The correlated evolution of biomechanics, gait and foraging mode in lizards

Eric J. McElroy*, Kristin L. Hickey and Stephen M. Reilly

Ohio Center for Ecology and Evolutionary Studies and Department of Biological Sciences, Ohio University, Athens, OH 45701, USA

*Author for correspondence (e-mail: em386403@ohio.edu)

SUMMARY

Foraging mode has molded the evolution of many aspects of lizard biology. From a basic sit-and-wait sprinting feeding strategy, several lizard groups have evolved a wide foraging strategy, slowly moving through the environment using their highly developed chemosensory systems to locate prey. We studied locomotor performance, whole-body mechanics and gaits in a phylogenetic array of lizards that use sit-and-wait and wide-foraging strategies to contrast the functional differences associated with the need for speed *vs* slow continuous movement during foraging. Using multivariate and phylogenetic comparative analyses we tested for patterns of covariation in gaits and locomotor mechanics in relation to foraging mode. Sit-and-wait species used only fast speeds and trotting gaits coupled with running (bouncing) mechanics. Different wide-foraging species independently evolved slower locomotion with walking (vaulting) mechanics coupled with several different walking gaits, some of which have evolved several times. Most wide foragers retain the running mechanics with trotting gaits observed in sit-and-wait lizards, but some wide foragers have evolved very slow (high duty factor) running mechanics. In addition, three evolutionary reversals back to sit-and-wait foraging are coupled with the loss of walking mechanics. These findings provide strong evidence that foraging mode drives the evolution of biomechanics and gaits in lizards and that there are several ways to evolve slower locomotion. In addition, the different gaits used to walk slowly appear to match the ecological and behavioral challenges of the species that use them. Trotting appears to be a functionally stable strategy in lizards not necessarily related to whole-body mechanics or speed.

Key words: lizards, biomechanics, gait, foraging behavior, locomotor function, correlated evolution.

INTRODUCTION

Foraging mode is a pervasive evolutionary force in lizards (reviewed in Reilly et al., 2007). Movement patterns have been used to categorize lizards as sit-and-wait or wide foraging (Perry, 2007; Pianka, 1966) and numerous physiological, morphological, behavioral, life history, dietary and other ecological traits have been shown to covary with foraging mode (Cooper, 1994; Reilly et al., 2007; Schwenk, 1993). For example, sit-and-wait foragers (SW) rely primarily on vision to ambush prey (and accordingly have poorly developed chemosensory abilities), and use large sticky tongues coupled with short broad skulls to capture and process prey. In wide foraging (WF), which has evolved several times (Fig. 1), lizards use an oscillating forked tongue to search for prey (with highly developed chemosensory systems), and use their long narrow jaws to capture and process prey (McBrayer and Corbin, 2007; Reilly and McBrayer, 2007).

Predator evasion and territorial defense in all lizards involves short bursts of fast locomotion (Husak, 2006; Husak and Fox, 2006; Irschick, 2000a; Irschick, 2000b). However, during foraging, locomotor speeds differ significantly between foraging types (Anderson, 2007; Cooper et al., 2005). During foraging, SW lizards remain motionless most of the time and then use short bursts of fast locomotion (~10% of activity period) to ambush passing prey. In contrast, WF lizards move slowly most of the time (~10–90% of activity period) over long distances, chemically sampling the environment to locate hidden caches of prey. Accordingly, performance studies have shown a trade-off between maximum speed and endurance such that SW foragers sprint faster than WF but WF have larger endurance capacities than SW foragers (Miles et al., 2007). Thus, maximum speed and endurance match the

locomotor demands of foraging mode. Although there have been many studies that have examined locomotor function when lizards move at their fastest speeds (Irschick and Jayne, 1999; Russell and Bels, 2001; Vanhooydonck et al., 2002; White and Anderson, 1994), far fewer have addressed function at slower speeds.

Two key functional aspects of locomotion that have likely evolved with foraging mode are center-of-mass (COM) biomechanics and gait. A variety of terrestrial animals, including lizards, move their COM using either running or walking mechanics (Biewener, 1998; Cavagna et al., 1977; Farley and Ko, 1997; Full and Tu, 1991; Full and Weinstein, 1992; Heglund et al., 1982; Reilly et al., 2006). Running mechanics involve the kinetic (KE) and gravitational potential (GPE) energies of the COM cycling in-phase and are usually associated with faster locomotion. Walking mechanics are characterized by out-of-phase oscillations of the KE and GPE of the COM, are usually associated with slow locomotion, and can involve mechanical energy savings *via* the inverted-pendular mechanism. Many animals, including lizards studied to date, use a trotting gait with small duty factors while moving fast and shift toward a single-foot (4-beat) gait with larger duty factors when moving slowly (e.g. Biknevicius and Reilly, 2006; Hildebrand, 1976; Sukanov, 1974; White and Anderson, 1994). Based on these patterns we predict that the speed demands of foraging mode should be related to biomechanics and gait. Sit-and-wait species rely on rapid locomotion during prey capture, which should involve running mechanics and trotting gaits. On the other hand, WF lizards predominantly use slower locomotion to locate prey, which should involve walking mechanics and a shift toward single-foot gaits (while retaining faster locomotion with running mechanics and trotting gaits for predator evasion and social interactions).
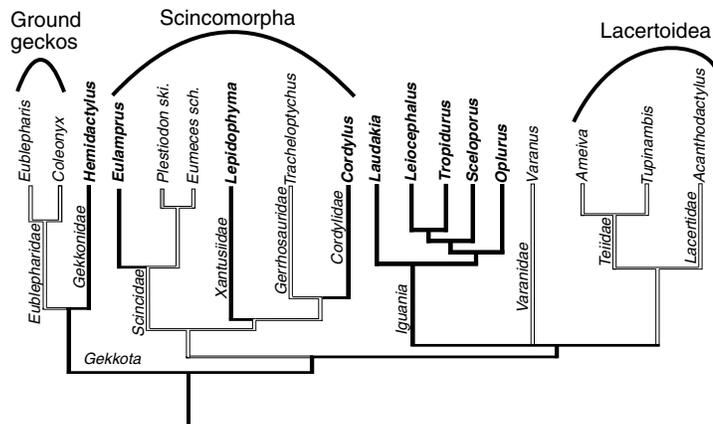
Fig. 1. Patterns of foraging mode evolution reconstructed on a molecular phylogeny for lizards (Townsend et al., 2004). Black branches (bold text) are sit-and-wait foragers, white branches are wide foragers, and foraging mode for *Plestiodon ski.* is unknown (grey). Note that WF evolved independently in the ground geckos, at the base of the Scincomorpha, and in the lineages leading to *Varanus* and the Lacertoidea. Foraging mode reconstruction (branch shading) is based on a larger sample of 110 species (Miles et al., 2007) and from Reilly and McBrayer (Reilly and McBrayer, 2007).

To test these hypotheses we examined the relationships among gait, mechanics and foraging mode in 15 lizard species with a phylogenetic history marked by numerous transitions in foraging mode (Fig. 1). Comparisons across species show a strong evolutionary correlation of gait and biomechanics with foraging mode and that not only have WF lizards evolved several different ways to walk slowly but some have also evolved very slow running.

MATERIALS AND METHODS
Study species

Locomotor biomechanics and gait were quantified in the following species, representing a phylogenetic sampling of foraging modes in nine families of lizards (Fig. 1): Agamidae (*Laudakia stellio* Linnaeus*)*, Iguanidae (*Leiocephalus schreibersi* Gravenhorst, *Oplurus cuvieri* Gray, *Sceloporus malachiticus* Cope, *Tropidurus torquatus* Wied-neuwied), Eublepharidae (*Eublepharis macularius* Blyth), Scincidae (*Eumeces schneideri* Daudin, *Eulamprus quoyii* Quoy and Gaimard), Cordylidae (*Tracheloptychus petersi* Peters, *Cordylus warreni* Boulenger), Xantusiidae (*Lepidophyma flavimaculatum* Dumeril), Teiidae (*Ameiva ameiva* Linnaeus, *Tupinambis teguixin* Linneaus), Lacertidae (*Acanthodactylus boskianus* Daudin) and Varanidae (*Varanus exanthematicus* Bosc). Additional data were taken from the literature for three species: *Coleonyx variegatus* (Eublepharidae) and *Plestiodon skiltonianus* (Scincidae) (Farley and Ko, 1996) and *Hemidactylus garnoti* (Gekkonidae) (Chen et al., 2006). Species foraging modes (Fig. 1) were based on literature accounts (Reilly et al., 2007) or our field data (for *Eulamprus quoyii*; E.J.M. and S.M.R., unpublished). Species were selected to sample SW taxa (Iguania), the major evolutionary transitions to WF mode (ground geckos, Scincomorpha, Lacertoidea and Varanidae), and three Scincomorphs that have reverted to SW foraging from WF ancestors (*Eulamprus*, *Cordylus* and *Lepidophyma*). All housing and experimental procedures followed approved animal use protocols.

