ONTOGENESIS OF CONTRACTILE PROPERTIES OF SKELETAL MUSCLE AND SPRINT PERFORMANCE IN THE LIZARD *DIPSOSAURUS DORSALIS*

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Summary

Ontogenetic allometries of running performance in 3- to 4-m burst runs (sprints) and of the contractile properties of the fast-twitch, glycolytic region of the iliofibularis muscle (FG-IF) were measured in *Dipsosaurus dorsalis*. This iguanid lizard hatches at a body mass of about 4 g and reaches adult masses of 40–70 g. Running velocity was little influenced by changes in body mass during development. Stride frequency (f) declines ontogenetically and was proportional to body mass (M_b) to approximately the -0.2 power (determined by regression analysis). Stride length (L_S) appeared to be related to the allometry of hindlimb length (L_{HL}); both L_S and L_{HL} were proportional to about $M_b^{0.28}$. Intrinsic shortening velocity of the FG-IF decreased only slightly with increasing body mass, and was consequently not proportional to f as has been assumed by various models of running dynamics. In contrast, twitch time lengthened markedly with increasing body mass, and the ratio of twitch time to stride time remained approximately constant. These results suggest that the intrinsic velocity of the muscles does not directly or solely determine maximal f, but instead limb kinematics are determined in part by other biomechanical constraints related to body dimensions. Further, the allometry of twitch kinetics supports the idea that the properties of the muscles are adjusted to allow ample time for full activation and deactivation within the biomechanically determined stride time.

Introduction

Most ectothermic vertebrates, including lizards, hatch at a small fraction of adult mass, and function without parental care during most of their growth to adult size. These circumstances require them to cope unaided with a host of physiological and morphological changes associated with increasing body size (Calder, 1984). Body mass (or weight) is a major influence on the biomechanics of locomotion (Hill, 1950; Alexander & Jayes, 1983; McMahon, 1984), and one might anticipate that ectotherms would show ontogenetic changes that adjust the features of the musculoskeletal system to the locomotor constraints imposed by changing body

mass. Somewhat surprisingly, these possible adaptations have received little attention. Parameters of locomotion and body dimensions have been measured during development in lizards (Huey & Hertz, 1982; Garland, 1985), but measurements of the contractile properties of locomotor muscles during post-hatching growth have not been made in any ectothermic vertebrate. The ontogenetic development of contractile properties has received attention in birds and mammals, but these studies have concentrated principally on the transition from slow- to fast-contracting fibres that occurs during early development in these endothermic vertebrates (Buller, Eccles & Eccles, 1960; Close, 1964; Gordon, Vrbová & Wilcock, 1981).

Despite their differences (see Discussion), most theoretical treatments of the biomechanics of running predict that stride frequency will decrease with increasing body size (Hill, 1950; McMahon, 1975; Alexander & Jayes, 1983), a prediction in accord with even casual observations on running animals of different size. As a corollary, most authors have assumed that the intrinsic rate of shortening of the fibres in homologous muscles of dimensionally similar animals will be directly related to stride frequency (Hill, 1950; McMahon, 1975; Lindstedt, Hoppeler, Bard & Thronson, 1985). Maintaining this relationship in a growing ectotherm would imply changes with growth in the properties of myosin (see Bárány, 1967) and/or the structure of the sarcomere. Not mentioned in any theoretical treatment of the allometry of running, but also worthy of attention, are possible relationships between stride frequency and the kinetics of the isometric twitch (see Marsh & Bennett, 1985, 1986b).

The present study examines the ontogenetic relationships between performance in sprint runs and the contractile properties of skeletal muscle in the iguanid lizard *Dipsosaurus dorsalis*. To avoid relying on somewhat controversial biomechanical models to predict the allometry of locomotor dynamics, observations of animal dimensions, contractile properties of isolated muscles and measurements of the kinematics of sprint running were integrated. Because of this approach, the results also provide a test of some of the predictions derived from the biomechanical models as they apply to ontogenesis in lizards. High-speed sprint runs were studied because many iguanid lizards, including *Dipsosaurus*, frequently use this type of locomotion and because Hill's (1950) well-known predictions of locomotor performance were framed in terms of maximal speed. *Dipsosaurus* can only support slow walking ($<0.3 \, \mathrm{m \, s^{-1}}$) aerobically (John-Alder & Bennett, 1981), and this mode of locomotion is used in foraging by these mainly herbivorous animals. In contrast, adults of this species have been observed to run bipedally at speeds as high as $5 \, \mathrm{m \, s^{-1}}$ during short burst runs (sprints) (Marsh & Bennett, 1985). These high-speed runs depend on anaerobic metabolism (Bennett & Dawson, 1972), and undoubtedly rely on fast-twitch glycolytic fibres that make up over 85% of the cross-sectional area of the hindlimb muscles (Putnam, Gleeson & Bennett, 1980). High-speed sprint runs can be readily elicited in the laboratory and lead to repeatable measures of performance (Bennett, 1980; Marsh & Bennett, 1985, 1986b).

Materials and methods

Animals

Most *Dipsosaurus dorsalis* used as experimental animals were collected in the vicinity of Palm Springs, CA and transported to Boston, MA for study. A few animals hatched from eggs laid in captivity. Captive animals were maintained on a diet of leafy green vegetables (primarily collard greens) supplemented weekly with a high-protein mix. Photoperiod was 16 h of light and 8 h of dark. A heat source was available for behavioural thermoregulation during the light phase.

Body mass (M_b), snout–vent length (L_{SV}) and hindlimb length (L_{HL}) were measured on live animals. L_{HL} was measured as the distance from the midline of the ventral body surface to the tip of the longest toe of the extended leg, not including the toenail.

Sprint runs

Lizards were used in measurements of locomotor performance within 2 weeks of capture or hatching. The animals weighed between 3·8 and 58 g. The sample sizes were 29 and 23 at body temperatures of 40°C and 35°C, respectively. In addition, data on nine animals weighing between 42 and 67 g from the study of Marsh & Bennett (1985) were included in the analysis. Methods for these measurements were similar to those of Marsh & Bennett (1985) with a few modifications. Animals were equilibrated overnight at 35°C or 40°C in individual containers in a constant-temperature cabinet. During the measurements, the race track used in the experiment was housed in a room regulated at approximately 35°C. On the 2 days immediately prior to filming, animals were run twice on the track to familiarize them with the experimental set-up. At each body temperature, measurements were based on the fastest of two 3- to 4-m sprint runs performed in rapid succession. These runs were filmed from above with a Bolex 16-mm camera. Framing rates, which averaged 75 Hz, were measured at the beginning and end of each roll of film by filming a digital stopwatch. Following each series of runs with an individual, body temperature (T_b) was measured with a type-K thermocouple connected to a Keithley thermocouple thermometer.

