

RESEARCH ARTICLE

Larger lacertid lizard species produce higher than expected iliotibialis muscle power output: the evolution of muscle contractile mechanics with body size

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ABSTRACT

Increases in body size can lead to alterations in morphology, physiology, locomotor performance and behaviour of animals. Most studies considering the effects of scaling on muscle performance have studied within-species effects, with few studies considering differences between species. A previous review of published data indicates that maximum muscle-shortening velocity decreases, but that maximum isometric stress does not change, with increased body mass across species of terrestrial animals. However, such previous analyses do not account for the phylogenetic relatedness of the species studied. Our aim was to use phylogenetically informed analysis to determine the effects of body size on isolated iliotibialis muscle performance across 17 species of lacertid lizards. Between one and five individuals were used to obtain mean performance values for each species. We analysed the relationship between each variable and body size, as estimated by snout–vent length (SVL), whilst taking into account the phylogenetic relationships between species. We found that isometric tetanus relaxation time, maximal tetanus stress (force per muscle cross-sectional area) and maximal work loop power output (normalised to muscle mass) all significantly increased with greater SVL. In contrast, fatigue resistance during repeated work loops significantly decreased with SVL and there was no effect of size on tetanus activation time. When we compare our findings with those that would be predicted by dynamic similarity, then as these lacertid species become bigger, there is a greater than expected increase in the normalised muscle power output, probably to counter the larger than expected increase in body mass.

KEY WORDS: Force, Power output, Scaling, Work loop

INTRODUCTION

Increases in body size can alter the functional and physiological demands placed upon animals which can result in changes in morphology, physiology and locomotor performance. Hill (1950) discussed such principles, outlining that animals of similar shape, made of similar materials, should be able to move at comparable speeds and jump similar heights, regardless of differences in body size. These principles of dynamic similarity were further developed with usage of Froude numbers (accounting for gravity, leg length and velocity) to demonstrate that many animals of different size move in a functionally equivalent way

(Alexander and Jayes, 1983). Hill (1950) also argued that as animals get larger their muscles become slower, yet the maximal force produced per cross-sectional area (stress) of the muscle remains virtually the same. If we assume that terrestrial animals have similar density and are subject to the same gravitational forces, then we can use the concept of dynamic similarity to estimate the likely changes in the mechanical properties of skeletal muscle that would occur with change in body length. According to dynamic similarity, body mass should scale as body length to the power of 3, muscle stress (maximal force divided by muscle cross-sectional area) to the power of 1, muscle tetanus activation and relaxation times to the power of 0.5, muscle power output (normalised to muscle mass) to the power of 0.5, whereas muscle fatigue resistance should be independent of body length.

Numerous studies have demonstrated that many measures of skeletal muscle performance differ across a body size range within species, with larger individuals having relatively slower muscle properties such as slower rates of isometric force generation and relaxation, lower length specific maximal shortening velocities and lower optimal cycle frequencies for work loop power output (Marsh, 1988; Bennett et al., 1989; Altringham and Johnston, 1990; Johnson et al., 1993; Altringham et al., 1996; James et al., 1998). In contrast, the force produced per cross-sectional area and the power output per unit muscle mass tends to be relatively constant across a body size range within a species (Marsh, 1988; Bennett et al., 1989; Johnson et al., 1993; Altringham et al., 1996; James et al., 1998).

There has been comparatively limited consideration of the changes in skeletal muscle mechanics between species of differing body size. A previous study found that the maximal shortening velocity of single muscle fibres decreased with increased body mass (M_b), from rat to rabbit to horse, scaling $M_b^{-0.18}$ in slow fibres and $M_b^{-0.07}$ in fast glycolytic fibres (Rome et al., 1990). Various scaling exponents have been found in other more recent studies comparing single fibres of the same fibre type between various mammalian species (Seow and Ford, 1991; Widrick et al., 1997; Pellegrino et al., 2003; Marx et al., 2006), with much of the variation in slope thought to be due to the species included in the study (Marx et al., 2006). A comprehensive review of published data demonstrated that maximal shortening velocity decreased with increased body mass, scaling across species $M_b^{-0.25}$, whereas maximal isometric stress showed no significant relationship with body mass (Medler, 2002). Indeed 85% of the between-species (terrestrial and flying animals) variation in maximal muscle shortening velocity could be explained by variation in body mass, once experimental temperature had been accounted for (Medler, 2002). However, such previous comparisons have compared very different species and have not accounted for phylogenetic relatedness of species in their analysis of variation in muscle mechanics between species. Moreover, a broad comparative study

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Table 1. Details of the 17 lacertid lizard species studied

