linear discriminant analysis for kinematic variables by both climbing ability and retreat site

Grouping factor	Wilk's lambda	*F*-statistic overall	*P*-value overall	Variable	LD1	LD2
Climbing ability	0.019	74.79	<0.001	Ankle angle (mid)	2.777	
				Femur rotation (mid)	−3.286	
				Femur adduction (mid)	3.959	
				Femur rotation change	1.240	
Retreat site	0.023	11.27	<0.001	Pelvic yaw change	3.028	−0.718
				Pelvic yaw (mid)	−2.304	0.010
				Knee angle change	0.235	1.423

Mid, midstance.

species, though the change in this rotation throughout the stance phase was greater for the terrestrial species.

Retreat site (three categories) returned two discriminant functions, which also showed clear separation among the groups (Fig. 4B). The first discriminant function separated species that retreat to burrows from those that retreat to rock/tree spaces. The second function separates only the single species that retreats to rock crevices from species that retreat to spaces in rocks and trees. The first function was positively loaded for the change in pelvic yaw during the stance phase of the stride. This suggests that species that retreat to spaces in rocks and trees show a greater degree of pelvic yaw, or alternatively species that retreat to burrows have reduced this movement. Positive values of pelvic yaw indicate that the anterior of the pelvis is directed toward the right hindlimb. The negative weighting of pelvic yaw at midstance suggests that species that retreat to burrows, despite moving the pelvis less during the stance phase, yaw the pelvis towards the hindlimb earlier than species that retreat to spaces in trees or rocks. Finally, the change in knee angle throughout the stance phase which separated species that retreat to spaces in rocks and trees from the single species that retreats to rock crevices, was reduced in this latter species.

When the first discriminant function from each analysis was regressed against S_{SL} there was a significant and positive relationship for both climbing ability ($R^2=0.51$, $P=0.021$) and retreat site ($R^2=0.42$, $P=0.043$) using both conventional regression techniques, and for the phylogenetically independent contrasts climbing ability

($R^2=0.48$, $P=0.025$) and retreat site ($R^2=0.69$, $P=0.002$). This indicates that these kinematic variables are likely responsible for the variation of stride length with speed.

DISCUSSION

Specialisation for one task can often prevent specialisation in others (e.g. Huey and Hertz, 1984; Futuyma and Moreno, 1988). For varanid lizards, we were unable to find a strong relationship between foraging mode and limb kinematics, as has been reported previously (McElroy et al., 2008). However, we did find strong evidence for a difference in the determinants of speed between climbing and non-climbing lizards, with climbing lizards varying speed predominately by stride frequency and terrestrial lizards predominately by stride length, reflecting results seen in geckos (Zaaf et al., 2001).

The major functional biomechanical differences between these climbing groups appear to be associated with a conflict between maintaining an upright posture on horizontal surfaces (and therefore increasing stride length) *versus* stability on vertical surfaces (by decreasing hip height). For example, increasing femur adduction, ankle angle, femur rotation and pelvic yaw at midstance would all significantly contribute to increasing the effective limb length of the hindlimb, allowing for longer stride length by terrestrial species. This effect, however, would be disadvantageous on vertical or narrow horizontal surfaces, where lengthening of the limbs would hinder climbing by moving the lizards' centre of mass away from the surface (Cartmill, 1985; Pounds, 1988; Losos and Sinervo, 1989).
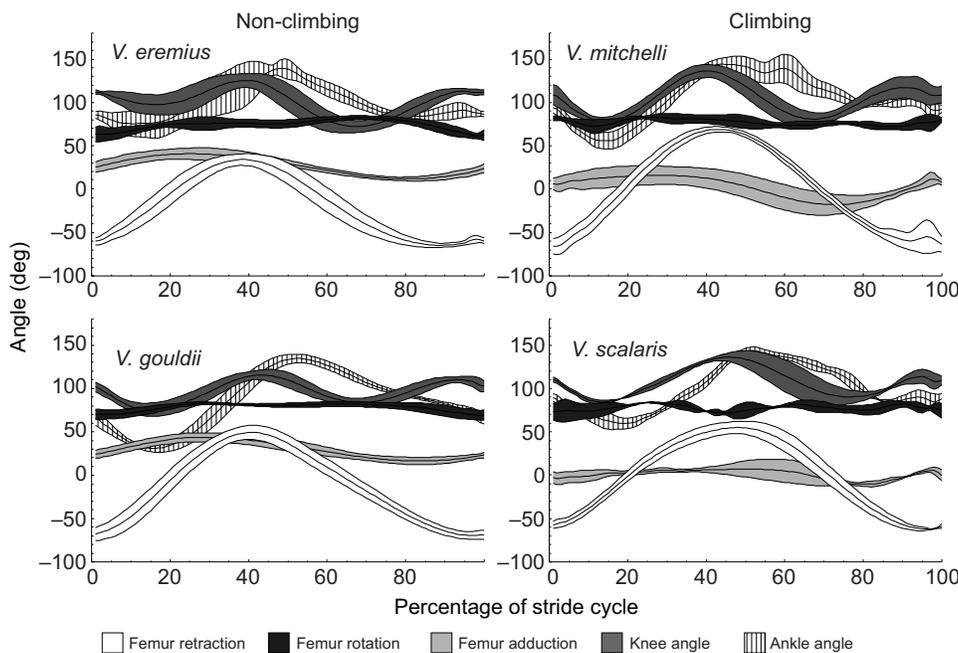

