RESEARCH ARTICLE

How muscles define maximum running performance in lizards: an analysis using swing- and stance-phase muscles

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SUMMARY

Maximum locomotor performance is crucial for capturing prey, escaping predators and many other behaviors. However, we know little about what defines maximum performance in vertebrates. Muscles drive the movement of the limbs during locomotion, and thus likely play a major role in defining locomotor capacity. For lizards, the iliofibularis, a swing-phase muscle, is often linked to ecology and/or performance. However, stance-phase muscles likely limit performance given that they propel the animal. Using a small semi-arboreal lizard (*Sceloporus woodi*), we compared how swing- and stance-phase muscles relate to maximum running speed and acceleration. We employed both a level and vertical trackway to elicit ecologically relevant locomotor performance. Six individuals were filmed at 250 frames s⁻¹ in lateral view. Following performance trials, upper and lower hindlimbs were sectioned and assessed using histochemistry. Fast glycolytic, fast oxidative and slow oxidative fibers were detected and counted in the gastrocnemius (GA; stance phase) and iliofibularis (IF; swing phase) muscles. In addition, the mean fiber diameter for each fiber type in each muscle was determined, as was the fiber cross-sectional area. We found that properties of the GA, but not the IF, were positively correlated with performance. Interestingly, certain attributes of the GA were correlated with maximum vertical locomotion whereas others were correlated with maximum level locomotion. We conclude that stance phase, not swing phase, limits maximum performance in this species of lizard. In addition, we highlight the need to include properties of stance-phase muscles and a spectrum of ecologically relevant behaviors when attempting to correlate locomotor physiology with ecology and/or performance.

Key words: muscle, performance, locomotion, lizard, *Sceloporus*, fiber type, cross-sectional area, running, acceleration.

INTRODUCTION

Movement is an essential part of survival and growth for most animals, and muscles drive this movement (Biewener, 2003). In particular, maximum locomotor performance is vital for escaping predators and capturing prey (Webb, 1976; Domenici and Blake, 1997; Walker et al., 2005; Higham, 2007). Maximum acceleration and velocity are key indicators of maximum locomotor performance, and have been studied widely in a variety of vertebrates (Domenici and Blake, 1997; Irschick and Jayne, 1998; Roberts and Scales, 2002; Vanhooydonck et al., 2005; Walker et al., 2005; Denny, 2008; Williams et al., 2009; McElroy and McBrayer, 2010). However, how muscle physiology might limit maximum acceleration and velocity has received much less attention. This is a significant missing link as variation in muscle characteristics likely has a profound influence on performance, and thus selection likely impacts various attributes of muscles depending on the ecology of the animal. Thus, understanding the physiological and morphological mechanisms underlying maximum performance is crucial to understanding how some animals can move faster than others.

A stride is comprised of stance (limb is in contact with the ground) and swing (limb is in the air) phases, and both theoretically could be modulated independently to alter running speed in terrestrial vertebrates. However, repositioning the limbs during swing at a faster rate does not appear to be a mechanism for increasing running speed (Weyand et al., 2000). For example, humans reach faster top speeds by applying greater support forces to the ground rather than moving the limbs more rapidly in the air (Weyand et al., 2000). By increasing the forces applied to the ground, an animal would increase its velocity at the end of the stance phase, which would increase both the distance traveled between strides and the time that the animal spends in the swing phase of the stride. To do this, it is likely the fast-twitch muscle fibers in the limbs that enable an animal to reduce the time that a foot spends on the ground relative to the entire stride (i.e. decrease in duty factor). Interestingly, recent work suggests that a limit to maximum running speed is likely imposed by the maximum rates at which the limbs can apply forces to the ground rather than how much force can be applied to the ground (Weyand et al., 2010). Taking this information into account, it is likely that animals with stance-phase limb muscles containing a greater number and size of fast-twitch fibers are able to run at higher maximum speeds, given that these fibers will enable an animal to produce sufficient impulse in a shorter contact time. However, this has not yet been tested.