Data collection

Gait and whole body mechanics were studied as lizards travelled down a racetrack towards a dark hide box. We induced the fastest speeds by gently pressing on the tail or hindlimb, medium speeds by tapping the ground near the animal or waving our hands above the animal, and slow speeds by allowing the animal to move down the track without any human stimulation. This procedure captured a wide range of speeds to represent the locomotor scope and foraging speeds for each species. Each individual was induced to move down the racetrack several times (usually 3–5 times) until we noticed signs of fatigue (uncoordinated limb movements, dragging belly, or refusal to move after 3 tail pinches). The total number of trials collected per species were roughly evenly distributed among individuals. Individuals were allowed to rest and recover for 24 h before subsequent trials. All diurnal species were maintained at ~ 36–40°C for the duration of each trial, except for the nocturnal *Eublepharis* and *Lepidophyma*, which were maintained at ~ 26–30°C. Lizards were warmed to these temperatures under heat lamps and temperature was checked *via* an infra-red thermal laser directed on the belly periodically throughout the experiments.

Ground reaction forces were quantified using a custom-made force platform based on a strain gauge, spring-blade design described in Bertram et al. (Bertram et al., 1997). Vertical (V), fore–aft (FA) and medio-lateral (ML) ground reaction forces were sampled at 500 Hz using National Instruments data acquisition hardware and a LABVIEW custom designed virtual data sampling instrument following Parchman et al. (Parchman et al., 2003). The 0.6 m long by 0.2 m wide force platform surface was flush with the racetrack surface and located 3–3.6 m along its 5.2-m length. The entire surface of the racetrack and platform was covered with fine grit sandpaper to prevent foot slippage.

Quantifying gait

During force data collection, lizards were filmed at 120 Hz or 500 Hz (small, fast lizards required higher frame rates) with high-speed video cameras mounted ~1 m above the surface of the force platform. Mirrors were mounted on angled walls along each side of the force platform to visualize footfalls. Kinematic analyses were conducted using APAS (version 1.0). First, we determined whether trials were steady speed by digitizing the tip of the snout as the lizard crossed seven evenly spaced lines along the surface of the racetrack. Next we calculated average speed across the entire field of view and discarded any trial that had >20% difference between any interval speed and the average speed. We recorded the timing of touch-down and lift-off for each limb in the steady speed trials. Lizards always used symmetrical gaits; therefore, we implemented the Hildebrand terminology to describe gait using two parameters, duty factor and limb phase (Hildebrand, 1976; Reilly and Biknevicius, 2003). We examined hindlimb duty factor, which is the amount of time that the reference hindlimb contacts the substrate divided by the total stride duration. Limb phase is the amount of time that the footfall of the ipsilateral forelimb follows the reference hindlimb divided by the stride duration. Duty factor and limb phase were multiplied by 100 to obtain percentages. A bivariate plot of limb phase *vs* duty factor (Hildebrand plot) was used to illustrate gaits following gait terminology of Biknevicius and Reilly (Biknevicius and Reilly, 2006). Trots were defined as gaits with

limb phases between 37.5% and 62.5%. Lateral sequence gaits were those with limb phases less than 50% and diagonal sequence gaits are those with limb phases greater than 50%. Single-foots were defined as those gaits on either side of the trot (limb phases 12.5%–37.5% and 62.5%–87.5%).

Quantifying mechanics

Whole-body mechanics were calculated by aligning steady speed steps with ground reaction forces using another custom LABVIEW virtual instrument. Following Willey et al. (Willey et al., 2004), we defined a step as the time from footfall of the first limb in one couplet to the footfall of the first limb in the opposite couplet. Three-dimensional force data were converted into KE and GPE profiles following published methods (Blickhan and Full, 1992; Donelan et al., 2002; Parchman et al., 2003). The integration constants for vertical and medio-lateral velocity were set as in Donelan et al. (Donelan et al., 2002); the integration constant for fore–aft velocity was set as the mean forward speed. Phase shift of the KE and GPE profiles was used to distinguish running from walking mechanical energy patterns. Phase shift was defined as the time difference between the minimum values of KE and GPE relative to step duration, multiplied by 360° (Farley and Ko, 1997; Parchman et al., 2003) and normalized to the range of 0–180°. Phase shifts from 135–180° were defined as walking mechanics and phase shifts of 0–45° were defined as running mechanics (Ahn et al., 2004; Reilly et al., 2006).

We used the ratio of total GPE to total KE over a step as an index of lumbering *vs* cursoriality following Reilly et al. (Reilly et al., 2006). Lumbering species are defined as having GPE:KE ratios significantly greater than one; cursorial species have ratios less than or equal to one.

Statistical analyses

Phylogeny

We ran all phylogenetic comparative analyses (phylogenetic ANOVA, maximum likelihood character reconstruction and independent contrasts, each described in the following sections) on two phylogenies for lizards (Estes et al., 1988; Townsend et al., 2004). Branch lengths were based on both fossil and biogeographic estimates (Estes, 1983; Evans, 2003; Krause et al., 2003; Wells, 2003) and the fossil-based methods (Vidal and Hedges, 2005). Root age was set to 225 mya (Vidal and Hedges, 2005). Branch lengths were not available for the relationships among skinks (*Eulamprus*, *Plestiodon* and *Eumeces*), geckos (*Eublepharis*, *Coleonyx* and *Hemidactylus*) or tropidurids (*Leiocephalus* and *Tropidurus*); these branches were arbitrarily assigned to 25 million years. We also ran analyses with all branch lengths set to one, which assumes a punctuational model where all change occurs at the nodes. All of the results from comparative analyses were qualitatively similar for both phylogenies and all branch lengths; thus, we report results of all phylogenetic comparative analyses for the Townsend et al. (Townsend et al., 2004) phylogeny and fossil-estimated branch lengths.

Correlated evolution of biomechanics, gait and foraging mode

We employed a phylogenetic ANOVA to test for correlated evolution among foraging mode and biomechanics or gait. We assigned phase shifts, limb phases and duty factors to each foraging mode while they were moving as they would while foraging. Values were assigned this way because it allowed us to test the hypothesis that the foraging behaviour and locomotor function have undergone correlated evolution. This resulted in values being assigned to SW foragers for running mechanics and to WF for walking mechanics. The phylogenetic ANOVA examines the difference between the F-

value obtained from a non-phylogenetic ANOVA and a critical F-value (F_{crit}) obtained from a null distribution of F-values calculated by simulating character evolution on the phylogeny (Garland et al., 1993). If the non-phylogenetic ANOVA is significant and its F-value is greater than the 95th percentile of F-values obtained from the null distribution then it can be concluded that the two traits have undergone correlated evolution. If the non-phylogenetic ANOVA is not significant then it is concluded that the two traits are not associated and there is no need for further analysis, i.e. the traits have not undergone correlated evolution. First, we ran a non-phylogenetic ANOVA with phase shift, limb phase and duty factor as response variables and foraging mode as the main effect. Next, we generated 1000 data sets that simulated the evolution of phase shift, limb phase and duty factor using PDSIMUL (Garland et al., 1993). We performed both bounded and unbounded simulations and used both Brownian motion and Ornstein–Uhlenbeck evolutionary models; however, these variations did not qualitatively alter the results. The simulated data sets were analyzed using PDANOVA to create a null distribution of F-values from which the F_{crit} was determined. We concluded foraging mode and biomechanics or gait to have undergone correlated evolution if the non-phylogenetic ANOVA was significant and its F-value was greater than F_{crit} determined from the 95th percentile of the null distribution of simulated F-values.

Evolutionary history

We employed ancestor character reconstruction to visualize the evolutionary relationships between foraging mode, biomechanics and gait. Foraging mode reconstruction was based on the larger sample of ~110 species (Miles et al., 2007) and interpretation from Reilly and McBrayer (Reilly and McBrayer, 2007). We reconstructed the ancestral character states for biomechanics (phase shift) and gait (limb phase and duty factor) using maximum likelihood in the computer program ANCML (Schluter et al., 1997). This program also outputs standard errors for reconstructed trait values. We used standard errors to generate 95% confidence intervals (CI) for each trait at each node. Then we determined significant evolutionary changes in each trait by comparing ancestor nodes to their associated descendent node or tip values. If the 95% CI overlapped then ancestor–descendent pairs were considered the same and the trait was not evolving; if the CI did not overlap then the pairs were considered significantly different and the trait was evolving. We used a modification of this approach to assign node values for phase shift. We assigned node values as running mechanics if the node's 95% CI was within the range of a running phase shifts (0–45°) but outside of the range of a walking phase shifts (135–180°). Likewise, we assigned node values as walking mechanics if the node's 95% CI was within the range of walking phase shifts (135–180°) but outside of the range of running phase shifts (0– 45°). If the CI did not overlap (46–134°) or overlapped both (contained values both ≥135° and ≤45°) running and walking phase shifts then the node was assigned as equivocal.