Species	Taxonomic authority	N_{sex}			N_{age}		SVL (mm)	Body mass (g)
		Male	Female	Unknown	Adult	Juvenile		
<i>Acanthodactylus</i> sp. ^a		1	3	–	4	–	53.6	3.78
<i>Lacerta agilis</i> ^b	Linnaeus 1758	1	2	–	3	–	73.7	10.2
<i>Psammmodromus algirus</i> ^c	Linnaeus 1758	1	1	–	2	–	69.9	8.34
<i>Australolacerta australis</i> ^d	Hewitt 1926	2	1	–	3	–	68.1	8.10
<i>Eremias acutirostris</i> ^a	Boulenger 1887	5	1	–	6	–	74.2	10.5
<i>Podarcis hispanica</i> ^c	Steindachner 1870	2	–	–	2	–	41.6	1.14
<i>Psammmodromus hispanicus</i> ^c	Fitzinger 1826	–	2	–	2	–	45.9	1.41
<i>Meroles knoxii</i> ^d	Milne-Edwards 1829	1	4	–	5	–	48.8	2.63
<i>Lacerta lepida</i> ^c	Daudin 1802	–	–	1	–	1	61.5	9.21
<i>Pedioplanis lineoocellata</i> ^d	Fitzinger 1843	2	2	–	4	–	50.5	2.82
<i>Latastia longicaudata</i> ^a	Reuss 1834	4	–	–	4	–	69.9	6.38
<i>Podarcis melisellensis</i> ^e	Braun 1877	–	1	–	1	–	53.7	2.72
<i>Podarcis muralis</i> ^{b,c}	Laurenti 1768	5	–	–	5	–	59.7	4.52
<i>Podarcis sicula</i> ^b	Rafinesque 1810	2	2	–	4	–	68.8	7.90
<i>Takydromus sexlineatus</i> ^a	Daudin 1802	3	2	–	5	–	56.5	3.70
<i>Nucras tessellata</i> ^d	Smith 1838	1	–	–	1	–	61.3	4.63
<i>Lacerta bilineata</i> ^{b,c}	Daudin 1802	1	–	2	2	1	84.3	18.5

Origins of lizards are as follows: ^apet trade; ^bCroatia; ^cFrance; ^dSouth Africa; ^elaboratory bred.

of closely related species would be of interest to tease apart effects of phylogeny versus size on skeletal muscle mechanics.

The aim of the present study was to compare skeletal muscle performance between species of lacertid lizards, using the known phylogenetic relationships of those species to correct for the fact that, as they share common ancestors, they are not truly independent data points. Therefore, we compare lizards from the same family that have similar life histories. We believe that this is the first such phylogenetic analysis of differences in muscle mechanics between species allowing us to investigate the co-evolution of skeletal muscle mechanics with size in these animals. We also compare the scaling relationships observed with those predicted by the concept of dynamic similarity.

MATERIALS AND METHODS

Animals

Individuals of 17 lacertid species were either lab bred, wild caught or bought via the pet trade (Table 1). Once each individual was brought into the laboratory, we measured snout–vent length (SVL) to the nearest 0.01 mm, using digital callipers (CD-15DC, Mitutoyo, Andover, UK). Details on place of origin, sample size per species, sex, age class and average SVL per species are given in Table 1.

Lizards were housed in a room with a light:dark cycle of 10 h:14 h and a temperature of 28°C. *L. bilineata*, *L. lepida*, and *P. algirus* were kept individually in terraria of 0.6×0.6 m. Other species were maintained with up to five individuals in each cage of 0.5×1 m. Each cage was fitted with a 75 W or 100 W light bulb, to deliver extra light and heat for 10 h day⁻¹. Rocks, branches and leaf litter were scattered on the base of each cage to offer opportunities to bask and hide. Lizards were fed crickets (*Achetus domesticus*) dusted with calcium and vitamin supplements three times per week. Each cage was sprayed twice weekly with water and water was available in water bowls for individuals to drink *ad libitum*.

Muscle mechanics

Each animal was killed by decapitation and transection of the spinal cord in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Each individual was then immediately transferred to a bath containing refrigerated (1–3°C), oxygenated (95% O₂; 5% CO₂) lizard Ringer solution (Johnson et al., 1993) and iliotibialis, a knee extensor, was isolated from the left hind-limb. A few muscle fibres were removed to ensure that the muscle fibres in the resultant muscle preparation had a low angle of pennation. Bone and tendon was left intact at the origin and the insertion of each muscle.

For each muscle preparation, the bone at one end was clamped using crocodile clips to a calibrated load cell (UF1, Pioden Controls Ltd, Canterbury, UK) and the bone at the other end was clamped to a motor arm (V201, Ling Dynamics Systems, Royston, UK) attached to a calibrated linear variable displacement transducer (DFG 5.0, Solartron Metrology, Bognor Regis, UK). Each muscle was kept at constant length in circulating oxygenated lizard Ringer solution maintained at 34.0±0.5°C. A series of twitches was generated in response to square-wave stimuli, of 2.0 ms pulse width and 150 mA, delivered via parallel platinum electrodes. Muscle length and/or stimulus voltage were adjusted after each twitch response until maximal isometric twitch force was achieved. The muscle length that yielded maximal twitch force (L_0) was measured to the nearest 0.1 mm using a dissecting microscope fitted with an eyepiece graticule. An isometric tetanic force response was generated by delivery of a 200 ms train of stimulation, with an individual pulse width of 2 ms, using the muscle length and stimulation voltage that elicited maximal twitch height. Maximal tetanic force was determined by changing stimulation frequency, within the range of 180–280 Hz, after each tetanic response, whilst allowing a 5 min rest period after each tetanus. Measurements of time to half peak tetanus force (THPT; time from stimulus to half maximal tetanic force) and time from last stimulus to half tetanus force relaxation (LSHR) were made using a digital storage oscilloscope for the maximal tetanus for each individual.

