RESEARCH ARTICLE

The need for speed: functional specializations of locomotor and feeding muscles in *Anolis* lizards

Christopher V. Anderson[1],* and Thomas J. Roberts[2]

ABSTRACT

Muscles often perform diverse mechanical roles within an organism. Tuning of contractile properties may therefore provide an opportunity for muscles to better perform their different roles and impact their associated whole-organism performance. Here, we examined the muscle contractile physiology of a jaw and a leg muscle in five *Anolis* species to determine whether consistent physiological differences are found in these muscles. We found that these jaw and leg muscles exhibited consistent patterns of variation across species, which may be related to the functional use of each muscle. In particular, we found that each muscle had differentially increased different measures of muscle speed. Although the jaw muscles had faster peak contractile velocities than the leg muscles, the leg muscles had faster twitch times and faster contractile velocities under intermediate loads. We also found that the jaw muscles exerted higher specific tensions and had a greater curvature to their force–velocity relationship. The consistent patterns across five species suggest that these jaw and leg muscles have specialized in different ways. Examination of these contractile property variations may help illuminate important features relating to performing their individual functional roles.

KEY WORDS: Muscle contraction, Contractile velocity, Twitch time, Tension, Power

INTRODUCTION

An organism's fitness and survival is directly affected by its ability to perform various behaviors effectively. Variations in feeding or escape performance, for instance, produce fitness gradients because these performance parameters affect survival and reproductive output (Arnold, 1983). Morphological specializations are often-studied examples of adaptation because of their measurable effect on an organism's performance, which ultimately affects fitness (Arnold, 1983). Many physiological processes, such as muscle contraction, however, similarly form part of the mechanistic basis of whole-organism performance, and variation within those may also impact performance and, therefore, fitness (Bennett and Huey, 1990).

Muscle, the biological motor of movement in animals, is used to perform a diverse range of tasks. Muscles can be employed, for instance, to produce the mechanical energy used to power a movement, or can act as a sink to dissipate that energy (Dickinson et al., 2000). However, even when muscle is powering movement through the production of mechanical energy, it can be tasked for different mechanical outputs. For example, while fast motions may

[1]Department of Biology, University of South Dakota, 414 East Clark Street, Vermillion, SD 57069, USA. [2]Department of Ecology and Evolutionary Biology, Brown University, Box G-B205, Providence, RI 02912, USA.

*Author for correspondence (Christopher.V.Anders@usd.edu)

C.V.A., 0000-0001-7411-3102

necessitate rapid muscle contraction rates, other movements may conversely demand high forces, which muscles produce at the expense of contractile velocity (Katz, 1939).

As a result of the differing mechanical demands imposed on muscle and the importance of muscle contractile physiology on whole-organism performance and, ultimately, fitness, muscle contractile properties may specialize for different mechanical outputs. Some muscle contractile properties, such as specific tension and twitch time, for instance, are known to vary among muscles used for different tasks (Josephson, 1973). Sprinting, for example, is a rapid, cyclical movement that may be limited by the twitch time of muscles (Marsh and Bennett, 1985; Marsh, 1988). Biting, in contrast, is a more episodic movement that may not be as dependent on cycling rate. Owing to these differing functions, locomotor and feeding muscles may therefore vary in muscle contractile properties. However, few such comparisons have been made.

Lizards are a commonly used model for studies examining whole-organism performance and muscle contractile physiology. Both within and among closely related species, lizards often inhabit a wide variety of habitats, which can vary in both their structural composition and environmental conditions. Lizards also vary considerably in their body size and structure. These and other sources of variation produce performance demands on the animal that vary considerably, making lizards a particularly good model for examining adaptive patterns of whole-organism performance and muscle contractile physiology (e.g. Marsh and Bennett, 1985; Marsh, 1988; Garland, 1994; Garland and Losos, 1994; Aerts et al., 2000; Meyers et al., 2002). *Anolis* lizards, in particular, are frequently studied with regard to their connection between anatomy and whole-organism performance (e.g. Losos, 1990; Lailvaux et al., 2004; Lailvaux and Irschick, 2007; Herrel et al., 2008).

We examined the contractile physiology of a locomotor and a jaw adductor muscle in *Anolis* lizards to test the hypothesis that the contractile properties of these muscles vary according to their functional demands. We hypothesized that because of the rapid force-generating events associated with sprinting, limb muscles would be faster than jaw muscles. Further, we predicted that jaw muscles would produce proportionately higher forces owing to the crushing and force-generating actions associated with feeding and defensive behaviors. Alternatively, given the likely importance of rapid jaw closure during prey capture, jaw muscles may also be under selective pressure for speed, potentially complicating this dichotomy. Ultimately, however, observed variation in muscle contractile physiology measurements among jaw and leg muscles may provide insight into what functional traits of muscle performance are important to how these different muscles are used *in vivo*.

MATERIALS AND METHODS

Specimens and muscle preparations

We collected 10–20 adult male specimens each of *Anolis bonairensis* Ruthven 1923, *A. carolinensis* Voigt 1832, *A.*

List of symbols

L_0	optimal muscle length for tetanic contractions
$L_{0,twitch}$	optimal muscle length for twitch contractions
L_{20}	normalized muscle length at which passive tension reached 20% of P_0
P_0	peak isometric force
V_{40}	contractile velocity at 40% of P_0
V_{max}	peak contractile velocity
W_{max}	peak power

cristatellus Duméril and Bibron 1837, *A. lineatus* Daudin 1802 and *A. sagrei* Duméril and Bibron 1837. Specimens of *A. bonairensis*, *A. carolinensis* and *A. lineatus* were collected from native populations in Bonaire, south Florida and Curaçao, respectively, while specimens of *A. cristatellus* and *A. sagrei* were collected from introduced populations in south Florida. All individuals were housed individually in glass terrariums with UVB and incandescent lighting, with *ad libitum* water and misting two to three times daily, and were fed gutloaded crickets three times per week. The use of animals and the experimental protocol for this study were approved by the Institutional Animal Care and Use Committees of the University of South Dakota and Brown University. Permits and declarations for collection, exportation and/or importation of specimens used in this study were obtained from all necessary sources prior to their collection.

Immediately prior to *in vitro* muscle experiments, lizards were euthanized by isoflurane overdose followed by decapitation and double pithing. For each individual, *in vitro* muscle contractile

experiments were performed on a jaw muscle and a leg muscle to compare the contractile properties of muscles with different functional demands. For these experiments we used the m. adductor mandibulae externus superficialis anterior (Fig. 1A), a superficial jaw adductor muscle originating on the quadrate and inserting onto the lower jaw (Wittorski et al., 2016), and the m. ambiens pars ventralis (Fig. 1B), a swing-phase hindlimb muscle originating on the ilium and inserting onto the tibia (Herrel et al., 2008). For each muscle, the bony origin was secured to the rigid base of an experimental chamber and the bony insertion was attached to a dual mode servomotor (Models 300B and 305B, Aurora Scientific Inc., Ontario, Canada) above via silver chain. Because of the fiber orientation of the jaw adductor muscle (Fig. 1A), a chain from the posterior end of the jaw insertion point and a second chain from the anterior end of the insertion point were attached to a single chain leading to the muscle motor at links that maintained the approximate *in vivo* fiber orientations. The leg muscle (Fig. 1B) was able to be attached to the muscle motor via a single chain in a more standard *in vitro* preparation.