Species differences in gait

We found that foraging mode evolution was strongly associated with phase shift and duty factor; however, there was a much weaker relationship with limb phase (see Results). In addition, the analysis of species differences for gait is inherently multivariate. Thus, to further probe patterns of gait evolution within Hildebrand gait space, we employed a repeated-measures (RM) MANOVA and CART (classification and regression tree). For the RM-MANOVA, species was a fixed effect, limb phase and duty factor were response variables, and repeated trials per individual was the repeated

Table 1. Species means for gait (duty factor and limb phase) and biomechanical (phase shift, percent recovery, and the ratio of potential to kinetic energy) variables

	Mechanics	Duty factor	Limb phase	Phase shift	% R	PE/KE	Speed (m s^{-1})
Tracheloptychus petersi	Walk[C]	72±1.4	43±1.0	158±3.6	47±3.0	1.48	0.16 (0.15–0.20)
	Run[1]	50±4.1	57±3.8	30±4.9	8±3.5	0.11	0.92 (0.24–1.41)
Varanus exanthematicus	Walk[C]	69±1.7	40±1.5	158±5.0	35±5.0	1.18	0.29 (0.24–0.34)
	Run[1]	41±0.9	50±0.6	18±1.3	5±0.7	0.11	1.37 (0.28–2.62)
Ameiva ameiva	Walk[B]	53±1.5	38±1.4	157±5.1	44±4.8	0.84	0.43 (0.29–0.49)
	Run[1]	50±1.9	41±1.1	21±3.2	9±1.4	0.22	0.75 (0.41–2.11)
Eublepharis macularius	Walk[C]	72±2.1	44±1.1	151±6.6	32±4.3	0.98	0.24 (0.20–0.27)
	Run[2]	70±0.7	43±1.8	8±3.2	16±3.9	0.97	0.29 (0.29–0.32)
Eumeces schneideri	Walk[C]	73±1.2	50±0.6	156±2.4	38±4.0	1.86	0.24 (0.10–0.47)
	Run[2]	71±3.7	50±0.6	23±2.1	18±4.0	0.58	0.19 (0.10–0.28)
Tupinambis teguixin	Walk[C]	64±1.6	46±1.1	151±4.4	35±7.2	0.80	0.22 (0.13–0.29)
	Run[2]	67±1.5	49±0.9	19±4.7	16±5.6	1.38	0.23 (0.16–0.43)
Acanthodactylus boskianus	Walk[A]	53±8.0	57±1.0	152±17	18±0.5	0.14	1.02 (0.82–1.23)
	Run[1]	37±1.5	56±0.7	16±2.5	1±0.4	0.12	1.84 (0.69–3.57)
Laudakia stellio	Run[1]	40±1.5	53±1.0	17±3.3	4±0.8	0.20	1.56 (0.82–2.40)
Leiocephalus schreibersi	Run[1]	47±0.8	52±0.6	16±1.1	6±0.5	0.54	0.80 (0.48–1.48)
Cordylus warreni	Run[1]	44±1.6	52±2.2	20±2.4	7±1.9	0.25	0.86 (0.53–1.23)
Eulamprus quoyii	Run[1]	50±1.7	59±1.4	19±2.0	4±0.8	0.62	1.10 (0.29–1.92)
Lepidophyma flavimaculatum	Run[1]	41±1.2	53±1.3	18±3.6	6±1.4	0.29	0.57 (0.42–0.73)
Oplurus cuvieri	Run[1]	48±0.6	52±0.6	10±1.0	5±0.6	0.50	1.14 (0.59–1.84)
Sceloporus malachiticus	Run[1]	45±0.8	51±0.5	20±1.4	4±0.5	0.45	0.81 (0.24–1.49)
Tropidurus torquatus	Run[1]	33±1.1	53±1.2	15±2.5	5±0.9	0.25	1.64 (1.13–2.88)
*Plestiodon skiltonianus**	Run	–	50	20			
	Walk	–	50	180			
*Coleonyx variegatus**	Run	–	50	10			
	Walk	–	40	170			
*Hemidactylus garnoti***	Run	43	48	0			

R, recovery; PE, potential energy; KE, kinetic energy.
Values are means ± s.e.m. Ranges of species speed means are given in parentheses.
Different superscripts after walk/run values indicate significant groups in Hildebrand gait space (letters, walking groups; numbers, running groups).
*(Farley and Ko, 1997); **(Chen et al., 2006).

measure. We ran separate analyses on walking and running mechanics because all species could run, but only seven species could walk (Table 1). We assigned species to *post-hoc* groupings by comparing their 95% CIs on the first canonical axis output from the RM-MANOVA (Mardia et al., 1980). Species whose 95% CIs overlapped are placed in the same group; species whose CIs did not overlap are placed in different groups. Our data violated some of the assumptions of MANOVA (unequal group sizes and variances; slight deviation from multivariate normality); therefore, we used non-parametric CART analysis to verify species groupings.

We also analyzed how lizards that used both running and walking COM mechanics shifted from running to walking in Hildebrand gait space. We ran separate MANOVAs for each species with COM mechanics as the main effect and duty factor and limb phase as response variables. A significant MANOVA would indicate that a species has shifted its position in gait space, whereas a non-significant finding would indicate no shift in gait space. A sequential Bonferroni correction was applied to account for multiple hypothesis testing. These analyses were performed in JMP 5.0 (SAS Institute Inc., Cary, NC, USA).

Locomotor integration
Finally, we wanted to assess the degree of evolutionary integration of the locomotor system (Dickinson et al., 2000; Reilly et al., 2007). We tested the hypothesis that gait and biomechanics have undergone correlated evolution by examining the correlations between phylogenetically independent contrasts for phase shift, limb phase, and duty factor. A significant correlation between independent contrasts would indicate that two traits have undergone correlated

evolution (Garland et al., 1992). This analysis was performed in the PDAP module of Mesquite (Maddison and Maddison, 2007; Midford et al., 2002). All regressions were computed through the origin and adequate standardization of contrasts was checked using diagnostics tests in the PDAP module of Mesquite (Garland et al., 1992).

RESULTS
Species means for variables describing biomechanics and gait are presented in Table 1 and sample sizes are indicated on Figs 2 and 3. Eight of our 15 focal species only used running COM mechanics (Fig. 2) while the remaining seven used both walking and running COM mechanics (Fig. 3).

Biomechanics and foraging mode
Phase shifts observed in our focal species are presented in Fig. 4B in relation to the foraging mode reconstruction in Fig. 4C. The non-phylogenetic ANOVA found that WF and SW species significantly differ in phase shift ($F_{1,15}$=43.28, P<0.0001). When testing for the effects of phylogeny, the PDANOVA on phase shift calculated a F_{crit}=5.73 much smaller than that of the non-phylogenetic ANOVA, indicating that phase shift and foraging mode have undergone correlated evolution. This is also clearly illustrated in Fig. 4 by comparing the appearance of walking phase shifts (Fig. 4A) to the appearance of WF mode (Fig. 4C).