The work loop technique was used to determine the power output of muscles during cyclical length changes (Josephson, 1993). Each muscle preparation was subjected to a set of four sinusoidal length changes, starting from L_0 , and the stimulation frequency and voltage found to elicit maximal isometric force. Length changes and electrical stimulation were controlled

Table 2. Phylogenetic analyses of the relationships between independent contrasts for body length data and independent contrasts for the mechanical properties of lacertid iliotibialis muscle

	Adjusted r^2	Slope	CI	F	P
M_b	0.95	3.64	3.16 to 4.13	256	<0.0001
THPT	0.21	0.465	–0.028 to 0.96	4.05	0.063
LSHR	0.39	0.710	0.22 to 1.20	9.69	0.007
Stress	0.29	1.11	0.16 to 2.07	6.13	0.026
NPO	0.37	1.41	0.39 to 2.42	8.73	0.01
FR	0.57	–0.466	–0.69 to –0.24	19.9	<0.0001

CI, 95% confidence limits; M_b , body mass; THPT, time to half peak tetanus force; LSHR, time from last stimulus to half peak tetanus force relaxation; Stress, maximal isometric stress; NPO, maximal mass-specific muscle power output; FR, fatigue resistance after 12 work loops. $N=17$ species in each case. i.e. 16 contrasts.

Table 3. Non-phylogenetic scaling relationships of log–log data of the mechanical properties of lacertid iliotibialis muscle against snout–vent length and body mass

	Adjusted r^2	Slope	95% confidence limits of slope	Intercept	95% confidence limits of intercept	F	P	Range or mean \pm s.d.*
A. Iliotibialis muscle against SVL								
M_b (g)	0.931	3.76	3.22 to 4.30	−6.00	−6.97 to −5.03	218	<0.0001	1.14–18.5
THPT (ms)	0.119	0.539	−0.107 to 1.18	0.308	−0.842 to 1.46	3.17	0.095	19.0 \pm 4.6
LSHR (ms)	0.463	0.862	0.384 to 1.34	−0.289	−1.14 to 0.563	14.8	0.0016	12.0–27.3
Stress (kN m $^{-2}$)	0.223	1.05	0.103 to 1.99	0.619	−1.06 to 2.30	5.59	0.032	120–526
NPO (W kg $^{-1}$)	0.386	1.52	0.546 to 2.50	−0.760	−2.50 to 0.981	11.0	0.0046	24.0–134
FR (%)	0.470	−0.414	−0.641 to −0.188	2.62	2.22 to 3.03	15.2	0.0014	58.6–93.4
B. Iliotibialis muscle against body mass								
SVL (mm)	0.931	0.249	0.213 to 0.285	1.61	1.58 to 1.64	218	<0.001	41.6–84.3
THPT (ms)	0.118	0.138	−0.028 to 0.304	1.17	1.05 to 1.30	3.13	0.097	19.0 \pm 4.6
LSHR (ms)	0.429	0.215	0.0879 to 0.341	1.10	1.00 to 1.19	13.0	0.0026	12.0–27.3
Stress (kN m $^{-2}$)	0.272	0.291	0.0562 to 0.526	2.28	2.10 to 2.46	6.97	0.019	120–526
NPO (W kg $^{-1}$)	0.483	0.432	0.202 to 0.663	1.65	1.48 to 1.83	15.9	0.0012	24.0–134
FR (%)	0.373	−0.0964	−0.160 to −0.033	1.95	1.90 to 2.00	10.5	0.0055	58.6–93.4

*Mean \pm s.d. is given where there was no significant regression relationship. M_b , body mass; THPT, time to half peak tetanus force; LSHR, time from last stimulus to half peak tetanus force relaxation; Stress, maximal isometric stress; NPO, maximal mass-specific muscle power output; FR, fatigue resistance after 12 work loops; SVL, snout–vent length. $N=17$ species in each case.