Films were analysed with a Lafayette motion analyser. Stride frequency (f) was determined by counting frames for at least three strides during the fastest portion of the run. Owing to high stride frequencies, a minimum of five strides was used for animals weighing less than 15 g. For the same portion of the run, stride length (L_S) and maximal running velocity (V_R) were determined from the frame counts and markings at 2-cm intervals on the track.

Contractile properties

The lizards from which muscles were removed for contractile studies had been in captivity for up to 6 months before use. These animals weighed between 3·6 and 60·4 g. Sixteen animals were studied and, for most, suitable data were obtained for

both isometric and isotonic contractile parameters at 40°C. In the analysis of isometric twitch properties, data from nine animals studied by Marsh & Bennett (1985) were also included. The preparation of the fast-twitch glycolytic region of the iliofibularis muscle (FG-IF) has been described previously (Gleeson, Putnam & Bennett, 1980; Marsh & Bennett, 1985). The pelvis with attached FG-IF was secured to the bottom of a Plexiglas chamber by two stainless-steel clips. The distal end of the muscle was attached with 000-gauge surgical silk to a light silver chain, which in turn was connected to the lever arm of a Cambridge Technology model 300H servo-controlled ergometer. Muscles were maintained at 40°C by immersion in a bath of recirculating Ringer's solution (in $mmol\,l^{-1}$: NaCl, 145; KCl, 4; imidazole, 20; $CaCl_2$, 2·5; glucose, 11) at pH 7·47 and saturated with 100% oxygen. Platinum plate electrodes delivered supramaximal stimuli consisting of 0·2 ms square wave pulses from a d.c.-coupled power amplifier driven by a Grass S-44 stimulator. Stimulus frequency was 300–400 Hz during tetanic contractions. Length was adjusted to achieve maximum active force in isometric tetanic contractions, and this length taken as resting length (L_0). Isometric twitch and tetanic contractions were recorded, followed by 8–10 isotonic tetanic contractions. Maximum isometric force (P_0) was measured before, in the middle, and the end of the isotonic series, and P_0 at the time of each isotonic contraction was estimated by linear extrapolation from the measurements of P_0. The decline in P_0 was never more than 10% during the series of isotonic contractions (see Marsh & Bennett, 1985). Three minutes elapsed between successive tetanic contractions. Twitch parameters reported are those for twitches recorded immediately following a tetanus.

After the contractile measurements, resting muscle length (L_0) was measured in place. The muscle was then removed from the apparatus and, with the aid of a dissecting microscope, the distal tendon, pelvis and any fragments of fibres resulting from the initial dissection were removed before weighing the remaining bundle to the nearest 0·1 mg. As fibres run the entire length of the parallel-fibred FG-IF, active cross-sectional area was estimated by dividing the mass of the muscle by L_0.

Force and position outputs from the ergometer were recorded using an R.C. Electronics eight-bit A/D converter run by an Apple II+ microcomputer. Signals from the ergometer were offset and amplified to achieve a maximum error in the A/D conversion of 1% of the peak force or position during an individual contraction. The A/D conversion rate was 14–28 kHz for twitch contractions and 7–10 kHz for tetani. Time to peak tension of the twitch (tP_{TW}), time from peak twitch force to 50% relaxation of the twitch (t50%R), peak twitch force (P_{TW}) and P_0 were determined with oscilloscope emulation software provided by the manufacturer of the A/D converter. Shortening velocity during isotonic contractions was measured with a custom program that averaged and differentiated the position trace. The portion of the position trace over which peak shortening velocity occurred was selected manually from a graphics display of the differen tiated trace.

Force–velocity curves were fitted to data from individual muscles using methods described and justified in detail by Marsh & Bennett (1986a). The following equation was used to describe the data:

$$V = \frac{B(1 - P/P_0)}{A + P/P_0} + C(1 - P/P_0),$$

where V is velocity in $L_0 s^{-1}$, P/P_0 is force as a fraction of maximum isometric force, B and C have dimensions of velocity, and A is dimensionless. This equation, developed by Marsh & Bennett (1986a), was chosen in preference to the exponential–linear equation used for *Dipsosaurus* muscles by Marsh & Bennett (1985) because it resulted in a 25 % lower mean residual sums of squares. Both these equations were greatly superior to Hill's (1938) characteristic equation. Maximum velocity of shortening at zero force (V_{max}) was predicted from the fitted curve.

Statistics

Of major importance to this study are comparisons of allometric scaling relationships: $y = aM_b{}^b$, or $\log y = \log a + b \log M_b$. Assuming linearity on log–log coordinates simplified comparisons among the statistically determined relationships and with theoretical predictions. There is no reason to expect that all the ontogenetic relationships will necessarily follow this simple form (e.g. see below, $\log t50\%R$ *versus* $\log M_b$). To predict these relationships $\log_{10}$-transformed data were analysed in two ways. First, standard least-squares regression lines (y on x, $x = \log M_b$) were derived. The resulting statistics were used to judge whether a statistically significant relationship existed between the two variables (F-test).

However, several authors have argued persuasively that the slope of the regression line should not be used to estimate the underlying functional (or structural) relationship between y and x (see Rayner, 1985). Ideally, the distribution of errors in both variables should be known and formulae for the general structural relationship used (Rayner, 1985). When the relative magnitudes of the errors of both variables are not known, the use of the reduced major axis (RMA) has been suggested to be the least biased estimator of the underlying relationship (Rayner, 1985). Consequently, this line was also calculated. The RMA slope was calculated as the geometric mean of the slope of y on x and the reciprocal of the slope of x on y (Sokal & Rohlf, 1981; Rayner, 1985). Rayner (1985) recently derived appropriate confidence limits for the RMA slope. In practice, these confidence limits can be calculated as the geometric means of the confidence limits of the slope of y on x and the reciprocals of the confidence limits of the slope of x on y. The confidence interval derived is asymmetrical around the RMA estimate of the slope and is not equal in size to the confidence limits of the regression slope as has been sometimes assumed (Sokal & Rohlf, 1981).