The reconstructed pattern of locomotor mechanics based on the maximum likelihood analysis of phase shift (Fig. 4A) assigned running mechanics to the ancestral node of lizards. Reconstructed phase shift values show that walking mechanics independently evolved four times at the nodes leading to following five groups:
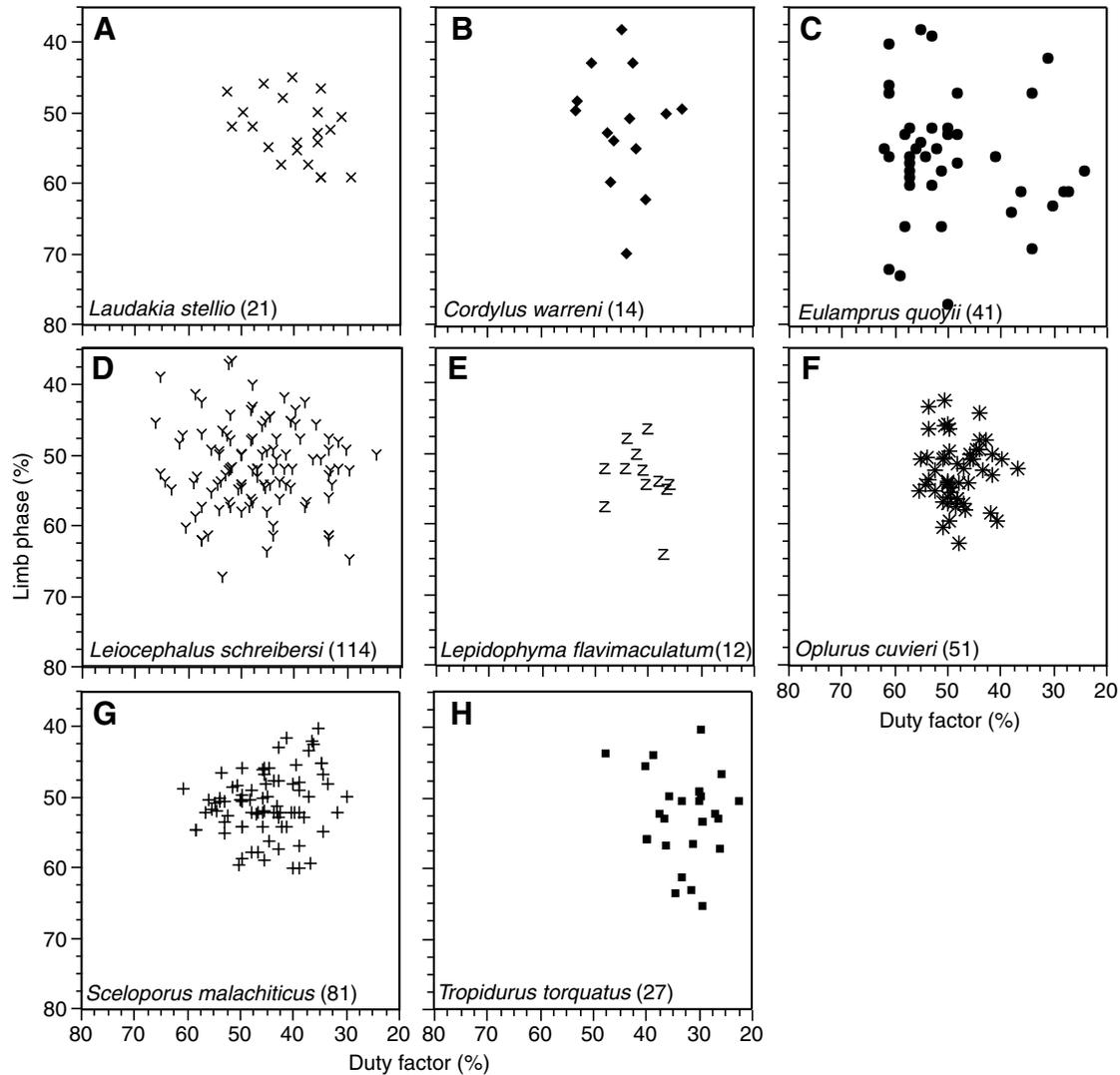
Fig. 2. (A–H) Locomotor gaits for lizards that only used running mechanics. Gait is expressed as limb phase *vs* duty factor (Hildebrand, 1976). Species and sample size are indicated on each panel. Note that by convention the numerical scale on each axis is reversed (Hildebrand, 1976).

(1) *Eublepharis* and *Coleonyx*, (2) the *Eumeces-Plestiodon* group, (3) *Tracheloptychus*, (4) *Varanus* and (5) the Lacertoidea (there was a single evolutionary transition to walking mechanics at the node leading to the Scincomorpha). Walking mechanics was independently lost three times in the Scincomorpha (*Cordylus*, *Eulamprus*, *Lepidophyma*).

Gait and foraging mode

To test for correlated evolution of gait with foraging mode we examined duty factor and limb phase separately. For duty factor, the non-phylogenetic ANOVA found that WF and SW species were significantly different ($F_{1,15}$=51.01, P<0.0001). The PDANOVA on duty factor calculated a F_{crit}=5.46, much smaller than that of the non-phylogenetic ANOVA, indicating that duty factor and foraging mode have undergone correlated evolution. Character reconstruction *via* maximum likelihood analysis on duty factor assigned values of ~54–49% across most of the ancestral nodes with significant relative increases in duty factor in the branches leading to four taxa (*Eublepharis*, *Tracheloptychus*, *Tupinambis* and *Varanus*).

For limb phase, the non-phylogenetic ANOVA was not significant ($F_{1,15}$=2.99, P=0.104), indicating that limb-stepping pattern did not vary to a significant extent across these lizards when tip values alone were considered. Based on this analysis we concluded that foraging mode and limb phase did not undergo correlated evolution. However, character reconstruction *via* maximum likelihood analysis revealed some significant changes in limb phase. Ancestral nodes had values ~50% while the branches leading to four species had significant changes in limb phase away from ~50% (decrease: *Tracheloptychus*, *Ameiva* and *Varanus*; increase: *Acanthodactylus*). This indicates that even though foraging mode and limb phase have not undergone correlated evolution, limb phase has undergone some evolutionary change.

Multivariate species differences in running and walking gaits

Observed gaits for all 15 species are plotted in Hildebrand gait space in Fig. 5 with running mechanics in Fig. 5A and walking mechanics in Fig. 5B. Species were significantly different in Hildebrand gait space for both walking and running mechanics (RM-MANOVA: running mechanics, $F_{42,472}$=8.8499, P<0.0001; walking mechanics,

$F_{9,55}$=20.65, *P*<0.0001). Based on both the 95% confidence ellipses on the first canonical axis and the CART analysis, species clustered in significantly different portions of Hildebrand gait space. Species clustered into two groups when using running mechanics (Table 1; Fig. 5A). *Tupinambis*, *Eumeces sch.* and *Eublepharis* used high duty factor trots; all other species used low duty factor trots. While walking, species clustered into three groups (Table 1; Fig. 5B). All species used trotting limb phases. Most of the wide foragers used trots with high duty factors while walking. Two species exhibited significantly different gaits. *Ameiva* walked using a significantly lower limb phase with relatively low duty factors. *Acanthodactylus* walked using a significantly higher limb phase with relatively low duty factors.

To examine how WF species alter the timing of limb contact when shifting from running to walking mechanics we plotted their running and walking gaits on the same Hildebrand plot (Fig. 6) and used MANOVA to test for significant differences in contact kinematics between the mechanically running *vs* walking gaits for each species. This analysis revealed four patterns of gait transition used by WF lizards when they shift from running to walking mechanics.

Gait 1 (G1): a large increase in duty factor shifting the gait toward a slower speed (0.16–0.29 m s^{-1}) and a significantly lower limb phase (i.e. a more lateral sequence trot; *Varanus*, $F_{2,79}$=43.15, *P*<0.0001; *Tracheloptychus*, $F_{2,23}$=20.04, *P*<0.0001).

Gait 2 (G2): a small increase in duty factor with moderate speed (0.43 m s^{-1}), shifting to a significantly lower limb phase (i.e. a more lateral sequence trot; *Ameiva*, $F_{2,25}$=6.83, *P*=0.004).

Gait 3 (G3): no change in a fast speed (1.02 m s^{-1}) and high limb phase gait (i.e. a more diagonal sequence trot; *Acanthodactylus*, $F_{2,33}$=2.96, *P*=0.06). Although duty factor exhibits a large increase when switching from running to walking, the increase is not statistically significant, probably due to the large standard error produced by the small walking sample (*N*=2) for this species.

Gait 4 (G4): no change in gait with slow (0.22–0.24 m s^{-1}) trotting walks and runs (*Tupinambis*, $F_{2,14}$=2.98 *P*=0.08; *Eumeces sch.*, $F_{2,16}$=0.25 *P*=0.78; *Eublepharis*, $F_{2,7}$=0.36, *P*=0.71). This pattern is also unique in having a significantly lower speed and higher duty factor during running (Fig. 5A).