For all experiments, reptilian Ringer's solution (Marsh, 1988) saturated with 100% oxygen was recirculated from a container suspended in a temperature-controlled water bath. Temperatures were set to maintain the muscle chamber at either the published field active body temperatures for each species (28.2–33.4°C) (Hertz et al., 2013) or, in the case of *A. lineatus*, to the average field active body temperature measured from specimens in the field (32.2°C) (A. Herrel and J. Losos, CNRS/MNHN and Washington University in St Louis, MO, USA, personal communication). Muscles were allowed to equilibrate to the experimental temperature and setup for 30 min prior to the beginning of *in vitro* muscle contractile experiments.
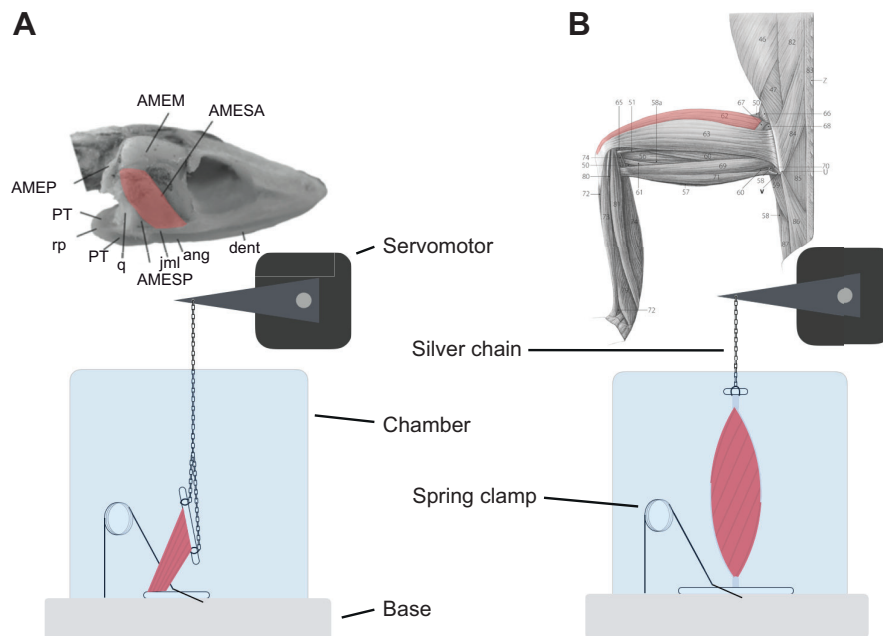

Fig. 1. *Anolis* **anatomical drawings and schematic of muscle preparations for muscles used to measure** *in vitro* **contractile properties.** (A) Superficial jaw musculature in lateral view with the m. adductor mandibulae externus superficialis anterior highlighted in red. The bony origin (quadrate) of the muscle was secured to the ridged base of the experimental chamber with a spring clamp, while the bony insertion (lower jaw) was attached to the muscle lever via two silver chains converging into a single chain at an adjustable point so as to mimic *in vivo* fiber orientations. (B) Superficial hindlimb musculature in dorsal view drawing with the m. ambiens pars ventralis highlighted in red. The bony origin (ilium) was secured to the ridged base of a chamber filled with Ringer's solution, while the bony insertion (tibia) was attached to the muscle lever via silver chain. Anatomical drawings adapted from (A) Wittorski et al. (2016; © 2015 Anatomical Society) and (B) Herrel et al. (2008; Museum of Comparative Zoology, Harvard University, © President and Fellows of Harvard College). AMEM, m. adductor mandibulae externus medialis; AMEP, m. adductor mandibulae externus profundus; AMESA, AMESP, m. adductor mandibulae externus superficialis anterior and posterior, ang, angular; dent, dentary; jml, jugal mandibular ligament; PT, m. pterygoideus; q, quadrate; rp, retroarticular process.

In vitro measurements

Muscles were supramaximally stimulated (0.2 ms square wave pulses) using custom-made platinum electrode bars attached to a stimulator and amplifier (Grass S48 stimulator, Grass Medical Instruments, Quincy, MA, USA; Crown DC-300A II amplifier, Crown International Inc., Elkhart, ID, USA). Muscle force and length were sampled from the servomotor at 10 kHz (PCI-MIO-16, National Instruments, Austin, TX, USA; Igor Pro 6, Wavemetrics Inc., Lake Oswego, OR, USA).

For each muscle, length was initially set to a short muscle length and twitch contractions were performed at incrementally increasing lengths to construct a length–tension curve. Passive force was measured as the tension prior to the onset of muscle stimulation, and maximal force was measured as the maximum tension recorded during stimulation. Active force at each length was calculated as the difference between passive and maximal force in a contraction. Optimal length for twitch contractions ($L_{0,\text{twitch}}$) was defined as the length corresponding to maximal force produced. Twitch kinetics were measured from two twitch stimulations approximately 200 ms apart with the muscle held at $L_{0,\text{twitch}}$. Twitch times were measured as the duration from the onset of each stimulus to the time of 50% relaxation of each twitch (Fig. 2) and the shortest duration for each muscle was then used as the twitch time for that muscle in each individual.

The stimulation frequency required to achieve fused tetanus was determined for each muscle, and a tetanic length–tension curve was constructed by incrementally increasing muscle length. Optimal length for tetanic contractions (L_0) was defined as the length corresponding to maximal active force produced. To construct a force–velocity curve, an isometric contraction at L_0 was performed to measure the peak isometric force (P_0). This was followed by a series of isotonic contractions of sequentially decreasing force values from P_0 as the muscles contracted through L_0. All tetanic contractions were performed with 5-min rest intervals between subsequent contractions.

At the end of each experiment, muscle length at L_0 and muscle mass were measured. For the jaw muscle, the length of anterior and posterior muscle fibers were measured so that an average fiber length at L_0 could be calculated. For the leg muscle, muscle length and fiber length at L_0 were measured, and the muscle was

photographed at L_0 so that fiber angle could be measured using ImageJ (National Institutes of Health, Bethesda, MD, USA). These data were used to characterize the physiological cross-sectional area in order to compare maximum specific tension of muscles and to normalize length data (Table S1).

Curve fitting

Tetanic length–tension curve data for each muscle were normalized by dividing extracted force values by the maximal active force value collected for that muscle across all muscle lengths (i.e. the peak isometric force, P_0). Muscle length was normalized by dividing the muscle lengths at which contractions were performed by the length at P_0 (L_0). For each muscle in every individual, a curve was fit to the passive and active force–length data based on equations from Otten (1987). The passive force–length curve was derived via a best fit to the equation:

$$f(L) = P_0 \times e^{c_1 + c_2 \times L}, \tag{1}$$

where L represents the muscle length, P_0 represents peak isometric force, and c_1 and c_2 are constants. The active force–length curve was derived from the best fit to the equation:

$$f(L) = e^{-\left|\frac{L^b - 1}{s}\right|^a}, \tag{2}$$

where L similarly represents muscle length, and a, b and s are constants. From these fitted curves, the normalized muscle length at which passive tension reached 20% of P_0 (termed L_{20}) was determined as a measure of muscle stiffness.