with a D/A board (KUSB3116, Keithley Instruments, Cleveland, OH, USA) and a customised program produced using Testpoint software (CEC Testpoint version 7, Measurement Computing, Norton, MA, USA). Muscle force was plotted against muscle length to generate a work loop, the area of which equated to the net work output from the muscle during the length change cycle (Josephson, 1993). Instantaneous power output was calculated for every data point in each work loop (1000 data points per work loop) by multiplying instantaneous velocity by instantaneous force. Instantaneous power output values (including some negative and many positive values) were averaged across the entire length change cycle to generate an average power output for each length change cycle. After each set of work loops a 5 min recovery period was allowed. The length (cycle

frequency and total strain) and stimulation (frequency, phase and duration) parameters imposed on the muscle were altered between each set of work loops to maximise net work output at each cycle frequency and to produce a power output–cycle frequency curve optimised for each individual. Each preparation was subjected to a set of control sinusoidal length change and stimulation parameters, every four to five sets of work loops, to measure any changes in ability to produce power. Any change in net power output was matched by an alteration in force generation. Therefore, the power produced by each preparation was corrected to the control run that yielded the highest power output, assuming that alterations in power generating ability were linear over time. All muscles still produced over 94.9 \pm 6.9% (mean \pm s.d.) of maximal control run power output by the end of each experiment. Typically each experiment lasted between 2.5 and 3 h in duration. We used the maximum power produced by any of the runs, as an estimate of an individual's maximum muscle power output.

One hundred and twenty consecutive work loop cycles were delivered at a cycle frequency of 8 Hz, using the stimulation parameters that had yielded peak power at that cycle frequency, to determine the pattern of fatigue. A set cycle frequency of 8 Hz was used as it yielded close to maximal power output in all species. Net power output at loop 12 was expressed as a percentage of net power output at loop 1 to provide an estimate of muscle fatigue resistance for each individual. Twelve work loops were sufficient to cause substantial power reduction in each species.

At the end of muscle mechanics experiments, the bones and tendons were removed and each muscle was blotted on absorbent paper to remove excess Ringer solution. Wet muscle mass was determined to the nearest 0.01 mg using an electronic balance (B204-S, Mettler-Toledo, Greifensee, Switzerland). Mean muscle cross-sectional area was calculated from muscle length and mass assuming a density of 1060 kg m $^{-3}$ (Méndez and Keys, 1960). The maximum average power output per work loop cycle was divided by wet muscle mass (W kg $^{-1}$ muscle mass) to calculate the individual's muscle mass-specific power output.

Statistical analyses

Average values of SVL and muscle performance traits (i.e. time to half peak tetanus, time from last stimulus to half tetanus relaxation, maximal isometric tetanic stress, maximal mass-specific net muscle power output and work loop fatigue resistance) were calculated for each species. Each of these values, except for fatigue resistance, were logarithmically (log $_{10}$) transformed prior to subsequent analyses. We used arcsine transformation on fatigue resistance data as it is expressed as a percentage (Sokal and Rohlf, 1995). We used least-squares regression analysis for scaling relationships.

As the species used in this study share parts of their evolutionary history they cannot be treated as independent data points, such that traditional statistical analyses would have been inappropriate (Felsenstein, 1985, 1988;

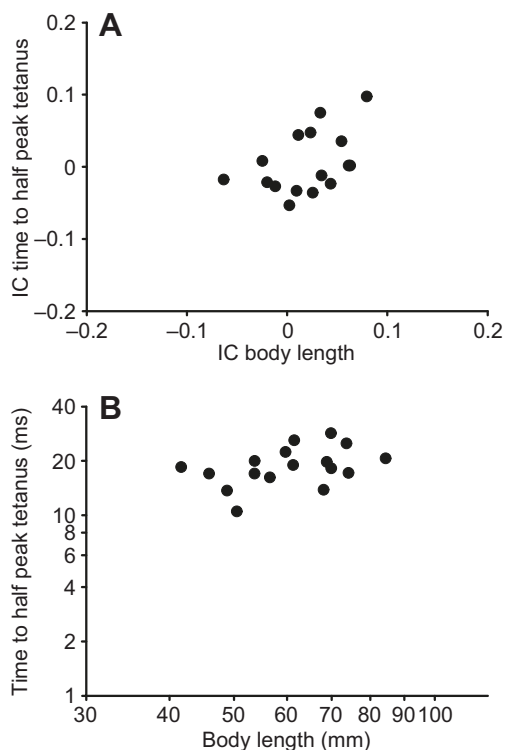


Fig. 1. Relationship between iliotibialis time to half peak tetanus force and snout–vent length in lacertids. (A) Phylogenetic independent contrasts. (B) Non-phylogenetic log–log plots. $N=17$ species.