Correlated evolution of biomechanics and gait

Finally, the degree of integration between biomechanics and gait was tested using phylogenetic independent contrasts between biomechanics (phase shift) and gait (expressed as both duty factor and limb phase). The independent contrasts for phase shift were
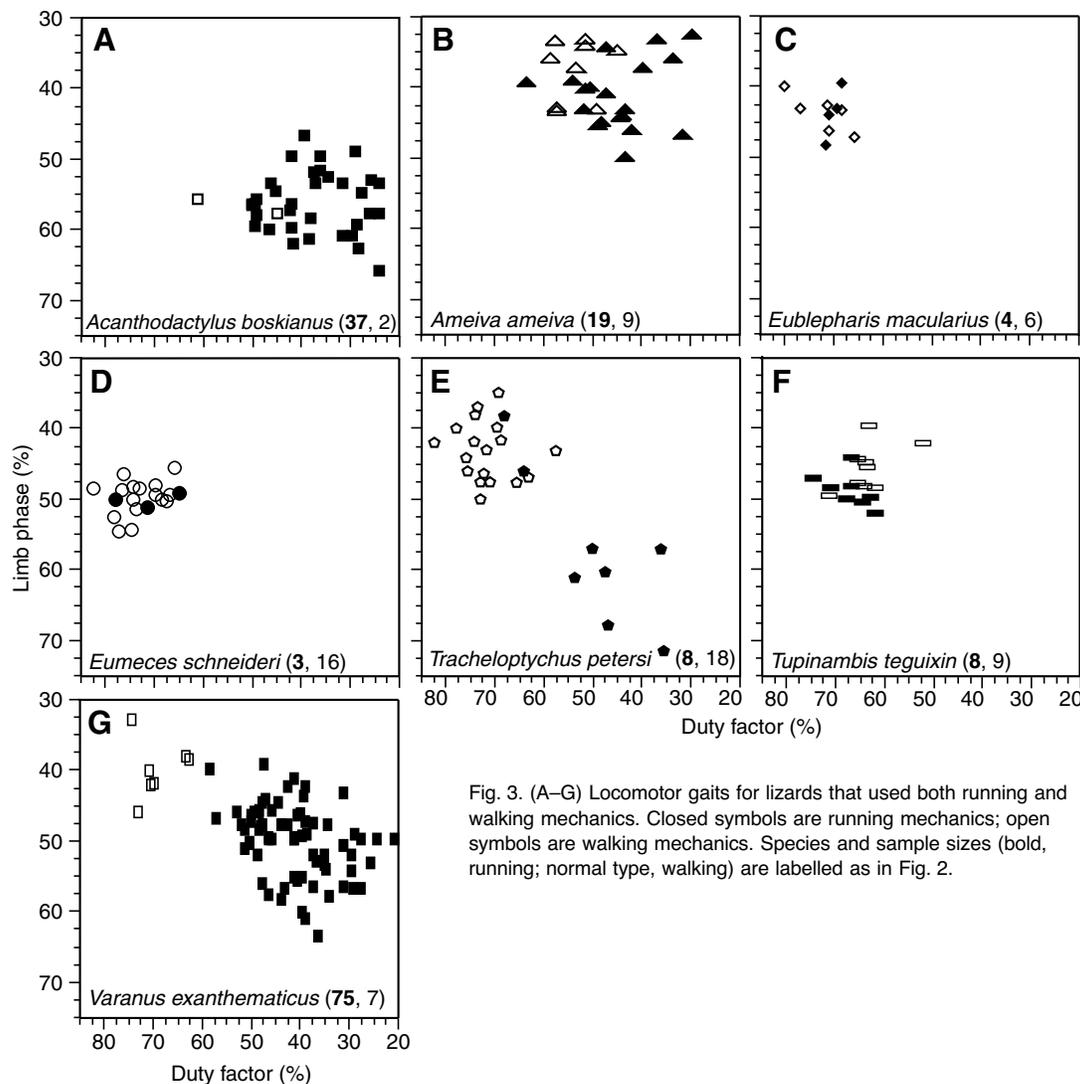

Fig. 3. (A–G) Locomotor gaits for lizards that used both running and walking mechanics. Closed symbols are running mechanics; open symbols are walking mechanics. Species and sample sizes (bold, running; normal type, walking) are labelled as in Fig. 2.
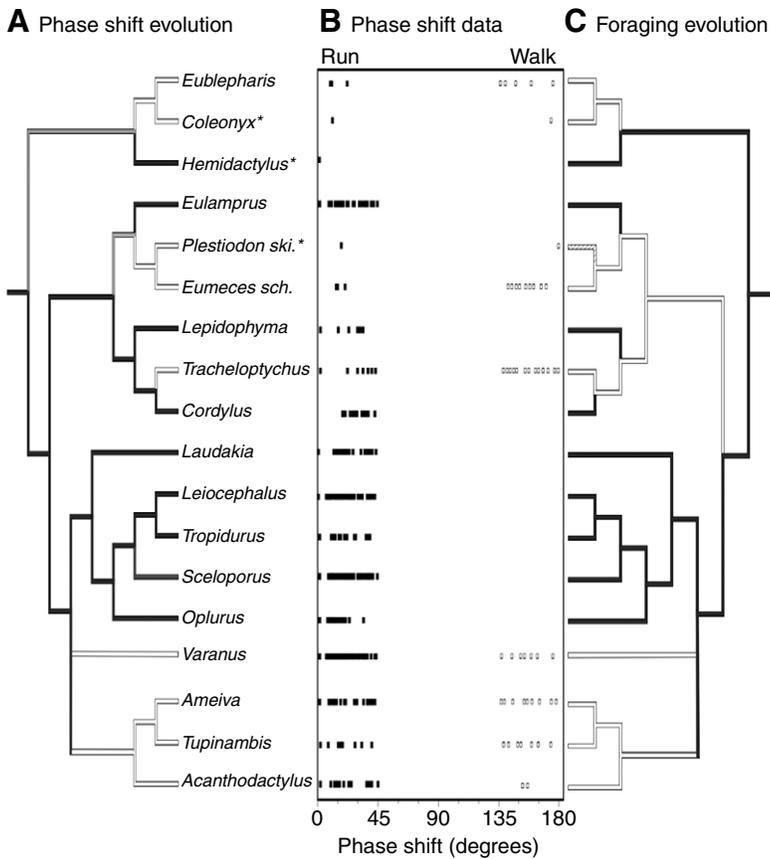
Fig. 4. The evolution of locomotor biomechanics in relation to foraging mode in lizards. (A) Ancestral character reconstruction of phase shift values (from B) are mapped onto the lizard phylogeny from Townsend et al. (Townsend et al., 2004) in A. Branch shading indicates running (black), walking (white), and the grey branches leading to the base of the Gekkota and the Scincidae are equivocal for running or walking. (B) Raw phase shift data indicating running (closed symbols, phase shift ≤45) and walking (open symbols, phase shift ≥135) mechanics. (C) Reconstructed patterns of foraging mode evolution from Fig. 1 (black branches are SW lineages, white branches are WF lineages, hatched branch is unknown). Note that walking mechanics evolved each time WF evolved. Asterisks indicate species taken from the literature.

significantly related to duty factor ($r^2=0.78$, $F_{1,14}=51.04$, $P<0.0001$) and limb phase ($r^2=0.479$, $F_{1,16}=14.68$, $P=0.002$). By explicitly accounting for phylogenetic patterns within these traits these results show that gait and biomechanics have undergone correlated evolution.

DISCUSSION
Patterns of running in lizards

In all lizards, rapid locomotion is an essential behavior for predator evasion and social interactions. We found that the lizards examined in this study used a trotting gait (limb phase ~50%) with running mechanics when moving at fast speeds (Table 1; Fig. 5A). Animals may use trotting gaits while running because the line of support generated by diagonal couplets is optimally aligned under the COM, thus offering good stability at high speed (Cartmill et al., 2002; Chen et al., 2006; Hildebrand, 1988). In addition, running mechanics coupled with a trotting gait may enhance maneuverability (Chen et al., 2006) and provide a simple template for the neural control of fast locomotion (Full and Koditschek, 1999). Given our observation that all species in this study used mechanical runs with a trotting gait it appears that these species achieve the advantages of stability, maneuverability and simplicity of neural control of fast locomotion.

Although all lizards in this study used a trotting gait while running we found that species cluster into two distinct groups in Hildebrand gait space (Fig. 5A) separated by a significant difference in duty factor. Most species (all of the SW and most of the WF) consistently used low (50–30%) duty factor trotting gaits (limb phase 40–60%) at high speeds (0.8–1.64 m s⁻¹; Table 1). This is similar to the high-speed gaits used by most cursorial animals when moving fast (Hildebrand, 1976; Reilly and Biknevicius, 2003). However, three WF species (*Eublepharis*, *Eumeces sch,* and *Tupinambis*) shift to

high duty factor (67–72%) and low-speed (0.16–0.24 m s⁻¹) running (Table 1). During mechanical runs, these species overlap the gait space used by tuataras, salamanders and frogs (Ahn et al., 2004; Reilly et al., 2006).

The evolution of mechanics and gait with foraging mode in lizards

A summary of patterns of evolution of locomotor traits in our sample of lizards based on our analyses is presented in Fig. 7. Ancestral reconstructions of foraging mode (Miles et al., 2007) show that SW is ancestral for all lizards. We suggest that running mechanics are ancestral for lizards based on three pieces of evidence: (1) all lizards examined in this study use running mechanics; (2) ancestral character reconstruction *via* maximum likelihood, based on our sample of 18 species, shows that running mechanics is ancestral; and (3) additional comparative analyses show a tight evolutionary coupling between foraging and mechanics. Based on this evidence we suggest that the SW ancestor of lizards only used running mechanics. However, when we compare species locomotor function when moving at their foraging speed, the most obvious pattern is that COM biomechanics and foraging behavior have undergone correlated evolution. Three lines of evidence support this pattern. First, foraging mode and biomechanics have a one-to-one pattern when mapped onto the phylogeny; all SW species only use running mechanics and every evolutionary transition to WF behavior is accompanied by the appearance of walking mechanics (Figs 4, 7). Second, the three examples of evolutionary reversals from WF back to the SW strategy (*Eulamprus quoyii*, *Lepidophyma flavimaculatum* and *Cordylus warreni*) have independently lost walking mechanics (Figs 4, 7). Our interpretation of these three species having lost walking mechanics is based on the evidence that the basal

Scincomorph was WF (Miles et al., 2007) and thus probably used walking mechanics. Third, our results were robust to variations in phylogenetic topology, branch lengths and evolutionary models; we consistently found that foraging mode and biomechanics have undergone correlated evolution.