Lizards are particularly adept at moving in complex three-dimensional habitats (Higham et al., 2001), and they have been extensively studied with respect to their ecomorphology (Losos, 1990; Irschick and Losos, 1999; Mattingly and Jayne, 2004; Higham and Russell, 2010) and muscle histochemistry (Bonine et al., 2001; Scales et al., 2009). Whether for escaping, finding a basking spot or

capturing prey, lizards utilize limb muscles to satisfy their locomotor demands. The types of muscle fibers that make up a muscle determine what type of activity in which that muscle will be used. Fast-twitch glycolytic fibers are used for very fast acceleration and short bursts of speed, but they fatigue quickly (Bonine et al., 2001; Biewener, 2003; Kohlsdorf et al., 2004). Thus, quick attacks for prey capture or quick escapes from a predator likely rely on fast-twitch glycolytic muscle fibers. Slow-twitch oxidative fibers are useful for sustained activity, movements requiring endurance or postural support (Smith et al., 1977; Biewener, 2003). In addition to the type of fibers in a muscle, the cross-sectional area (CSA) of a muscle impacts peak force-generating capabilities (Powell et al., 1984). Although much is known regarding lizard locomotor performance and a little is known about lizard muscle morphology and physiology, we lack a thorough understanding of how these traits are linked.

Among lizards, a considerable amount of research has focused on the iliofibularis (IF), a swing-phase muscle in the upper hindlimb (Putnam et al., 1980; Gleeson, 1983; Gleeson and Johnston, 1987; Jayne et al., 1990; Mutungi, 1990; Kohlsdorf et al., 2004). Depending on the lifestyle of the lizard, the proportions of each fiber type within the IF muscle can vary greatly (Kohlsdorf et al., 2004; Scales et al., 2009). Although swing-phase muscles are important for locomotion (Marsh et al., 2004), stance-phase muscles are responsible for generating the forces necessary to propel an animal forward and off of the ground. Thus, understanding the mechanisms underlying locomotor performance in lizards requires an assessment of both swing- and stance-phase muscles. One stance-phase muscle, the gastrocnemius (GA), contributes to propulsion during a crucial portion of the stride in a wide variety of vertebrates (Walmsley et al., 1978; Reilly, 1995; Roberts et al., 1997; Daley and Biewener, 2003; Higham and Biewener, 2008; Higham et al., 2008; Nelson and Roberts, 2008). For example, the lateral GA is primarily responsible for generating the increased forces necessary for running at higher speeds in guinea fowl (Higham et al., 2008).

We focused on a species of lizard from the genus *Sceloporus* because this genus has a demonstrated capacity for sprinting on a trackway or treadmill and a well-defined IF muscle morphology, making members of this genus ideal subjects for muscle parameter and performance studies (Bonine et al., 2001; Bonine et al., 2005). In addition to quantifying maximum locomotor performance, we quantified fiber type, fiber diameter, muscle cross-sectional area (CSA) and fiber cross-sectional area (FCSA) of both the IF and the GA (Fig. 1). These attributes have been identified as important aspects of muscle function (e.g. Bodine et al., 1987). We addressed the following questions in this study: (1) are aspects (morphology, dimensions and/or histochemical properties) of stance-phase muscles better at predicting maximum locomotor performance compared with swing-phase muscles and (2) are certain aspects of a muscle better at predicting maximum locomotor performance than others? Because of their roles during a stride, we expected that the GA would be a better predictor of maximum locomotor performance than the IF. In addition, we expected all aspects of the muscles (fiber type and size) to be equally good at predicting performance.

MATERIALS AND METHODS
Study animals
We collected male Florida scrub lizards, *Sceloporus woodi* Stejneger 1918 (mean snout vent length=50.9±0.8 mm), from the Ocala National Forest, FL, USA (US Department of Agriculture Forest Service permit SEM451 and Florida Fish and Wildlife Conservation
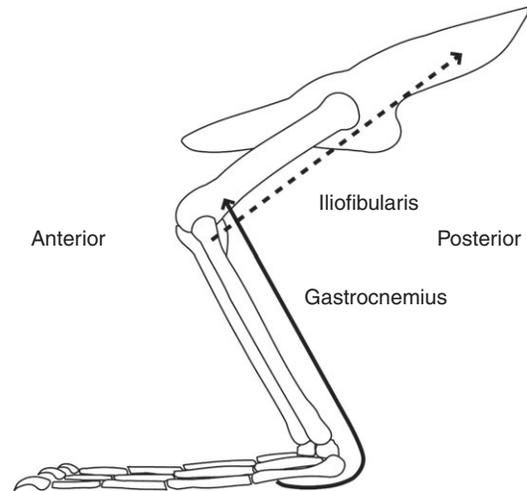

Fig. 1. Lateral schematic of the left hind leg of a lizard showing the locations of the iliofibularis (dashed line; a knee flexor in the upper hindlimb) and the gastrocnemius (solid line; an ankle extensor in the lower hind limb).