In my view the use of RMA is not without its disadvantages in the case of the present data. For example, the kinematic and physiological variables measured in this study are almost certainly subject to larger measurement errors than the

morphological measures. Also, some uncertainty exists as to how the RMA slopes of the three interrelated variables describing running dynamics should be treated. In simple linear regression, with the assumption that all the errors are in the y-variable, the line derived by regressing V_R on M_b is exactly equivalent to the line predicted by multiplying the regression equations for L_S on M_b and f on M_b. Because the RMA model assumes a particular distribution of errors in both x and y, calculating the RMA slope for V_R versus M_b does not result in an equation equivalent to that derived by multiplying the RMA equations for L_S versus M_b and f versus M_b. Because biomechanical models of the effects of body mass on running dynamics (Hill, 1950; McMahon, 1975) treat V_R as a value derived from separate predictions of L_S and f, this procedure was applied to the RMA equations. Finally, I was interested in comparing values of stride times and twitch times predicted at given values of body mass. Regression, not RMA, is appropriate when predicting y from x (Sokal & Rohlf, 1981). Fortunately for the present study, consistent comparisons within the regression slopes or the RMA slopes yield similar conclusions. Therefore, in most cases the more familiar regression slopes are emphasized in the text. The RMA slopes are given in Table 1, and in the text when comparing empirically and theoretically derived slopes (see Rayner, 1985).

Results

Morphological measures

If an animal remains geometrically similar with growth, its linear dimensions are expected to be proportional to the 1/3 power of body mass. Geometrical similarity appeared to hold for snout–vent length of Dipsosaurus (regression slope of $\log L_{SV}$ versus $\log M_b = 0.324$; Table 1). However, the length of the hindlimb deviated significantly from geometric similarity (regression slope of $\log L_{HL}$ versus $\log M_b = 0.289$; Table 1). This deviation means that smaller animals have relatively longer limbs than expected from geometric scaling.

Sprint running

As expected from previous studies (Marsh & Bennett, 1985, 1986b), increasing T_b from 35 to 40°C had little effect on the kinematics of sprint running in Dipsosaurus (Figs 1, 2). Stride length during sprint runs scaled with body mass approximately as expected from the length of the hindlimbs (Table 1; Figs 1A, 2A) (regression slopes of 0.273 and 0.271 at T_b values of 35 and 40°C, respectively). Stride frequency declined with increasing body mass (Figs 1B, 2B) with regression slopes on log–log coordinates of −0.238 and −0.187 at T_b values of 35 and 40°C, respectively. The regression equations predicted decreases in stride frequency of 40–48% during growth from 4 g to 60 g.

At a T_b of 35°C V_R was not influenced significantly by body mass (Fig. 1C). At 40°C larger lizards ran significantly faster (Fig. 2C; Table 1). The regression equation describing V_R at 40°C as a function of M_b had a slope of 0.093 and predicted a V_R of 3.12 m s^{-1} at a body mass of 4 g and 4.01 m s^{-1} at a body mass of

Table 1. *Allometric relationships (logy = loga + blogM$_b$; M$_b$ in g) of body dimensions, sprint running performance at 35 and 40°C, and contractile properties at 40°C of fast-twitch skeletal muscle during growth in the lizard Dipsosaurus dorsalis*

y	Regression (logy on logM$_b$)				Reduced major axis		
	r	a	b	95% confidence interval	a	b	95% confidence interval
Body dimensions							
L$_{SV}$ (mm)	0·988	32·9	0·324	0·309–0·338	32·5	0·327	0·313–0·342
L$_{HL}$ (mm)	0·987	28·9	0·289	0·274–0·304	28·6	0·292	0·277–0·308
Sprint running							
L$_S$ (40°C) (m)	0·838	0·109	0·273	0·213–0·333	0·094	0·326	0·261–0·408
L$_S$ (35°C) (m)	0·883	0·109	0·271	0·216–0·325	0·098	0·306	0·250–0·376
f (40°C) (Hz)	−0·745	25·39	−0·187	−0·130−−0·244	30·89	−0·251	−0·181−−0·354
f (35°C) (Hz)	−0·881	26·72	−0·238	−0·186−−0·282	29·36	−0·265	−0·216−−0·327
V$_R$ (40°C) (m s^{-1})	0·488	2·741	0·093	0·037–0·149			(see text)
V$_R$ (35°C) (m s^{-1})	0·267	NS					
Contractile properties							
L$_0$ (mm)	0·987	6·19	0·303	0·275–0·331	6·128	0·307	0·280–0·337
P$_0$ (kN m^{-2})	0·270	NS					
P$_{TW}$/P$_0$	0·083	NS					
tP$_{TW}$ (ms)	0·809	5·51	0·194	0·133–0·254	4·79	0·239	0·173–0·330
t50%R (ms)	0·644	6·67	0·114	0·055–0·172	5·51	0·177	0·101–0·311
V$_{max}$ (L$_0$ s^{-1})	−0·614	25·71	−0·084	−0·022−−0·146	29·8	−0·137	−0·053−−0·352
V$_{0·3P_0}$ (L$_0$ s^{-1})	−0·730	8·64	−0·068	−0·032−−0·105	9·27	−0·094	−0·052−−0·174
Ẇ$_{max}$/V$_{max}$P$_0$	0·172	NS					

