

Is the whole more than the sum of its parts? Evolutionary trade-offs between burst and sustained locomotion in lacertid lizards

B. Vanhooydonck, R. S. James, J. Tallis, P. Aerts, Z. Tadic, K. A. Tolley, G. J. Measey and A. Herrel

Proc. R. Soc. B 2014 **281**, 20132677, published 8 January 2014

Supplementary data

["Data Supplement"](#)

<http://rspsb.royalsocietypublishing.org/content/suppl/2014/01/02/rspsb.2013.2677.DC1.html>

References

[This article cites 63 articles, 22 of which can be accessed free](#)

<http://rspsb.royalsocietypublishing.org/content/281/1777/20132677.full.html#ref-list-1>

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)



CrossMark
click for updates

Research

Cite this article: Vanhooydonck B, James RS, Tallis J, Aerts P, Tadic Z, Tolley KA, Measey GJ, 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* **281**: 20132677. <http://dx.doi.org/10.1098/rspb.2013.2677>

Received: 14 October 2013

Accepted: 19 November 2013

Subject Areas:

biomechanics, evolution

Keywords:

trade-off, locomotion, whole-organism, muscle

Author for correspondence:

B. Vanhooydonck

e-mail: bieke.vanhooydonck@ua.ac.be

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2013.2677> or via <http://rsob.royalsocietypublishing.org>.

Is the whole more than the sum of its parts? Evolutionary trade-offs between burst and sustained locomotion in lacertid lizards

B. Vanhooydonck¹, R. S. James², J. Tallis², P. Aerts¹, Z. Tadic³, K. A. Tolley^{4,5}, G. J. Measey⁶ and A. Herrel^{7,8}

¹Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Antwerpen, Belgium

²Department of Biomolecular and Sport Sciences, Coventry University, Coventry, UK

³Division of Biology, University of Zagreb, Zagreb, Croatia

⁴Applied Biodiversity Research Division, South African National Biodiversity Institute, Cape Town, South Africa

⁵Department of Botany and Zoology, University of Stellenbosch, Matieland, South Africa

⁶Department of Zoology, Nelson Mandela Metropolitan University, Port Elizabeth, South Africa

⁷UMR7179, CNRS/MNHN, Paris, France

⁸Department of Biology, University of Ghent, Ghent, Belgium

Trade-offs arise when two functional traits impose conflicting demands on the same design trait. Consequently, excellence in one comes at the cost of performance in the other. One of the most widely studied performance trade-offs is the one between sprint speed and endurance. Although biochemical, physiological and (bio)mechanical correlates of either locomotor trait conflict with each other, results at the whole-organism level are mixed. Here, we test whether burst (speed, acceleration) and sustained locomotion (stamina) trade off at both the isolated muscle and whole-organism level among 17 species of lacertid lizards. In addition, we test for a mechanical link between the organismal and muscular (power output, fatigue resistance) performance traits. We find weak evidence for a trade-off between burst and sustained locomotion at the whole-organism level; however, there is a significant trade-off between muscle power output and fatigue resistance in the isolated muscle level. Variation in whole-animal sprint speed can be convincingly explained by variation in muscular power output. The variation in locomotor stamina at the whole-organism level does not relate to the variation in muscle fatigue resistance, suggesting that whole-organism stamina depends not only on muscle contractile performance but probably also on the performance of the circulatory and respiratory systems.

1. Introduction

Performance trade-offs are fundamental to understanding the large phenotypic diversity found among individuals, populations or species. Apart from originating because of physiological reasons [1], performance trade-offs may also arise when two functional traits cannot be optimized simultaneously as they pose conflicting demands on the same design feature. Consequently, excellence in one trait will come at the cost of performance in the other. Thus, a trade-off may result in a compromise phenotype (i.e. an individual performing sub-optimally for both traits), and as such may represent a constraint on evolution [2,3], or conversely, an organism may specialize in one trait at the expense of the other, in which case a trade-off may facilitate phenotypic differentiation [4–6].

Locomotor performance trade-offs have been extensively studied as the locomotor system is typically involved in executing multiple tasks (e.g. sprinting, manoeuvring, long-distance running, fighting), which may be dependent on the same design features (e.g. limb length, muscle mass, metabolic rates) in opposing ways (e.g. [7–10]). Of these trade-offs, the one between sprint speed and endurance is probably the most widely studied (e.g. mammals

[9,11–13]; reptiles [7,14–16]; frogs [17,18]; fish [19,20]). Results of these studies are mixed despite the fact that a negative relationship between burst and sustained locomotion would seem intuitive owing to the contrasting biochemical and physiological features associated with these types of locomotion. Whereas fast-contracting, easily fatigued fast-twitch glycolytic (FG) muscle fibres are associated with sprinting and acceleration [21–24], fast-twitch oxidative glycolytic (FOG) muscle fibres, conferring both speed and endurance, are associated with lower-intensity activity over more extended periods of time [25–27]. In addition, in a single muscle, the proportion of FG fibres is negatively correlated with the proportion of FOG fibres [26,28,29]. Also, at the whole-muscle level, a trade-off exists whereby muscles producing high-power output are less fatigue-resistant and fatigue-resistant muscles produce less power [17,30–32].

Because muscles power movement, performance at the whole-animal level can be expected to be the net result of the performance of the underlying traits of skeletal muscle and the supporting cardiovascular and respiratory systems. However, it remains unclear whether and how physiological and muscular trade-offs translate into a performance trade-off at the whole-organism level. The one study explicitly testing for the existence of a trade-off at both the isolated-muscle and whole-organism levels reported incongruent results: whereas power output and fatigue resistance of the peroneus muscle in individual *Xenopus laevis* frogs were negatively correlated, individual burst and sustained swimming capacity of the whole animal were not [17]. Also, the hypothesized link between whole-organism locomotor performance and muscle mechanics often remains unclear. Apart from a few cases in which muscle power output was correlated directly to whole-animal locomotor speed and acceleration [23,33], other studies have failed to link inter-individual variation in muscle mechanics with differences in whole-organism performance [17,22,32].

Here, we explicitly investigate the fast versus sustained locomotion trade-off at two levels of organization (i.e. at the isolated-muscle and at the whole-organism levels) by comparing data among 17 lacertid lizard species. By linking muscular with organismal performance, we aim to elucidate the mechanistic basis of the hypothesized performance trade-off. We do so in an interspecific comparison, as the variation among species is typically greater than among individuals (cf. [17]) and may reflect the range of potential functional trade-offs that limit the evolution of performance [32]; we specifically opted to use lacertid lizards as a trade-off between speed and endurance has been shown to exist in locomotor performance in a subset of these species [7].

In addition to stamina and sprint speed, we also quantify acceleration capacity as a second type of fast explosive locomotion. Organisms in general, and lizards more specifically, typically use intermittent locomotion, during which they continuously accelerate and decelerate as their primary mode of movement. Acceleration capacity may therefore be of prime ecological relevance [34,35]. In addition, acceleration capacity has been previously shown to correlate with the proportion of FG fibres in a muscle [24] and with muscular power output [23,33]. Because the physiological and muscle mechanical correlates of burst acceleration may conflict with those of sustained locomotion (see above), a trade-off between acceleration and stamina at the whole-organism level can be expected. Empirical evidence for the existence of an acceleration–stamina trade-off, however, is scarce and ambiguous [18].

