

RESEARCH ARTICLE

Sprint sensitivity and locomotor trade-offs in green anole (*Anolis carolinensis*) lizards

Erik A. Sathe and Jerry F. Husak*

ABSTRACT

How well an organism completes an ecologically relevant task – its performance – is often considered a key factor in determining individual fitness. Historically, ecomorphological studies have examined how morphological traits determine individual performance in a static manner, assuming that differential fitness in a population is due indirectly to differences in morphological traits that determine a simple measure of performance. This assumption, however, ignores many ecological factors that can constrain performance in nature, such as substrate variation and individual behavior. We examined some of these complexities in the morphology–performance–fitness paradigm, primarily the impact that substrate variation has on performance. We measured maximal sprint speed of green anole lizards on four substrates that varied in size and complexity and are used by or available to individuals in nature. Performance decreased significantly from a broad substrate to a narrow substrate, and lizards were three times slower on a complex substrate than the broadest substrate. We also detected trade-offs in running on substrates with different diameters and in cluttered versus uncluttered environments. Furthermore, morphological predictors of performance varied among substrates. This indicates that natural selection may act on different morphological traits, depending on which substrates are used by individuals, as well as an individual's ability to cope with changes in substrate rather than maximal capacities.

KEY WORDS: Locomotion, Sprint speed, Trade-off**INTRODUCTION**

The standard paradigm for ecomorphological studies has centered on the presumption that the morphology of an individual directly determines its performance capacities, which, through tasks such as foraging and predator evasion, determine its fitness for a given environment (Arnold, 1983; Garland and Losos, 1994; Irschick and Garland, 2001; Vanhooydonck and Van Damme, 2003; Irschick et al., 2008). Therefore, natural selection acting on performance ultimately shapes morphological traits that favor individuals' abilities to perform these tasks (Irschick and Losos, 1999). However, the presumed link between Darwinian fitness and performance has been simplistic at best, with performance measured under presumably ideal conditions. This practice either implicitly assumes that an individual's performance capacity is invariant among the many different microenvironments within an individual's environment or that performance across all microenvironments is strongly and positively correlated. Studies measuring performance in an isolated laboratory context on one specific substrate have provided an

important base for comparative analyses (Bauwens et al., 1995; Bonine and Garland, 1999; Vanhooydonck et al., 2001, 2002; Irschick et al., 2005b; Vanhooydonck et al., 2014) and an accurate estimate of 'maximum performance' on an ideal substrate. However, such studies may not accurately represent variation in performance that exists in nature as a result of a range of environmental factors, such as substrate variation, that individuals may encounter (Garland and Losos, 1994; Irschick and Garland, 2001; Husak, 2006a; Husak and Fox, 2006). Since selection 'sees' performance in nature, performance variation due to environmental variation may be key to our understanding of morphological evolution. Individuals may regularly move across various substrates in pursuit of prey or during predator evasion, so an individual's locomotor performance is not necessarily a static trait, and performance measurements should aim to replicate this diversity (Losos and Irschick, 1996; Irschick and Losos, 1999; Jones and Jayne, 2012; Collins et al., 2013). In addition, the extent to which different substrates decrease an individual's performance (Irschick and Losos, 1999; Calsbeek and Irschick, 2007), can vary among individuals and may become a key factor in determining fitness where a slight change in substrate could cause a detrimental decrease in performance in individuals that are highly sensitive to substrate changes (Calsbeek and Irschick, 2007; Collins et al., 2013).

An important, but often overlooked, consequence of habitat complexity on intra-individual performance variation is that morphological predictors of performance may also vary with substrate properties such as surface material, shape or openness (Herrel et al., 2008; Calsbeek, 2008). That is, maximal sprint speed on one substrate may be predicted by different morphological traits compared with maximal sprint speed on another substrate. Different morphological properties are advantageous for different activities and both intra- and inter-specific studies have shown that different morphologies may be optimized in different habitats (Melville and Swain, 2000; Van Damme et al., 2003; Husak and Rouse, 2006; Losos, 2009; Wynn et al., 2015). For example, short relative limbs are favored in arboreal lizard species that occupy narrow perches or terrestrial lizard species that maneuver often through grass (Irschick and Losos, 1999; Newbold, 2005; Jones and Jayne, 2012). Conversely, long relative limbs are favored in arboreal lizard species that occupy broad perches or terrestrial lizard species that live in open habitats with few obstacles (Losos and Sinervo, 1989; Bauwens et al., 1995). If there is one preferred microhabitat on which locomotion is maximized, as in Caribbean *Anolis* lizards (reviewed in Losos, 2009), then selection will favor performance specialization, and locomotor trade-offs may result across species (Vanhooydonck et al., 2001; Angilletta et al., 2003; Wilson et al., 2014). However, if many substrates are frequently used, then the phenotype may be a compromise, because fitness may depend on sufficient locomotion on a variety of substrates. For example, in Caribbean *Anolis sagrei*, selection favored the long-limbed lizards that were fast on broad surfaces and preferred broad substrates in

Department of Biology, University of St. Thomas, 2115 Summit Avenue, St Paul, MN 55105, USA.