After log transformation, data were described with both standard least-squares regression (logy on logM$_b$) and reduced major axis. L$_{SV}$, snout–vent length; L$_{HL}$, hindlimb length; L$_S$, stride length; f, stride frequency; V$_R$, running velocity; L$_0$, muscle length at which P$_0$ is maximal; P$_0$, maximal isometric tetanic force; P$_{TW}$, peak isometric twitch force; tP$_{TW}$, time to P$_{TW}$; t50%R, time from P$_{TW}$ to 50 % of P$_{TW}$ during relaxation; V$_{max}$, shortening velocity predicted at zero force; V$_{0·3P_0}$, shortening velocity predicted at 0·3P$_0$; Ẇ$_{max}$, maximal power output; NS, not significant.
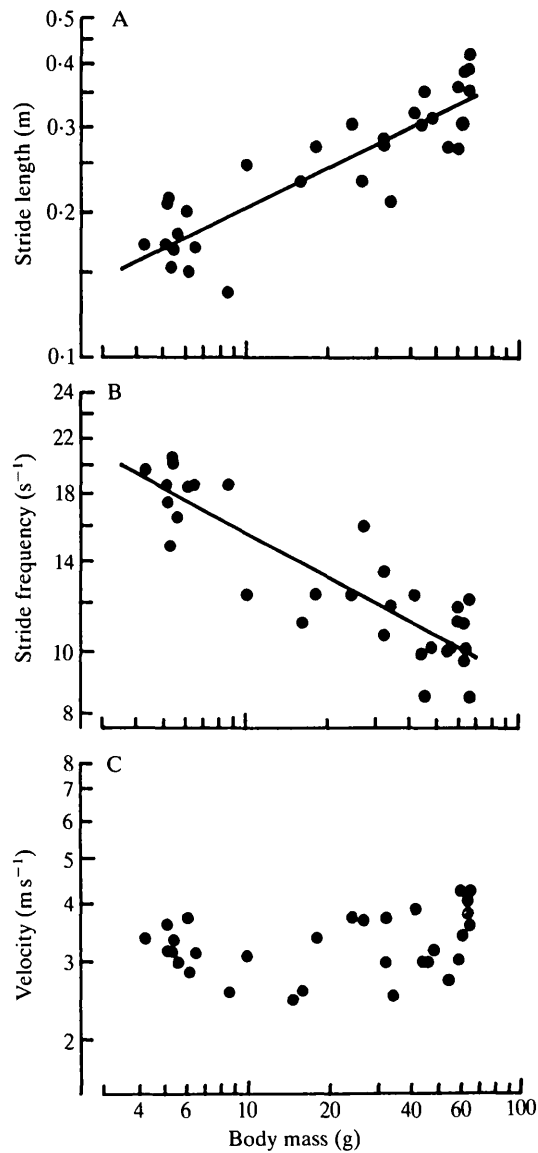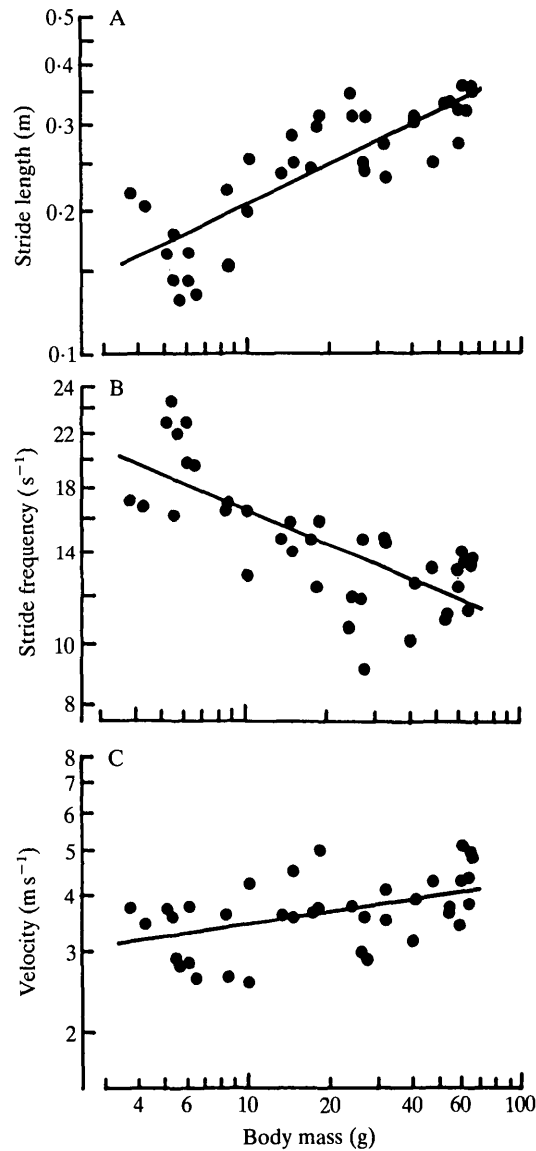
Fig. 1. Stride length (A), stride frequency (B) and running velocity (C) as functions of body mass during sprint runs with a body temperature of 35°C in the lizard *Dipsosaurus dorsalis*. Data are plotted on log–log coordinates. Solid lines are the regression lines.

60 g. Multiplying the RMA equations for f and L_S at a T_b of 40°C yielded a predicted slope of $logV_R$ on $logM_b$ of 0·074 (see Materials and methods for justification of this indirect estimate). Whatever the statistical uncertainties, clearly body mass had little effect on V_R of *Dipsosaurus* sprinting with T_b values of 35°C or 40°C.

Fig. 2. Stride length (A), stride frequency (B) and running velocity (C) as functions of body mass during sprint runs with a body temperature of 40°C in the lizard *Dipsosaurus dorsalis*. Data are plotted on log–log coordinates. Solid lines are the regression lines.

Isometric contractile properties

The length of the FG-IF at which isometric force is maximal (L_0) scaled with body mass, approximately as expected from the scaling of L_{HL} (regression slope of $\log L_{HL}$ *versus* $\log M_b$ of 0·303; Table 1). Mean P_0 was 214 ± 10 (s.e.m.) kN m^{-2} and was not significantly correlated with body mass ($r = 0.27$, F = 0·15, $N = 25$ for
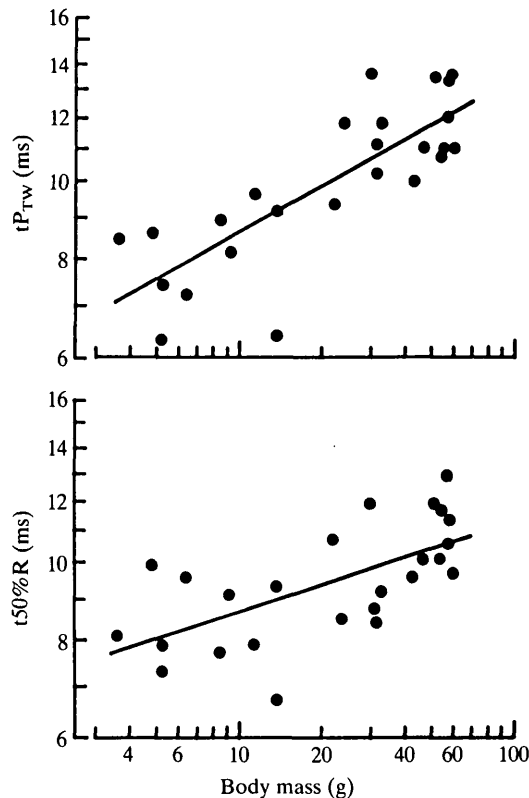
Fig. 3. Time to peak tension (tP_{TW}) and time from peak tension to 50 % relaxation ($t50\%R$) in isometric twitch contractions as functions of body mass in the lizard *Dipsosaurus dorsalis*. Data are plotted on log–log coordinates. Solid lines are the regression lines.