Commission permit WX07348 to L.D.M.). Lizards were brought back to the laboratory and housed individually in 10 gallon aquaria with a sandy substrate. In the laboratory, lizards were fed crickets every other day and provided with water *ad libitum*. Lighting was maintained on a 12 h:12 h light:dark cycle.

Laboratory performance trials
Prior to laboratory trials, individual lizards were warmed to their optimal running temperature (31–35°C). Once warmed, each lizard was encouraged to run up a vertical tree trunk. Following these trials, and on a different day, lizards were encouraged to run on a 1 m level wooden trackway that was lined with 600 grit sandpaper for increased traction. The trackway had Plexiglas® sides and was approximately 10 cm wide. Lizard movement was recorded using a Photron APX RS camera (Photron USA, San Diego, CA, USA) operating at 250 frames s^{-1}, an appropriate frame rate for quantifying maximum velocity and acceleration (Walker, 1998). Using gentle taps on the tail or sudden hand movements towards the animal, each lizard was run on each substrate (vertical tree, horizontal track) four to six times; only the maximum trial on each substrate was retained for further analysis. Each lizard was rested for at least 30 min between trials.

Tissue preparation and histochemical analyses
Following the performance trials, the lizards were killed *via* an intramuscular injection of ketamine. The upper and lower hind limbs were sectioned at the mid region of the limb segment (midpoint between the joints) and the two blocks (approximately 5 mm long) were placed in frozen section medium. These samples were immediately floated on a bath of liquid nitrogen and then placed in a −80°C freezer. Samples were stored in the freezer for a short period of time prior to sectioning. Serial sections (10 or 12 μm) taken from the proximal section of the muscle block were then stained for myosin ATPase and NADH/SDH following previously published protocols (Gleeson, 1983) in order to categorize fibers as fast glycolytic (FG), fast oxidative glycolytic (FOG) or slow oxidative (SO). Once the slides were prepared, images (resolution= 2048×1536 pixels) of the GA and IF were captured using a

QImaging Micropublisher digital camera (Quantitative Imaging Corporation, Burnaby, BC, Canada) and ACDSee software (5.0, ACDSee Systems of America, Inc., Miami, FL, USA) and then imported into ImageJ (version 1.42q, National Institutes of Health, Bethesda, MD, USA) for analysis.

Data analysis
For both level and vertical trials, we digitized the tip of the lizard's snout in each frame using DLTdv3 custom MATLAB software (The MathWorks, Natick, MA, USA) (Hedrick, 2008). Videos were scaled and *x*-coordinate data were extracted from MATLAB. Displacement data were smoothed using a smoothing spline in Igor Pro version 5 (WaveMetrics, Inc, Lake Oswego, OR, USA). Instantaneous velocity was calculated as the first derivative of displacement (from the *x*-coordinate data) and acceleration was calculated as the first derivative of velocity.

Muscle fibers were categorized using the following criteria: myosin ATPase assays, following an acid preincubation, result in fast fibers staining darkly, whereas NAHD/SDH assays result in oxidative fibers staining darkly. Each fiber was categorized and counts were performed using ImageJ and then the relative proportion of fibers (%) was determined by relative counts. Fiber diameter data were obtained from 15 randomly selected fibers of each type (in each muscle) in ImageJ and averaged using Microsoft Excel. We initially tested more than 15 fibers, but found that the results did not change if more were included. Thus, 15 randomly selected fibers suitably represents the overall population of the fibers of each type. Muscle CSA and FCSA were measured with the ImageJ area function. To account for differences in muscle CSA, FCSA was scaled to CSA.

Statistical analyses
Our final sample size was limited to five or six individuals as we only included individuals with paired histochemistry and performance data on both the vertical and horizontal surfaces. Our criteria for inclusion included: (1) running performance trials in which the lizard (with intact tail) ran in a straight line along the racetrack and tree trunk; and (2) histochemistry data with complete muscles, i.e. no folding, tearing or missing segments in the section (these can occur during the removal of the segment from the limb or during sectioning for histochemical staining). It is worth noting that the performance data among all individuals tested fell within the range found among the five individuals included in the analyses and in another study (McElroy and McBrayer, 2010). Thus, we feel that the data set presented is highly representative of the species. Data were log-transformed to normalize variances and were then analyzed using linear regressions (SYSTAT 13, Systat Software, Chicago, IL, USA). Linear regressions were performed between all combinations of the muscle (fiber type, fiber diameter, CSA and FCSA) and performance data (maximum acceleration and maximum velocity). Non-size-corrected performance and muscle data were used because the differences in size between individuals were not significantly different. A *P*-value of <0.05 was used for the criterion for statistical significance. Data are presented as means ± s.e.m.