For force–velocity curve data from each muscle, muscle contractile force was normalized by dividing the force values by P_0, and velocity was normalized by dividing velocity measures by average fiber length at L_0 for the jaw muscle and fiber length at L_0 for the leg muscle. For each muscle in every individual, we then fit Hill's 'characteristic equation' (Hill, 1938) to the data to create a force–velocity curve. The force–velocity curve was fit to the equation:

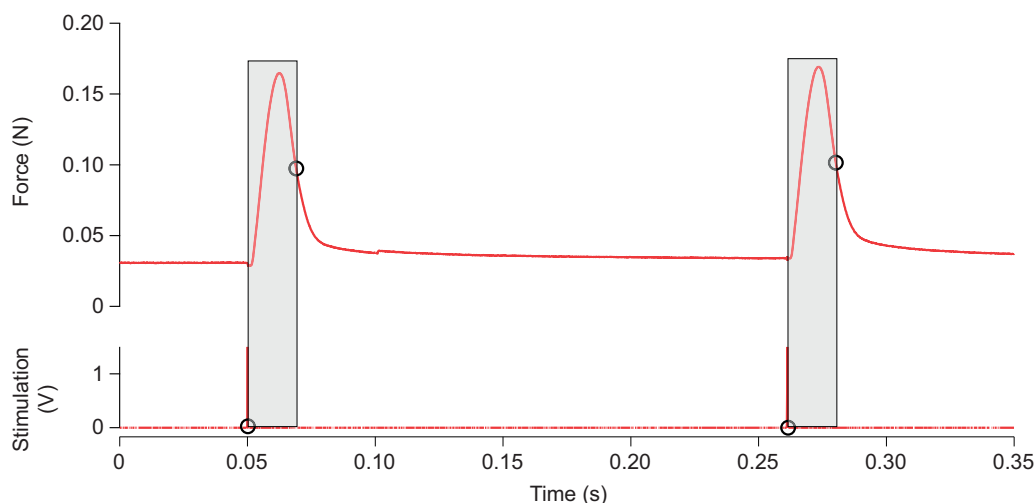$$f(V) = \frac{V \times a - b}{-b - V}, \tag{3}$$

Fig. 2. Muscle twitch contractions showing the calculation of twitch times. Twitch times were calculated as the duration from the onset of stimulus (circles in bottom panel) to the time of 50% relaxation (circles in top panel), as indicated by the shaded boxes.

where V represents velocity, and a and b are constants. From this curve, we recorded the peak contractile velocity (i.e. the peak contractile speed under no load; V_{max}) as the x-intercept of the fitted force–velocity relationship, as well as the contractile velocity at 40% of P_0 (V_{40}; Rummel et al., 2018).

From these fitted data, a mass-specific power curve was calculated based on force and velocity values of the fitted curve, and peak power (W_{max}) was recorded as the peak of the fitted mass-specific power curve. Normalized velocity at W_{max} was determined from these fitted data. Power ratio, a dimensionless measure of how curved the force–velocity relationship is, was calculated for each muscle based on Marsh and Bennett (1986).

Statistical analysis

Statistical analyses were performed in JMP Pro 14.1.0 (SAS Institute Inc., Cary, NC, USA). To ensure that only data from undamaged muscle preparations were included in the dataset, data on L_{20}, specific tension, V_{max}, V_{40}, W_{max} and power ratio from muscles with specific tensions measured below $10 \, N \, cm^{-2}$ or from muscles that experienced ≥20% decline in active force between tetanic trials and the end of force–velocity trials were excluded from analyses (Table S2). All response variables (twitch time, L_{20}, specific tension, V_{max}, V_{40}, W_{max} and power ratio) were log transformed prior to model fitting. A three-factor nested random effects model was used to test

for an effect of species and muscle, with individual nested within species as a random effect. Significance levels were then adjusted to control for false discovery rates in multiple comparisons (Benjamini and Hochberg, 1995).

RESULTS

Across all species examined, leg muscles had significantly faster (shorter) twitch times than jaw muscles (Fig. 3A, Tables 1 and 2). Comparisons of representative twitch traces show that leg muscles consistently reached peak tension and returned to 50% relaxation faster than jaw muscles from the same individual (Fig. 3B,C). Significant differences among species in muscle twitch time were found (Table 2), with jaw twitch times being particularly variable among species (Fig. 3A, Table 1).

Raw length–tension relationship data in jaw and leg muscles were relatively consistent across individuals (Fig. 4A). Although there were significant differences in L_{20} values between species, no significant difference was found between L_{20} values in jaw and leg muscles within species (Table 2), indicating that neither the jaw nor the leg muscles generate passive tension at significantly shorter muscle lengths than the other.

Raw force–velocity relationship data were similarly quite consistent across individuals and well represented by the fitted curves (Fig. 5A, Table S3). On average, jaw muscles had a significantly higher V_{max} and a more curved force–velocity
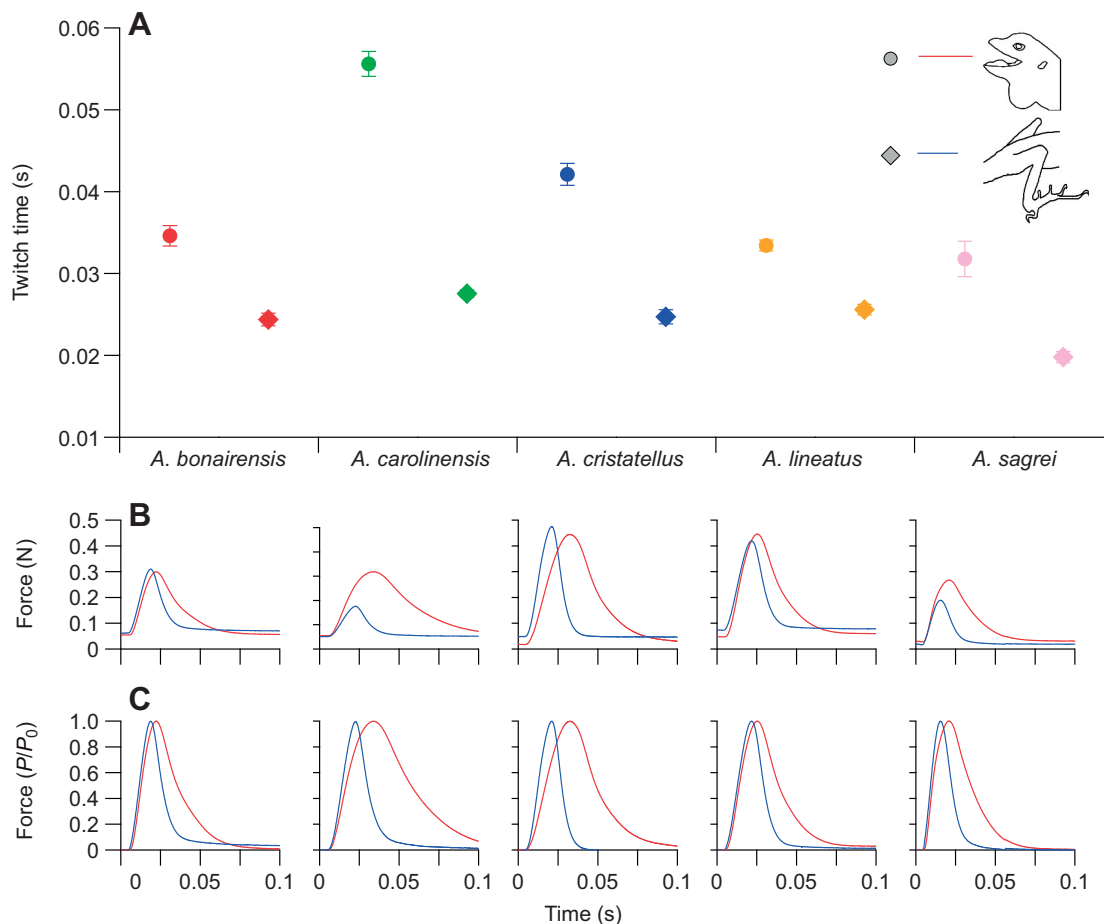

Fig. 3. Comparison of muscle twitch times between jaw and leg muscles. (A) Mean±s.e.m. twitch times for jaw (circles) and leg (diamonds) muscles in five *Anolis* species. (B) Representative twitch traces from jaw (red lines) and leg (blue lines) muscles for each species, corresponding to the species label in A above. Zero time point corresponds with the onset of stimulation. (C) Twitch traces depicted in B normalized by the peak force attained in each respective contraction.