$logP_0$ *versus* $logM_b$). The ratio of peak twitch force to P_0 was 0.303 ± 0.023 and was also unrelated to M_b [$r = 0.08$, $F = 0.15$, $N = 25$ for $log(P_{TW}/P_0)$ *versus* $logM_b$]. The time to peak force in the twitch (tP_{TW}) and the time from peak force to 50 % relaxation ($t50\%R$) increased with increasing body mass (Table 1; Fig. 3). The regression slopes for $logtP_{TW}$ and $logt50\%R$ *versus* $logM_b$ were 0.194 and 0.114, respectively. Note that $logt50\%R$ may not be strictly a linear function of $logM_b$.

Force–velocity curves

Fig. 4A,B illustrates a typical isotonic contraction recorded digitally. One potential problem with differentiating digitally acquired position information to determine velocity is the tendency to amplify greatly a small amount of signal noise. By averaging 1- to 2-ms portions of the position trace before differentiation, this problem was avoided and the peak shortening velocity could be specified with some precision (Fig. 4C).

Force–velocity data from the FG-IF were fitted quite precisely using nonlinear techniques (Fig. 5). Significant decreases were found in the velocity of contraction
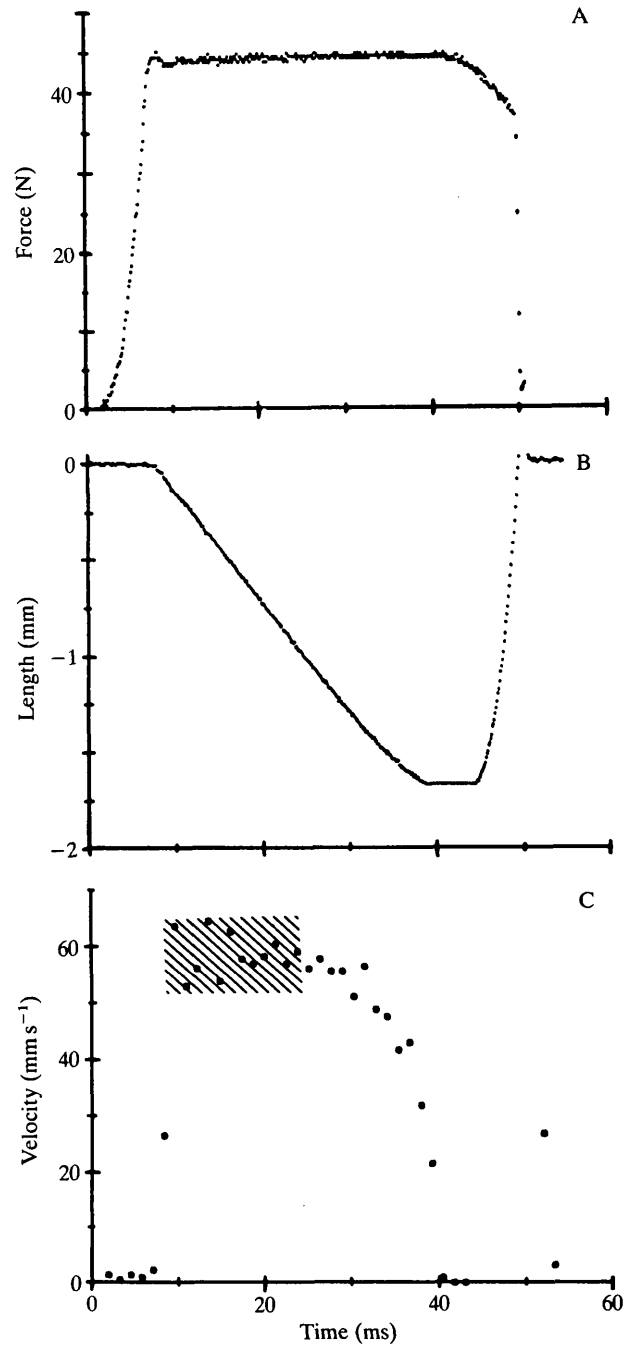
Fig. 4. Typical record of an isotonic contraction of the fast-glycolytic region of the iliofibularis muscle of the lizard *Dipsosaurus dorsalis*. (A) Force; (B) length; (C) shortening velocity calculated by averaging and differentiating the length trace. The velocity reported for this contraction was the mean of the points within the hatched area.
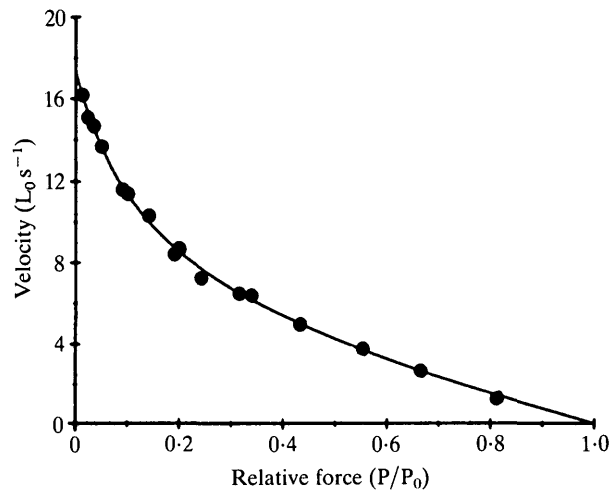
Fig. 5. Typical force–velocity curve of the fast-glycolytic region of the iliofibularis muscle of the lizard *Dipsosaurus dorsalis*. Solid line was fitted by nonlinear least squares (see Materials and methods).

with increasing body mass. These changes were relatively small as can be seen from the relationship between $\log V_{max}$ and $\log M_b$ (Fig. 6A), which yields a regression slope of -0.084 (Table 1). Because V_{max} is derived by extrapolation from the steeply rising force–velocity curve, this characteristic velocity is subject to relatively large errors in estimation compared with other points on the curve. For this reason I also calculated the velocity at $0.3 P_0$ ($V_{0.3P_0}$), which can be estimated accurately for a given muscle (Fig. 5). $V_{0.3P_0}$ also declined significantly with increasing body mass (Fig. 6B). The regression slope of this relationship was -0.068 for log-transformed data, and the error limits for this estimate of the slope were narrower than those for V_{max} (Table 1). The degree of curvature of the force–velocity curves was estimated using the power ratio: $\dot{W}_{max}/V_{max}P_0$, where $\dot{W}_{max}$ is the maximum power output (Marsh & Bennett, 1986a). This ratio averaged 0.112 ± 0.003 for all muscles used and did not vary with body mass ($r = 0.172$, $F = 0.424$, $N = 16$ for log-transformed data).