*Author for correspondence (jerry.husak@stthomas.edu)

Received 30 October 2014; Accepted 30 April 2015

nature, but also short-limbed lizards that were less sprint sensitive on narrow surfaces and preferred narrow perches (Calsbeek and Irschick, 2007). This shows that selection can favor combinations of morphology, performance traits and behavior that may be missed when only considering one maximal performance trait on one substrate. We know surprisingly little about how the performance of an individual on different substrates is predicted by morphological traits or what the evolutionary phenotypic response to selection is on performance across diverse substrates.

Lizards have been model subjects for locomotor studies, and locomotion has been shown to be essential for their survival as potential prey and as predators themselves (Sinervo et al., 2000; Miles, 2004; Husak, 2006a,b; Husak and Fox, 2006), with a multitude of studies demonstrating the morphology–performance link in lizards (Pounds, 1988; Losos and Sinervo, 1989; Huey et al., 1990; Losos, 1990a,b; Sinervo and Losos, 1991; Bauwens et al., 1995; Elphick and Shine, 1998; Irschick and Losos, 1999; Vanhooydonck et al., 2001; Irschick et al., 2005a; Newbold, 2005; Herrel et al., 2008; Foster and Higham, 2012; Jones and Jayne, 2012; Tulli et al., 2012; Collins et al., 2013). Caribbean *Anolis* lizards in particular are ideal subjects for these kinds of studies because their morphology tends to optimize performance on the substrate on which they run most often (Irschick and Losos, 1999). This specialization has led to the divergence of species, as well as evolutionary trade-offs both within and among species (Losos, 1990a,b, 2009; Calsbeek, 2008; Calsbeek and Irschick, 2007). By contrast, the green anole (*Anolis carolinensis*), which is native to North America, is an arboreal species that evolved until recently with no competing congeners, so it historically experienced high variation in its habitat and substrate availability. Theoretically, since the green anole currently uses many substrates (Irschick et al., 2005a), it should have a morphology that reflects a compromise among optimal performances on the different substrates that the species uses. A compromise morphology in an arboreal species would likely favor stability on many substrates, because stability, in regards to balance, is one of the most influential factors in optimizing locomotion, since a loss of balance decreases surefootedness and reduces speed (Jones and Jayne, 2012; Wynn et al., 2015). Consequently, maximal speeds should be fastest on the broadest substrates, which provide sufficient surface area for foot placement and therefore optimize stability, but as the diameter of the substrate decreases, the lizard's stability and speed also decrease. The decrease in speed due to stability is primarily the result of an increased likelihood of making mistakes, such as stumbling or falling, as a result of changes in kinematics (Foster and Higham, 2012). Furthermore, a substrate littered with obstacles also forces a change in kinematics causing a decrease in maximal sprint speed (Jones and Jayne, 2012; Tucker and McBrayer, 2012; Wynn et al., 2015).

We determined the impact of perch diameter and substrate openness on maximal sprint speed in green anoles and explored morphological predictors of performance on each substrate. Green anoles tend to perch frequently on both the broadest and narrowest substrates rather than on substrates of intermediate width (Irschick and Losos, 1999; Irschick et al., 2005a); hence, based on habitat use, we predicted that maximal sprint speeds would be high on those substrates. Alternatively, sprint speed may be compromised on one of these substrates, most likely the narrowest, as was shown by Losos and Irschick (1996). However, the broadest substrate in the Losos and Irschick (1996) study was 5.1 cm, excluding the much broader substrates on which green anoles often perch (Irschick and Losos, 1999). Further, we predicted that sprint speeds on open substrates would be greater than on a cluttered substrate, which may decrease speed by altering kinematics, body posture, behavior, or a

combination of these while navigating obstacles (Jones and Jayne, 2012; Tucker and McBrayer, 2012). Given that long limbs provide greater acceleration and velocity on broad surfaces than do short limbs, but decrease stability on narrow surfaces (Herrel et al., 2008), we expected that our exploratory analysis of morphological predictors would reveal that lizards fastest on the broad substrates would have longer hind-limbs, whereas lizards fastest on small and complex substrates would have shorter limbs (Calsbeek, 2008). We also quantified sprint sensitivity to changing substrate diameter and complexity (Irschick and Losos, 1999; Calsbeek and Irschick, 2007; Collins, 2012; Collins et al., 2013) to determine how much performance decreases across substrates and whether there are morphological predictors of sprint sensitivity. Finally, we tested for trade-offs among performance traits, predicting as an alternative that if speeds were in fact different among substrates because of differences in stability, then speed on a broad substrate would trade-off for speed on narrower substrates.

RESULTS

Performance differed significantly among substrates ($F_{3,48}=33.93$, $P<0.001$; Fig. 1), with speed being fastest on the flat surface and the 5 cm substrate (no significant difference between these two, $P=0.40$), but significantly decreasing on the 2.5 cm substrate and the flat substrate with pegs ($P<0.001$ for all comparisons).

Sprint speed was not predicted on any substrate by SVL, body mass or residual hindlimb length, but speed on the 5 cm substrate was negatively related to residual forelimb length (Table 1). The lack of any correlation of performance with size is likely due to the small variation in body size (mean SVL=63.8±0.45; mass=4.85±0.09). Sprint speed on the 2.5 cm substrate was not predicted by any of the morphometric or muscle measures that we quantified (Table 1). Sprint speed on the 5 cm substrate was negatively related to limb PC1 and limb PC2. This can be interpreted as faster speeds on the 5 cm substrate being due to smaller limbs in general, but smaller humerus and manus in particular (Table 2). Sprint speed on the flat substrate was negatively predicted by limb PC3 (Table 1), meaning that lizards with short hindtoes, but large radius and pes were faster on a flat substrate. Sprint speed on the peg substrate was negatively predicted by limb PC2 and positively predicted by muscle PC3 (Table 3), meaning that lizards with small forelimbs, iliofibularis and biceps, but large pectoralis, puboischiotibialis and trapezius, were faster running through pegs.