Table 1. Summary of contractile data from jaw and leg muscles of five *Anolis* species

Species	Twitch time (s)	L_{20} ($L\,L_0^{-1}$)	V_{max} ($L_0\,s^{-1}$)	V_{40} ($L_0\,s^{-1}$)	W_{max} ($W\,kg^{-1}$)	Velocity at W_{max} ($V\,V_{max}^{-1}$)	Specific tension (N cm^{-2})	Power ratio
A. bonairensis								
Jaw	0.0346±0.0013 (9)	1.37±0.04 (8)	11.4±1.0 (8)	2.65±0.15 (8)	263±25 (8)	0.31±0.01 (8)	25.1±1.8 (8)	0.10±0.01 (8)
Leg	0.0244±0.0008 (9)	1.28±0.04 (8)	11.3±1.0 (8)	4.44±0.36 (8)	204±48 (8)	0.40±0.02 (8)	13.2±1.5 (8)	0.17±0.02 (8)
A. carolinensis								
Jaw	0.0556±0.0015 (14)	1.28±0.02 (12)	9.2±0.6 (12)	1.97±0.14 (12)	230±19 (12)	0.30±0.01 (12)	30.0±2.5 (12)	0.09±0.01 (12)
Leg	0.0276±0.0004 (13)	1.31±0.02 (12)	6.0±0.5 (12)	2.95±0.17 (12)	199±24 (12)	0.45±0.01 (12)	17.9±1.6 (12)	0.20±0.01 (12)
A. cristatellus								
Jaw	0.0421±0.0013 (15)	1.34±0.04 (8)	12.5±0.9 (8)	3.04±0.32 (8)	205±25 (8)	0.30±0.01 (8)	17.5±1.6 (8)	0.10±0.01 (8)
Leg	0.0247±0.0008 (15)	1.42±0.03 (8)	10.8±0.9 (8)	5.24±0.21 (8)	427±34 (8)	0.40±0.02 (8)	22.6±1.7 (8)	0.17±0.01 (8)
A. lineatus								
Jaw	0.0334±0.0007 (10)	1.37±0.04 (10)	14.2±1.0 (10)	3.38±0.22 (10)	239±20 (10)	0.32±0.01 (10)	18.1±1.1 (10)	0.10±0.01 (10)
Leg	0.0256±0.0006 (10)	1.37±0.03 (9)	11.8±0.7 (9)	4.76±0.37 (9)	219±39 (9)	0.41±0.02 (9)	13.1±1.2 (9)	0.17±0.01 (9)
A. sagrei								
Jaw	0.0318±0.0022 (5)	1.24±0.03 (5)	9.4±1.1 (5)	1.97±2.23 (5)	170±28 (5)	0.30±0.01 (5)	21.5±2.7 (5)	0.09±0.01 (5)
Leg	0.0198±0.0007 (5)	1.26±0.03 (5)	7.7±0.9 (5)	3.29±0.43 (5)	145±36 (5)	0.41±0.01 (5)	14.2±1.9 (5)	0.17±0.01 (5)

All values are presented as means±s.e.m. (*n*).
L_{20}, length at which passive force reaches 20% of P_0; L_0, muscle length at P_0; V_{max}, peak contractile velocity; V_{40}, contractile velocity at 40% of P_0; W_{max}, peak power; P_0, peak isometric force.
The total number of individuals (muscles) from which data were used for each variable in each species is presented as *n*.

relationship, represented by a lower power ratio, than leg muscles (Figs 5B and 6A,E, Tables 1 and 2). Associated with the more curved relationship was a lower V_{40} (Fig. 6B) in the jaw muscles. The mass-specific power of jaw muscles was maximized at a lower normalized velocity than in leg muscles (Fig. 5B), although on average W_{max} values did not differ in magnitude across muscle type (Figs 5B and 6C, Tables 1 and 2). Additionally, specific tensions for peak isometric contractions were significantly higher for jaw muscles than for leg muscles (Fig. 6A, Tables 1 and 2).

DISCUSSION

The results of the present study show that the jaw and leg muscles examined in *Anolis* lizards differ in various aspects of their muscle contractile physiology. Given the disparate functional roles these muscles play in the day-to-day life of these animals and the importance of optimizing performance in survival and fitness (Arnold, 1983; Bennett and Huey, 1990), muscle contractile properties may specialize to better perform their contrasting functions. The observed variation in muscle contractile physiology measurements in these jaw and leg muscles may therefore provide insight into what functional traits of muscle performance are important to how these different muscles are used.

We found, for instance, that although this *Anolis* leg muscle exhibits muscle twitch times that are 1.3 to 2.0 times faster than the jaw muscle of the same species and faster contractile velocities at

Table 2. Results of a three-factor nested random effects model examining effects on muscle contractile variables from jaw and leg muscles of five *Anolis* species

Variable	Species *P*-value	Muscle *P*-value
Twitch time	**<0.0001**	**<0.0001**
L_{20}	**0.0006**	0.5461
V_{max}	**<0.0001**	**0.0022**
V_{40}	**<0.0001**	**<0.0001**
W_{max}	**0.0079**	0.6306
Specific tension	**0.0015**	**<0.0001**
Power ratio	0.6524	**<0.0001**

Bold *P*-values indicate significance after adjustment to correct for false discovery rate (Benjamini and Hochberg, 1995).

40% of peak force, their peak contractile velocity is slower. This apparent discrepancy in patterns of specialization for increased muscle speed may be related to the specific functional role these performance parameters play in producing movement. Leg muscle, for instance, may naturally be expected to be faster than jaw muscles owing to their contribution to producing the rapid cyclical motions associated with sprinting. Rapid cyclical movements, however, likely rely not only on rapid contraction and force development, but also on the rapid dissipation of that tension so as not to inhibit antagonist muscles involved in the alternating stance and swing phases of sprinting. Jaw muscles, in contrast, generally contribute to more episodic movements such as chewing and biting behaviors, and may benefit from rapid length changes to speed the interaction between the jaws and their prey or adversary. Similarly, muscles associated with biting and chewing may not rely as much on rapid dissipation of forces but rather on sustained tension. Different measures of muscle speed have similarly been shown to exhibit dissimilar patterns of variation between muscles used to power actions of disparate speed. Male katydids, for example, have co-opted one of their two pairs of flight muscles to produce the rapid stridulations responsible for their singing or calling behavior (Josephson and Halverson, 1971). These muscles have been shown to have faster muscle twitch times than the flight muscle pair not co-opted for this use in males, which shows similar muscle twitch times to the flight muscles of females that do not call (Josephson, 1973). Despite these faster twitch times in their stridulatory muscles, peak contractile velocities are similar between stridulatory and flight muscle pairs in katydids (Josephson, 1984). This suggests that different measures of muscle speed are commonly capable of independently specializing to the functional demands.