Discussion

Allometry of running performance and limb dimensions

Dipsosaurus are capable of effective high-speed locomotion throughout growth from hatching masses of 3–4 g to adult masses of 40–70 g (Figs 1, 2). These sprint runs were performed predominantly bipedally at all body masses. Maximal sprint velocities of hatchlings are not significantly different from the values for adults at a T_b of 35 °C. At a T_b of 40 °C there is a slight but significant increase in V_R with increasing body mass. These two T_b values bracket the preferred body tempera-ture and are within the field-active range of body temperatures of this species (Norris, 1953). Interspecific data on iguanids using similar techniques to this study
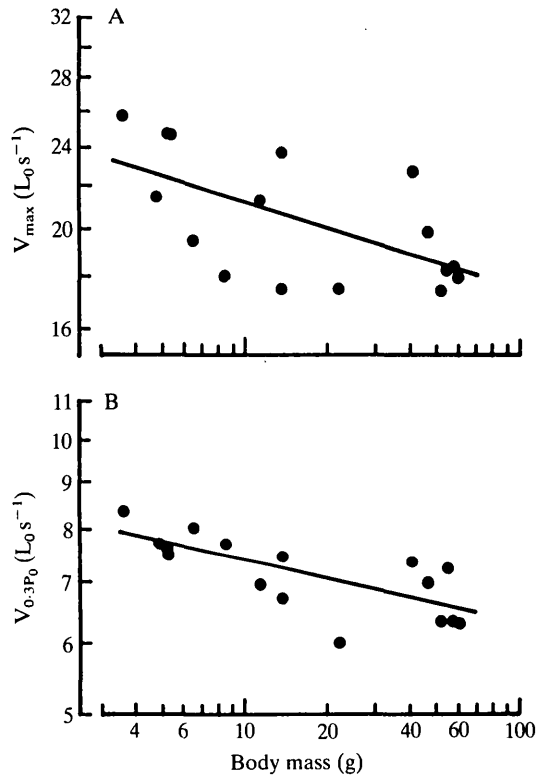
Fig. 6. Predicted shortening velocities of the fast-glycolytic region of the iliofibularis muscle at zero force (V_{max}) and at $0.3P_0$ as functions of body mass in the lizard *Dipsosaurus dorsalis*. Data are plotted on log–log coordinates. Solid lines are the regression lines.

indicate that V_R is influenced slightly by body mass ($V_R \propto M_b^{0.11}$; R. L. Marsh, unpublished observations), and that young *Dipsosaurus* run as fast as, or faster than, would be predicted for adults of similar size (compare also the data on small lizards in Huey, Bennett, John-Alder & Nagy, 1984).

Hindlimb length in *Dipsosaurus* scales with the 0.29 power of body mass (Table 1). Similar exponents for hindlimb span have been noted during ontogenesis in other species of lizards (Garland, 1984, 1985). These exponents differ significantly from the expectations of geometric similarity in length measurements ($L \propto M_b^{0.333}$). The scaling of L_S is close to what would be expected based on L_{HL}. Nevertheless, the deviation of L_S from geometric similarity is slight enough to make it clear that young *Dipsosaurus* achieve high speeds more by having considerably higher stride frequencies than adults than by taking relatively longer strides (Figs 1, 2).

The allometric exponents for stride frequency of *Dipsosaurus* running at maximal speed (−0.19 to −0.24 based on regression; −0.25 to −0.27 based on RMA) differed from the exponents predicted either by geometric similarity

criteria ($b = -0\cdot333$) as assumed by Hill (1950) or by McMahon's (1975, 1984) elastic similarity model ($b = -0\cdot125$). The 95% confidence intervals of both the regression and RMA exponents at T_b values of 35°C and 40°C exclude the slope predicted by elastic similarity. The slope expected from geometric similarity was outside the 95% confidence intervals of the regression slopes at both T_b values, and was excluded by the 95% confidence intervals of the RMA slope at a T_b of 35°C, but not at 40°C (Table 1). The lack of agreement between the measured and predicted values for the allometric scaling of f may not be due to any inherent flaw in the mechanical principles upon which the models are based, but instead may depend on differences between the actual scaling of the lizards' morphological dimensions and the scaling predicted or assumed by the models (see below). Few data exist to compare with the allometry of f in this lizard. I know of no comparable observations for other bipedal runners. McMahon (1975) has cited information on f at the trot–gallop transition of quadrupedal mammals in support of his elastic similarity model, but Alexander & Jayes (1983) have pointed out that the determinants of running dynamics at top speed differ from those acting at the trot–gallop transition. Alexander, Langman & Jayes (1977) examined the effect of body mass on stride frequency during high-speed galloping by free-living ungulates, and found that the allometric exponent for maximal stride frequency was $-0\cdot18$, based on regression analysis. Their data yielded an RMA slope of $-0\cdot23$ (calculated as b/r from their regression data). These data, collected at near top speed in mammals, thus reveal an allometry of stride frequency similar to that found during high-speed sprint runs in *Dipsosaurus*.

Shortening velocity and stride frequency

Hill (1950) in his widely cited, if rather speculative, review assumed that natural selection would have optimized contractile properties to suit the functions of muscles *in vivo*. The primary focus of his discussion was the isotonic force–velocity curve. Based on this curve, measured for frog muscles *in vitro*, he predicted that muscles producing natural movements will do so at a shortening speed that optimizes power output and efficiency. As a consequence of this prediction, he further hypothesized that the maximal velocity of shortening of homologous muscles in dimensionally similar animals will be directly related to the frequency of contraction (see also McMahon, 1984, p. 274). In addition to dimensional similarity, this hypothesis assumes force–velocity curves of similar shape in animals of different sizes. Hill reasoned that the intrinsic shortening velocity should be adjusted to allow the muscles to drive the limbs at their optimum frequency. Because Hill assumed geometric similarity, his hypothesis resulted in the prediction that V_{max} will be proportional to $M_b^{-0\cdot333}$. Although subsequent authors have adopted different scaling rules for body dimensions, they have retained the prediction that V_{max} will be proportional to contractile frequency (e.g. McMahon, 1975).