To measure whole-muscle performance, we here use the work loop technique, which evaluates the work and power output of the whole muscle *in vitro* while simulating its movement *in vivo* [36–38]. We chose to use the iliotibialis muscle, a cylindrical, parallel-fibred thigh muscle that functions primarily as knee extensor in reptiles [39–43]. Specifically, knee extensors have been shown to be of prime importance for the locomotor performance traits under study here, as the variation in knee extensor muscle mass is the principal determinant of variation in acceleration capacity and sprint speed in *Anolis* lizards [34].

Using an interspecific comparative approach, we ask (i) whether the variation in fast, explosive locomotor performance (sprint speed, acceleration) among lacertid lizard species is negatively correlated with the variation in sustained locomotor performance (stamina); (ii) whether the variation in iliotibialis muscular power output among lacertid lizard species is negatively correlated with the variation in iliotibialis muscle fatigue resistance; (iii) whether the variation in fast, explosive locomotor performance among lacertid lizard species is positively correlated with the variation in iliotibialis muscle power output; and (iv) whether the variation in sustained locomotor performance among lacertid lizard species is positively correlated with the variation in iliotibialis muscle fatigue resistance.

2. Material and methods

(a) Animals

Between November 2010 and June 2012, we quantified whole-organism locomotor performance and muscle performance in 17 lacertid species that were wild-caught, laboratory-bred or obtained through the pet trade. Upon arrival in the laboratory, we measured snout–vent length (SVL) of each individual to the nearest 0.01 mm, using digital callipers (Mitutoyo CD-15DC). We started the locomotor performance trials within two to six weeks after the animals arrived in the laboratory, with the exception of the laboratory-bred individual of *Podarcis melisellensis*. During experimentation, we did not observe any noticeable difference in performance capacity or behaviour between wild-caught or pet trade species.

Lizards were kept in the animal room of the Lab of Functional Morphology at the University of Antwerp, set at a 10 D: 14 L cycle and at a temperature of 28°C. *Lacerta bilineata*, *Lacerta lepida* and *Psammotromus algirus* were housed individually in terraria of 0.6 × 0.6 m. Other lizard species were housed with a maximum of five individuals per cage in cages of 0.5 × 1 m. Cages were equipped with a 75 or 100 W light bulb, according to the cage size, providing extra light and heat for 10 h per day. Rocks, branches and leaf litter were scattered on the cage bottom to provide basking and hiding spots. Lizards were fed crickets (*Achetus domesticus*) dusted with calcium and vitamin supplements three times per week. In addition to providing water *ad libitum* in water bowls, cages were sprayed with water twice a week.

(b) Locomotor performance

(i) Sprint speed

We quantified sprint speed following standard procedures (cf. [7,44]). Each individual was chased five times down a 2 m long electronic racetrack, with a cork substrate, equipped with photocells every 25 cm. Each run was scored as ‘good’ or ‘bad’ depending on the individual’s willingness to run (cf. [45,46]). We would call a run a ‘good’ run if the lizard ran continuously, in the middle of the track, over a distance of at least 50 cm; in a bad run the animal frequently stopped and turned, and/or

jumped from side to side. Bad runs were not included in further analyses. Prior to the start of the experiment, and between trials, lizards were placed in an incubator set at the species-selected body temperature (i.e. at 35°C for all species, except for *Latastia longicaudata*, in which case the incubator was set at 38°C [7]) for at least an hour. These temperatures are within the range of field-active body temperatures (cf. review in [47]). As an estimate of a lizard's maximal sprinting capacity, we used maximal speed attained over any 25 cm interval of the five trials.

(ii) Acceleration

We quantified acceleration capacity of each lizard on a 2 m long racetrack with a cork substrate. A dot was painted dorsally, at the level of the hip on each individual lizard, using a non-toxic white paint pen, to facilitate subsequent digitization. Each individual was tested five times. Prior to the start of the experiment and between trials, lizards were placed in an incubator set at 35°C (38°C for *L. longicaudata*) for at least an hour. A high-speed video camera (Redlake Motionscope) set at 500 Hz was placed 1 m above the track so that the first 0.5 m of the track was in view, filming the lizards dorsally when accelerating from a standstill. A scaling grid of known dimensions was filmed before the start of each filming session and was digitized (PROANALYST v. 1.5.3.8) to calculate the scaling factor. Clips were subsequently digitized at 500 Hz, using the (semi-)automatic tracking option in PROANALYST. Digitization started at least 20 frames prior to any movement by the lizard and stopped when the lizard ran out of view. We subsequently exported the scaled (in m) displacement coordinates to EXCEL (Microsoft Corp.) and filtered the data using a fourth-order zero-phase shift Butterworth low-pass data noise filter (VBA for EXCEL [48]) at 40 Hz. Based on the filtered data, the first and second derivatives of displacement against time were calculated and the latter was used as a measure of instantaneous acceleration. In 99% of the cases, the acceleration profile (i.e. instantaneous acceleration plotted over time) showed a clear peak before the end of the sequence. Cases in which this was not true were not withheld for further analyses. As an estimate of an individual's maximum acceleration capacity, we used the maximum instantaneous acceleration out of any of the five trials.

(iii) Stamina

We quantified stamina on a circular track with a cork substrate and a circumference of 2.5 m (cf. [18,49]). Each individual was tested three times with trials spread over two consecutive days. A trial was considered terminated if a lizard did not respond to multiple taps on the tail base and did not show a righting response when placed on its back (cf. [50]). Prior to the start of the experiment, lizards were placed in an incubator set at 35°C (38°C for *L. longicaudata*) for at least an hour. Between trials on the same day, this period was extended to at least two hours. As an estimate of a lizard's maximal stamina, we used the maximal time to exhaustion (in s) out of the three trials.

Our estimates of maximum performance are based on the performance of a limited number of individuals in a limited number of trials. We acknowledge the fact that some of the estimates may in fact represent submaximal performance.

(c) Muscle mechanics

(i) Muscle preparation

After quantification of all locomotor performance traits (i.e. maximum sprint speed, acceleration and stamina), one to six individuals of each species were transported to the laboratory at Coventry University (see electronic supplementary material, table S1). Animals were killed by decapitation and transection of the spinal cord in accordance with the British Home Office

Animals Scientific Procedures Act 1986, Schedule 1. Immediately after having been killed, lizards were transferred to a bath with oxygenated (95% O₂; 5% CO₂) lizard Ringer's solution [51] where the m. iliobtibialis was isolated from the left hind-limb. For each muscle, bone was left intact at the origin and the insertion.

(ii) Twitch and tetanic kinetics

For each muscle preparation the bone at one end was clamped via crocodile clips to a calibrated load cell (UF1, Pioden Controls, Canterbury, UK) and the bone at the other end was clamped to a motor arm attached to a calibrated linear variable displacement transducer at the other. Each muscle was maintained at 34.0 ± 0.5°C in circulating oxygenated lizard Ringer solution. Each preparation was stimulated via parallel platinum electrodes while being held at constant length to generate a series of twitches. Stimulus amplitude and muscle length were adjusted to maximize isometric twitch force. Square wave stimulus pulse width was set at 2.0 ms. The muscle length that yielded maximal twitch force was measured to the nearest 0.1 mm using a dissecting microscope fitted with an eyepiece graticule. An isometric tetanic force response was elicited by subjecting the muscle to a 200 ms train of stimulation (individual pulse width: 2 ms). Stimulation frequency was then altered to determine maximal tetanic force. A rest period of 5 min was allowed between each tetanic response (cf. [51,52]). For the maximal tetanus of each individual muscle measurements were made of the time taken from stimulus to maximal tetanus force and the time from last stimulus to half force relaxation.