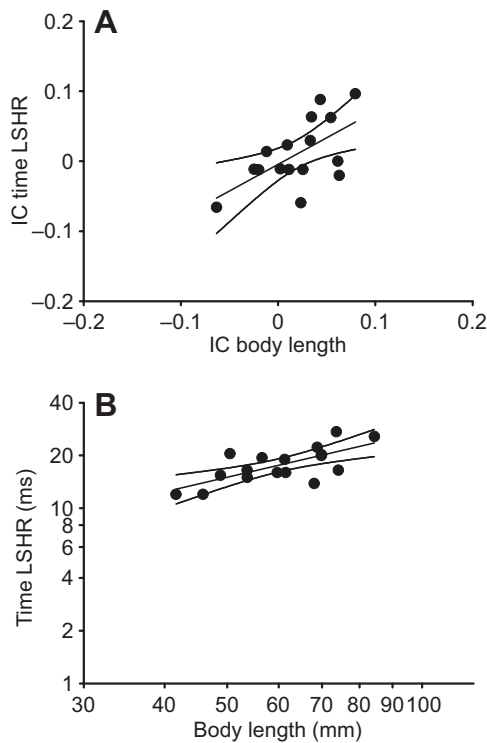


Fig. 2. Relationship between iliotibialis time from last stimulus to half tetanus force relaxation and snout-vent length in lacertids. (A) Phylogenetic independent contrasts. (B) Non-phylogenetic log-log plots. The lines represent a first-order polynomial fitted to the data using a least-squares regression and the 95% confidence limits of this line. $N=17$ species. See Tables 1 and 2 for scaling relationships.

Harvey and Pagel, 1991). Therefore, a phylogenetic independent contrast approach was used to take into consideration the phylogenetic relationships between the 17 lacertid species studied (Felsenstein, 1985, 1988). We used the current best phylogenetic tree for the 17 species used (see fig. S2 in Vanhooydonck et al., 2014). We calculated independent contrasts (IC) for all variables using the PDAP module in Mesquite v2.74 (Maddison and Maddison, 2010; Midford et al., 2009).

Independent contrasts were used as input for bivariate regressions (through the origin) between muscle performance traits and body length to test for scaling relationships. We used body length as the measure of body size, in these species, as body mass can rapidly change after feeding (Massot and Aragón, 2013). In addition, we calculated the amount of phylogenetic signal present in all traits (Blomberg et al., 2003).

RESULTS

Body length and body mass were significantly related across species (Table 2; $r^2=0.95$, $P<0.0001$).

As body length changed across species there were significant effects on almost all iliotibialis mechanical properties measured (Tables 2 and 3A), with the exception of tetanus activation time (time to half peak tetanus force; Fig. 1; Tables 2 and 3A). As body length increased across lizard species there were significant increases in: the time taken for muscle relaxation (time from last stimulus to half peak tetanus force relaxation; Fig. 2); maximal isometric muscle stress (force per muscle cross-sectional area; Fig. 3); and maximal mass-specific muscle power output (power output per muscle mass; Fig. 4). However, muscle fatigue resistance significantly decreased as body length increased across species (Fig. 5). Taking phylogeny into account modified the relationships between mechanical properties of iliotibialis and body length; however, the slope of the relationship that accounted for phylogeny

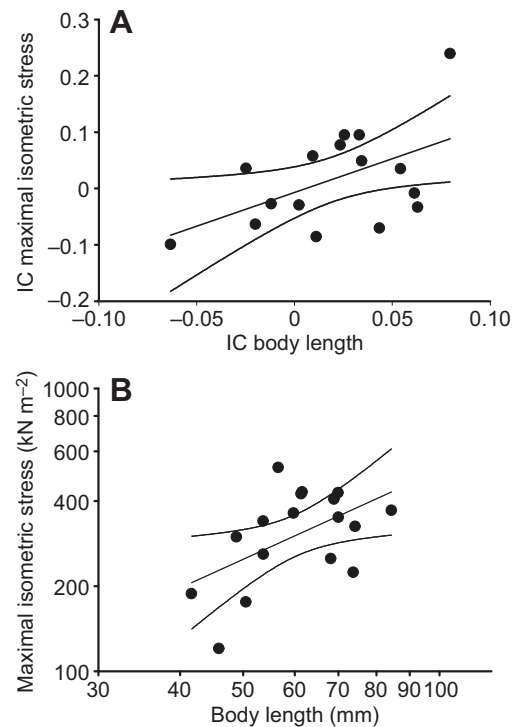


Fig. 3. Relationship between maximal iliotibialis isometric stress and snout-vent length in lacertids. (A) Phylogenetic independent contrasts. (B) Non-phylogenetic log-log plots. The lines represent a first-order polynomial fitted to the data using a least-squares regression and the 95% confidence limits of this line. $N=17$ species. See Tables 1 and 2 for scaling relationships.

was always within the 95% confidence limits of the slope for the non-phylogenetic relationship.

Of all of the traits studied only time to half peak tetanus showed a significant amount of phylogenetic signal (Table 4), suggesting that only time to half peak tetanus is evolutionarily conserved.

When compared with the scaling relationships predicted from the concept of dynamic similarity, body mass had a higher than predicted phylogenetic scaling exponent and fatigue resistance had a lower than predicted phylogenetic scaling exponent: in each of these cases, the predicted scaling exponent fell outside the confidence intervals of the scaling exponent for the parameter measured in this study.