The present data on *Dipsosaurus* clearly indicate that this prediction is incorrect for this species. Based on regression analysis, stride frequency was

proportional to about $M_b^{-0.2}$, but velocity of shortening scaled with an exponent of approximately -0.07. Apparently, the intrinsic shortening velocity of the muscles does not directly or solely determine maximal stride frequency. Instead, frequency appears to be determined, at least partially, by biomechanical constraints imposed by body mass independently of the intrinsic shortening velocity. This conclusion is supported by studies of the effects of temperature on sprint dynamics and contractile properties of skeletal muscles in adult lizards (Marsh & Bennett, 1985, 1986a,b). These studies demonstrated that, over a large range of T_b values, shortening velocity is affected by temperature to a much greater extent than is stride frequency. This lack of correspondence in thermal effects can also be noted in the present study, which demonstrated almost no difference in stride frequency at T_b values of 35°C and 40°C (Figs 1, 2), whereas shortening velocity is expected to change by 30% over this interval (Marsh & Bennett, 1985). As indicated above, Hill's original predictions were based on the isotonic force–velocity curve; however, muscles shortening *in vivo* probably rarely, if ever, contract isotonically. The various muscles used in running animals undergo a variety of patterns of lengthening and/or shortening when activated during a stride (e.g. Goslow *et al.* 1981). This more complicated pattern of contractions may damp the effects of the intrinsic shortening velocity of the muscles on the actual velocities achieved as segments of the body are accelerated and decelerated during the stride. Further empirical and theoretical studies should help resolve this question.

The mechanisms responsible for the small decrement in V_{max} with increasing mass are unknown. Sarcomere length can potentially influence intrinsic shortening velocity (Josephson, 1975), but ultrastructural studies indicate that thick filament lengths are constant during development in *Dipsosaurus* (R. L. Marsh, unpublished results). Shortening velocity may also be correlated with myosin ATPase activity (Bárány, 1967). Data on fish muscle (Witthames & Walker, 1982) show a negative correlation between myofibrillar ATPase and body length, and indicate that this enzymatic activity can change developmentally.

Kinetics of isometric twitches and stride frequency

Theoretical treatments of the allometry of running have not considered the kinetics of isometric twitch contractions. This type of contraction may yield information regarding the time course of cross-bridge binding and contractile deactivation following a single stimulus. The time to peak tension in the twitch is sometimes considered a measure of the time course of activation (e.g. Akster, Granzier & ter Keurs, 1985), but this notion is probably mistaken. The time course of activation, caused by Ca^{2+} release from the sarcoplasmic reticulum and binding to troponin, is generally considered to be very rapid and is not rate-limiting for twitch kinetics (Josephson, 1975; Cannell, 1986). The classic two-component model of muscle (Hill, 1970) attributes the delay in tension development in the twitch to the presence of series elastic elements, which take time to be stretched by

the contractile elements. However, within rather large limits, the amount of series elasticity influences the rate of rise in tension but not tP_{TW} (Hill, 1951). According to Hill's model the peak twitch force represents a point determined by the steep curve representing the action of deactivating processes (e.g. see Hoyle, 1983, pp. 229–236). Recent interpretations assign some of the series elasticity to the cross-bridges themselves, and evoke a relatively slow time constant for cross-bridge attachment as the cause for the delay in force development (e.g. Julian & Moss, 1976; also Woledge, Curtin & Homsher, 1985, pp. 35–39, 85). Again, however, cross-bridge attachment rate should mainly influence the rate of rise in tension. Experimental manipulations that result in changes in tP_{TW} lend empirical support to the correlation of twitch time and the systems responsible for deactivation (Heilman & Pette, 1979; Fitts *et al.* 1980). Therefore, regardless of the possibly complex determinants of tP_{TW}, twitch kinetics are probably dominated by how quickly a muscle is turned off, not how quickly it is turned on, and this is assumed to be true in the subsequent discussion. In this context, tP_{TW} and contraction time $(t_C = tP_{TW} + t50\%R)$ are measures related to the rate of deactivation. The term 'deactivation' is used in preference to 'relaxation', which commonly refers to the actual decline in force measured in *in vitro* contractions. In twitch contractions the cross-bridges are actually beginning to be deactivated before peak force is reached. The twitch kinetics reported in this study are for twitches immediately following a period of activity, because these contractions are assumed to be more relevant to the repetitive activation that occurs during running.

The time course of the twitch is lengthened considerably with increasing body mass in *Dipsosaurus* (Fig. 3). The allometry of twitch kinetics matches approximately the scaling of stride time $(t_S = 1/f)$, and results in a constant relationship between the time for deactivation of the muscles and the time required for a stride. This constancy is illustrated by calculating ratios of the times predicted by the regression equations. Because a given muscle only operates for approximately one-half of the stride, $t_S/2$ is used in the calculated ratios. The predicted values of $2t_{TW}/t_S$ are 0·282 and 0·287 for a 4-g and 60-g animal, respectively. If one includes $t50\%R$ in the estimate of twitch kinetics, the predicted values of $2t_C/t_S$ are 0·552 and 0·579 for a 4-g and 60-g animal, respectively.

Optimum twitch kinetics may be determined by two opposing influences. Keeping the twitch contraction time shorter than $1/2t_S$ would allow the muscles to be activated by short tetanic bursts of stimuli and be deactivated rapidly before the antagonistic muscles are activated in the next phase of the stride. Marsh & Bennett (1985, 1986b) have suggested that as t_C approaches values equal to $1/2t_S$ (because of the effects of low T_b) stride frequency may be directly limited by twitch kinetics. Conversely, muscles with short twitch times probably pay a penalty in lowered economy of force production because of the energy required for Ca^{2+} pumping by the sarcoplasmic reticulum (for general estimates of this cost see Woledge *et al.* 1985). Consequently, an advantage exists for decreasing the rate of deactivation in concert with the lowered stride frequency as body mass increases with growth. To

the extent that tP_{TW} also reflects cross-bridge binding kinetics, more rapid twitches may also allow more rapid force development.