RESULTS
Performance
The mean maximum acceleration on the horizontal surface was $52.3\pm11.6\,\mathrm{m\,s^{-2}}$ and the highest maximum acceleration was $122.9\,\mathrm{m\,s^{-2}}$. Maximum acceleration from a standstill occurred in every case in the first one or two strides. The mean maximum velocity was $2.3\pm0.4\,\mathrm{m\,s^{-1}}$ and the highest maximum velocity was $4.1\,\mathrm{m\,s^{-1}}$.

Mean maximum acceleration on the vertical tree trunk was $103.1\pm14.1\,\mathrm{m\,s^{-2}}$ and the highest maximum acceleration observed was $158.9\,\mathrm{m\,s^{-2}}$. The mean maximum velocity was $1.8\pm0.7\,\mathrm{m\,s^{-1}}$ and the highest maximum velocity was $2.0\,\mathrm{m\,s^{-1}}$. Maximum acceleration for vertical treatments also occurred during the first two steps starting from a stop.

Histochemistry
Muscle fibers of different types in the IF were found in segregated regions, with a small central pocket of SO fibers, a layer of FOG fibers and then a layer of FG fibers at the outer boundary of the muscle. The fibers of the IF were always in this arrangement. The fibers of the GA were not as consistently distributed from individual to individual as those of the IF. Whereas the GA exhibited a greater proportion of FG fibers relative to the IF, the IF contained a greater proportion of SO fibers relative to the GA (Table 1). Although the mean fiber diameters were comparable between the IF and GA, the mean total area of the GA was approximately twice that of the IF. The rank order of fiber diameters within each muscle was always FG>FOG>SO (Table 1).

Performance and histochemistry
Three muscle characteristics, all associated with the GA, were positively correlated with maximum acceleration. Maximum level acceleration was significantly and positively correlated with the diameter of the FG fibers in the GA (linear regression, $r^2=0.78$, $P<0.05$; Fig. 2, Table 2). In contrast, there was no significant relationship between the diameter of the FG fibers and maximum acceleration in the IF ($r^2=0.02$, $P>0.05$; Table 2). Maximum level running speed was significantly and positively correlated with the percentage of FG fibers in the GA ($r^2=0.75$, $P<0.05$; Fig. 3, Table 2) but not in the IF ($r^2=0.01$, $P>0.05$; Table 2). Maximum acceleration on the vertical surface was positively correlated with FG FCSA for the GA ($r^2=0.82$, $P<0.05$; Fig. 4, Table 2) but not in the IF ($r^2=0.13$, $P>0.05$; Table 2). Finally, the percentage of FG fibers within the GA was significantly correlated with maximum acceleration on the

Table 1. Mean (±s.e.m.) percentage of fiber types, fiber diameter and total area for a swing-phase (Iliofibularis) and stance-phase (gastrocnemius) muscle in the Florida scrub lizard, *Sceloporus woodi*

Muscle	Relative fiber type (%)			Fiber diameter (mm)			Total area (mm²)
	FG	FOG	SO	FG	FOG	SO	
Iliofibularis	43.7±7.6	36.6±5.9	19.8±2.4	0.08±0.003	0.07±0.002	0.04±0.004	1.42±0.14
Gastrocnemius	62.1±3.9	33.4±2.8	4.5±1.6	0.07±0.003	0.05±0.002	0.02±0.001	3.11±0.15