Fig. 3. Example strides for four species of varanid lizard averaged from multiple strides. Lines indicate means ± 1 s.d. for each kinematic parameter. Non-climbing terrestrial species are shown on the left, and climbing species are shown on the right.
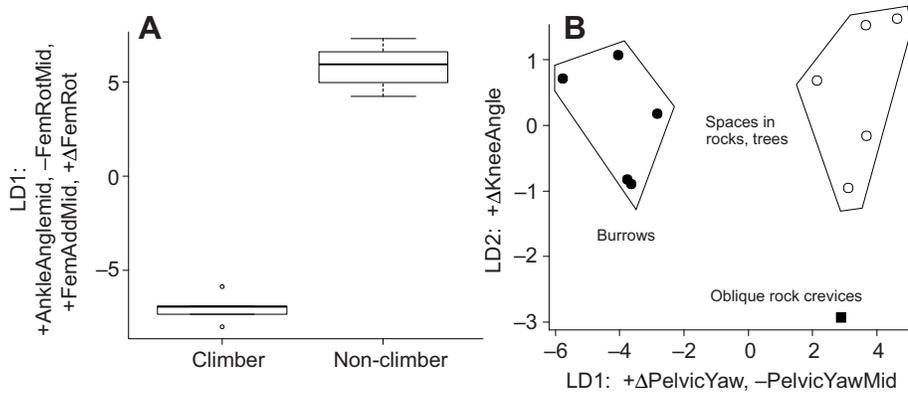
Fig. 4. Results from linear discriminant analysis on kinematic stride variables separated using climbing ability (A) or retreat sites (B) as a grouping factor. For the box plots in A, the bold lines indicate the 50th percentile of the data, while the lower and upper bounds of the box represent the 1st and 3rd quartiles, respectively. Whiskers represent the 1st and 3rd quartiles ± (1.5× inter-quartile range), and circles represent outliers. Δ, change in variable during the stride; + and −, positive and negative effects, respectively.

For *Anolis*, shorter limbed species (with a corresponding lower centre of gravity) had greater 'sure-footedness' on narrower structures (Losos and Sinervo, 1989). Here we see a similar association, although instead of morphological modification of the hindlimbs we find that the joint angles employed during the stride were altered to lower the effective height of the body. Similar results were reported for geckos, where changes in kinematics were more pronounced than changes in morphology, and followed a similar pattern of upright locomotion for running species, but a more crouched posture in climbing species (Fuller et al., 2011). A lower posture may also be advantageous when climbing on vertical structures, such as tree trunks, because friction from the under body and tail can resist the downward pull of gravity. Supporting this hypothesis, the tail scales of arboreal varanids were reported to be coarser than those of terrestrial or aquatic varanids (Bedford and Christian, 1996), suggesting a link between tail skin ultrastructure and biomechanics within varanids.

Our biomechanical analysis of varanids and previous studies in geckos (Zaaf et al., 2001; Fuller et al., 2011) indicate that increasing stride length is constrained in arboreal habitats. Instead of increases in stride length, movement of the hindlimb reflects adaptations to stability on vertical or narrow surfaces. Stride length is typically a major contributor to speed for lizards (Rewcastle, 1983; Reilly and Delancey, 1997), so we might therefore expect that this association would also result in a trade-off with speed for climbing lizards. Trade-offs between sprinting and climbing performance have been previously reported for diverse groups of lizards including *Anolis* (Losos and Sinervo, 1989), *Chameleo* (Losos et al., 1993) and *Sceloporus* (Sinervo and Losos, 1991). However, a lack of a trade-off for sprint speed with climbing ability among lizards has also been reported previously among lygosomine skinks (Goodman et al., 2007) and among lacertid lizards (Vanhooydonck and Van Damme, 2001). Further, there was no difference in speed between climbing and non-climbing varanids (Clemente et al., 2009b), suggesting that the predicted performance difference between climbing and non-climbing lizards may not be as general as previously thought. While the reason underlying this lack of trade-off among lygosomids and lacertids remains to be explored, the lack of difference in speed between climbing and non-climbing varanids is probably best explained by speed modulation *via* stride frequency in climbing species.

By modulating stride frequency, arboreal varanids appear to have avoided lower sprint speeds, normally associated with biomechanical changes to the stride to increase stability on narrow or vertical surfaces. This solution to the lack of difference in sprint speed on level ground among climbing lizards is only evident when examining locomotion at a level below whole-body performance traits (e.g.

sprint speed), by examining the kinematic patterns of limb movement. Our study thus provides an interesting example of multiple solutions that lizards have evolved to deal with an ecological problem, and to circumvent potential trade-offs in nature.