DISCUSSION

The between-species changes in mechanical properties with body size observed in the current study are different to what might be expected from previous literature comparing scaling relationships across species from widely different groups of animals (Seow and Ford, 1991; Rome et al., 1990; Medler, 2002; Pellegrino et al., 2003; Marx et al., 2006) or between individuals within a species. In general, the previous literature indicates that larger individuals have skeletal muscle mechanical properties indicative of slower fibre type. In contrast, the current study indicates that whilst isometric relaxation times become slower in larger species of lizards, changes in other key skeletal muscle mechanical properties, such as increased stress and relative power along with decreased fatigue resistance, are indicative of faster muscle fibre types in these larger species. Previous studies within species of lizards have shown no significant effect of body size on muscle stress or power output (Marsh, 1988; Johnson et al., 1993). However, in the present study there were increases in muscle stress and normalised muscle power

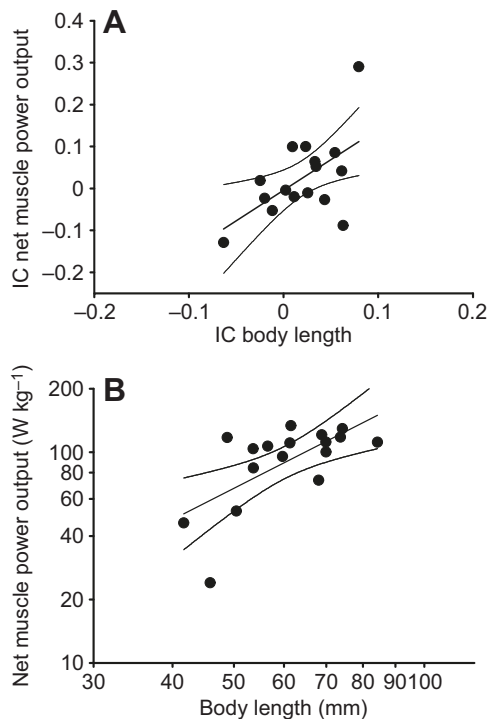


Fig. 4. Relationship between maximal mass-specific iliotibialis work loop power output and snout-vent length in lacertid lizards. (A) Phylogenetic independent contrasts. (B) Non-phylogenetic log-log plots. The lines represent a first-order polynomial fitted to the data using a least-squares regression and the 95% confidence limits of this line. $N=17$ species. See Tables 1 and 2 for the scaling relationships.

output with increased species snout-vent length, which increased with slopes (phylogenetic analysis) of 1.11 and 1.41, respectively (Table 2). It is possible that such increases in muscle stress and normalised power output could have been achieved via mechanisms such as alterations in muscle fibre density and/or changes in muscle fibre type. Previous studies, some using phylogenetic analysis, have demonstrated that both the relative proportions of fast glycolytic and fast oxidative glycolytic fibres and the cross-sectional area of individual muscle fibres vary widely in iliofibularis muscle across closely related lizard species, but that this variation is not necessarily well explained by variation in species body size (Bonine et al., 2005). However, as iliofibularis (used in previous studies) is a knee flexor and iliotibialis (used in the present study) is a knee extensor it is possible that such different functions could mean that scaling could have differential effects on their morphology and biochemistry, and it is likely that iliotibialis is a key muscle used in powering locomotion. Previous work has demonstrated that iliotibialis has a far higher proportion of fast glycolytic fibres than iliofibularis in the Brazilian sand lizard *Tropidurus psammonastes* (Pereira et al., 2015). A possible change to a higher proportion of fast glycolytic fibres in the larger lizard species in this study is partly supported by the decreased fatigue resistance with increased body size along with the increases in muscle stress and normalised power output. No previous study has analysed the effects of body size on the fatigue resistance of lizard muscle. Such an amongst-species trade-off between fatigue resistance and power output has previously been reported for this set of lizard species (Vanhooydonck et al., 2014). Interestingly, a previous study on the locomotor performance of a similar set of lizard species to those used in the current study also found a

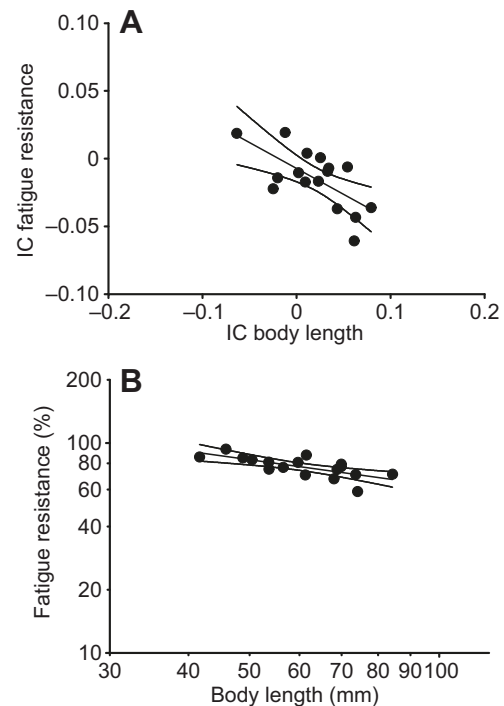


Fig. 5. Relationship between iliotibialis work loop fatigue resistance and snout-vent length in lacertid lizards. (A) Phylogenetic independent contrasts. (B) Non-phylogenetic log-log plots. The lines represent a first-order polynomial fitted to the data using a least-squares regression and the 95% confidence limits of this line. $N=17$ species. See Tables 1 and 2 for scaling relationships.

significant trade-off between sprint speed and endurance when phylogenetic analysis was undertaken (Vanhooydonck et al., 2001).