(iii) Work loop measurements

The work loop technique was used to determine the power output of muscles during cyclical length changes [36]. Each muscle preparation was subjected to a set of four sinusoidal length changes, starting from the length that was optimal for maximal twitch force production. The muscle stimulation parameters (stimulation frequency and amplitude) found to yield maximal isometric force were used. Electrical stimulation and length changes were controlled via a D/A board (KUSB3116) and a customized program produced using TESTPOINT software (CEC TESTPOINT v. 7). For each work loop cycle muscle force was plotted against muscle length to generate a work loop, the area of which equated to the network produced by the muscle during the cycle of length change [36]. 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 were averaged to generate an average power output for each length change cycle. The total strain of length change cycles was optimized at each cycle frequency. The cycle frequency of length change was altered, up and down, within the range of 2–26 Hz (the range varied depending on the species) to generate power-output cycle frequency curves. During these length changes, the muscle was subjected to phasic stimulation (active work loop cycles). Every 5 min the muscle was subjected to a further set of four work loop cycles with stimulation duration and stimulation phase parameters being altered until power was maximized at each cycle frequency. Before the fatigue run, a set of control sinusoidal length change and stimulation parameters were imposed on the muscle every four to five sets of work loops to monitor variation in the muscles ability to produce power/force. Any variation in power was found to be because of a matching change in ability to produce force. Therefore, the power produced by each preparation, prior to the fatigue run, was corrected to the control run that yielded the highest power output, assuming that alterations in power-generating ability were linear over time. At the end of experimentation muscles produced 94.9 ± 6.9% (mean ± s.d.)

of their maximal control run power output. We used the maximum power produced by any of the runs as an estimate of an individual's maximum muscle power output.

Each muscle, on completion of the power-output cycle frequency curve, was subjected to a fatigue run consisting of 120 work loop cycles at a cycle frequency of 8 Hz using the stimulation pattern that had yielded peak power at that cycle frequency. As an estimate of an individual's muscle fatigue resistance, we used the proportion of power output at loop 12 to power output at loop 1. An example of a work loop of a non-fatigued versus a fatigued muscle is shown in electronic supplementary material, figure S1.

At the end of the muscle mechanics experiments, the bones and tendons were removed and each muscle was blotted on absorbent paper to remove excess Ringer's solution. Wet muscle mass was determined to the nearest 0.01 mg using an electronic balance (Mettler-Toledo). Normalized muscle power output (i.e. maximum average power output per length change cycle divided by wet muscle mass, measured in $W\text{ kg}^{-1}$ muscle mass) was used as an estimate of an individual's muscle-mass-specific power output.

(d) Statistical analyses

Mean values of SVL, whole-organism performance traits (i.e. sprint speed, acceleration and stamina) and muscle traits (i.e. absolute muscle power output, mass-specific muscle power output and fatigue resistance) were calculated for each species. Raw data of all traits measured (means \pm s.e. per species) are presented in the electronic supplementary material, table S1. All, except fatigue resistance, were logarithmically (\log_{10}) transformed prior to subsequent analyses. As fatigue resistance is expressed as a proportion, we transformed it using an arcsine transformation [53].

To test for trade-offs at both levels of organization, we performed bivariate correlations between the whole-organism performance traits (i.e. sprint speed versus stamina, acceleration versus stamina) and between muscle performance traits (i.e. absolute power output versus fatigue resistance, mass-specific power output versus fatigue resistance). To test which (of the variation in) muscle performance traits best explained the variation in whole-organism performance traits, we performed a multiple regression analysis (backward elimination method), with speed or acceleration as a dependent variable, and absolute power output and mass-specific power output as independent variables in each case. For stamina, we used regression analysis with stamina as the dependent variable and fatigue resistance as an independent variable. We re-did these analyses using the residual values of all traits (i.e. regressed against SVL) as input variables.

Because species share parts of their evolutionary history, they cannot be regarded as independent data points, as is required when performing traditional statistical analyses [54–56]. We used the phylogenetic independent contrast approach to take into account the phylogenetic relationships among the 17 lacertid species under study here. The independent contrast approach requires input of the topology and branch lengths of the phylogenetic tree. We combined results from several mitochondrial DNA studies [57–61] to compile a current best tree for the 17 species used (see electronic supplementary material, figure S2). Because divergence times among these species remain largely unknown, all branch lengths were set to unity. To check whether branch lengths were adequate, we plotted the diagnostic graphs (PDAP module in MESQUITE [62]) and tested whether absolute values of standardized contrasts were correlated with their standard deviations [63]. Because in some cases the absolute values were correlated with their standard deviations (indicating that branch lengths were not adequate), we transformed branch lengths using Pagel's arbitrary branch length transformation, followed by Grafen's transformation with ρ set to 0.5.

We calculated independent contrasts (ICs) for all variables using the PDAP module in MESQUITE v. 2.74 [62,64]. Using these ICs as input variables, we performed the bi-variate correlation and multiple regression analyses (through the origin) as described above. Residuals of the IC of all whole-organism and muscle performance traits against the IC of SVL were calculated. All analyses were rerun using the residual ICs as input variables. Reported p -values are of one-tailed tests.

In addition, we calculated the amount of phylogenetic signal present in all traits (absolute and size-corrected), as outlined by Blomberg *et al.* [65].

3. Results

Because none of the *Lacerta agilis* and *P. melisellensis* runs was scored as 'good' in the whole-organism performance trials, they were discarded from further analyses. Also, the muscle performance of the only *L. lepida* individual tested was very distinct (i.e. high mass-specific power output) from that of the 16 other species, and was not used in further analyses.

Non-phylogenetic bivariate correlations were non-significant for sprint speed and stamina, both in absolute and relative (to SVL) terms (table 1; figure 1*a*). The relationship between acceleration capacity and stamina tended to be positive, both in absolute and relative terms (table 1; figure 1*c*).

Results from phylogenetic correlation analyses using the IC and residual IC of the whole-organism performance traits as input variables were similar, although there was a slight tendency for residual sprint speed and residual stamina to be negatively correlated (table 1; figure 1*b,d*).

As for the non-phylogenetic correlations between muscle performance traits, power output was negatively correlated to fatigue resistance, both in absolute and relative (to SVL) terms. Absolute mass-specific power output was negatively correlated to absolute fatigue resistance, but the relationship between the residuals was non-significant (table 1; figure 2*a*). Results for the phylogenetic analyses, using the IC and residual IC as input variables, were similar, except for the relationship between residual power output and residual fatigue resistance, which showed only a slightly negative trend (table 1; figure 2*b*).

The multiple regression analysis with sprint speed as the dependent variable, and power output and mass-specific power output as independent variables, resulted in a highly significant model in which only the variation of absolute muscle power output was withheld. The same analysis using the residuals resulted in a similar model (table 2; figure 3*a*). This was also true for the phylogenetic analyses, using the IC and residual IC of sprint speed, absolute and mass-specific power output (table 2; figure 3*b*). A multiple regression analysis using acceleration as the dependent variable, and power output and mass-specific power output as independent variables, did not result in a significant model. Neither did the regression analyses using residuals, IC or residual IC of acceleration, power output and mass-specific power output (table 2). The multiple regression analysis with stamina as the dependent variable and fatigue resistance as the independent variable did not result in a significant model, although the variation in stamina tended to be negatively correlated to the variation in fatigue resistance (table 2). When using IC and residual IC of stamina and fatigue resistance, we did not obtain any significant models (table 2).

None of the traits showed a significant amount of phylogenetic signal, suggesting that these traits are not evolutionarily conserved (see electronic supplementary material, table S2).