N=6 for iliofibularis data and *N*=5 for gastrocnemius data.
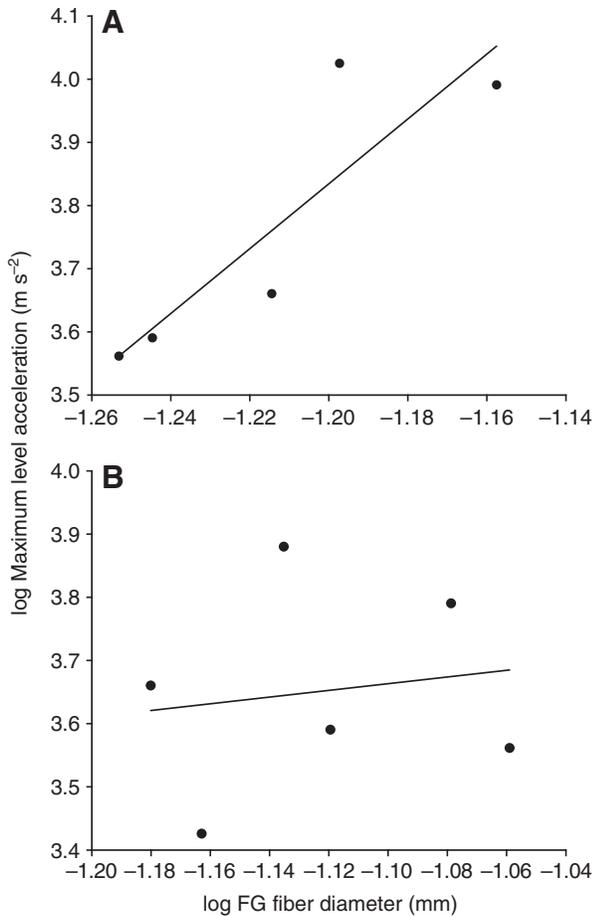FG, fast glycolytic; FOG, fast oxidative; SO, slow oxidative.

Fig. 2. Relationships between log maximum level acceleration and log fast glycolytic (FG) fiber diameter for the gastrocnemius (A) and the iliofibularis (B) in *Sceloporus woodi*. The r^2-values for the gastrocnemius and iliofibularis are 0.78 ($P<0.05$) and 0.02 ($P>0.05$), respectively.
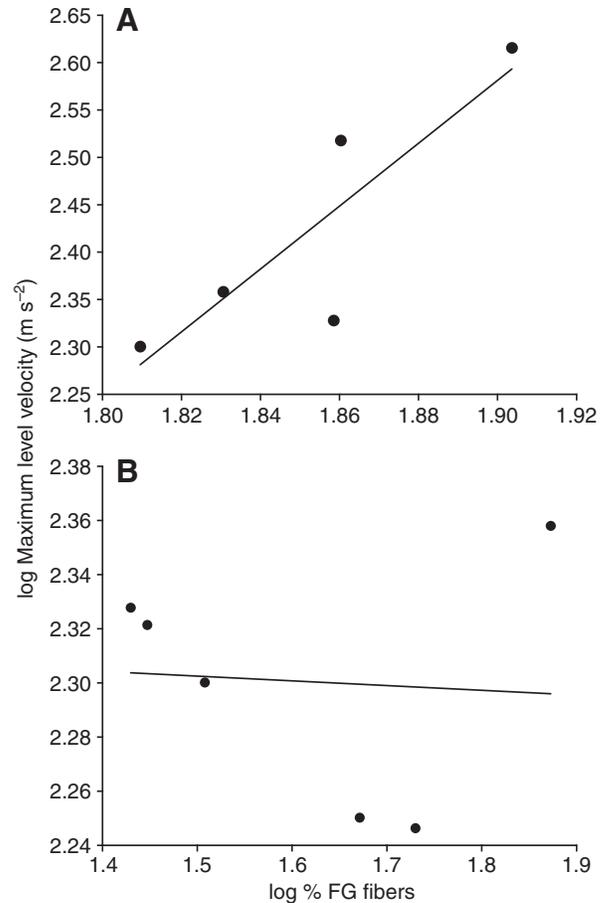

Fig. 3. Relationships between log maximum level velocity and log % FG fibers for the gastrocnemius (A) and the iliofibularis (B) in *Sceloporus woodi*. The r^2-values for the gastrocnemius and iliofibularis are 0.75 ($P<0.05$) and 0.01 ($P>0.05$), respectively.

vertical surface ($r^2=0.99$, $P<0.01$; Fig. 4, Table 2). We found no correlations between maximum vertical running speed and any of the muscle variables.

Table 2. Results from linear regressions of maximum velocity and maximum acceleration for various characteristics of hindlimb muscles in *S. woodi*

Muscle	Incline	FG diameter	FG FCSA	% FG fibers	Muscle CSA
Velocity					
GA	Level	0.60	0.03	**0.75***	0.04
IF	Level	0.13	0.28	0.01	0.01
GA	Vertical	0.23	0.45	0.28	0.54
IF	Vertical	0.51	0.03	0.16	0.66
Acceleration					
GA	Level	**0.78***	0.08	0.62	0.08
IF	Level	0.02	0.04	0.01	0.51
GA	Vertical	0.24	**0.82***	**0.99****	0.42
IF	Vertical	0.15	0.13	0.11	0.31

Data are r^2-values. Bold values indicate those that were statistically, and positively, correlated. *$P<0.05$; **$P<0.01$.
CSA, cross-sectional area; FCSA, fiber cross-sectional area; FG, fast glycolytic; GA, gastrocnemius; IF, iliofibularis.