Another obvious pattern from our results is that gait evolution, too, is correlated with foraging mode evolution (Figs 5, 7). Based on our sample, we show that the ancestral lizard gait used a low duty factor trotting gait at high speeds when running (Fig. 5A). Each of the WF species used one of three walking gaits that were significantly different from the ancestral low duty factor running region occupied by most lizards (Figs 5, 6). In addition, the three
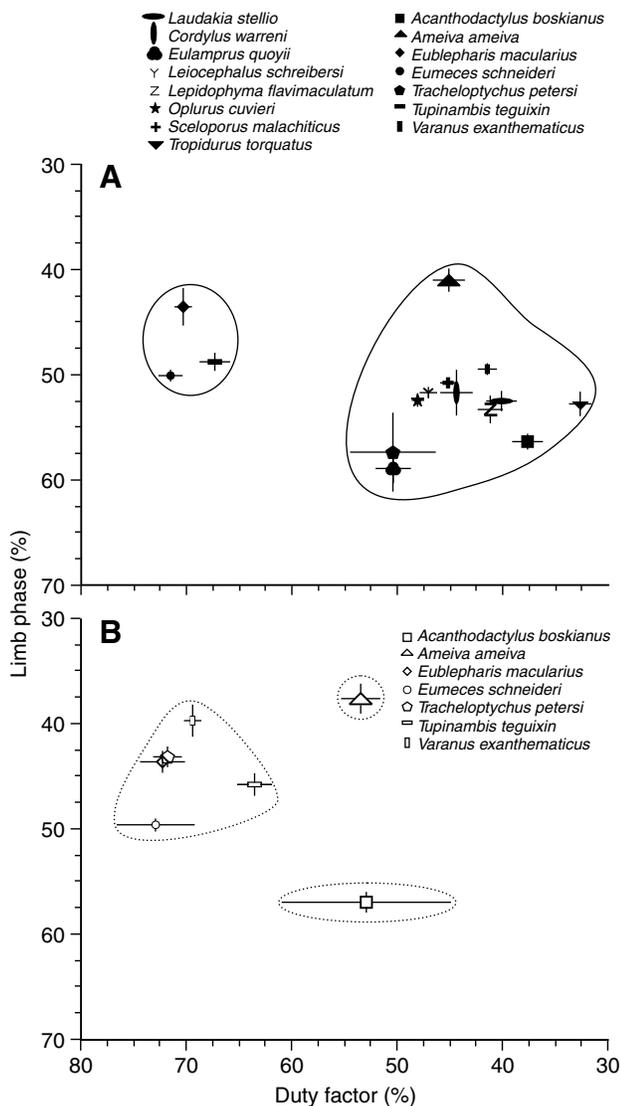
species that have undergone an evolutionary reversal back to SW foraging retained the ancestral high-speed low duty factor trot (Figs 5, 7). Patterns of gait shift when changing from running to walking also evolve with foraging mode (Fig. 7). In fact, two of the patterns of gait change (G1 and G4) have evolved more than once with the evolution of WF. Clearly the evolution of WF and walking is accompanied by evolutionary changes in both biomechanics and gait.

Why change mechanics and gait when moving at a slower foraging speed?

The tight evolutionary correlation between mechanics and foraging mode suggests that walking mechanics may have been a key innovation in the evolution of slower locomotion in WF lizards. One possible benefit of walking mechanics is that they may decrease the total mechanical energy needed to move the COM because of the pendulum-like exchange of KE and GPE (Biewener, 2006; Cavagna et al., 1977; Farley and Ko, 1997). Pendular savings, measured as % recovery of external mechanical energy, ranged from 18–47% in WF species during walking compared to 1–18% during running (Table 1). Thus, walking in WF lizards requires less external mechanical energy than running, suggesting that it is an energetic adaptation for long periods of slow locomotion. However, while WF lizards do reduce the amount of external mechanical energy used during locomotion it does not necessarily follow that this results in a relevant reduction in metabolic energy, for two reasons. First, lizards have body masses that are too small to realize relevant metabolic savings from mechanical energy savings during walking because their actual metabolic costs are two orders of magnitude greater than their total mechanical energy costs (Reilly et al., 2007). Therefore, no matter how much percent recovery of

Fig. 5. Gaits (means ± s.e.m.) and multivariate differences between species during running (A, for all species) and walking (B, for WF) mechanics. Ellipses surround species means that are not significantly different. (A) When using running mechanics *Tupinambis*, *Eumeces sch.* and *Eublepharis* differed in using significantly larger high duty factor trots than the remaining species. (B) During walking mechanics species clustered into three statistically distinct gait groups. *Ameiva* and *Acanthodactylus* use a fast walking gait (lower duty factors) but diverge toward more lateral (lower limb phase) and diagonal (higher limb phase) sequence gaits, respectively. The remaining species clustered into a single group that uses a higher duty factor trotting gait while walking.
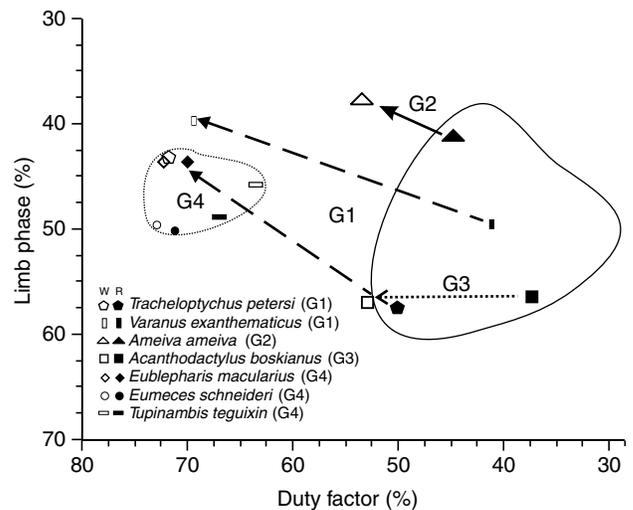

Fig. 6. Patterns of gait change when shifting from running to walking mechanics in WF lizards. White symbols, walking mechanics (W); black symbols, running mechanics (R). Solid cloud is the ancestral running gait cloud from Fig. 5A. When shifting from running to walking mechanics lizard exhibited four ways of changing position in gait space, based on MANOVAs comparing running to walking gaits for each species: (G1, broken arrows) *Varanus* and *Tracheloptychus* switch from the ancestral trotting run to a higher duty factor and lower limb phase trot while walking, (G2, solid arrow) *Ameiva* exhibits small shifts in limb phase and duty factor in a lateral sequence trot. (G3, dotted arrow) *Acanthodactylus* maintained a fast diagonal sequence trot and (G4, stippled cloud) *Tupinambis*, *Eumeces sch.* and *Eublepharis* maintained a slow speed high duty factor trot when switching from running to walking.

external mechanical energy the lizards attain, it is insignificant in relation to the actual metabolic cost of locomotion. Second, the cost of locomotion during walking has been shown to be greater than running, both on a per stride basis and on an absolute basis, because WF actually spend the majority of their activity budget walking slowly (Anderson and Karasov, 1981; Reilly et al., 2007). Thus, it is difficult to support the idea that walking mechanics is a key adaptive innovation to reduce the metabolic cost of locomotion in WF. In fact, it has been proposed that walking and running mechanics may actually be spandrels (*sensu* Gould and Lewontin, 1979) of legged locomotion in small animals (Reilly et al., 2006; Reilly et al., 2007). Resolution of the true energetic relevance of mechanical energy savings in small animals awaits future integrative studies.

Another argument for why animals switch mechanics with speed relates to the relationship between centripetal and gravitational forces acting on the COM (Kram et al., 1997). When animals increase speed centripetal force increases until it exceeds the gravitational force (occurring at a Froude number ~1), which prevents the animal from walking with an inverted pendulum and necessitates the switch to running mechanics. This probably explains why many lizards switch from walking to running mechanics with increasing speed (Fig. 6; G1–G3). However, this argument does not explain the reverse; when slowing down, animals are not physically required to switch back to walking at a given speed. This is clearly illustrated by studies showing that animals actually prefer to switch from walking to running mechanics at a Froude number ~0.5 (Alexander, 1989), well below the Froude number ~1, which requires the switch. Thus, animals appear to be capable of running at any speed, but can only walk up to a critical speed corresponding to a Froude number of ~1. It follows that when lizards slow down they are not switching from running to walking mechanics due to a physical requirement. The difference in speeding up *vs* slowing down is not trivial because lizards have evolved slower locomotor speeds as a necessity for WF behavior. Thus, neither energy savings nor physical constraints explain the evolutionary transition from fast running to slow walking mechanics in WF lizards.