Table 1. Bivariate correlations between burst and sustained locomotion at the whole-animal (i.e. sprint speed and acceleration versus stamina) and isolated-muscle levels (i.e. absolute power output and muscle mass-specific power output versus fatigue resistance). Both the results of non-phylogenetic traditional analyses and phylogenetic analyses, using the independent contrast approach, are shown. Also, results are given for both absolute values and residuals (against SVL). CI: 95% confidence intervals for the unstandardized coefficient. All tests are one-tailed. Significant relationships are shown in bold; trends in italic.

whole-animal performance					
		stamina			
		non-phylogenetic ($n = 15$)		phylogenetic ($n = 14$)	
		absolute	residual	absolute	residual
sprint speed	r	-0.10	-0.06	-0.19	-0.25
	F	0.14	0.045	0.49	0.85
	p	0.36	0.42	0.25	0.19
	CI	-0.58 to 0.41	-0.46 to 0.38	-0.63 to 0.32	-0.54 to 0.22
acceleration	r	0.45	0.50	0.43	0.47
	F	3.34	4.61	2.87	3.68
	p	<i>0.09*</i>	<i>0.058*</i>	0.11*	<i>0.08*</i>
	CI	-0.06 to 0.67	-0.01 to 0.67	-0.08 to 0.69	-0.04 to 0.63
muscle mechanics					
		fatigue resistance			
		non-phylogenetic ($n = 16$)		phylogenetic ($n = 15$)	
		absolute	residual	absolute	residual
absolute power	r	-0.49	0.14	-0.41	0.20
	F	4.3	0.26	2.85	0.59
	p	0.029	0.62*	0.06	0.46*
	CI	-3.64 to 0.06	-1.55 to 2.53	-2.94 to 0.35	-1.29 to 2.73
mass-specific power	r	-0.69	-0.44	-0.58	-0.24
	F	12.93	3.37	7.04	0.87
	p	0.001	0.044	0.01	0.17
	CI	-2.00 to -0.51	-1.97 to 0.15	-1.83 to -0.19	-1.71 to 0.68

* p -value for two-tailed test.

4. Discussion

(a) Trade-offs

We find evidence, at the isolated muscle level, for a trade-off between fast and sustained locomotion among the 17 lacertid lizard species under study here. The trade-off between muscle power output and fatigue resistance, however, is not convincingly mirrored by a trade-off at the whole-animal level. Similarly, a trade-off between isolated muscle power production and muscle fatigue resistance was not translated into a trade-off between burst and sustained sprinting/swimming performance at the whole-organism level in previous studies on individual variation within species of frogs and mice [17,30,31]. The complex interplay with other muscles involved in locomotion, or with other non-muscular physiological factors associated with aerobic capacity, motivational and behavioural differences, were all factors previously hypothesized to

mask the speed–endurance trade-off at the level of the whole animal [17].

We note, however, that the absence of a trade-off between sprint speed and stamina contradicts an earlier study, which showed that speed and endurance were negatively correlated among 12 lacertid lizard species [7]. However, whereas in the previous study we measured endurance (i.e. time till exhaustion when lizards were running at a low constant speed on a treadmill), in the present study we measured stamina (i.e. time till exhaustion when lizards were running at their ‘preferred’ speed(s) on a circular track). In fact, for the four species that were used in both studies (i.e. *L. longicaudata*, *Podarcis muralis*, *Podarcis sicula* and *L. bilineata*), the relationship between stamina and endurance, if anything, tends to be negative ($r = -0.70$, $p = 0.30$). We acknowledge the fact that endurance and stamina might represent two different measures of sustained locomotion, encompassing different demands upon the

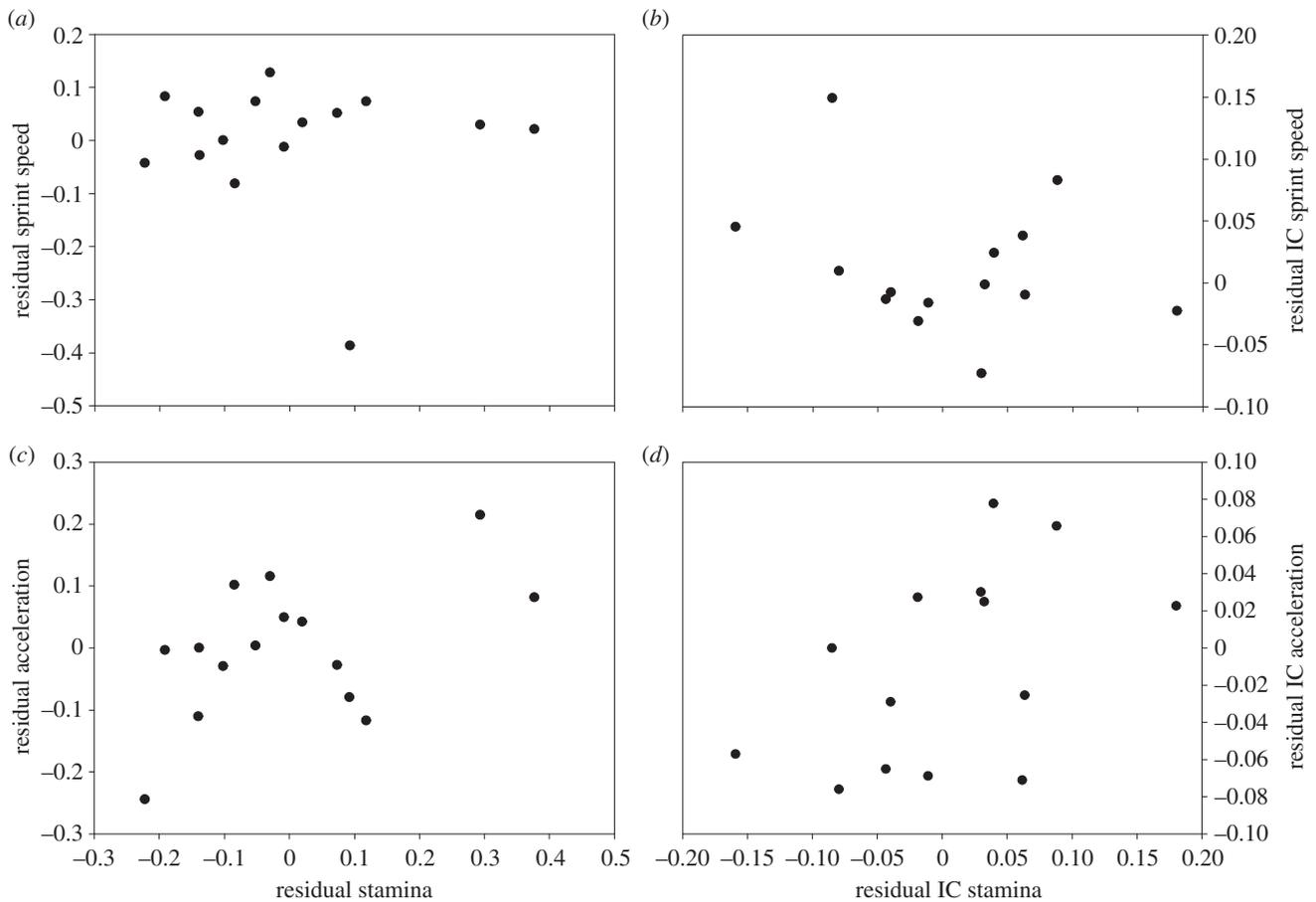


Figure 1. Bivariate correlation between burst and sustained locomotion at the whole-organism level with the variation in body size (SVL) taken into account. (a,c) Results for traditional analyses, not taking phylogeny into account; (b,d) results for their phylogenetic counterparts. (a,b) residual sprint speed is not significantly correlated to residual stamina ($r = -0.06$, $p = 0.42$ and $r = -0.25$, $p = 0.19$, respectively) and (c,d) residual acceleration capacity tends to be positively correlated to residual stamina ($r = 0.50$, $p = 0.06$ and $r = 0.47$, $p = 0.08$, respectively).