However, there may still be a conflict among these performance traits. We might imagine that speed modulation *via* stride frequency is more energetically costly than that *via* stride length. Do climbing varanids incur a higher metabolic cost? Climbing varanids do appear to have higher maximum metabolic rates than non-climbing species (Thompson and Withers, 1997b; Clemente, 2006). While this increased metabolic rate may be to support the increased metabolic cost of climbing (Thompson and Withers, 1997b), our study suggests that increased metabolic rates may also reflect a more energetic running style, even when running on level ground. This is a fruitful area for future research.
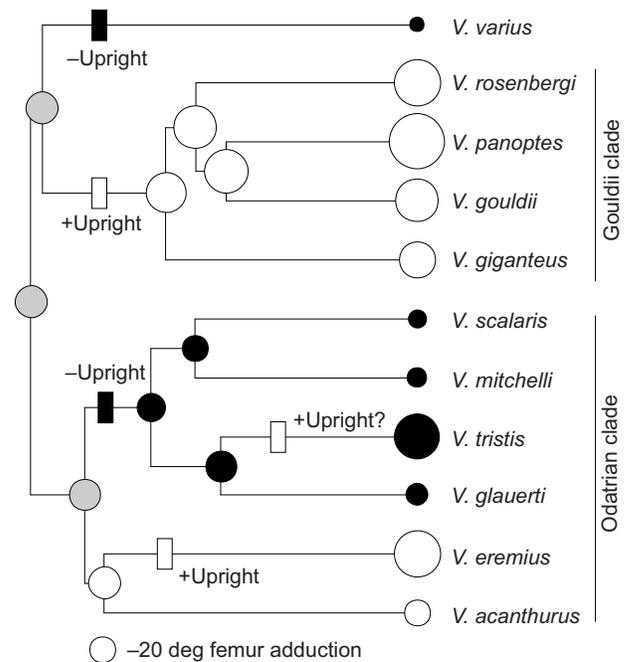

Fig. 5. Phylogeny for 11 species of *Varanus* used in present study (based on Thompson et al., 2009). Circles at species tips represent the magnitude of femur adduction at midstance, with larger circles representing a more upright posture. Ancestral states for nodes were estimated using the ape package in R. Climbing species are represented by closed circles and terrestrial species by open circles. Grey circles indicate that climbing habit status is ambiguous. Rectangles indicate likely transitions in posture.

The underlying morphological basis for kinematic differences between climbing and running lizards remains unclear. The differences do not appear to be based on length of the limb bones, as has been reported for other lizards (Losos and Sinervo, 1989). Instead, kinematic differences may reflect other morphological changes, for example differences in muscle morphology or activation. When Zaaf et al. (Zaaf et al., 1999) compared the musculature in the hind and fore limbs of a climbing and a terrestrial gecko, muscle origins, insertions and general shape were highly similar. Only when examining functional features such as muscle mass, fibre length, pennation angle and muscle moment arms were differences evident. The climbing species had greater moment arms in the hip and shoulder retractors (Zaaf et al., 1999), reflecting differences in femur retraction seen between climbing and non-climbing varanids. Alternatively, the terrestrial species of gecko showed higher moment arms for the knee and ankle extensors, allowing animals to elevate their body (Zaaf et al., 1999), similarly reflecting the upright posture seen in terrestrial varanids.

Given the complex morphological changes required to affect these changes in kinematics, it is interesting to note that these differences may have evolved at least twice independently within climbing varanids. Fig. 5 shows the phylogenetic relationships among varanids with the degree of femur adduction (a measure of upright posture) shown for tips and nodes. Ancestral node states were estimated using the function anc.R from the ape package (Paradis et al., 2004) in R, using the maximal likelihood method, and assuming Brownian motion. While there was still partial overlap for confidence intervals between adjacent ancestral states, it suggests that the ancestral condition had an intermediate posture, with upright posture evolving independently in the gouldii clade and *V. eremius*, while a more sprawling posture evolved for *V. varius* independently from the odatrian clade (*V. tristis* being a noticeable exception). This suggests that morphological variability associated with the upright posture of terrestrial lizards or the sprawling posture of climbing lizards probably evolved multiple times, but it is unclear whether the same morphological modifications have evolved in each instance. Examination of a broader varanid phylogeny (Thompson et al., 2009) suggests that the number of transitions between climbing and terrestrial habits may be higher still, making varanids an ideal group to understand the evolution of morphological associations with biomechanics.

Transitions to upright posture in mammals have previously been suggested to reduce size-related stress on limb bones by decreasing the proportion of bending stress and increasing the proportion of stress borne by compression along bones (Biewener, 2005). However, this was not the case for varanids, as size was not related to posture, and instead size-related stress is likely to be modulated by changes in duty factor and femur rotation (Clemente et al., 2011). In the present study we have shown that variation in posture within varanids, and possibly other groups [e.g. felids (Day and Jayne, 2007)], may better reflect differences in habitat than differences in size. The effects these changes in posture have on limb bone stress in varanids remain to be explored, but are likely complex.

In summary, we have shown by measuring the relevant biomechanics of the lizard stride that we can advance the study of ecomorphological associations among species. We have shown an example of how kinematic differences among species can mask higher-level performance traits typically associated with habitat variation, illustrating that form–function relationships can be more complex than previously thought.

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