The concept of dynamic similarity would predict a slope of 3.0 for body mass against body length, yet the slope of 3.64 (phylogenetic analysis) determined in the present study, and the fact that 3.0 is outside of the 95% confidence intervals for this slope, indicates that the larger lizard species are getting relatively heavier than would be predicted if their lengths and masses were changing in proportion. Previous work, that has accounted for the relatedness of species in their analyses, has demonstrated that sprint performance varies widely across related lizard species, but that such variation is not related to overall body size, including work on a very similar set of species to those used in the present study (Losos, 1990; Bauwens et al., 1995; Bonine and Garland, 1999; Vanhooydonck et al., 2001). So, if larger lizards are able to maintain locomotor performance they would need

Table 4. Descriptive statistics for the phylogenetic signal (Blomberg et al., 2003) present in the traits used in the analyses

	K	P	MSE	Observed ratio
SVL	0.67	0.56	0.009	0.77
M_b	0.72	0.39	0.13	0.83
THPT	1.09	0.005	0.009	1.25
LSHR	0.78	0.24	0.012	0.89
Stress	0.62	0.67	0.04	0.72
NPO	0.69	0.47	0.05	0.79
FR	0.62	0.77	0.03	0.71

M_b , body mass; SVL, snout-vent length; THPT, time to half peak tetanus force; LSHR, time from last stimulus to half peak tetanus force relaxation; Stress, maximal isometric stress; NPO, maximal mass-specific muscle power output; FR, fatigue resistance after 12 work loops. $N=17$ species in each case.

to counteract the extra mass by some other disproportionate change such as greater muscle mass, higher muscle stress or higher normalised power output. The present study indicates that some of the maintenance of performance between species can be due to significant changes in the mechanical properties of muscle that would not have been predicted from previous intraspecific studies. Thus, the evolution of larger body size in lacertids appears to go hand in hand with the evolution of greater stress and normalised muscle power output than would have been expected from previous muscle mechanics studies. Although the phylogenetic scaling exponent for normalised power output is much higher than would have been predicted from dynamic similarity – 1.41 compared with 0.5, respectively – the predicted value is just within the lower end of the 95% confidence intervals found in this study. Previous work that has accounted for phylogeny has also demonstrated that species with greater sprint speed have relatively longer hind limbs (Bauwens et al., 1995; Bonine and Garland, 1999), although this relationship is not always significant (Vanhooydonck et al., 2001). A previous study found that species with higher sprint speed also had significantly higher physiological optimum temperatures for sprinting (Bauwens et al., 1995). Although the mechanical properties of the muscles were not measured by Bauwens et al. (1995), an increase in physiological optimum temperature for sprinting would be expected to increase skeletal muscle power output (James, 2013). Previous studies within species have demonstrated that varying solutions are used to maintain similar locomotor performance across a range of body sizes. For instance, in the desert iguana *Dipsosaurus dorsalis*, hind-limb length is relatively longer in smaller individuals (Johnson et al., 1993), whereas in the Australian lizard *Amphibolurus nuchalis*, thigh muscle mass is relatively higher in larger individuals (Garland, 1985).

Conclusions

Our results show that the evolution of larger body size in lizards studied here resulted in the evolution of greater normalised muscle power output to compensate for relative increases in body mass. The increase in body mass was greater than would have been predicted by the concept of dynamic similarity and this occurred alongside a significant increase in normalised muscle power output and isometric stress, which was unexpected in comparison to previous lizard scaling studies. However, meeting the functional demand of moving an increased body mass had an associated cost, causing a decrease in fatigue resistance that was greater than would have been predicted by dynamic similarity. Therefore, data from the current study combined with previously published data demonstrating between-species variation in fibre type and limb morphology helps to explain some of the previously observed patterns of between-species variation in locomotor performance. This study is the first to undertake a comparative phylogenetic analysis of between-species variation in skeletal muscle performance, thereby adding to the current literature to clarify how changes in morphology and physiology between related species help to explain observed variation in locomotor performance and the potential relationships between these changes during evolution.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

A.H., B.V. and R.S.J. conceived and designed the experiments; all authors conducted the experiments; R.S.J. and B.V. analysed the data; R.S.J. wrote the manuscript; A.H., B.V. and J.A.T. edited the manuscript.