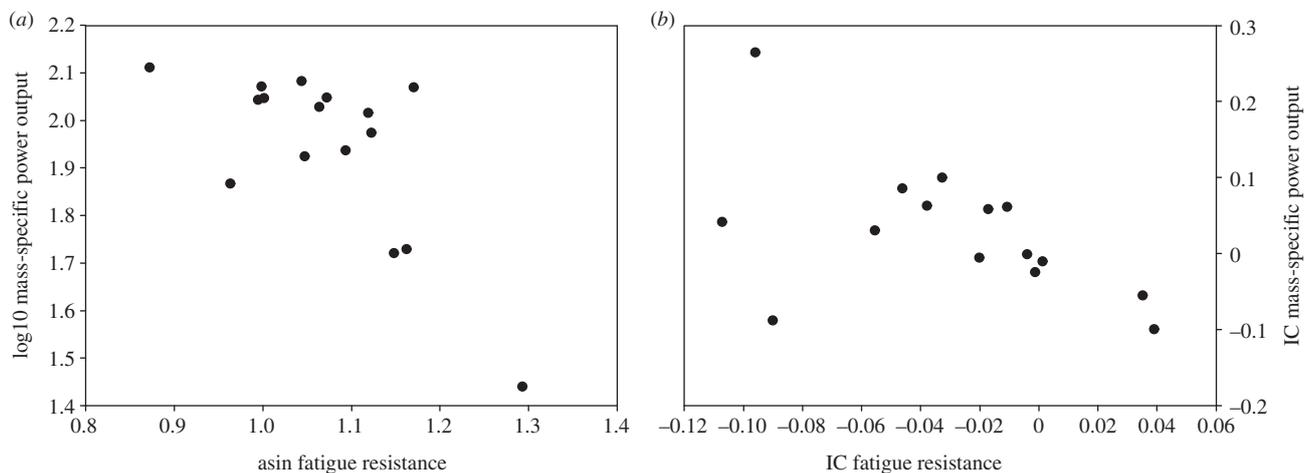


Figure 2. Bivariate correlation between iliotibial muscle mass-specific power output and iliotibial muscle fatigue resistance. Mass-specific power output is negatively correlated to fatigue resistance when using both (a) traditional non-phylogenetic analyses ($r = -0.69$, $p = 0.001$) and (b) phylogenetic analyses ($r = -0.58$, $p = 0.01$).

locomotor system. In addition, in the same previous study [7], ecological differences among species seemed to explain some of the variation in locomotor performance. Ecological differences between species could also account for some of the unexplained variation between species in locomotor performance observed in the present study.

Contrary to our expectations, however, the variation in acceleration capacity tends to be positively correlated to the

variation in stamina, in both absolute and relative terms. This is surprising, as acceleration capacity (an explosive anaerobic locomotor event) and stamina (a sustained aerobic locomotor event) are predicted to pose conflicting demands on the fibre-type composition and mechanics of the muscles involved. Whereas burst acceleration has been shown to correlate to the proportion of FG fibres [30] and muscle power output [23,33] (this study), stamina is assumed to correlate

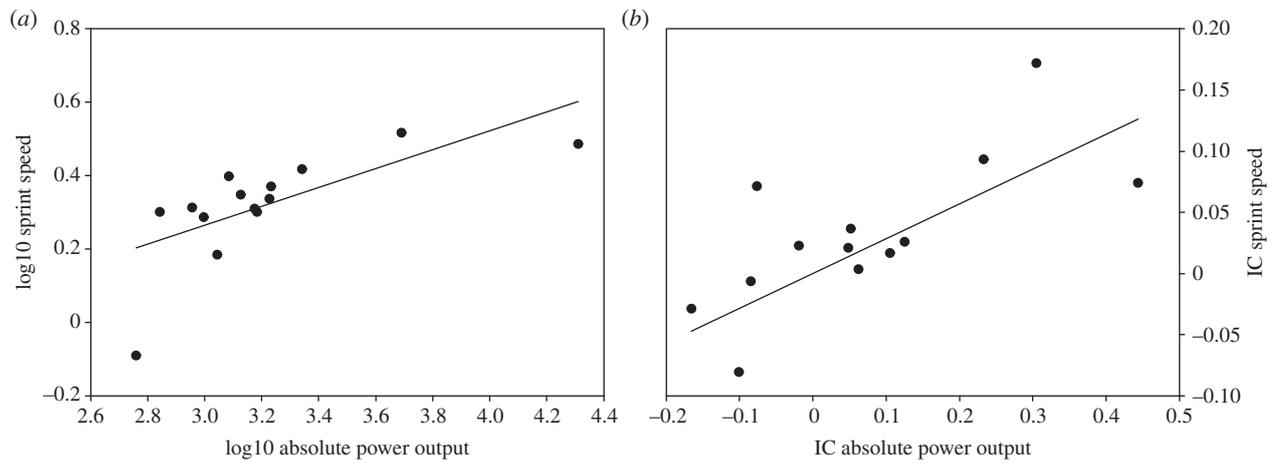


Figure 3. Regression between sprint speed and iliopsoas muscle power production. The variation in sprint speed is explained by the variation in absolute power output when using both (a) traditional non-phylogenetic analyses ($r = 0.69$, $p = 0.0035$) and (b) phylogenetic analyses ($r = 0.77$, $p = 0.0005$).

Table 2. Multiple regressions (backward elimination method) with locomotor performance traits (i.e. sprint speed, acceleration, stamina) as dependent variables, and relevant isolated muscle performance traits (i.e. muscle absolute power output, muscle mass-specific power output, muscle fatigue resistance) as independent variables. The results of both non-phylogenetic traditional analyses and phylogenetic analyses, using the independent contrast approach, are shown. Also, results are given for both absolute values and residuals (against SVL). All tests are one-tailed. CI: 95% confidence intervals for the unstandardized coefficient. Significant models, and the variable withheld, are shown in bold; trends in italic. If none of the models was significant, the results are given for the full model (CI not shown).

dependent	independent		non-phylogenetic		phylogenetic	
			absolute	residual	absolute	residual
sprint speed	absolute power	<i>R</i>	0.69	0.52	0.77	0.63
		<i>r</i> ²	0.47	0.27	0.59	0.40
		<i>F</i> _(d.f.,d.f.)	10.73 _(1,13)	4.49 _(1,13)	17.13 _(1,12)	7.9 _(1,12)
		<i>p</i>	0.0035	0.028	0.0005	0.008
		CI	0.09 to 0.43	−0.01 to 0.51	0.14 to 0.44	0.06 to 0.48
acceleration	absolute power	<i>r</i>	0.36	0.14	0.33	0.07
		<i>r</i> ²	0.13	0.02	0.11	0.006
		<i>F</i> _(d.f.,d.f.)	0.84 _(2,13)	0.11 _(2,13)	0.66 _(2,12)	0.03 _(2,12)
		<i>p</i>	0.23	0.45	0.27	0.49
		<i>F</i> _(d.f.,d.f.)	0.88 _(1,13)	3.99 _(1,13)	0.28 _(1,12)	1.23 _(1,12)
stamina	fatigue resistance	<i>r</i>	0.26	−0.50	−0.15	−0.31
		<i>r</i> ²	0.07	0.25	0.02	0.09
		<i>F</i> _(d.f.,d.f.)	0.88 _(1,13)	3.99 _(1,13)	0.28 _(1,12)	1.23 _(1,12)
		<i>p</i>	0.18	0.072*	0.61*	0.29*