The differences in curvature of the force–velocity relationship between these limb and jaw muscles illustrate how the maximum speed of shortening is not the only determinant of the speed of muscle shortening during movement. For instance, we found that although this jaw muscle has higher peak contractile velocities than the leg muscle, the increased curvature of their force–velocity relationship results in their contractile velocity at 40% peak force being lower than that of the leg muscle (Figs 5 and 6, Table 1). Although higher peak contractile velocities may benefit jaw
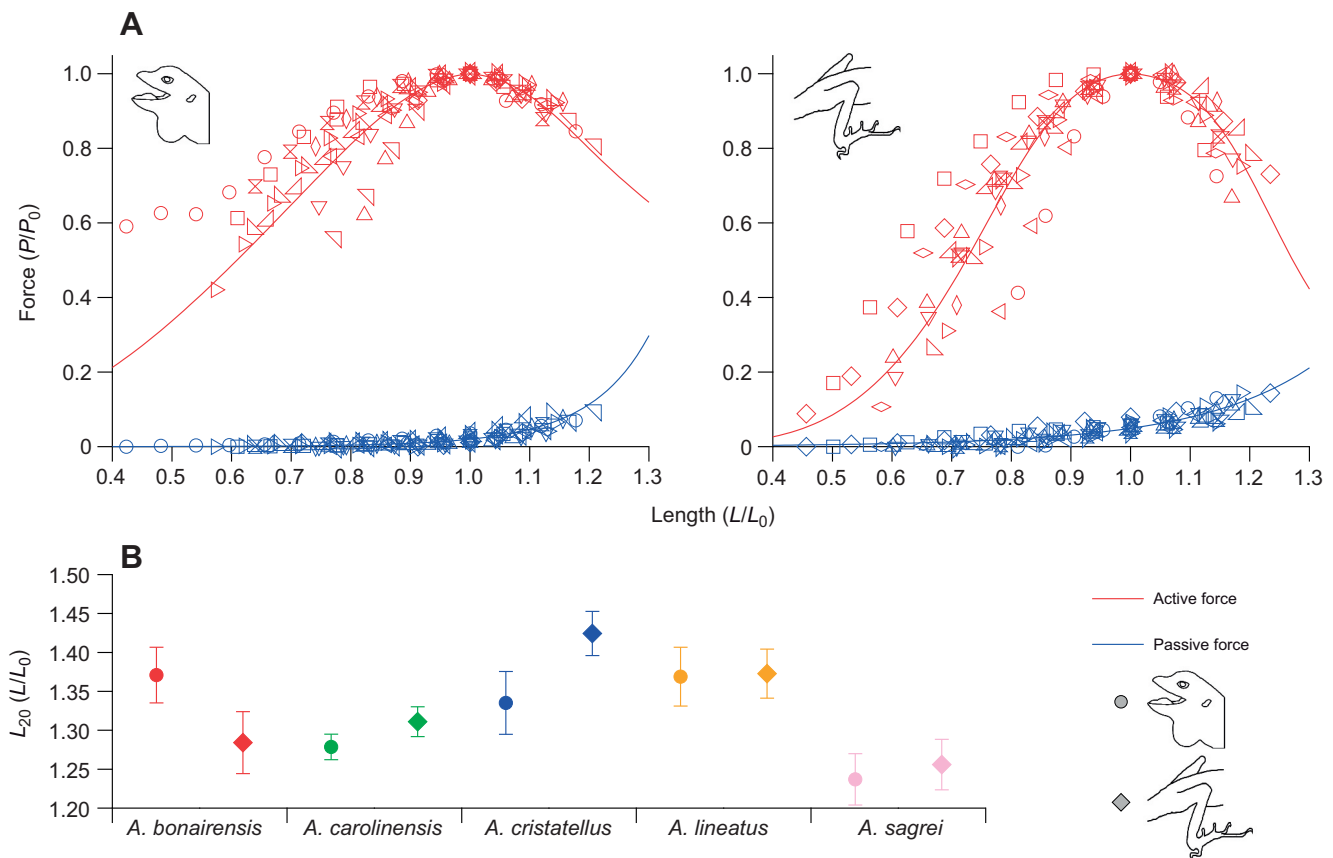
Fig. 4. Comparisons of length–tension relationships between jaw and leg muscles. (A) Average active and passive force curves (red and blue solid lines, respectively) for the jaw (left) and leg (right) muscles of a single *Anolis* species constructed using the averaged coefficients from fitted curves for each individual preparation (raw data for each individual represented by different symbols). Force values are normalized to P_0 and length values are normalized to L_0. (B) Mean±s.e.m. L_{20} values (muscle length normalized to L_0 where passive tension corresponds to 20% of P_0 for jaw (circles) and leg (diamonds) muscles in five *Anolis* species.

muscles operating under low loads, such as by imparting more rapid length changes to speed the interaction between the jaws and their prey during gape closure, leg muscles may benefit from increased contractile velocities at intermediate loads. Higher top sprint speeds in humans, for instance, are known to be achieved by increasing the forces imparted to the ground rather than the speed at which the limbs are cycled during the alternating stance and swing phases of sprinting (Weyand et al., 2000). At intermediate contractile velocities, leg muscles in *Anolis* are able to impart relatively larger forces than jaw muscles, potentially as a mechanism favoring sprint speed. Further, the jaw and leg muscles of *Anolis* produce similar peak power output values, but because of their differing force–velocity relationship curvatures, this power is maximized at different relative contractile velocities. Muscles with a greater curvature of their force–velocity relationship have lower power ratio values and, as a result, their power output is maximized at proportionately lower contractile velocities (Marsh and Bennett, 1986). With a lower power ratio, and thus a more curved force–velocity curve, jaw muscles maximize their power at 30–32% of their peak contractile velocity, whereas leg muscles maximize their power at 40–45% of their peak contractile velocity (Figs 5 and 6, Table 1). This also means that leg muscles maximize their power and are able to contract more rapidly at higher relative forces than jaw muscles (Fig. 5).

In contrast, these jaw and leg muscles do not necessarily differ in all their muscle contractile properties. We found, for instance, that

neither the jaw nor the leg muscles of *Anolis* lizards generate passive tensions at shorter muscle lengths than the other. Variation in passive tension among muscles is common (Brown et al., 1996; Azizi and Roberts, 2010), and it has been proposed that this variation relates to mechanical demand (Azizi, 2014). Although hindlimb muscles in toads, for example, are responsible for powering aerial hopping behaviors, their forelimbs are responsible for controlling their landing. A forelimb muscle involved in landing generates passive tension at shorter lengths when compared with a hindlimb muscle involved in jumping, suggesting a shift in passive tension (lower L_{20} values) that may reduce the risk of damage during eccentric loading (Azizi, 2014). We expected that jaw muscles might have higher L_{20} values than limb muscles, owing to the reduced chance of eccentric loading in jaw muscles. However, without data on muscle length changes during movement, it is difficult to know whether significant differences in eccentric loading exist between the jaw and limb muscles studied here.