As with the change in V_{max}, the mechanisms responsible for lengthening the twitch in the fast-twitch glycolytic fibres of *Dipsosaurus* during growth are unknown. Obvious candidates include those characteristics that influence deactivation, such as increases in the surface area of sarcoplasmic reticulum membranes and the activity of the Ca^{2+}-ATPase per unit of membrane surface area. Kinetic properties of the contractile proteins that influence cross-bridge binding may also change. Further studies should address these issues and also the intriguing question of what feedback mechanisms might trigger alterations in muscle structure and biochemical characteristics as body mass changes.

Ontogenesis of contractile properties in other animals

I am aware of no other developmental data for an ectotherm with which the ontogenetic changes in contractile properties of *Dipsosaurus* can be compared. The development of contractile properties has been studied in several species of mammals and birds (Buller *et al.* 1960; Close, 1964; Gordon *et al.* 1981). These studies have concentrated on the transition from slow to fast contractions that occurs in the fast-twitch fibres of these groups of animals. This transition occurs before hatching or birth in precocially developing species (Gordon *et al.* 1981), and during postnatal development in species with altricial development (Close, 1964). Although this developmental change is well documented, its functional significance has received little attention. Perhaps slow muscles allow energy savings at a time during development when rapid movements are not required. Two altricial mammals, the laboratory rat and the domestic cat, have been most thoroughly investigated. In these species, the twitch kinetics of fast-twitch fibres change little after the transition from slow to fast contractions (Buller *et al.* 1960; Close, 1964). However, predominantly slow-twitch soleus muscles in these species do show increases in twitch time after decreases early in development. This ontogenetic slowing of the soleus has been suggested to be due to decreases in the proportion of fast-twitch fibres (Kugelberg, 1976), and not to changes in the contraction time of a single fibre type, as occurs in *Dipsosaurus*.

Mammals and birds, particularly those with altricial development, are dependent on parental care for a significant fraction of their growth, and may not require effective high-speed locomotion early in life. In contrast, most ectotherms rely on their own locomotor skills for foraging and predator avoidance almost from the time of hatching, and may be assumed to have been selected for effective locomotion at an early age. Of course, because the mechanisms that trigger the developmental change noted in field-collected *Dipsosaurus* are not known, caution must be used in interpreting the results obtained with cage-reared mammals and birds that are never given the opportunity to run at high speed. Levels of activity may be too low to trigger a slowing of the muscle as body mass increases. Further data on precocial endotherms would be useful in this context.

Comparison of predicted and measured stride frequencies

Although the allometry of sprint stride frequencies of *Dipsosaurus* does not agree with the predictions from geometric or elastic similarity, deviations in the observed direction can be predicted using the reasoning of Hill (1950) or McMahon (1984) combined with observed dimensions of lizards during growth. Hill's arguments were based on considerations of inertial acceleration and predict the following proportionality:

$$t \propto \left(\frac{lm}{F}\right)^{0.5}, \tag{1}$$

where t is the time to accelerate a mass m through a distance l, which is assumed to be proportional to the characteristic length dimension of the limbs, and F is the force developed by the muscles. Because muscle force per cross-sectional area is constant (see data above for P_0), F is proportional to the cross-sectional area of the muscle fibres. Consequently, if strict geometric similarity is maintained, the proportionality reduces to:

$$t \propto M_b^{0.333}, \quad \text{or} \quad 1/t \propto f \propto M_b^{-0.333}. \tag{2}$$

However, the actual dimensions of lizards during growth show significant departures from geometric similarity. The present study and others (Garland, 1984, 1985) indicate that, considered intraspecifically, the length of the hindlimbs of lizards is proportional to $M_b^{0.29}$. Data from this study also indicate that this proportionality probably holds for muscle fibre lengths as well. Additionally, Garland (1984, 1985) has shown that the mass of the muscles in the hindlimb during growth scales to a power of body mass greater than 1·0. Given the observed scaling of length, and taking the mean exponent from Garland's studies of 1·1 for muscle mass, muscle cross-sectional area is predicted to be proportional to $M_b^{0.81}$ If m in proportionality 1 is assumed to be the mass of a limb, we then have:

$$t \propto \left(\frac{M_b^{0.29}M_b^{1.1}}{M_b^{0.81}}\right)^{0.5} \propto M_b^{0.29}. \tag{3}$$

If instead the acceleration of the body mass is important then the proportionality becomes:

$$t \propto \left(\frac{M_b^{0.29}M_b}{M_b^{0.81}}\right)^{0.5} \propto M_b^{0.24}. \tag{4}$$

This predicted allometry is in substantial agreement with the data on stride frequency in *Dipsosaurus*.

McMahon (1984, pp. 220–272) derived the following proportionality based on considerations of resonant rebound:

$$f \propto rl^{-2}, \tag{5}$$

where r is the characteristic radius and l is the characteristic length of the limb. For

geometrically similar animals this proportionality yields the same prediction as considerations of inertial loading and acceleration, i.e. $f \propto M_b^{-0.333}$. However, for elastically similar structures, where $r \propto M_b^{0.375}$ and $l \propto M_b^{0.25}$, resonant rebound predicts $f \propto M_b^{-0.125}$. Interestingly, an animal structured and moving its limbs as predicted by elastic similarity criteria would not also preserve similarity of inertial loading because proportionality 1 above would predict $f \propto M_b^{-0.25}$.

If we again take the approximate dimensions of lizards based on measurements rather than similarity models, proportionality 5 becomes approximately: $f \propto M_b^{-0.2}$. These arguments suggest that the hindlimbs of lizards are dimensioned and move dynamically in a manner that provides approximate similarity of inertial loading based on body mass and that also nearly satisfies the requirements of resonant rebound given the actual dimensions of the limbs. Of course, any investigator who makes forays into the realm of allometry must be aware that the congruence of theoretically and empirically derived exponents does not provide hard evidence of underlying mechanisms. Nevertheless, the series of calculations above illustrates at least one important point. Any biomechanical model of running should predict a coherent set of exponents for both animal dimensions and running dynamics. If the model does not accurately predict the physical dimensions of a series of animals of different mass, agreement between measured exponents for running dynamics and exponents derived from the predicted dimensions should not be taken as support for the model.

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