Sprint sensitivities (represented hereafter as $\Delta_{\text{speed substrate}}$) were analyzed to determine how each substrate individually reduced speed from the flat substrate to the substrate of interest. Sprint sensitivity was thus calculated for the 2.5 cm substrate ($\Delta_{\text{speed 2.5}}$),

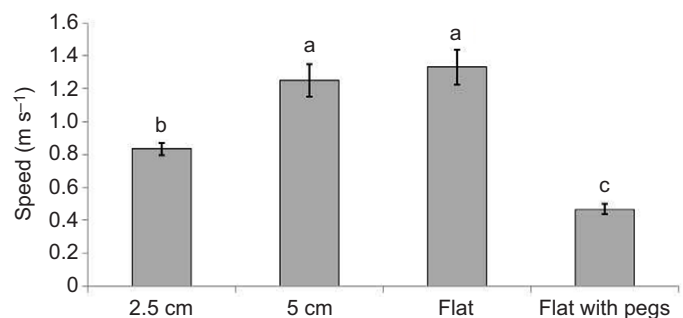


Fig. 1. Maximal sprint speeds of green anole lizards on four substrates. Values represent means±s.e.m. Bars with different letters are significantly different ($P<0.005$).

Table 1. Predictors of green anole maximal sprint speed on four different substrates

Predictor	Substrate			
	2.5 cm	5 cm	Flat	Flat with pegs
SVL	0.47	0.91	0.19	0.48
Mass	0.97	0.54	0.08	0.47
Residual HL	0.14	0.10	0.65	0.15
Residual FL	0.45	0.008 (-)	0.41	0.92
Limb PC1	0.24	0.03 (-)	0.75	0.10
Limb PC2	0.99	0.02 (-)	0.19	0.05 (-)
Limb PC3	0.53	0.13	0.046 (-)	0.32
Limb PC4	0.99	0.33	0.72	0.63
Muscle PC1	0.62	0.66	0.82	0.43
Muscle PC2	0.42	0.07	0.67	0.06
Muscle PC3	0.80	0.21	0.13	0.04 (+)

Results are from exploratory analyses of potential predictors of speed on each substrate.

Numbers are *P*-values from regressions. SVL, mass, residual hindlimb length (HL), and residual forelimb length (FL) were analyzed separately. Two additional regressions were conducted, one with PC scores from a PCA on limb morphometrics, and one with PC scores from a PCA on muscle masses (see Tables 2 and 3 for interpretation of PCAs). Significant predictors are bold, with the direction of the relationship given in parentheses.

the 5 cm substrate ($\Delta_{\text{speed } 5}$) and the peg substrate ($\Delta_{\text{speed pegs}}$). More negative values mean a greater reduction in speed compared with running on the flat substrate. There was no significant predictor of $\Delta_{\text{speed } 2.5}$ ($P > 0.07$ for all variables). Residual hindlimb length was the only significant predictor of $\Delta_{\text{speed } 5}$ ($P = 0.03$; Fig. 2), meaning that lizards with relatively longer hindlimbs had greater reductions in speed on the narrower substrate. Limb PC3 was positively related to $\Delta_{\text{speed pegs}}$ ($P = 0.03$), meaning that lizards with shorter toes and a larger radius and pes had the highest reduction in speed on pegs.

When considering raw performance values, sprint speed on the 5 cm substrate, and the flat substrate were significantly positively correlated with each other, but other performance values were uncorrelated (Table 4). After correcting for individual quality, there were significant correlations between speed on 2.5 cm substrate and the 5 cm substrate, as well as between speed on the flat substrate and the substrate with pegs (Table 4). When examining correlations among sprint sensitivities, we found that $\Delta_{\text{speed pegs}}$ was positively correlated with both $\Delta_{\text{speed } 2.5}$ ($r = 0.91$, $P < 0.001$) and $\Delta_{\text{speed } 5}$ ($r = 0.59$, $P = 0.02$), suggesting that sensitivity to increasingly narrow substrates is associated with sensitivity to obstacles. There was no correlation between $\Delta_{\text{speed } 2.5}$ and $\Delta_{\text{speed } 5}$ ($r = 0.49$, $P = 0.06$).

Table 2. Principal components analysis matrix of residual limb morphometrics, showing the factor loadings of each measured variable and the direction in which they contribute towards the components

Principal component	PC1	PC2	PC3	PC4
Eigenvalue	2.98	1.80	1.24	0.82
% Variance	37.2	22.5	15.5	10.3
Femur	0.81	-0.32	-0.15	-0.06
Tibia	0.74	-0.24	0.15	0.48
Pes	0.71	0.22	-0.46	-0.33
Hind toe	0.60	0.04	0.70	0.12
Humerus	0.27	0.78	0.12	-0.33
Radius	0.54	0.33	-0.59	0.41
Manus	0.04	0.91	0.23	0.23
Fore toe	0.75	-0.21	0.26	-0.37

PC scores were used as potential predictors of performance.