Variation in muscle speed is often associated with differences in muscle fiber type composition. Muscle fiber types differ in their myosin isoforms and are known to exhibit different contractile properties as a result. For example, Type I (slow oxidative) muscle fibers are known to develop slower peak contractile velocities than Type II fibers, with Type IIB (fast glycolytic) fibers having higher peak contractile velocities than Type IIA (fast oxidative-glycolytic) fibers (Larsson and Moss, 1993). This pattern is associated with higher myofibrillar ATPase activity in Type IIB fibers and low
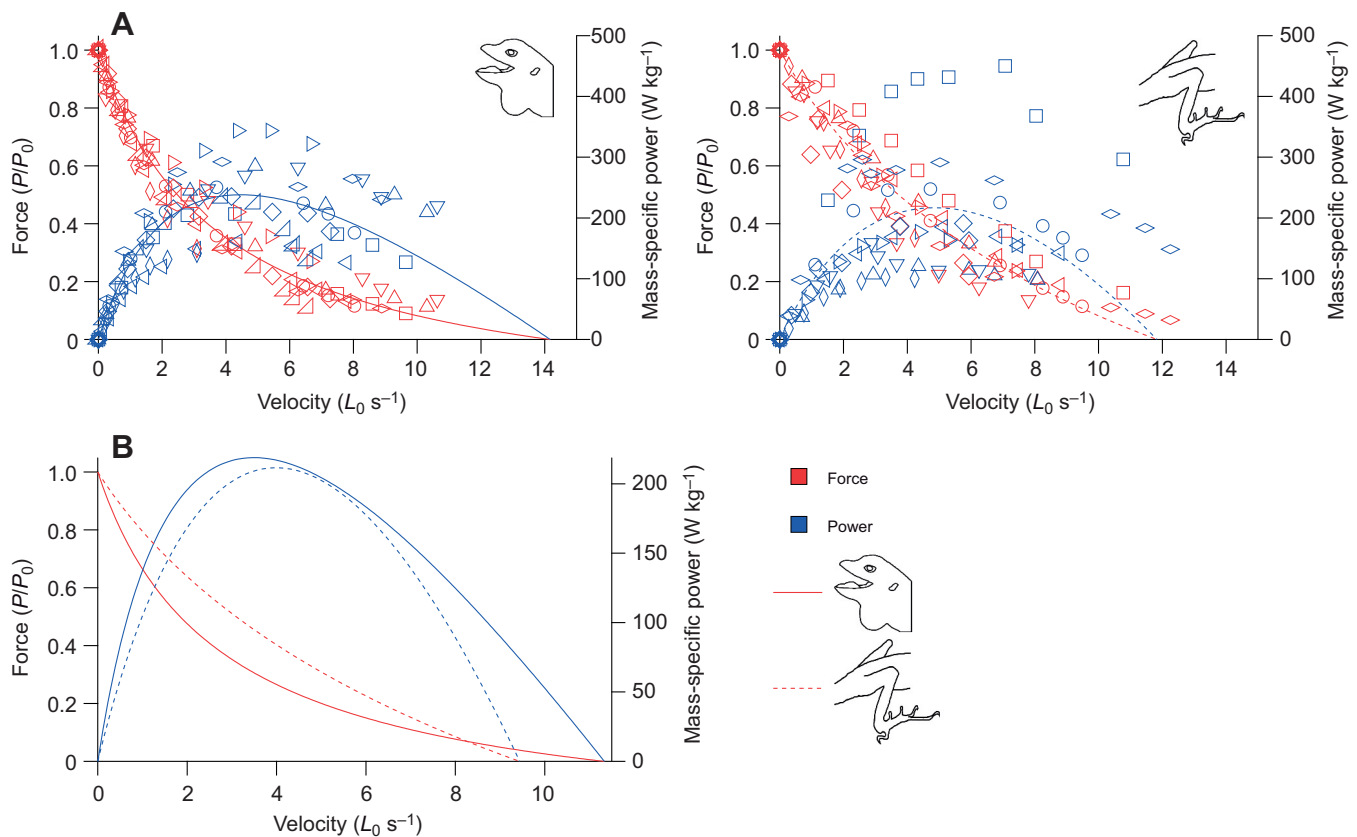
Fig. 5. Comparisons of force–velocity and power relationships between jaw and leg muscles. (A) Average force and power curves (red and blue lines, respectively) for the jaw (left, solid lines) and leg (right, dashed lines) muscles of a single *Anolis* species constructed using the averaged coefficients from fitted curves for each individual preparation (raw data for each individual represented by different symbols). Force values are normalized to P_0 and velocity values normalized L_0. (B) Average force (red) and power (blue) curves for the jaw (solid) and leg (dashed) muscles of five *Anolis* species constructed using averaged coefficients from average fitted curves for each species.

myofibrillar ATPase activity in Type I fibers (Stienen et al., 1996), as increasing myofibrillar ATPase activity is correlated with faster muscle shortening speeds (Bárány, 1967). Twitch kinetics also generally vary across fiber type, with Type II fibers having faster relaxation times and shorter twitch durations (Close, 1972; Schiaffino and Reggiani, 2011). A similar pattern is known for parvalbumin concentrations and relaxation speed, with Type I fibers having lower parvalbumin concentrations and relaxation speeds than Type II fibers, and Type IIB fibers having higher parvalbumin concentrations and relaxation speeds than Type IIA fibers (Celio and Heizmann, 1982; Heizmann et al., 1982). These patterns result in a close correlation between twitch kinetics and peak contractile velocity across fiber types, with Type II fibers generally having both shorter (faster) twitch parameters and faster peak contractile velocities (Reggiani and te Kronnie, 2006; Schiaffino and Reggiani, 2011). Additionally, different muscle fiber types are also known to differ in the curvature of their force–velocity relationships, with Type I fibers having been found to have the most curved force–velocity relationship and Type IIB fibers tending to have a flatter relationship (Bottinelli et al., 1991; Schiaffino and Reggiani, 2011). Further, Type I fibers tend to maximize power at lower contractile velocities than Type II fibers, with Type IIB fibers maximizing power at higher contractile velocities than Type IIA fibers (Bottinelli et al., 1996; Gilliver et al., 2009; Schiaffino and Reggiani, 2011). Finally, Type II fibers typically have greater peak isometric tension than Type I fibers (Linari et al., 2004; Schiaffino and Reggiani, 2011). Variation in the types and relative

concentrations of fibers within individual muscles can therefore produce shifts in the muscle contractile properties at the whole muscle level (Larsson and Moss, 1993; Heizmann et al., 1982). These general patterns, however, suggest that Type IIB fibers would tend to have faster peak contractile velocities, faster muscle twitch times, a flatter force–velocity relationship, higher contractile velocities at maximum power and greater peak isometric tension than Type I fibers. However, with *Anolis* jaw muscles expressing faster peak contractile velocities and greater peak isometric tension, and leg muscles expressing faster twitch times, a flatter force–velocity relationship and higher contractile velocities at maximum power, each muscle appears to show characteristics that might be associated with high Type IIB fiber concentrations. This could be driven by differences in fiber type concentrations or, alternatively, by differences in the respective sizes of different fiber types in these muscles. The m. ambiens in the lizard *Tropidurus psammonastes*, for instance, is composed of over 60% Type IIB fibers, approximately 30% Type IIA fibers and less than 10% Type I fibers (Pereira et al., 2015). Further, following either sprint or endurance training, specific muscle fiber types in the mixed fiber type muscle of *A. carolinensis* have been shown to increase in size to meet the demands of their training type (Husak et al., 2015). Variation in either fiber type concentrations or the size of specific fiber types between these muscles may therefore provide a mix in muscle contractile property features.