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References

- Alexander, R. McN. and Jayes, A. S. (1983). A dynamic similarity hypothesis for the gaits of quadrupedal mammals. *J. Zool.* **201**, 135–152.
- Altringham, J. D. and Johnston, I. A. (1990). Scaling effects on muscle function: power output of isolated fish muscle fibres performing oscillatory work. *J. Exp. Biol.* **151**, 453–446.
- Altringham, J. D., Morris, T., James, R. S. and Smith, C. I. (1996). Scaling effects on muscle function in fast and slow muscles of *Xenopus laevis*. *Exp. Biol. Online* **1**, 1–8.
- Bauwens, D., Garland, T., Jr., Castilla, A. M. and van Damme, R. (1995). Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioral covariation. *Evolution* **49**, 848–863.
- Bennett, A. F., Garland, T. and Else, P. L. (1989). Individual correlation of morphology, muscle mechanics and locomotion in a salamander. *Am. J. Physiol.* **256**, R1200–R1208.
- Blomberg, S. P., Garland, T. and Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717–745.
- Bonine, K. E. and Garland, T. (1999). Sprint performance of phrynosomatid lizards, measured on a high-speed treadmill, correlates with hindlimb length. *J. Zool.* **248**, 255–265.
- Bonine, K. E., Gleeson, T. T. and Garland, T., Jr. (2005). Muscle fiber-type variation in lizards (Squamata) and phylogenetic reconstruction of hypothesized ancestral states. *J. Exp. Biol.* **208**, 4529–4547.
- Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15.
- Felsenstein, J. (1988). Phylogenies and quantitative characters. *Annu. Rev. Ecol. Syst.* **19**, 445–471.
- Garland, T. (1985). Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus nuchalis*. *J. Zool. A* **206**, 425–439.
- Harvey, P. H. and Pagel, M. D. (1991). *The Comparative Method in Evolutionary Biology*. Oxford, UK: Oxford University Press.
- Hill, A. V. (1950). The dimensions of animals and their muscular dynamics. *Sci. Prog. Lond.* **38**, 209–230.
- James, R. S. (2013). A review of the thermal sensitivity of the mechanics of vertebrate skeletal muscle. *J. Comp. Physiol. B.* **183**, 723–733.
- James, R. S., Cole, N. J., Davies, M. L. F. and Johnston, I. A. (1998). Scaling of intrinsic contractile properties and myofibrillar protein composition of fast muscle in the fish. *J. Exp. Biol.* **201**, 901–912.
- Johnson, T. P., Swoap, S. J., Bennett, A. F. and Josephson, R. K. (1993). Body size, muscle power output and limitations on burst locomotor performance in the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **174**, 199–213.
- Josephson, R. K. (1993). Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* **55**, 527–546.
- Losos, J. B. (1990). Ecomorphology, performance capability, and scaling of West Indian *Anolis* lizards: an evolutionary analysis. *Ecol. Monogr.* **60**, 369–388.
- Maddison, W. P. and Maddison, D. R. (2010). Mesquite: a modular system for evolutionary analysis, v. 2.74. <http://mesquiteproject.org>.
- 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.
- Marx, J. O., Olsson, M. C. and Larsson, L. (2006). Scaling of skeletal muscle shortening velocity in mammals representing a 100,000-fold difference in body size. *Pflügers Arch.* **452**, 222–230.
- Massot, M. and Aragón, P. (2013). Phenotypic resonance from a single meal in an insectivorous lizard. *Curr. Biol.* **23**, 1320–1323.
- Medler, S. (2002). Comparative trends in shortening velocity and force production in skeletal muscles. *Am. J. Physiol.* **283**, R368–R378.
- Méndez, J. and Keys, A. (1960). Density and composition of mammalian muscle. *Metabolism* **9**, 184–188.
- Midford, P. E., Garland, T., Jr. and Maddison, W. P. (2009). PDAP:PDTree package for Mesquite, v. 1.15. http://mesquiteproject.org/pdap_mesquite/.
- Pellegrino, M. A., Canepari, M., Rossi, R., D'Antona, G., Reggiani, C. and Bottinelli, R. (2003). Orthologous myosin isoforms and scaling of shortening

- velocity with body size in mouse, rat, rabbit and human muscles. *J. Physiol.* **546**, 677–689.
- 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.
- Rome, L. C., Sosnicki, A. A. and Goble, D. O.** (1990). Maximum velocity of shortening of three fibre types from horse soleus muscle: implications for scaling with body size. *J. Physiol.* **431**, 173–185.
- Seow, C. Y. and Ford, L. E.** (1991). Shortening velocity and power output of skinned muscle fibers from mammals having a 25,000-fold range of body mass. *J. Gen. Physiol.* **97**, 541–560.
- Sokal, R. R. and Rohlf, F. J.** (1995). *Biometry: The Principles and Practice of Statistics in Biological Research*. New York, NY: Freeman and Company.
- Vanhooydonck, B., Van Damme, R. and Aerts, P.** (2001). Speed and Stamina trade-off in lacertid lizards. *Evolution* **55**, 1040–1048.
- 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.
- Widrick, J. J., Romatowski, J. G., Karhanek, M. and Fitts, R. H.** (1997). Contractile properties of rat rhesus monkey and human type I muscle fibers. *Am. J. Physiol.* **272**, R34–R42.