**p*-value for two-tailed test.

to the proportion of FOG fibres [25–27]. Although at the muscular level, absolute and mass-specific power output trade off with fatigue resistance, this does not seem to be paralleled by a locomotor performance trade-off at the whole-organism level between acceleration and stamina. As we have no (bio)mechanical grounds to explain the positive relationship between acceleration and stamina, it seems plausible that the relationship is indirect, and influenced by other (non-muscular) physiological factors. The fact that the variation in acceleration capacity is not explained by the variation in absolute nor mass-specific power output, and the variation in stamina is not explained by the variation in muscular fatigue resistance, further corroborates this idea (see below). Finally, our measure of stamina on a circular track involves

animals moving in bursts, which may represent some sort of sustained acceleration capacity; as such this may be correlated to absolute whole-organism acceleration performance.

(b) Mechanistic link between whole-organism and isolated muscle performance

The mechanistic link between our measures of whole-organism performance and isolated muscle performance remains partially unresolved. As for sprint speed, our results show that muscles of fast sprinters seem to produce high muscle power output in both absolute and relative terms. Although a positive correlation between speed and muscle power production has been predicted based on theoretical grounds, we believe our study is

one of the few providing empirical evidence corroborating this hypothesis (cf. [23]). A comparison of locomotor performance (i.e. jumping and sprinting), fibre-type composition and muscle mechanics in three tropidurid lizard species showed that the documented differences in locomotor capacities were only partially explained by the muscular physiological attributes, and not by the muscle mechanical properties [22]. Similarly, Wilson and co-authors [17] did not find a significant relationship between swimming speed and muscle power production in individual frogs. In both cases, however, we believe the choice of experimental muscle may not have been ideal. The peroneus muscle in frogs is a small knee extensor muscle, not contributing much to locomotion [17]; the iliofibularis muscle used in the tropidurid study [22] (see also [66,67]) is a knee flexor, and a swing phase muscle. Recently, however, it has been demonstrated that locomotor performance (i.e. speed, acceleration) in lizards is not correlated to the biochemical and physiological properties of swing phase muscles. Rather, maximum performance is limited by those of the stance phase muscles [24]. Besides the fact that the muscle used in the present study (i.e. the iliotibialis muscle) is a knee extensor, shown to be a major determinant of speed and acceleration capacity [7], it is also a stance phase muscle (see also [68]). The difference in functional relevance in the context of locomotor capacity between the iliotibialis and the iliofibularis may explain the difference between the results in this and Kohlsdorf's study [22].

The variation in acceleration capacity, on the other hand, was not explained by the variation in absolute or mass-specific muscle power output either in absolute or in relative terms. This finding contradicts results from studies on frogs and lizards, where it has been shown that explosive burst locomotor events are associated with high mass-specific (muscular) power output [23,33]. However, note that in the first study measurements were done *in vivo* by means of electromyography, sonomicrometry and force measurements of the plantaris muscle while frogs were swimming voluntarily [23], whereas in the latter *in vitro* work measurements of isolated fibre bundles were performed with subsequent extrapolation of the measurements to the whole muscle [33].

Lastly, the variation in locomotor stamina was not explained by the variation in muscle fatigue resistance in either absolute or relative terms, among the 17 lacertid lizard species. We therefore do not find evidence of a muscle mechanistic basis for the capacity to sustain locomotion. Several explanations are plausible. First, stamina, like most locomotor performance traits, is determined by a whole suite of morphological, biochemical and physiological (muscular and non-muscular) traits, and the

interplay among them. For instance, both at the interspecific and intraspecific level, time to exhaustion has been shown to correlate to the energetic cost of locomotion, enzymatic activity, respiratory and/or cardiovascular properties (e.g. [69–72]). Quantifying fatigue resistance in only one limb muscle may be too simplistic an approach as other (non-muscular) physiological factors may override the effect of muscle mechanics on sustained locomotor capacity. Second, our measure of fatigue resistance at the muscular level may not perfectly mimic our measure of locomotor stamina at the whole-organism level, and *in vitro* fatigue runs were much shorter than *in vivo* stamina trials. Whereas the entire fatigue run consisted of 120 cycles at 8 Hz, thus lasting 15 s in total, and our measure of fatigue resistance is estimated as the proportion of power output at cycle 12 (i.e. after 1.5 s) against power output at the first cycle, the maximum time to exhaustion in the whole animal ranged from 57 to 223 s. During the muscle experiments, all parameters were set to elicit maximum power output (see Material and methods), as measured in non-fatigued muscles, thereby recruiting all muscle fibres simultaneously. This may not reflect *in vivo* locomotor conditions as it is unlikely all fibres are recruited at once during extended bouts of locomotion. Instead, individual fibres or fibre bundles may alternate between rest and work [17].

In conclusion, in our dataset of 17 lacertid lizard species, we find evidence for a trade-off between burst and sustained locomotion at the isolated muscle level. However, this trade-off does not seem to be convincingly paralleled at the whole-organism level. Variation in locomotor sprint speed can be explained by variation in isolated muscle power output. In contrast, we did not find strong evidence to support the hypothesis that variation in locomotor acceleration capacity and stamina can be explained by variation in muscle mechanics.

Animals were captured under permit numbers 0011-AAA004-00333 and 532-08-01-01/1-11-04. Experiments were carried out in accordance with procedures stipulated by the ethics committees of Coventry University and Antwerp University.

Acknowledgements. We would like to thank Duje Lisičić, Shelley Edwards, Buyisile Makhubo, Deon Visser, Wolfgang Böhme, Le Fras Mouton and Jan Scholliers for help with fieldwork and experiments.

Funding statement. Funding was provided by the European Community's Seventh Framework Programme FP7/2007–2013, Future Emerging Technologies, Embodied Intelligence (grant no. 231688), to P.A., the National Research Foundation of South Africa (KISC funding) to K.A.T. and A.H., and an ANR grant (MOBIGEN, programme 6ieme extinction) to A.H.