These patterns suggest that differences in fiber type concentrations may not be the sole explanation for the observed differences between
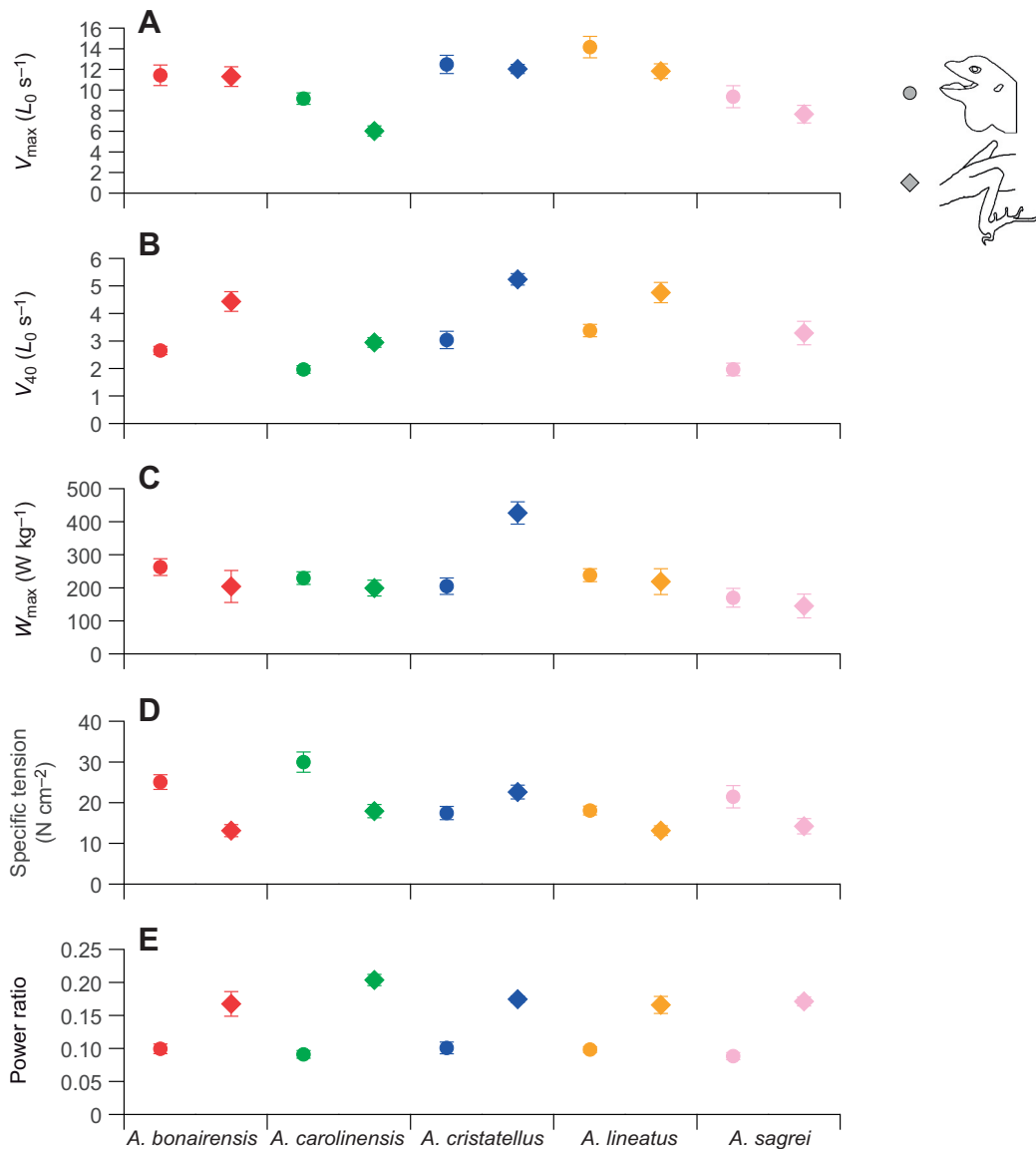
Fig. 6. Comparison of performance values extracted from force–velocity and power relationships for jaw (circles) and leg (diamonds) muscles in five
Anolis **species.** Comparisons are presented for (A) peak contractile velocity (V_{max}) normalized to L_0, (B) contractile velocity at 40% peak contractile force (V_{40}) normalized to L_0, (C) peak power (W_{max}), (D) specific tension and (E) power ratio.

jaw and leg muscles. These conflicting speed records may be driven by variation in contractile properties within individual fiber types or by variation in other muscular properties. Parvalbumin concentrations and their associated muscle relaxation rates, for instance, are known to vary within the same histochemical fiber type (Celio and Heizmann, 1982), while sarcoplasmic reticulum (SR)-Ca^{2+}-ATPase (SERCA) pump isoform makeup within fiber types, and the resultant affinity of Ca^{2+} uptake, differs across species (Lamboley et al., 2014). Therefore, variation in muscular properties related to calcium cycling frequency, such as variation in parvalbumin concentrations or SERCA pumps, or alterations of troponin affecting off-rates of Ca^{2+} from troponin C (e.g. Rome, 2006), could also produce increased twitch times.

Conclusions

Whereas some contractile properties of the jaw and limb muscles examined in *Anolis* lizards do not vary, we found that others show consistent patterns of variation across species. In particular, the jaw

muscles have faster peak contractile velocities, and slower muscle twitch times and contractile velocities under intermediate loads, and are able to maximize their power output at lower relative contractile velocities and tensions than the leg muscles. This suggests that both muscles appear to be built for speed, but in different ways. Leg muscles have an increased ability to produce very rapid force generation and then dissipate that force, as well as an increased ability to exert force under intermediate contractile velocities, which may be associated with the cyclical movements and ground reaction forces of sprinting. Jaw muscles, in contrast, have higher peak contractile velocities, allowing for rapid shortening under low loads, perhaps enabling them to quickly engage prey items. The exact mechanism by which these contractile properties vary is unknown; however, these variations in muscle contractile physiology within individuals may be promoted by the disparate functional demands of each muscle. Examining and understanding a muscle's contractile properties can thus help illuminate important features relating to the task at hand that they power.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/doi/10.1242/jeb.213397.supplemental

References
Aerts, P., Van Damme, R., Vanhooydonck, B., Zaaf, A. and Herrel, A. (2000). Lizard locomotion: how morphology meets ecology. *Neth. J. Zool.* **50**, 261-277. doi:10.1163/156854200505865

Arnold, S. J. (1983). Morphology, performance and fitness. *Am. Zool.* **23**, 347-361. doi:10.1093/icb/23.2.347

Azizi, E. (2014). Locomotor function shapes the passive mechanical properties and operating lengths of muscle. *Proc. R. Soc. B* **281**, 20132914. doi:10.1098/rspb.2013.2914

Azizi, E. and Roberts, T. J. (2010). Muscle performance during frog jumping: influence of elasticity on muscle operating lengths. *Proc. R. Soc. B* **277**, 1523-1530. doi:10.1098/rspb.2009.2051

Bárány, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. Gen. Physiol.* **50**, 197-216. doi:10.1085/jgp.50.6.197

Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**, 289-300. doi:10.1111/j.2517-6161.1995.tb02031.x

Bennett, A. F. and Huey, R. B. (1990). Studying the evolution of physiological performance. *Oxford Surveys Evol. Biol.* **7**, 251-284.

Bottinelli, R., Schiaffino, S. and Reggiani, C. (1991). Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle. *J. Physiol.* **437**, 655-672. doi:10.1113/jphysiol.1991.sp018617

Bottinelli, R., Canepari, M., Pellegrino, M. A. and Reggiani, C. (1996). Force-velocity properties of human skeletal muscle fibres: myosin heavy chain isoform and temperature dependence. *J. Physiol.* **495**, 573-586. doi:10.1113/jphysiol.1996.sp021617

Brown, I. E., Liinamaa, T. L. and Loeb, G. E. (1996). Relationships between range of motion, L_0, and passive force in five strap-like muscles of the feline hindlimb. *J. Morphol.* **230**, 69-77. doi:10.1002/(SICI)1097-4687(199610)230:1<69::AID-JMOR6>3.0.CO;2-I

Celio, M. R. and Heizmann, C. W. (1982). Calcium-binding protein parvalbumin is associated with fast contracting muscle fibres. *Nature* **297**, 504-506. doi:10.1038/297504a0

Close, R. I. (1972). Dynamic properties of mammalian skeletal muscles. *Physiol. Rev.* **52**, 129-197. doi:10.1152/physrev.1972.52.1.129

Dickinson, M. H., Farley, C. T., Full, R. J., Koehl, M. A. R., Kram, R. and Lehman, S. (2000). How animals move: an integrative view. *Science* **288**, 100-106. doi:10.1126/science.288.5463.100

Garland, T., Jr (1994). Phylogenetic analyses of lizard endurance capacity in relation to body size and body temperature. In *Lizard Ecology. Historical and Experimental Perspectives* (ed. L. J. Vitt and E. R. Pianka), pp. 237-259. Princeton: Princeton University Press.