Table 3. Principal components analysis matrix of residual muscle masses, showing the factor loadings of each measured variable and the direction in which they contribute towards the components

Principal component	PC1	PC2	PC3	PC4
Eigenvalue	6.51	1.02	0.83	0.57
% Variance	65.1	10.2	8.3	5.7
Ambiens	0.83	0.06	0.16	-0.42
Biceps	0.85	0.09	-0.38	0.09
Caudofemoralis	0.83	-0.06	-0.29	-0.31
Gastrocnemius	0.88	-0.32	-0.06	0.03
Iliofibularis	0.83	0.20	-0.34	0.07
Latissimus dorsi	0.93	-0.26	-0.07	0.11
Pectoralis	0.84	-0.13	0.35	-0.14
Puboischiotibialis	0.61	0.61	0.42	-0.06
Trapezius	0.66	-0.48	0.39	0.29
Triceps	0.76	0.43	0.03	0.40

PC scores were used as potential predictors of performance.

DISCUSSION

We found that maximal sprint speeds within individuals varied dramatically across substrates (Fig. 1). While individuals may choose to run at different speeds on different substrates, we forced them to run at maximal levels, showing that when not given a choice, individuals vary in their maximal speed across substrate types. Lizards were fastest on the broad substrates and were significantly slower on the narrow (2.5 cm) substrate, which is contrary to previous results where no significant difference was found between the 5 and 2.5 cm substrates (Losos and Irschick, 1996). Further, lizards were over three times slower on the cluttered substrate than on the broadest substrate. We also found that performance capacities on different substrates were not predicted by the same morphological traits in our exploratory analyses. We detected no morphological predictors for maximal sprint speeds or sprint sensitivity on the 2.5 cm substrate. On the 5 cm substrate, small forelimbs predicted faster sprint speeds, whereas hindlimb length predicted how much speed decreased on this diameter substrate. Here, longer hindlimbs made individuals more sensitive to a decrease in substrate diameter. On the flat substrate, short toes and large forelimbs predicted faster sprint speeds. On the peg substrate, large humeral retractor muscles predicted faster sprint speeds whereas longer toes and smaller forelimb elements decreased sensitivity. We expected to find trade-offs in speed among different substrates because of differences in stability and/or kinematics and posture, and we found trade-offs due to both substrate diameter (2.5-cm substrate vs. 5-cm substrate) and the presence of obstacles (flat substrate versus peg substrate). However, these were only detectable after correcting for overall performance quality.

Contrary to what we predicted based on results from past studies of microhabitat use by green anoles (Irschick and Losos, 1999; Irschick et al., 2005a), among the four substrates tested, only the flat surface and the 5 cm substrate provided similar sprint speeds. This means that performance differs dramatically among substrates of various sizes that are typically used by green anoles in nature, indicating that the instability and/or change in posture/kinematics associated with small-diameter substrates is more detrimental to green anole sprint speed than we anticipated. Since green anoles encounter and use many substrates on a regular basis (Irschick and Losos, 1999; Irschick et al., 2005a), it is likely that they need to run on substrates similar to those we tested. Simple, standard measurements of performance on one particular substrate implicitly assume that either individuals predominantly use one substrate in nature (i.e. where they run fastest) and that most selective pressure

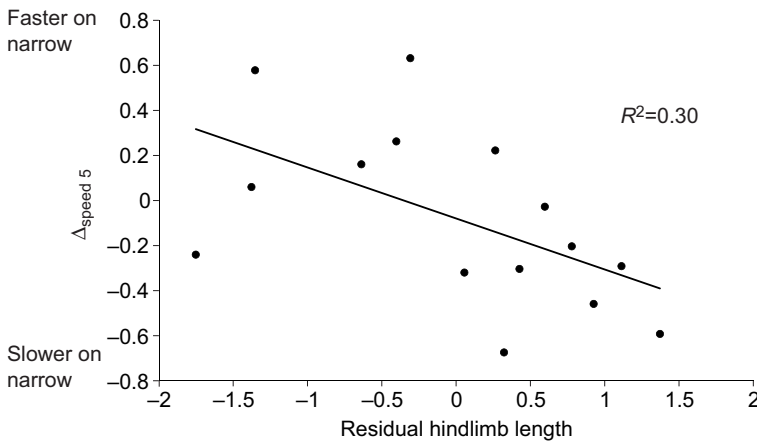


Fig. 2. Lizards with relatively longer limbs are more sensitive to decreases in substrate diameter. $\Delta_{\text{speed } 5}$ is the decrease in speed from a flat substrate to a 5 cm substrate.

occurs there, or individuals that are good (or poor) performers on one substrate are good (or poor) performers on all substrates. Performance trade-offs suggest that this is not fully true, although our results were mixed. Performance levels on larger-diameter substrates were positively correlated, but there were detectable negative relationships. Therefore such measures of only maximal performance on one ideal substrate may not fully reveal the potential targets of natural selection. Instead, the decrease in sprint speed across substrates suggests that natural selection may act on an individual's ability to cope with substrate variation, as has been proposed by others (Macrini and Irschick, 1998; Calsbeek and Irschick, 2007). For example, if a lizard is running from a predator and crosses from a broad substrate to a narrow substrate, the individual that can maintain a faster speed is more likely to escape the predator and survive better than an individual who slows more dramatically. Perhaps it is beneficial for green anoles to move into suboptimal habitat, such as cluttered microenvironments where speed is slowed, because they can hide more effectively or their predators' relative performance is also decreased. These are important empirical questions that have not been addressed to our knowledge.