References

- Careau VC, Garland Jr T. 2012 Performance, personality, and energetics: correlation, causation, and mechanism. *Phys. Biochem. Zool.* **85**, 543–571. (doi:10.1086/666970)
- Lewontin RC. 1978 Adaptation. *Scient. Am.* **239**, 156–169. (doi:10.1038/scientificamerican0978-212)
- Arnold SJ. 1992 Constraints on phenotypic evolution. *Am. Nat.* **140**, S85–S107. (doi:10.1086/285398)
- DeWitt TJ, Scheiner SM. 2004 *Phenotypic plasticity: functional and conceptual approaches*. Oxford, UK: Oxford University Press.
- Konuma J, Chiba S. 2007 Trade-offs between force and fit: extreme morphologies associated with feeding behavior in carabid beetles. *Am. Nat.* **170**, 90–100. (doi:10.1086/518182)
- Herrel A, Podos J, Vanhooydonck B, Hendry AP. 2009 Force-velocity trade-off in Darwin's finch jaw function: a biomechanical basis for ecological speciation? *Funct. Ecol.* **23**, 119–125. (doi:10.1111/j.1365-2435.2008.01494.x)
- Vanhooydonck B, Van Damme R, Aerts P. 2001 Speed and stamina trade-off in lacertid lizards. *Evolution* **55**, 1040–1048. (doi:10.1554/0014-3820(2001)055[1040:SASTO]2.0.CO;2)
- Pasi BM, Carrier DR. 2003 Functional trade-offs in the limb muscles of dogs selected for running vs. fighting. *J. Evol. Biol.* **16**, 324–332. (doi:10.1046/j.1420-9101.2003.00512.x)
- Dlugosz EM, Chappell MA, McGillivray DG, Syme DA, Garland Jr T. 2009 Locomotor trade-offs in mice selectively bred for high voluntary wheel running. *J. Exp. Biol.* **212**, 2612–2618. (doi:10.1242/jeb.029058)

10. Ellerby DJ, Gerry SP. 2011 Sympatric divergence and performance trade-offs of Bluegill ecomorphs. *Evol. Biol.* **38**, 422–433. (doi:10.1007/s11692-011-9130-y)
11. Van Damme R, Wilson RB, Vanhooydonck B, Aerts P. 2002 Performance constraints in decathletes. *Nature* **415**, 755–756. (doi:10.1038/415755b)
12. Chappell MA, Garland Jr T, Robertson GF, Saltzman W. 2007 Relationships among running performance, aerobic physiology and organ mass in male Mongolian gerbils. *J. Exp. Biol.* **210**, 4179–4197. (doi:10.1242/jeb.006163)
13. Garland Jr T, Geiser F, Baudinette RV. 1988 Comparative locomotor performance of marsupial and placental mammals. *J. Zool. Lond.* **215**, 505–522. (doi:10.1111/j.1469-7998.1988.tb02856.x)
14. Huey RB, Bennett AF, John-Alder HB, Nagy KA. 1984 Locomotor capacity and foraging behaviour of Kalahari lacertid lizards. *Anim. Behav.* **32**, 41–50. (doi:10.1016/S0003-3472(84)80322-X)
15. Sorci G, Swallow JG, Garland Jr T, Clobert J. 1995 Quantitative genetics of locomotor speed and endurance in the lizard *Lacerta vivipara*. *Phys. Zool.* **68**, 698–720.
16. Stephens PR, Wiens JJ. 2008 Testing for evolutionary trade-offs in a phylogenetic context: ecological diversification and evolution of locomotor performance in emydid turtles. *J. Evol. Biol.* **21**, 77–87. (doi:10.1111/j.1420-9101.2007.01467.x)
17. Wilson RS, James RS, Van Damme R. 2002 Trade-offs between speed and endurance in the frog *Xenopus laevis*: a multi-level approach. *J. Exp. Biol.* **205**, 1145–1152.
18. Herrel A, Bonneaud C. 2012 Trade-offs between burst performance and maximal exertion capacity in a wild amphibian, *Xenopus tropicalis*. *J. Exp. Biol.* **215**, 3106–3111. (doi:10.1242/jeb.072090)
19. Reidy SP, Kerr SR, Nelson JA. 2000 Aerobic and anaerobic swimming performance of individual Atlantic cod. *J. Exp. Biol.* **203**, 347–357.
20. Langerhans RB. 2009 Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *J. Evol. Biol.* **22**, 1057–1075. (doi:10.1111/j.1420-9101.2009.01716.x)
21. Esbjörnsson M, Sylvé C, Holm I, Jansson E. 1993 Fast twitch fibres may predict anaerobic performance in both females and males. *Int. J. Sports Med.* **14**, 257–263. (doi:10.1055/s-2007-1021174)
22. Kohlsdorf T, James RS, Carvalho JE, Wilson RS, Dal Pai-Silva M, Navas CA. 2004 Locomotor performance of closely related *Tropidurus* species: relationships with physiological parameters and ecological divergence. *J. Exp. Biol.* **207**, 1183–1192. (doi:10.1242/jeb.00870)
23. Richards CT, Biewener AA. 2007 Modulation of *in vivo* muscle power output during swimming in the African clawed frog (*Xenopus laevis*). *J. Exp. Biol.* **210**, 3147–3159. (doi:10.1242/jeb.005207)
24. Higham TE, Korchari PG, McBrayer LD. 2011 How muscles define maximum running performance in lizards: an analysis using swing- and stance-phase muscles. *J. Exp. Biol.* **214**, 1685–1691. (doi:10.1242/jeb.051045)
25. James RS, Altringham JD, Goldspink DF. 1995 The mechanical properties of fast and slow skeletal muscles of the mouse in relation to their locomotory function. *J. Exp. Biol.* **198**, 491–502.
26. Bonine KE, Gleeson TT, Garland Jr T. 2001 Comparative analysis of fiber-type composition in the iliofibularis muscle of phrynosomatid lizards (Squamata). *J. Morph.* **250**, 265–280. (doi:10.1002/jmor.1069)
27. Biewener AA. 2003 *Animal locomotion*. Oxford, UK: Oxford University Press.
28. Bonine KE, Gleeson TT, Garland Jr T. 2005 Muscle fiber-type variation in lizards (Squamata) and phylogenetic reconstruction of hypothesized ancestral states. *J. Exp. Biol.* **208**, 4529–4247. (doi:10.1242/jeb.01903)
29. Scales JA, King AA, Butler MA. 2009 Running for your life or running for your dinner: what drives fiber-type evolution in lizard locomotor muscles. *Am. Nat.* **173**, 543–553. (DOI10.1086/597613)
30. Wilson RS, James RS, Kohlsdorf T, Cox VM. 2004 Interindividual variation of isolated muscle performance and fibre-type composition in the toad *Bufo viridis*. *J. Comp. Physiol. B* **174**, 453–459. (doi:10.1007/s00360-004-0431-7)
31. Wilson RS, James RS. 2004 Constraints on muscular performance: trade-offs between power output and fatigue resistance. *Biol. Lett.* **271**, S222–S225. (doi:1098/rsbl.2003.0143)
32. Navas CA, James RS, Wilson RS. 2006 Interindividual variation in the muscle physiology of vertebrate ectotherms: consequences for behavioral and ecological performance. In *Ecology and biomechanics: a mechanical approach to the ecology of animals and plants* (eds A Herrel, T Speck, NP Rowe), pp. 231–252. Boca Raton, FL: CRC Press.
33. Curtin NA, Woledge RC, Aerts P. 2005 Muscle directly meets the vast power demands in agile lizards. *Proc. R. Soc. B* **272**, 581–584. (doi:10.1098/rsbp.2004.2982)
34. Vanhooydonck B, Herrel A, Van Damme R, Irschick DJ. 2006 The quick and the fast: the evolution of acceleration capacity in *Anolis* lizards. *Evolution* **60**, 2137–2147.
35. Huey RB, Hertz PE. 1984 Effects of body size and slope on acceleration of a lizard (*Stellio stellio*). *J. Exp. Biol.* **110**, 113–123.
36. Josephson RK. 1993 Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* **55**, 527–546. (doi:10.1146/annurev.ph.55.030193.002523)
37. Caiozzo VJ. 2002 Plasticity of skeletal muscle phenotype: mechanical consequences. *Muscle Nerve* **26**, 740–768. (doi:10.1002/mus.10271)
38. James RS, Tallis J, Herrel A, Bonneaud C. 2012 Warmer is better: thermal sensitivity of both maximal and sustained power output in the iliobtibialis muscle isolated from adult *Xenopus tropicalis*. *J. Exp. Biol.* **215**, 552–558. (doi:10.1242/jeb.063396)
39. Snyder RC. 1962 Adaptations for bipedal locomotion of lizards. *Am. Zool.* **2**, 191–203.
40. Bennett AF, Garland Jr T, Else PL. 1989 Individual correlation of morphology, muscle mechanics, and locomotion in a salamander. *Am. J. Physiol. Integr. Comp. Physiol.* **256**, R1200–R1208.
41. Blob RW, Biewener AA. 2001 Mechanics of limb bone loading during terrestrial locomotion in the green Iguana (*Iguana iguana*) and American alligator (*Alligator mississippiensis*). *J. Exp. Biol.* **204**, 1099–1122.
42. Higham TE, Jayne BC. 2004 *In vivo* muscle activity in the hindlimbs of the arboreal lizard, *Chamaleo calytratus*: general patterns and the effects of incline. *J. Exp. Biol.* **207**, 249–261. (doi:10.1242/jeb.00745)
43. Butcher MT, Blob RW. 2008 Mechanics of limb bone loading during terrestrial locomotion in river cooter turtles (*Pseudemys concinna*). *J. Exp. Biol.* **211**, 1187–1202. (doi:10.1242/jeb.012989)
44. Bauwens D, Garland Jr T, Castilla AM, Van Damme R. 1995 Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioral covariation. *Evolution* **49**, 848–863. (doi:10.2307/2410408)
45. van Berkum FH, Tsuji JS. 1987 Inter-familial differences in sprint speed of hatchling *Sceloporus occidentalis* (Reptilia: Iguanidae). *J. Zool. Lond.* **212**, 511–519. (doi:10.1111/j.1469-7998.1987.tb02921.x)
46. Tsuji JS, Huey RB, van berkum FH, Garland Jr T, Shaw RG. 1989 Locomotor performance of hatchling fence lizards (*Sceloporus occidentalis*): quantitative genetics and morphometric correlates. *Evol. Ecol.* **3**, 240–252. (doi:10.1007/BF02270725)
47. Castilla AM, Van Damme R, Bauwens D. 1999 Field body temperatures, mechanisms of thermoregulation and evolution of thermal characteristics in lacertid lizards. *Natura Croatica* **8**, 253–274.
48. Van Wassenbergh S. 2007 *VBA Excel add-in utilities and worksheet templates*. See <https://www.vantwerp.be/en/staff/sam-vanwassenbergh/my-website/excel-vba-tools>.
49. Huyghe K, Vanhooydonck B, Herrel A, Tadić Z, Van Damme R. 2007 Morphology, performance, behavior and ecology of three color morphs in males of the lizard *Podarcis melisellensis*. *Int. Comp. Biol.* **47**, 211–220. (doi:10.1093/icb/icm043)
50. Huey RB, Dunham AE, Overall KL, Newman RA. 1990 Variation in locomotor performance of demographically known populations of the lizard *Sceloporus merriami*. *Physiol. Zool.* **63**, 845–872.
51. Johnson TP, Swoap SJ, Bennett AF, Josephson RK. 1993 Body size, muscle power output and limitations on burst locomotor performance in the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **174**, 199–213.
52. Herrel A, James RS, Van Damme R. 2007 Fight versus flight: physiological basis for temperature-dependent behavioral shifts in lizards. *J. Exp. Biol.* **210**, 1762–1767. (doi:10.1242/jeb.003426)
53. Sokal RR, Rohlf FJ. 1995 *Biometry: the principles and practice of statistics in biological research*. New York, NY: Freeman and Company.
54. Felsenstein J. 1985 Phylogenies and the comparative method. *Am. Nat.* **125**, 1–15. (doi:10.1086/284325)