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. M. Reilly), pp. 240-302. Chicago: The University of Chicago Press.

Gilliver, S. F., Degens, H., Rittweger, J., Sargeant, A. J. and Jones, D. A. (2009). Variation in the determinants of power of chemically skinned human muscle fibres. *Exp. Physiol.* **94**, 1070-1078. doi:10.1113/expphysiol.2009.048314

Heizmann, C. W., Berchtold, M. W. and Rowlerson, A. M. (1982). Correlation of parvalbumin concentration with relaxation speed in mammalian muscles. *Proc. Natl. Acad. Sci. USA* **79**, 7243-7247. doi:10.1073/pnas.79.23.7243

Herrel, A., Vanhooydonck, B., Porck, J. and Irschick, D. J. (2008). Anatomical basis of differences in locomotor behaviour in *Anolis* lizards: a comparison between two ecomorphs. *Bull. Mus. Comp. Zool. Harvard Univ.* **159**, 213-238. doi:10.3099/0027-4100-159.4.213

Hertz, P. E., Arima, Y., Harrison, A., Huey, R. B., Losos, J. B. and Glor, R. E. (2013). Asynchronous evolution of physiology and morphology in *Anolis* lizards. *Evolution* **67**, 2101-2113. doi:10.1111/evo.12072

Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. B* **126**, 136-195. doi:10.1098/rspb.1938.0050

Husak, J. F., Keith, A. R. and Wittry, B. N. (2015). Making Olympic lizards: the effects of specialized exercise training on performance. *J. Exp. Biol.* **218**, 899-906. doi:10.1242/jeb.114975

Josephson, R. K. (1973). Contraction kinetics of the fast muscles used in singing by a katydid. *J. Exp. Biol.* **59**, 781-801.

Josephson, R. K. (1984). Contraction dynamics of flight and stridulatory muscles of tettigoniid insects. *J. Exp. Biol.* **108**, 77-96.

Josephson, R. K. and Halverson, R. C. (1971). High frequency muscles used in sound production by a katydid. I. Organization of the motor system. *Biol. Bull. Mar. Boil. Lab., Woods Hole* **141**, 411-433. doi:10.2307/1540258

Katz, B. (1939). The relation between force and speed in muscular contraction. *J. Physiol. Lond.* **96**, 45-64. doi:10.1113/jphysiol.1939.sp003756

Lailvaux, S. P. and Irschick, D. J. (2007). The evolution of performance-based male fighting ability in Caribbean *Anolis* lizards. *Am. Nat.* **170**, 573-586. doi:10.1086/521234

Lailvaux, S. P., Herrel, A., VanHooydonck, B., Meyers, J. J. and Irschick, D. J. (2004). Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proc. R. Soc. Lond. B* **271**, 2501-2508. doi:10.1098/rspb.2004.2891

Lamboley, C. R., Murphy, R. M., KcKenna, M. J. and Lamb, G. D. (2014). Sarcoplasmic reticulum Ca^{2+} uptake and leak properties, and SERCA isoform expression, in type I and type II fibres of human skeletal muscle. *J. Physiol. Lond.* **592**, 1381-1395. doi:10.1113/jphysiol.2013.269373

Larsson, L. and Moss, R. L. (1993). Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. *J. Physiol. Lond.* **472**, 595-614. doi:10.1113/jphysiol.1993.sp019964

Linari, M., Bottinelli, R., Pellegrino, M. A., Reconditi, M., Reggiani, C. and Lombardi, V. (2004). The mechanism of the force response to stretch in human skinned muscle fibres with different myosin isoforms. *J. Physiol.* **554**, 335-352. doi:10.1113/jphysiol.2003.051748

Losos, J. B. (1990). The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. *Evolution* **44**, 1189-1203. doi:10.1111/j.1558-5646.1990.tb05225.x

Marsh, R. L. (1988). Ontogenesis of contractile properties of skeletal muscle and sprint performance in the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **137**, 119-139.

Marsh, R. L. and Bennett, A. F. (1985). Thermal dependence of isotonic contractile properties of skeletal muscle and sprint performance of the lizard *Dipsosaurus dorsalis*. *J. Comp. Physiol. B* **155**, 541-551. doi:10.1007/BF00694443

Marsh, R. L. and Bennett, A. F. (1986). Thermal dependence of contractile properties of skeletal muscle from the lizard *Sceloporus occidentalis* with comments on methods for fitting and comparing force-velocity curves. *J. Exp. Biol.* **126**, 63-77.

Meyers, J. J., Herrel, A. and Birch, J. (2002). Scaling of morphology, bite force and feeding kinematics in an iguanian and a scleroglossan lizard. In *Topics in Functional and Ecological Vertebrate Morphology* (ed. P. Aerts, K. D'Août, A. Herrel and R. Van Damme), pp. 47-62. Maastricht: Shaker Publishing.

Otten, E. (1987). A myocybernetic model of the jaw system of the rat. *J. Neurosci. Methods* **21**, 287-302. doi:10.1016/0165-0270(87)90123-3

Pereira, A. G., Abdala, V. and Kohlsdorf, T. (2015). Function and position determine relative proportions of different fiber types in limb muscles of the lizard *Tropidurus psammonastes*. *Zoology* **118**, 27-33. doi:10.1016/j.zool.2014.09.001

Reggiani, C. and te Kronnie, T. (2006). RyR isoforms and fibre type-specific expression of proteins controlling intracellular calcium concentration in skeletal muscles. *J. Muscle Res. Cell. Motil.* **27**, 327-335. doi:10.1007/s10974-006-9076-3

Rome, L. C. (2006). Design and function of superfast muscles: new insights into the physiology of skeletal muscle. *Annu. Rev. Physiol.* **68**, 193-221. doi:10.1146/annurev.physiol.68.040104.105418

Rummel, A. D., Swartz, S. M. and Marsh, R. L. (2018). Low thermal dependence of the contractile properties of a wing muscle in the bat *Carollia perspicillata*. *J. Exp. Biol.* **221**, jeb180166. doi:10.1242/jeb.180166

Schiaffino, S. and Reggiani, C. (2011). Fiber types in mammalian skeletal muscles. *Physiol. Rev.* **91**, 1447-1531. doi:10.1152/physrev.00031.2010

Stienen, G. J. M., Kiers, J. L., Bottinelli, R. and Reggiani, C. (1996). Myofibrillar ATPase activity in skinned human skeletal muscle fibres: fibre type and temperature dependence. *J. Physiol.* **493**, 299-307. doi:10.1113/jphysiol.1996.sp021384

Weyand, P. G., Sternlight, D. B., Bellizzi, M. J. and Wright, S. (2000). Faster top running speeds are achieved with greater ground forces not more rapid leg movements. *J. Appl. Physiol.* **89**, 1991-1999. doi:10.1152/jappl.2000.89.5.1991

Wittorski, A., Losos, J. B. and Herrel, A. (2016). Proximate determinants of bite force in *Anolis* lizards. *J. Anat.* **228**, 85-95. doi:10.1111/joa.12394