Interindividual Differences in Leg Muscle Mass and Pyruvate Kinase Activity Correlate with Interindividual Differences in Jumping Performance of *Hyla multilineata*

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ABSTRACT

Frog jumping is an excellent model system for examining the structural basis of interindividual variation in burst locomotor performance. Some possible factors that affect jump performance, such as total body size, hindlimb length, muscle mass, and muscle mechanical and biochemical properties, were analysed at the interindividual (intraspecies) level in the tree frog *Hyla multilineata*. The aim of this study was to determine which of these physiological and anatomical variables both vary between individuals and are correlated with interindividual variation in jump performance. The model produced via stepwise linear regression analysis of absolute data suggested that 62% of the interindividual variation in maximum jump distance could be explained by a combination of interindividual variation in absolute plantaris muscle mass, total hindlimb muscle mass (excluding plantaris muscle), and pyruvate kinase activity. When body length effects were removed, multiple regression indicated that the same independent variables explained 43% of the residual interindividual variation in jump distance. This suggests that individuals with relatively large jumping muscles and high pyruvate kinase activity for their body size achieved comparatively large maximal jump distances for their body size.

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Introduction

Natural selection acts within populations by favouring survival in those individuals with variations of phenotypic traits that improve a major component of fitness, increasing the likelihood of those traits being passed on to the next generation (Arnold 1983; Jones 2001). Locomotor performance, particularly in the context of predator avoidance, is considered important in the survival of vertebrates and is often assumed to correlate with lifetime fitness (Arnold 1983). The links between phenotypic traits, fitness, and locomotor performance can be investigated by analysing the effects of a phenotypic trait on locomotor performance and then analysing the effect of locomotor performance on fitness (Arnold 1983). Previous studies have demonstrated that escape from predators by tadpoles can depend on morphological traits (Van Buskirk and McCollum 1999), burst swimming speed, and evasiveness (Watkins 1996). Jayne and Bennett (1990) found that garter snakes with higher sprint speed were more likely to survive from one year to the next. Survival in hatchling lizards has been found to be positively correlated with body size, sprint speed, and stride length but negatively correlated with growth rate (Warner and Andrews 2002; Miles 2004). Studies of interindividual variation in locomotor performance are, therefore, important to determine the functional basis of differences in performance (because if a trait and performance are functionally associated, they should also be significantly correlated) and their ecological and evolutionary consequences (Bennett 1987).

Evidence of functional links between morphological traits and locomotor performance comes mainly from interspecific comparisons, although both interspecific and interindividual relationships clearly show that many differences in anuran locomotor performance are related to body size (Emerson 1978; Zug 1978; Marsh 1994; Tejedo et al. 2000; Wilson et al. 2000). However, the scatter within each allometric scaling relationship suggests that variables other than body size may also affect burst locomotor performance in reptiles and amphibians. For example, interspecific differences in frog takeoff speed have been positively correlated with body-mass-specific hindlimb

thigh muscle mass and with body-length-specific hindlimb length (Choi et al. 2003). Although many studies have demonstrated that differences in locomotor patterns and performance between anuran species are also linked with differences in the morphological, enzymatic, and mechanical properties of muscles (Marsh 1994), these need not necessarily apply to intraspecific correlates of locomotor performance. A few studies have examined size-independent interindividual variation in the underlying factors explaining variation in adult locomotor performance within species of lizards and salamanders (e.g., Garland 1984; Bennett et al. 1989) but very few within adult anuran species (e.g., Longphre and Gatten 1994; Tejedo et al. 2000). Although previous studies on adult anurans have demonstrated interindividual differences in locomotor performance and hindlimb muscle mechanics (Navas et al. 1999; Wilson et al. 2002), they have not attempted to correlate the two findings.

Frog jumping is an ideal system for examining the structural basis of interindividual variation in locomotor performance because the contractile properties of frog hindlimb muscle have been extensively studied (Marsh 1994). Ranid species have highly adapted musculosketal systems designed for maximal power production during jumping (Lutz and Rome 1994), and hylids (tree frogs), which often exhibit typical jump-specialist morphology, produce relatively long jumps when compared with many other frog taxa (Zug 1978; Marsh 1994).