The widely accepted paradigm holds that morphological traits should determine an individual's maximal performance capacities (Arnold, 1983). But if maximal speed varies among individuals across substrates, then natural selection may act upon different traits on different substrates, if selection is operating on maximal speed (s). For example, on the flat substrate, individuals with large forelimbs have faster sprint speeds, whereas the opposite occurs on the 5 cm substrate. Therefore, on a flat substrate, natural selection acts on short forelimbs, driving the population to have longer forelimbs, whereas forelimb size would be driven the opposite direction on the 5 cm substrate-like substrate. Both substrates are common in nature and used by lizards, so it is difficult to predict the

optimal morphology in a given environment where natural selection is potentially acting on multiple traits on different surfaces without knowing what substrates are available to individuals, in what proportions they are used, and on which substrates fitness-related tasks happen. The phenotypic consequences of selection are complicated further if natural selection is also acting on sprint sensitivity (Calsbeek and Irschick, 2007). Our study shows that individuals with relatively long hindlimbs are significantly slowed on substrates of decreasing size, whereas short toes and a long radius are unfavorable on substrates with increasing obstacles. If natural selection is acting on phenotypic variation in sprint sensitivity, compromised phenotypes are expected that result from combined selection for: (1) relatively short hind-limbs, which provide balance where substrate size is widely variable, and (2) relatively short radii, which allow for an increase in maneuverability where substrates are complex (Jones and Jayne, 2012).

Since performance is clearly affected by substrate, it seems that there would be performance trade-offs among the substrates, so it was expected, based on substrate use, that exceptionally fast individuals on one substrate would be exceptionally slow on another. This is indeed what we found, with trade-offs occurring when moving to a smaller-diameter substrate and to a substrate with obstacles. This implies that there is variation among individuals in how they deal with complex habitats, a phenotypic trait upon which natural selection may act, albeit in a complex way. The correlation between sensitivity on pegs and sensitivity on the narrow substrates further supports this notion. The trade-off between speeds on the flat and cluttered substrates suggests that there may be an inverse relationship between maximal speed and maneuverability on a similar substrate. While this is consistent with theoretical predictions (e.g. Wilson et al., 2014; Wynn et al., 2015), more research must be done to verify a relationship. We note that there may be a change in limb posture to bring the limbs closer to the body and reduce sprawl while navigating obstacles such as the pegs, and we noticed lizards adopt such a strategy. Such a strategy may contribute to the trade-off between moving on cluttered and uncluttered substrates.

Because the fitness of an individual is broadly defined as the result of its performance – whether it be maximal sprint speeds (Irschick et al., 2008) or sprint sensitivity (Calsbeek and Irschick, 2007) – and since performance trait(s) are a direct result of an individual's morphology, investigators should approach these links in a much more complex way compared with how the morphology–performance–fitness paradigm has been used in previous ecomorphological studies (see also Lailvaux and Husak, 2014). A particular set of morphological traits will not necessarily predict

Table 4. Correlation matrix of maximal sprint speeds of green anoles on four substrates, using raw values and quality-corrected values

	2.5 cm	5 cm	Flat	Flat with pegs
2.5 cm	–	–0.62	–0.22	–0.05
5 cm	–0.02	–	–0.29	–0.26
Flat	0.33	0.51	–	–0.52
Flat with pegs	0.17	0.14	0.12	–

Numbers below the diagonal are *r*-values from correlation analysis on raw performance values, and numbers above the diagonal are *r*-values from correlation analysis on quality-corrected values (see text for details). Bolded values are statistically significant ($P < 0.04$ for all).

maximal performance on all substrates, but rather certain traits will predict performance on one or a suite of substrates. This raises questions about how we measure the link between morphology and a performance trait that we presume to be important to fitness. These findings may explain why some studies do not find a performance–fitness relationship (reviewed in Irschick et al., 2008), because it is possible that in these studies, a performance trait that was not ecologically relevant was measured. Thus, although studies using ‘standard’ methods to measure performance in the lab yield results that are comparable to other studies, future studies on performance should consider the classic definition of performance as ‘a measure of how well an organism accomplishes a given task’ and measure performance in a relevant way (Irschick and Garland, 2001; Irschick et al., 2008). Maximal sprint speed should be clearly defined to include the substrate on which that speed was measured. Furthermore, some individuals are more sensitive to a changing substrate than others, so it cannot always be assumed that an individual’s speed on various substrates is consistent relative to other individuals, which standard measures of performance often overlook. In a complex environment where individuals regularly encounter many substrates, resistance to decreases in speed, as well as other aspects of performance, such as acceleration or jump distance, may be essential for survival.