55. Felsenstein J. 1988 Phylogenies and quantitative characters. *Ann. Rev. Ecol. Syst.* **19**, 445–471. (doi:10.1146/annurev.es.19.110188.002305)
56. Harvey PH, Pagel MD. 1991 *The comparative method in evolutionary biology*. Oxford, UK: Oxford University Press.
57. Harris DJ, Arnold EN, Thomas RH. 1998 Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. *Proc. R. Soc. B* **265**, 1939–1948. (doi:10.1098/rspb.1998.0524)
58. Oliverio M, Bologna MA, Mariottini P. 2000 Molecular biogeography of the Mediterranean lizards *Podarcis* Wagler, 1830 and *Teira* Gray, 1838 (Reptilia, Lacertidae). *J. Biogeogr.* **27**, 1403–1420. (doi:10.1046/j.1365-2699.2000.00517.x)
59. Godinho R, Crespo EG, Ferrand N, Harris DJ. 2005 Phylogeny and evolution of the green lizards, *Lacerta* spp. (Squamata: Lacertidae) based on mitochondrial and nuclear DNA sequences. *Amphibia-Reptilia* **26**, 271–285. (doi:10.1163/156853805774408667)
60. Mayer W, Pavlicev M. 2007 The phylogeny of the family Lacertidae (Reptilia) based on nuclear DNA sequences: convergent adaptations to arid habitats within the family Eremiinae. *Mol. Phyl. Evol.* **44**, 1155–1163. (doi:10.1016/j.ympev.2007.05.015)
61. Edwards S, Vanhooydonck B, Herrel A, Measey GJ, Tolley KA. 2012 Convergent evolution associated with habitat decouples phenotype from phylogeny in a clade of lizards. *PLoS ONE* **7**, e51636. (doi:10.1371/journal.pone.0051636)
62. Maddison WP, Maddison DR. 2010 *Mesquite: a modular system for evolutionary analysis*, v. 2.74. See <http://mesquiteproject.org>.
63. Garland Jr T, Midford PE, Ives AR. 1999 An introduction to phylogenetically based statistical methods, with a new method for confidence intervals on ancestral values. *Am. Zool.* **39**, 374–388.
64. Midford PE, Garland Jr T, Maddison WP. 2009 *PDAP:PDTree package for Mesquite*, v. 1.15. See http://mesquiteproject.org/pdap_mesquite/.
65. Blomberg SP, Garland Jr T, Ives AR. 2003 Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**, 717–745.
66. Marsh RL, Bennett AF. 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.
67. Marsh RL. 1988 Ontogenesis of contractile properties of skeletal muscle and sprint performance in the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **137**, 119–119.
68. Marsh RL, Ellerby DJ, Carr JA, Henry HT, Buchanan CI. 2004 Partitioning the energetic of walking and running: swinging the limbs is expensive. *Science* **303**, 80–83. (doi:10.1126/science.1090704)
69. Garland Jr T. 1984 Physiological correlates of locomotory performance in a lizard: an allometric approach. *Am. J. Physiol.* **247**, R806–R815.
70. Garland Jr T, Losos JB. 1994 Ecological morphology of locomotor performance in squamate reptiles. In *Ecological morphology: integrative organismal biology* (eds PC Wainwright, SM Reilly), pp. 240–302. Chicago, IL: University of Chicago Press.
71. Kolok AS. 1999 Interindividual variation in the prolonged locomotor performance of ectothermic vertebrates: a comparison of fish and herpetofaunal methodologies and a brief review of the recent fish literature. *Can. J. Fish. Aquat. Sci.* **56**, 700–710. (doi:10.1139/f99-026)
72. Cano JM, Nicieza AG. 2006 Temperature, metabolic rate, and constraints on locomotor performance in ectotherm vertebrates. *Funct. Ecol.* **20**, 464–470. (doi:10.1111/j.1365-2435.2006.01129.x)