Although there is not a clear energetic or biomechanical benefit of walking mechanics in WF lizards, there may be other benefits associated with evolving slower speeds and new gaits. Our study shows that WF species move at slower speeds than SW species when considering speeds that they likely use while foraging [see also Cooper et al. (Cooper et al., 2005) for the same pattern in field movement speeds]. Wide foraging lizards have evolved entire suites of characters related to their shift to derived chemosensory systems (Cooper, 1994; McBrayer and Corbin, 2007; Reilly et al., 2007; Schwenk, 1993). From the brain to olfactory receptors to forked air sampling tongues, WF lizards exhibit a number of characters that enhance their ability to slowly search for food [Reilly et al. (Reilly et al., 2007) and references therein]. While foraging, slower locomotor movements may enhance wide foraging by allowing the chemosensory apparatus to meticulously sample a complex heterogeneous habitat for prey chemicals (Anderson, 2007; Cooper, 1994). The prey items that WF chemically search for reward them with a higher energy pay-off (Gasnier et al., 1994). Fast locomotor movements, while foraging, would preclude WF from being able to sample chemicals thoroughly and follow them in the environment. Thus, simply moving slower while foraging is of adaptive value to WF lizards because it affords them the ability to effectively locate and discriminate energy-rich prey. Our findings suggest that the convergent evolution of slower foraging locomotion in WF lizards is an important correlate of effective predatory chemosensory behavior.

Wide foraging lizards couple slower speeds while foraging with a change in gait characterized by an increase in duty factor. Evolutionarily, the lizards examined in this study appear to shift from the ancestral high-speed low duty factor trot while running to slower-speed higher duty factor trots while walking (Fig. 6). Thus, our findings show that duty factor is the principal functional parameter that changes when lizards evolve slower speed locomotion and WF behavior. Previous studies of lizard gait have also shown that duty factor increases with decreasing speed (Hildebrand, 1976; Sukanov, 1974). The increase in duty factor associated with moving slowly may have two benefits for a WF lizard. First, larger duty factors, i.e. longer ground contact time, are actually energetically less expensive because they allow muscle force to be produced at a slower rate, which is more energetically economical (Kram and Taylor, 1990; Pontzer, 2007). Thus, WF lizards may realize metabolic energy benefits by increasing duty factor when moving slowly. However, all animals appear to increase duty factor as they slow down, suggesting that the energetic benefits are not an adaptation for WF *per se*, but rather a general feature of slower terrestrial locomotion. Second, moving with larger duty factors offers greater stability at slower speeds for animals that only trot (Hildebrand, 1976). When trotting animals slow down they move from aerial trots (alternating periods of support on 2 diagonal feet) at low duty factors to regions in Hildebrand gait space with periods of support by 2, 3 or 4 feet [fig. 7 in Hildebrand (Hildebrand, 1976)]. Thus, simply increasing duty factor enhances stability at lower speeds. Accordingly, WF lizards may realize both force-production energetic benefits and stability benefits as simple correlates of the increase in duty factor when moving at slower foraging speeds.
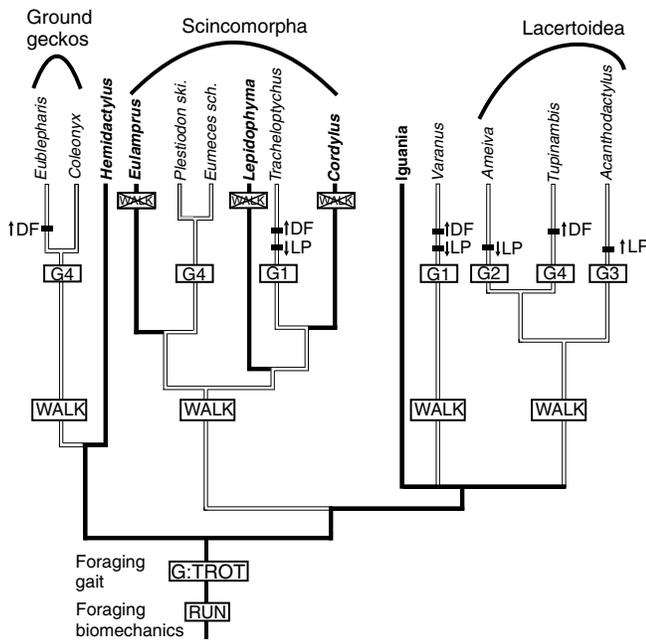

Fig. 7. Evolutionary patterns of foraging locomotor biomechanics and gait in relation to foraging mode in lizards (Fig. 1). From the ancestral condition of sit-and-wait foraging (black branches) with running mechanics (RUN) and a trotting gait (G:TROT), lizards have evolved walking mechanics (WALK) in concert with wide foraging (white branches) several times. Wide foraging species exhibit one of 4 patterns of gait shift (G1, G2, G3, G4, from Fig. 6) involving different shifts in limb phase (↑ or ↓ LP) and duty factor (↑ or ↓ DF). Note that walking mechanics was lost (WALK crossed out) each time foraging mode underwent an evolutionary reversal to SW.

Variation in ecological relevance of gait among WF lizards

Demonstration of the evolution of duty factor with foraging mode supports the general view that gait is a dynamic part of the locomotor system that is capable of responding to divergent ecological and behavioral challenges (Stevens, 2006). The WF lizards examined in this study exhibit four patterns of gait change when they switch from running to walking (Fig. 6) that may be related to details of their foraging ecology. These four patterns differ in the relative shifts in speed, duty factor limb phase (Fig. 6).

In terms of speed, the slowest G1 and G4 (Fig. 6) walkers are extreme WF (*Varanus*, *Tupinambis*, *Tracheloptychus*), have particularly short limbs and are fossorial (*Eumeces sch*.), or are cryptic and nocturnal (*Eublepharis*). The moderate speed G2 (*Ameiva*) and high speed G3 (*Acanthodactylus*) walkers both belong to lizard families that exhibit field movement patterns marked by frequent pauses and changes in direction (Anderson, 2007; Verwaijen and Van Damme, 2007) and many of the walks we recorded from *Ameiva* and *Acanthodactylus* fit this description. Interestingly, these two species used relatively small duty factors compared to the other species (Table 1; Fig. 6). Such low duty factor walking may be useful for foraging with frequent pauses because it has been hypothesized to allow numerous opportunities to change direction, thereby increasing maneuverability (Vanhooydonck et al., 2002), which may facilitate chemosensory tracking abilities. Thus, the speed that each lizard species uses while foraging appears to be related to the specific techniques or ecological context they use to forage.

In terms of patterns of gait change, G1 involved significant increases in duty factor (from 41–50% to 69–72%) and a shift to a more lateral sequence trot (limb phase from 50–57% to 40–43%; Fig. 6). Both species that evolved G1 (*Varanus* and *Tracheloptychus*) exhibited significant decreases in both duty factor and limb phase from their immediate ancestral nodes. G1 is also associated with a large decrease in speed (from running at 0.92–1.37 m s^{-1} to walking at 0.16–0.29 m s^{-1}). Although little is known about the field behavior of these species, they clearly follow the general tetrapod pattern of shifting towards a single-foot gait with larger duty factors when moving slowly (Biknevicius and Reilly, 2006; Hildebrand, 1976).

The G2 of *Ameiva* also had a small decrease in duty factor and limb phase, but was different in three ways. First, both running and walking limb phases were the lowest observed, and during walking, *Ameiva* occasionally utilized a single-foot gait (Fig. 3) (*sensu* Biknevicius and Reilly, 2006). Second, character reconstruction showed that limb phase was significantly lower in *Ameiva* than in its immediate WF ancestor. Third, *Ameiva* utilized moderate walking speeds (mean=0.43 m s^{-1}; Table 1). *Ameiva* appears to exhibit G2 for a number of reasons. In the wild, *Ameiva* travels widely and quickly between patches of resources (Magnusson et al., 1985; Anderson, 2007). In addition, *Ameiva* has comparatively longer feet than most lizards (E.J.McE., unpublished). Thus, both ecological and morphological factors may affect mechanics and gait in *Ameiva*. Clearly more comparative kinematic, morphometric and behavioral studies are needed to understand why *Ameiva* has a lower limb phase during walking.