MATERIALS AND METHODS

We obtained 15 adult, male *Anolis carolinensis* Voight 1832 from Candy’s Quality Reptiles (La Place, LA). Each male was housed individually in a 40.64×25.4×27.94 cm plastic cage that contained two bamboo dowels (substrate) and artificial foliage for perching. Lizards were housed in a temperature-controlled room (27–30°C), sprayed twice daily, and fed crickets three times per week (Fluker Farms, Port Allen, LA; dusted weekly with Fluker’s Repta-Vitamin dietary supplement). Each cage was placed under a fluorescent bulb and a Repti Glo 5.0 full-spectrum fluorescent bulb (5% UVB, Hagen, Montreal) for ultraviolet radiation. The lizards were allowed to acclimate to the cages for 7 days before performance testing began.

Sprint speed was measured using wood substrates, all of which were wrapped with a thin layer of natural cork liner. Four substrates were used: a 2.5-cm-diameter substrate (2D), a 5-cm-diameter substrate (5D), a flat board 14 cm wide and a flat board with wooden pegs (6.35 mm in diameter) spaced 30 mm from center to center and placed in a diamond pattern to prevent the lizards from running a clear path (supplementary material Fig. S1). The cork liner added 2 mm to the final diameter of the dowel substrates. All substrates were 2 m in length and fixed at an incline of 51 deg to the horizontal, as *A. carolinensis* tend to jump on horizontal surfaces. A 19-cm-wide, smooth, wooden frame held the substrates in place and provided walls 18 cm in height to discourage lizards from leaving the substrate and escaping. The frame had eight photo-sensor ‘gates’ spaced at 25 cm and were connected to a computer that directly measured and recorded the time that each gate was passed using the program TrackMate ScL Timer v6.8.1 (Trackmate Racing, Surrey, British Columbia, Canada) (Cox et al., 2009). Each lizard ran on every substrate a total of six times. Three trials per lizard were performed on one substrate per day, with an hour of rest in their cages between each trial. Lizards were inspected visually for health before and after each trial. The fastest time over a 25 cm interval on each substrate was considered the maximal speed on that substrate for each lizard (Cox et al., 2009). The lizards were encouraged to run by pursuing them with a hand or a paintbrush. If a lizard jumped off of the track or reversed direction and would not go the correct direction, the run was considered bad and was omitted from the analyses. We used the fastest speed for each individual on each substrate as its maximal sprint speed on that substrate.

We calculated sprint sensitivity for each substrate by subtracting speed on the flat substrate from the speed on each of the other substrates (e.g. $\Delta_{\text{speed pegs}} = \text{speed}_{\text{pegs}} - \text{speed}_{\text{flat}}$). More negative values are interpreted as a greater reduction in speed compared with running on the flat substrate.

Table 5. Principal components analysis matrix of the four performance traits, showing the factor loadings of each measured variable and the direction in which they contribute towards the components

Principal component	PC1	PC2	PC3	PC4
% Variance	42.0	26.3	22.4	9.4
2.5 cm	0.50	0.71	−0.43	0.25
5 cm	0.72	−0.57	0.14	0.37
Flat	0.85	−0.15	−0.26	−0.42
Flat with pegs	0.42	0.44	0.79	−0.07

PC1 scores were used to ‘quality correct’ standardized performance measures according to Wilson et al. (2014).

All subjects were measured to the nearest gram for body mass on a portable electronic balance, measured with digital calipers for snout–vent length (SVL), and then humanely killed by rapid decapitation. In order to preserve the subjects, they were fixed for 24 h in 10% aqueous formalin, rinsed with water and placed in 70% ethanol (Huyghe et al., 2010). All limb measurements were made posthumously. Length measurements for femur, tibia, foot, long toe on the foot, humerus, radius, hand and long toe on the hand were made using digital calipers. The measurements of femur, tibia, pes and long toe on the pes were summed to determine hind-limb length, and humerus, radius, manus and long toe on the manus were summed to determine forelimb length (Herrel et al., 2008). We dissected muscles following Herrel et al. (2008), with the muscle anatomy of *Anolis carolinensis* being most similar to *A. valencienni*. We removed the following muscles: m. ambiens (knee extension), m. biceps (elbow flexion), m. caudofemoralis longus (femoral retraction), m. gastrocnemius pars fibularis (ankle extension), m. iliofibularis (knee flexion), m. latissimus dorsi (humeral retraction), m. pectoralis pars superficialis (humeral retraction), m. puboischiotibialis (knee flexion), m. trapezius (shoulder rotation and stability) and m. triceps brachii (elbow extension). Once removed, we patted each muscle dry and weighed it to the nearest 0.001 mg (Mettler Toledo UMX2).

We used repeated-measures analysis of variance (ANOVA), followed by pair-wise Tukey honestly significant difference *post hoc* tests, to test for differences in maximal sprint speed among substrates. We regressed each limb measurement separately against SVL and used residuals from those regressions in a principal components analysis (PCA) to obtain multivariate measures of limb shape (Table 2). Similarly, we regressed muscle masses (separately) against body mass and used residuals in a second PCA to obtain multivariate measures of muscle morphology (Table 3). PC scores from the separate PCAs were used in separate exploratory regression analyses to determine predictors of speed.