DISCUSSION

What determines maximum running performance in vertebrates?

Many factors likely act in concert to limit maximum running performance, including those related to the neuromuscular, respiratory and circulatory systems (Jones and Lindstedt, 1993). Muscles are undoubtedly a key determinant of maximum performance and our study is the first to assess the relative importance of stance- *versus* swing-phase muscle physiology in contributing to maximum running performance in lizards. We found that a stance-phase muscle (gastrocnemius) is a better predictor of maximum performance than a swing-phase muscle (iliofibularis), despite the latter being the focus of most studies of lizards. We also found that different attributes of muscle fibers (e.g. percent fiber type, fiber diameter and FCSA) can predict different types of locomotor performance (e.g. maximum speed and maximum acceleration). In addition, maximum acceleration was greater during vertical running compared with level running [as found by Higham et al. (Higham et al., 2011)], and these different levels of performance were correlated with different attributes of the gastrocnemius. Ultimately, factors related to the stance phase of the stride, as suggested elsewhere (Weyand et al., 2000; Weyand et al., 2010), likely limit maximum running performance.
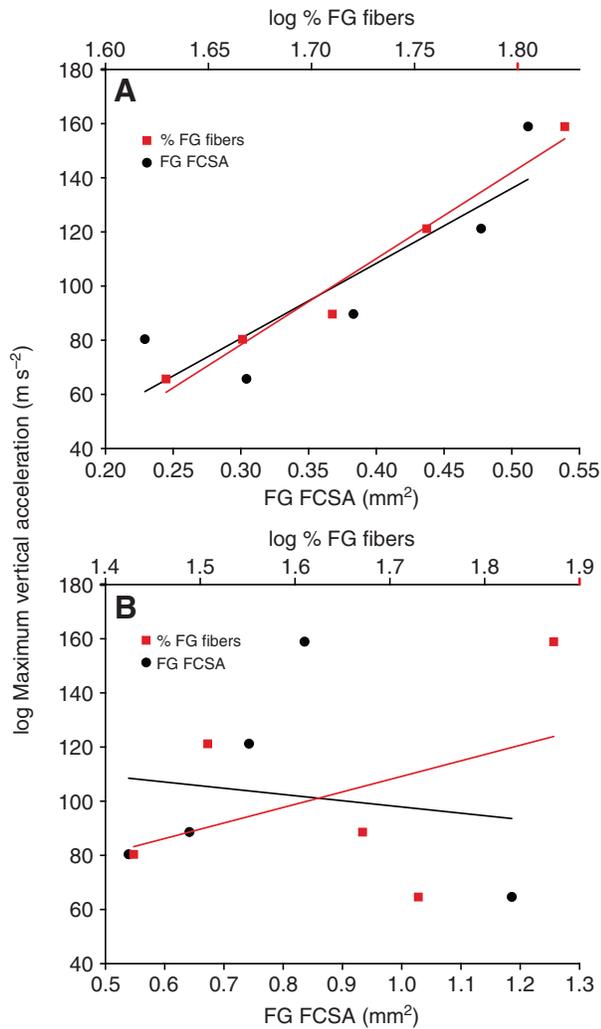
Fig. 4. Relationships between log maximum vertical acceleration and both FG fiber cross-sectional area (FCSA) and log % FG fibers for the gastrocnemius (A) and the iliofibularis (B) in *Sceloporus woodi*. The r^2-values for the relationship between FCSA and vertical acceleration for the gastrocnemius and iliofibularis are 0.82 ($P<0.05$) and 0.13 ($P>0.05$), respectively. The r^2-values for the relationship between % FG fibers and vertical acceleration for the gastrocnemius and iliofibularis are 0.99 ($P<0.01$) and 0.11 ($P>0.05$), respectively.