When standard projectile equations are applied to jumping, they suggest that increased hindlimb length and mass of jumping muscles and beneficial alteration of the origin and/or insertion of jumping muscles should improve animal jump performance (for a review, see Emerson 1985). These predictions have been empirically confirmed in studies between species of frogs, and the studies have concluded that, in comparison with nonjumping species, jumping specialists have longer hindlimbs, larger jumping muscles, and a more proximal insertion of hip extensor muscles in jumping (for a review, see Emerson 1985).

This investigation focuses on the relationships between morphology and performance in anuran amphibians and asks the following: What is the extent of interindividual variation in locomotor performance within a population of tree frogs that use jumping as the main locomotor mode? How does this variation depend on body size, and to what extent can individual animal performance be explained in terms of morphometry and the metabolic and biomechanical properties of the muscles supporting locomotion? Additionally, we investigate whether the generalisations about morphometrics and jumping performance that are evident at the interspecies level can also be detected at the interindividual level. To answer these questions, we explore in detail some relevant aspects of hindlimb morphology, biochemistry, and physiology that, independent of overall body size, might be used to predict differences in the jumping performance of conspecific individual frogs within the Neotropical tree frog *Hyla multilineata*. This species is a hylid with relatively long legs compared with snout-vent length (indicating it should produce relatively long jumps compared with frog species that are less specialised for jumping, such as *Bufo* spp.; Zug 1978). We studied traits that, according to published literature, are relevant to anuran locomotion including hindlimb length and muscle mass, force production of isolated plantaris muscle, and the metabolic profile of the hindlimb muscles as derived from enzyme activities. Such information would illustrate the magnitude of the variance in limb morphology and physiology that characterises a population and would allow for a better comprehension of how this variance relates to concomitant variance in locomotor performance.

Material and Methods

Animals

Calling male *Hyla multilineata* Boulenger, 1887 were captured between March and April 2002 from two different nature reserves (Anhanguera [$N = 14$] and Intervales [$N = 23$]) in the state of São Paulo, Brazil. Frogs were temporarily transferred to damp cloth bags containing plant material until they could be placed in glass tanks (0.29 m × 0.18 m × 0.13 m) at the University of São Paulo. Frogs were kept two to each tank at 24° ± 2°C in a moist environment with plant material, in a 14L : 10D cycle. Frogs were allowed to recover overnight. Jumping experiments were performed 1–3 d after capture and mechanical experiments 2–5 d after capture. The Anhanguera and Intervales populations were kept for 2–3 and 4–5 d, respectively, from capture to the end of the mechanics experiments. Such short time periods in captivity do not affect overall metabolic traits in hylid frogs (Navas and Gomes 2001).

Jumping Performance

The glass tanks containing the frogs were moved to a temperature-controlled room (air temperature maintained at 25° ± 0.5°C) at least 1 h before jumping measurements. This temperature is close to the upper extreme of active field temperatures for this species, which range between 19.1° and 26.4°C, according to agar models placed in the field. Immediately before jumping, a frog was removed from its tank and placed on the floor of the room. The frog was encouraged to jump along the floor of the experimental room by lightly touching its urostyle, with at least five jumps recorded from each individual. The distance of each jump was recorded by measuring the distance between each damp mark on the floor. Jumping performance measurements were repeated twice more to give a total of three trials for each individual. Each trial was separated by at least 5 h. The jump that covered the greatest distance of all the trials for each individual was used as a measure of maximum jumping performance.

Dissection

Frog snout-vent length was measured to the nearest 0.1 mm using vernier callipers. Frog body mass was measured to the nearest 0.01 g using an electronic balance (V-1mg, Acculab, Bradford, MA), and then the frog was cooled on ice for 2 min. Frogs were euthanised by pithing and transection of the spinal cord. Both frog legs were then pinned out in oxygenated (100% O_2) Ringer solution (composition [mM]: NaCl, 115; KCl, 2.5; Na_2HPO_4, 2.15; NaH_2PO_4, 0.85; sodium pyruvate, 5.0; $CaCl_2$, 1.8; pH 7.2 at 25°C) kept on ice. All muscles from the upper and lower right leg (excluding the toes) were removed and dabbed with blotting paper before being frozen in liquid nitrogen. Frozen muscle tissue was then stored at −85°C before biochemical measurements. The plantaris longus (gastrocnemius) muscle was dissected from the left leg, with a small piece of bone left at the end of both the proximal and distal tendons. Plantaris was chosen because it is one of the main muscles that powers jumping in frogs (Marsh 1994), representing 13.0% ± 0.95% (mean ± SD, $N = 37$) of total leg muscle mass of the individuals used in this study. The plantaris is a pennate muscle that originates from the aponeurosis covering the knee and inserts distally onto the plantar surface of the foot (Duellman and Trueb 1994). The primary function of plantaris is to extend the ankle. Aluminium foil clips were clamped around the whole length of the tendons at either end of the muscle to reduce problems of series elastic compliance as much as possible, leaving the sections of bone unwrapped to avoid tendon slippage in the clips.