We used Pearson correlation analyses to test for trade-offs among performance measures. Additionally, because individual quality may mask performance trade-offs, we corrected for overall quality using the technique described by Wilson et al. (2014). Briefly, when examining variation in performance among individuals, a significant portion of that variation may be due to among-individual variation in overall performance ability (i.e. some individuals are better at all measures, and others are worse at all). This possibility does not preclude there still being trade-offs, but it can mask their detection (Wilson et al., 2014). To account for this, standardized performance measures were put into a PCA, and scores from the first principal component were used in regressions with each original performance variable to obtain residuals that represent quality-corrected values (Table 5). These were then used in Pearson correlation analyses to test for trade-offs, with significant negative correlations suggesting trade-offs.

Acknowledgements

We thank A. Keith for help with lizard husbandry and members of the UST Lizard Lab for comments on previous versions of the manuscript.

Competing interests

The authors declare no competing or financial interests.

Author contributions

E.A.S. and J.F.H. designed and conducted the research, as well as analyzed the data. E.A.S. wrote the manuscript with input from J.F.H.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.116053/-DC1>

References

- Angilletta, M. J., Wilson, R. S., Navas, C. A. and James, R. S. (2003). Tradeoffs and the evolution of thermal reaction norms. *Trends Ecol. Evol.* **18**, 234-240.
- Arnold, S. J. (1983). Morphology, performance and fitness. *Am. Zool.* **23**, 347-361.
- Bauwens, D., Garland, T., Castilla, A. and Van Damme, R. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioral covariation. *Evolution* **49**, 848-863.
- Bonine, K. E. and Garland, T. Jr (1999). Sprint performance of phrynosomatid lizards, measured on a high-speed treadmill, correlates with hindlimb length. *J. Zool.* **248**, 255-265.
- Calsbeek, R. (2008). An ecological twist on the morphology-performance-fitness axis. *Evol. Ecol. Res.* **10**, 197-212.
- Calsbeek, R. and Irschick, D. J. (2007). The quick and the dead: correlational selection on morphology, performance, and habitat use in island lizards. *Evolution* **61**, 2493-2503.
- Collins, C. E. (2012). *Home field advantage: sprint sensitivity to ecologically relevant substrates in lizards*. MS Thesis, Georgia Southern University, Statesboro, GA, USA.
- Collins, C. E., Self, J. D., Anderson, R. A. and McBrayer, L. D. (2013). Rock-dwelling lizards exhibit less sensitivity of sprint speed to increases in substrate rugosity. *Zoology* **116**, 151-158.
- Cox, R. M., Stenquist, D. S., Henningsen, J. P. and Calsbeek, R. (2009). Manipulating testosterone to assess links between behavior, morphology, and performance in the brown anole *Anolis sagrei*. *Physiol. Biochem. Zool.* **82**, 686-698.
- Elphick, M. J. and Shine, R. (1998). Longterm effects of incubation temperatures on the morphology and locomotor performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biol. J. Linn. Soc.* **63**, 429-447.
- Foster, K. and Higham, T. E. (2012). How forelimb and hindlimb function changes with incline and perch diameter in the green anole, *Anolis carolinensis*. *J. Exp. Biol.* **215**, 2288-2300.
- Garland, T., Jr and Losos, J. B. (1994). Ecological morphology of locomotor performance in squamate reptiles. In *Ecological Morphology: Integrative Organismal Biology* (ed. P. C. Wainwright and S. Reilly), pp. 240-302. Chicago: University of Chicago Press.
- Herrel, A., Vanhooydonck, B., Porck, J. and Irschick, D. (2008). Anatomical basis of differences in locomotor behavior in *Anolis* lizards: a comparison between two ecomorphs. *Bull. Mus. Comp. Zool.* **159**, 213-238.
- Huey, R. B., Dunham, A. E., Overall, K. L. and Newman, R. A. (1990). Variation in locomotor performance in demographically known populations of the lizard *Sceloporus merriami*. *Physiol. Zool.* **63**, 845-872.
- Husak, J. F. (2006a). Does survival depend on how fast you can run or how fast you do run? *Funct. Ecol.* **20**, 1080-1086.
- Husak, J. F. (2006b). Does speed help you survive? A test with collared lizards of different ages. *Funct. Ecol.* **20**, 174-179.
- Husak, J. F. and Fox, S. F. (2006). Field use of maximal sprint speed by collared lizards (*Crotaphytus collaris*): compensation and sexual selection. *Evolution* **60**, 1888-1895.
- Husak, J. F. and Rouse, M. N. (2006). Population variation in escape behavior and limb morphology of collared lizards (*Crotaphytus collaris*) in Oklahoma. *Herpetologica* **62**, 156-163.
- Huyghe, K., Husak, J. F., Moore, I. T., Vanhooydonck, B., Van Damme, R., Molina-Borja, M. and Herrel, A. (2010). Effects of testosterone on morphology, performance and muscle mass in a lizard. *J. Exp. Zool. A Ecol. Genet. Physiol.* **313A**, 9-16.
- Irschick, D. J. and Garland, T. Jr (2001). Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annu. Rev. Ecol. Syst.* **32**, 367-396.