Stance- *versus* swing-phase muscles

We found that attributes of stance-phase muscles, but not swing-phase muscles, are positively correlated with maximum performance. The mechanics of locomotion are important for interpreting this result. During propulsion, the hindlimb of an animal is in contact with the substrate (stance phase) and propulsive muscles are active (Carlson-Kuhta et al., 1998; Higham and Jayne, 2004; Gillis et al., 2005). During the swing phase, the hindlimb is lifted off the ground and swings through the air in order to contact the ground forward of the animal's center of mass. Muscles are certainly active during the swing phase, and faster locomotion requires increases in swing-phase muscle recruitment and thus force production (Jayne et al., 1990; Marsh et al., 2004; Nelson and Roberts, 2008). However, the primary source for increased running speed can likely be attributed to the stance phase of the stride. The idea that the stance phase is more important for maximizing locomotor speed has been suggested by Weyand et al. (Weyand et

al., 2000; Weyand et al., 2010) for humans, in which minimizing contact time with the ground was linked to faster running speeds. Here, we argue that attributes of stance-phase muscles, being more important for pushing the mass of animal forward, likely limit maximum locomotor performance in lizards. Our results, coupled with those of Weyand et al. (Weyand et al., 2000; Weyand et al., 2010), suggest that increased rates of force generation (and increased power) from stance-phase muscles decreases ground contact time, thus increasing stride length, ultimately leading to increased running performance.

Apart from the work by Putnam et al. (Putnam et al., 1980), the IF, a swing-phase muscle, has been the focus of attention from researchers studying lizards over the past 30 years. For example, a recent study found that species of lizards relying on sprint locomotion to avoid predators have relatively higher proportions of FG fibers in their IF, whereas lizards relying predominantly on crypsis have higher proportions of FOG fibers in their IF (Scales et al., 2009). Although our study only assessed variation within a single species, we suggest that characteristics of stance-phase muscles might be a stronger proxy for locomotor performance. Our results suggest that future work, both inter- and intra-specific, should include stance-phase muscles, especially if the goal is to link ecology with locomotor performance, e.g. studies hoping to estimate fitness from physiological parameters (Fig. 5). In this case, one first has to correlate the physiological parameters with maximum performance, as we did in this study. However, it is also important to link maximum performance with the actual behavior in nature (Irschick et al., 2005) (Fig. 5). Doing this would enable the researchers to quantify the strength of the association between key aspects of locomotor physiology (e.g. muscle fiber type) and fitness (Fig. 5).

Muscle characteristics and level performance

Mean FG fiber diameter within the GA was positively correlated with maximum level acceleration for *S. woodi* (Fig. 2). Larger fiber diameters will result in greater force and power generation because of contractile properties (Widrick et al., 1996), potentially resulting in superior sprinting performance, given that running faster requires an increased rate of force generation and an increased magnitude of force (Higham et al., 2008). In addition to increased force, muscle fibers with larger diameters exhibit faster conduction velocities (Hakansson, 1956; Blijham et al., 2006). Compared with slow-twitch fibers, fast-twitch muscle fibers tend to be larger in diameter (Table 1) and have a faster maximum velocity of shortening (Rome et al., 1990). Ultimately, increased force, conduction velocity and shortening velocity in larger fast-twitch fibers likely results in greater locomotor performance. A key assumption is that FG motor units of the GA are being recruited during this behavior. Several lines of evidence support the idea that FG motor units are typically recruited during high-intensity behaviors (Henneman et al., 1965; Wakeling and Rozitis, 2005). For example, fishes often recruit white (FG) muscle at faster swimming speeds whereas red (SO) muscle exhibits a decrease in recruitment with increased swimming speed (Jayne and Lauder, 1994). Future studies combining *in vivo* muscle activity data and wavelet analyses would reveal the type of fibers being recruited during locomotion (Wakeling et al., 2002).

We found that mean FG fiber diameter predicted maximum level acceleration, but muscle CSA did not. However, the CSA of a muscle is positively correlated with peak tension (Powell et al., 1984). As mentioned above, CSA includes all types of fibers that differ in force capabilities (Bodine et al., 1987). Thus, if a certain behavior resulted in the recruitment of only a single type of motor unit, CSA might not predict the performance during that behavior. This is
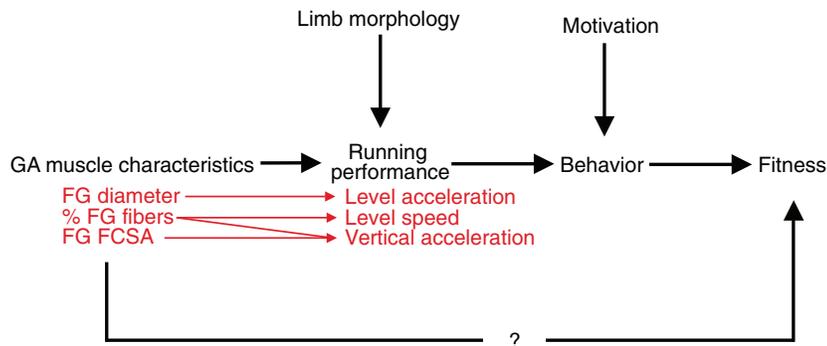
Fig. 5. Schematic showing the hypothetical relationship between various muscle characteristics and fitness. It is clear from our study that muscle characteristics directly influence running performance, and other studies have linked morphology with performance. Our results suggest that there may be a direct path from muscle characteristics to fitness, but this remains to be tested (represented by the question mark). Other components of this theoretical framework are from Garland and Losos (Garland and Losos, 1994). GA, gastrocnemius.