Acanthodactylus boskianus exhibited G3 with an increase in duty factor that was nearly significant (*P*=0.06). The G3 pattern had no change in limb phase with walking. This species employed the highest limb phase (57%) observed during walking in the locomotor sample we collected. Character reconstruction indicated a significantly higher limb phase in *A. boskianus* relative to that of its immediate ancestor, indicating it had evolved toward a more diagonal-sequence trot. In addition, *A. boskianus* adopted a strategy of significantly faster speed walking (1.02 m s^{-1}) than other lizards

in our study. We propose that very fast walking in *A. boskianus* may be related to WF on hot desert sands that are nearly devoid of vegetation (Belliure and Carrascal, 2002; Perry et al., 1990). *Acanthodactylus erythrurus* has been shown to heat up more slowly, cool down more quickly, and exhibit higher physiologically optimal and preferred temperatures than do most lizards. All of these thermal traits are posited to be adaptations to the scarcity of cover, and/or high predation risk in the xeric and thermally demanding environments they inhabit (Bauwens et al., 1995; Belliure and Carrascal, 2002; Belliure et al., 1996). Although *A. erythrurus* is a SW species, it seems likely that WF species (such as *A. boskianus*) would experience even stronger selection on thermal traits because they are presumably more exposed to predators and hot temperatures than are SW species. Thus, rapid walking and low duty factors during foraging in *A. boskianus* may be an adaptation for seeking prey on extremely hot sandy substrates found in deserts.

Species exhibiting the fourth pattern (G4) adopt the same walking gait (duty factor 64–73%, limb phase 43–46%) and speed range (0.22–0.24 m s^{-1}) as the G1 pattern. However, the G4 species are unique in utilizing running gaits with significantly higher duty factors (Fig. 5A). Thus, they have shifted both the walk and run to high duty factors at slow speeds (0.19 to 0.29 m s^{-1}). An additional skink (*Plestiodon skiltonianus*) and gecko (*Coleonyx variegatus*) appear to occupy a similar region in gait space (Farley and Ko, 1997) and likely experienced a similar evolutionary history because they are closely related to two species in this study (*Eumeces schneideri* and *Eublepharis macularius*). The phylogenetic reconstructions (Fig. 7) suggest that these species have independently evolved low-speed locomotion.

Why run slow?

The speeds exhibited by our *Tupinambis* match field and lab foraging speeds (Klein et al., 2003), so we are confident that the patterns of gait and mechanics we observed reflect their walking foraging mode. In terms of running, *Tupinambis* is known to employ the strategy of defensive and aggressive behavior rather than flight both in the lab (E.J.M., unpublished) (Klein et al., 2003) and the field (De Lema, 1983). However, *Tupinambis* are capable of moving more rapidly (Urban, 1965) and when they do, they exhibit high speed, low duty factor trots (White and Anderson, 1994). Thus, they may actually use the G1 pattern, although given the choice they appear to prefer to fight rather than high-speed running as an antipredatory behavior.

The remaining two species that exhibit slow running probably never have to run fast (at least as fast as other lizards). *Eumeces schneideri* has extremely small limbs and inhabits burrows, rarely venturing into the open (Disi and Amr, 1998). *Eublepharis* is a large, nocturnal, slow moving, WF ground gecko (Cooper, 1994) that does not rapidly flee but uses crypsis, posture, tail movement displays and tail autotomy as antipredatory behaviors (Marcellini, 1977). All other WF lizards in our sample were long limbed, diurnal, and preferred high-speed running as an antipredatory response (E.J.M., personal observation).

The G4 species exhibited a lack of relationship between speed and mechanics (Table 1). It has been argued that this is related to the basal condition of lumbering locomotion in tetrapods, and is found in a variety of sprawling animals such as salamanders, tuataras, alligators and frogs (Ahn et al., 2004; Reilly et al., 2006; Willey et al., 2004). Lumbering locomotion has been defined on the basis of having GPE greater than KE, whereas cursorial locomotion has been defined by KE being equal to or greater than GPE (Reilly et al., 2006). Interestingly, all lizards exhibited GPE/KE ratios that were either not significantly different from or far less

than one (Table 1). Thus, G4 lizards are cursorial even though they exhibit the same patterns of gait and mechanics as other sprawling tetrapods. This finding shows that the evolutionary shift to slow running in the G4 species does not include a shift to lumbering locomotor mechanics.

Lizards only trot

One interesting observation about the lizard gaits observed in this study is that they do not substantially deviate from trotting limb phase during running or walking. In general, the SW runners we studied exhibited the 'cleanest' trots (near 50% limb phase indicating coordination of diagonal limb couplets). During walking, most WF we studied exhibited lower limb phase values (Table 1). Although there are a few data points extending well into limb phases diagnostic of the lateral sequence single-foot (Figs 2, 3), all species means fell into the limb phase range of 37.5–62.5% that describes a trotting gait (*sensu* Biknevicius and Reilly, 2006). The lack of single-foot gaits was surprising given that lateral and diagonal sequence single-foots are predicted to improve stability in slow moving animals due to the larger polygons of support associated with these gaits (Cartmill et al., 2002; Hildebrand, 1988). Thus, when lizards shift to walking they may experience some enhanced stability afforded by more lateral or diagonal sequence trots but they do not fully move into the areas of single-foot gait space that take full advantage of hypothesized increases in stability (Cartmill et al., 2002; Hildebrand, 1976). This finding suggests the presence of some underlying neural or biomechanical constraint, which may limit lizards from routinely using lateral and diagonal sequence gaits that many mammals use when moving slowly. Primates also exhibit a lack of relationship between limb phase and speed or substrate type (Stevens, 2007). Thus, limb phase may show less of a response to functional or environmental requirements than previously envisaged.

Caveats

Our sample of 18 species is only a fraction of the ~4000 species of lizards and thus, like virtually all other comparative studies, our study suffers from limited taxon sampling. However, given the difficulty of obtaining data on lizard locomotor function (particularly center-of-mass mechanics of small animals), we feel that our study provides convincing insights into the evolutionary correlation between locomotor function and foraging ecology and provides an important starting point for future research in this area. Every comparative study has to address the issue of how the choice of species affects its results. We sampled species to maximize the number of evolutionary transitions to increase the power of statistical tests of trait-correlated evolution, based on an *a priori* evolutionary pattern of foraging mode evolution. However, sampling this way can provide results that appear at odds with accepted patterns of evolution. One such instance is the phase shift reconstruction at the base of the Scincomorpha (Fig. 4). Based on our focal sampling (focusing on reversals to test correlated evolution), the base of the Scincomorpha reconstructs as using running mechanics. In fact, the basal Scincomorph is known to be WF (Miles et al., 2007), which is the foundation of our interpretation that the basal Scincomorph would walk. Based on this interpretation we concluded that the three independent evolutionary reversals to SW foraging are accompanied by losses of walking mechanics in the Scincomorpha (Fig. 7). Clearly the Scincomorpha is a hot bed of foraging mode evolution and a complete understanding of functional evolution within the group requires additional sampling. However, given the strength of our findings and their robustness to phylogenetic uncertainty, differences in ecology among species, and the phylogenetic breadth of our sample, we feel that our results are robust and provide a general picture of correlated evolution of foraging ecology and locomotor function both within Scincomorpha and across lizard phylogeny.

Conclusions and future directions

The primary observation of this study is that locomotor mechanics and gait coevolve with foraging mode in lizards, at least within our sample of 18 species. In addition, different locomotor patterns have appeared (and sometimes convergently evolved) with WF strategies and subsequently disappear when lizards revert to SW foraging. The strong correlation of locomotion and foraging mode would be predicted given the similar pervasive patterns of correlated evolution and convergence in feeding biomechanics, skull and tongue morphology, chemosensory physiology and behavior in lizards (Cooper, 1994; McBrayer and Corbin, 2007; Reilly and McBrayer, 2007; Schwenk, 1993).

Many previous studies have focused on how animals move rapidly and the evolution of high-speed sprinting locomotion, particularly studies of locomotion in lizards. Our study differs, in formally showing the functional responses to the divergent speed demands of foraging mode and, in particular, how animals evolve slower locomotion. Overall, our study highlights the need to examine the ecological and behavioral relevance of the full spectrum of locomotor scope, both fast and slow.

Although our research supports a tight evolutionary coupling between biomechanics, gait and foraging behavior, several issues remain unresolved. First, although we show an evolutionary coupling between function and behavior we still have a poor understanding of how and when biomechanics, gait and speed are used in the field. Second, the utility of mechanical energy savings, particularly in small animals, remains unclear. Finally, it remains unknown how limb morphology is related to locomotor function in the context of foraging ecology. Given the pervasive effects of foraging behavior on lizard biology and the renewed and expanding interest in this subject (Reilly et al., 2007), the time seems ripe for additional detailed integrative studies of the functional and ecological basis of foraging locomotion in lizards.

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