- Irschick, D. J. and Losos, J. B. (1999). Do lizards avoid habitats in which performance is submaximal? The relationship between sprinting capabilities and structural habitat use in Caribbean anoles. *Am. Nat.* **154**, 293-305.
- Irschick, D. J., Carlisle, E., Elstrott, J., Ramos, M., Buckley, C., VanHooydonck, B., Meyers, J. and Herrel, A. (2005a). A comparison of habitat use, morphology, clinging performance and escape behaviour among two divergent green anole lizard (*Anolis carolinensis*) populations. *Biol. J. Linn. Soc.* **85**, 223-234.
- Irschick, D. J., Herrel, A., Vanhooydonck, B., Huyghe, K. and Van Damme, R. (2005b). Locomotor compensation creates a mismatch between laboratory and field estimates of escape speed in lizards: a cautionary tale for performance-to-fitness studies. *Evolution* **59**, 1579-1587.
- Irschick, D. J., Meyers, J. J., Husak, J. F. and Le Galliard, J. F. (2008). How does selection operate on whole-organism functional performance capacities? A review and synthesis. *Evol. Ecol. Res.* **10**, 177-196.
- Jones, Z. M. and Jayne, B. C. (2012). Perch diameter and branching patterns have interactive effects on the locomotion and path choice of anole lizards. *J. Exp. Biol.* **215**, 2096-2107.
- Lailvaux, S. P. and Husak, J. F. (2014). The life-history of whole-organism performance. *Q. Rev. Biol.* **89**, 285-318.
- Losos, J. B. (1990a). Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monogr.* **60**, 369-388.
- Losos, J. B. (1990b). The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. *Evolution* **44**, 1189-1203.
- Losos, J. B. (2009). *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. Berkeley, CA: University of California Press.
- Losos, J. B. and Irschick, D. J. (1996). The effect of perch diameter on escape behaviour of *Anolis* lizards: laboratory predictions and field tests. *Anim. Behav.* **51**, 593-602.
- Losos, J. B. and Sinervo, B. (1989). The effects of morphology and perch diameter on sprint performance of *Anolis* lizards. *J. Exp. Biol.* **145**, 23-30.
- Macrini, T. and Irschick, D. J. (1998). An intraspecific analysis of trade-offs in sprinting performance in a West Indian lizard species (*Anolis lineatopus*). *Biol. J. Linn. Soc.* **63**, 579-591.
- Melville, J. and Swain, R. (2000). Evolutionary relationships between morphology, performance and habitat openness in the lizard genus *Niveoscincus* (Scincidae: Lygosominae). *Biol. J. Linn. Soc.* **70**, 667-683.
- Miles, D. B. (2004). The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. *Evol. Ecol. Res.* **6**, 63-75.
- Newbold, T. A. S. (2005). Desert horned lizard (*Phrynosoma platyrhinos*) locomotor performance: the influence of cheatgrass (*Bromus Tectorum*). *Southwest. Nat.* **50**, 17-23.
- Pounds, J. A. (1988). Ecomorphology, locomotion, and microhabitat structure: patterns in a tropical mainland *Anolis* community. *Ecol. Monogr.* **58**, 299-320.
- Sinervo, B. and Losos, J. B. (1991). Walking the tight rope: arboreal sprint performance among *Sceloporus occidentalis* lizard populations. *Ecology* **72**, 1225-1233.
- Sinervo, B., Miles, D. B., Frankino, W. A., Klukowski, M. and DeNardo, D. F. (2000). Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Horm. Behav.* **38**, 222-233.
- Tucker, D. B. and McBrayer, L. D. (2012). Overcoming obstacles: the effect of obstacles on locomotor performance and behaviour. *Biol. J. Linn. Soc.* **107**, 813-823.
- Tulli, M. J., Abdala, V. and Cruz, F. B. (2012). Effects of different substrates on the sprint performance of lizards. *J. Exp. Biol.* **215**, 774-784.
- Van Damme, R., Vanhooydonck, B., Aerts, P. and De Vree, F. (2003). Evolution of lizard locomotion: context and constraint. In *Vertebrate Biomechanics and Evolution* (ed. V. L. Bels, J.-P. Gasc and A. Casinos), pp. 267-282. Oxford, UK: BIOS Scientific Publishers.
- Vanhooydonck, B. and Van Damme, R. (2003). Relationships between locomotor performance, microhabitat use and antipredator behaviour in lacertid lizards. *Funct. Ecol.* **17**, 160-169.
- Vanhooydonck, B., Van Damme, R. and Aerts, P. (2001). Speed and stamina trade-off in lacertid lizards. *Evolution* **55**, 1040-1048.
- Vanhooydonck, B., Van Damme, R. and Aerts, P. (2002). Variation in speed, gait characteristics, and microhabitat use in lacertid lizards. *J. Exp. Biol.* **205**, 1037-1046.
- Vanhooydonck, B., James, R. S., Tallis, J., Aerts, P., Tadic, Z., Tolley, K. A., Measey, G. J. and Herrel, A. (2014). Is the whole more than the sum of its parts? Evolutionary trade-offs between burst and sustained locomotion in lacertid lizards. *Proc. R. Soc. B Biol. Sci.* **281**, 20132677.
- Wilson, R. S., Niehaus, A. C., David, G., Hunter, A. and Smith, M. (2014). Does individual quality mask the detection of performance trade-offs? A test using analyses of human physical performance. *J. Exp. Biol.* **217**, 545-551.
- Wynn, M. L., Clemente, C., Amir Abdul Nasir, A. F. A. A. and Wilson, R. S. (2015). Running faster causes disaster: trade-offs between speed, manoeuvrability and motor control when running around corners in northern quolls (*Dasyurus hallucatus*). *J. Exp. Biol.* **218**, 433-439.