important when addressing maximum running performance, given that the rate of force generation (during stance) is likely more important than the maximum force generated (Weyand et al., 2010). Thus, it is not necessarily surprising that muscle CSA does not correlate with maximum performance. Ultimately, FG fibers are likely crucial for acceleration, whereas other types of fibers, making up the total CSA of the muscle, may not play a role at high levels of performance. It is well understood that fast-twitch fibers are better suited for high-powered movements, whereas SO fibers are better suited for conditions requiring low levels of force (Biewener, 2003). According to the 'size principle' of motor unit recruitment, SO motor units should still be recruited during high-intensity behaviors (Henneman et al., 1965). However, recent studies have found that SO fibers can be inhibited (de-recruited) during higher intensity movements (Jayne and Lauder, 1994) and that faster muscles fibers can be preferentially recruited over slow fibers (Gillespie et al., 1974). Thus, it is quite possible that the only active motor units are FG, although *in vivo* muscle recordings are required to test this hypothesis.

Muscle characteristics and vertical performance

We found that, in the GA, FG FCSA and the percentage of FG fibers predict maximum acceleration on the vertical, but not level, surface (Table 2). One potential explanation is that acceleration on a level surface, as opposed to a vertical surface, does not involve the recruitment of as many FG fibers within a muscle. Thus, the FCSA and percentage of FG fibers in the GA need not correlate with maximum level locomotor performance. In contrast, vertical locomotion requires more force (and power) from propulsive-phase muscles because of the forces exerted by gravity that act against forward movement (Roberts et al., 1997; Daley and Biewener, 2003; Higham and Jayne, 2004). An individual with a larger area of FG fibers and a greater percentage of FG fibers in their GA would thus be able to generate more force (and power), resulting in fast accelerations. On a large scale, it is well known that fiber type recruitment changes with demand (Smith et al., 1977; Jayne and Lauder, 1994; Nelson and Jayne, 2001; Higham and Jayne, 2004; Gillis et al., 2005; Wakeling and Rozitis, 2005), although we know little about how much of a muscle is recruited under different conditions. Given that many lizards are semi-arboreal or inhabit a variety of substrates (compliant sand to vertical running on rocks or trees), variation in muscle function *via* recruitment of different fiber types, or volumes of a given fiber type, may enable them to deal with dynamic challenges.

A puzzling result was that maximum acceleration on the vertical surface was not correlated with fiber diameter, in contrast to maximum acceleration on the level surface. The diameters of muscle fibers within a motor unit are likely constrained to be similar in size

(Andreassen and Arendt-Nielsen, 1987) in order to maintain a constant conduction velocity within the motor unit. However, different motor units of a given fiber type can exhibit variation in fiber diameter. Our study did not discriminate between motor units and we thus found variation in fiber diameter within a fiber type. The limiting factor for maximum acceleration on the vertical surface, which likely elicits maximum power output of the GA, could potentially be the largest diameter fibers. Future studies quantifying the recruitment of individual motor units (Hoffer et al., 1987) would help identify which motor units are being recruited during vertical and level running.

In conclusion, our results have broader implications for morphologists (who might quantify variables such as muscle CSA), physiologists (who might quantify fiber types and activation patterns) and biomechanists (who might quantify performance and mechanics) who aim to understand vertebrate ecology. We have highlighted that the addition of physiology to the general paradigm (morphology–performance–fitness) set forth originally by Arnold (Arnold, 1983) must be assessed in greater detail. For example, physiological parameters that can predict performance and, ultimately, fitness are likely limited to certain muscles and to certain characteristics of muscles (Fig. 5). Just as morphologists have highlighted the fact that only certain morphological traits are important for maximum performance (e.g. Miles et al., 1995), we have outlined which physiological parameters are likely to be important for maximum running performance in lizards. This is merely the beginning of what will likely be an extremely informative area of research that will reveal how physiology and ecology are related.

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