All other muscles of the upper and lower left hindlimb were removed, their wet mass was determined to the nearest 0.1 mg using a balance (FA1604, Shapping, Shanghai), and then these muscles were placed in a drying oven overnight before dry mass was measured. Total hindlimb muscle mass (excluding plantaris) was calculated by multiplying the mass of muscle in the left hind leg (excluding plantaris) by 2 (to estimate the total hindlimb muscle mass, excluding plantaris, of the whole frog). Soft tissue was removed from the bones of the right leg by beetles of the Dermestidae family. Femur, tibiofibula, and tarsus bone lengths were then measured to the nearest 0.1 mm using vernier callipers. Total leg bone length was estimated as the sum of these three bone lengths; that is, any possible interindividual variation in length of longest toe and potential effect on performance has not been measured.

Isometric Studies on Isolated Muscle

The muscle preparation was attached via the foil clips to a strain gauge (model 1030, UFI, Morro Bay, CA; calibrated to 283 mN V^{-1}) at one end and a displacement transducer (V201, LDS, Royston, UK) at the other. A linear variable-displacement transformer (DFG5.0, Solartron Metrology, Bognor Regis, UK; calibrated to 1.35 mm V^{-1}) was used for position detection. The muscle was maintained at 25°C in circulating oxygenated (100% oxygen) Ringer solution. The preparation was stimulated via parallel platinum electrodes while held at constant length to generate a series of isometric twitches. Stimulus amplitude (16–17 V), pulse width (1.2–1.8 ms), and muscle length were optimised to yield the maximum isometric twitch force. During optimisation of the twitch response, a number of parameters were repeated to ensure that the optimal conditions had been reached independently of the occasional slight improvement (recovery from dissection) that occurred at this stage of the experiment. Muscle stimulation and length changes were controlled using custom written software (Testpoint, CEC, Bedford, NH) via a D/A board (KPCI3108, Keithley Instruments, Cleveland). Force and length data were sampled at a rate of 10 kHz. Optimal muscle length was measured using a microscope fitted with an eyepiece graticule. An isometric tetanic response was elicited by subjecting the muscle to a 200-ms train of stimulation. Stimulation frequency was optimised (60–90 Hz) to yield the maximal isometric tetanic force. A 5-min recovery period was allowed between each tetanic response. The tetanus activation rate was calculated by fitting a regression line to the first 50 data points after force had started to increase. After the first tetanus, another twitch response was elicited to confirm that the twitch generation capacity of the muscle was maintained.

Power Output of Isolated Muscle

Each muscle preparation was subjected to a constant-velocity shortening ramp using a total strain of 0.20 (20%) muscle length (a similar approach to that used by Lutz and Rome [1994]). Although this pattern of length change differed from that recently measured during locomotion in other muscles in large frogs (Gillis and Biewener 2000; Roberts and Marsh 2003), the exact in vivo muscle length changes in *H. multilineata* cannot be accurately measured because of their small size. Therefore, a constant-velocity shortening ramp was used on the premise that individual variation in power output would be apparent regardless of the exact strain waveform imposed on the frogs.

Muscles were stimulated to contract, and timing and duration of stimulation were altered to yield the maximum net power output during shortening. The stimulation parameters yielding maximal power were typically a stimulation phase of between −80 and −95 ms (where time 0 ms corresponds to when shortening begins) and stimulation duration of 70–100 ms (sufficient duration to maintain activation during shortening). A recovery period of 5 min was allowed before the muscle was subjected to another constant-velocity shortening ramp at a different velocity. This protocol was continued with velocity varied around that expected to yield maximum power output (between 1.2 and 2.4 muscle lengths per second) until maximal power output was obtained. A plot of force against

length during shortening enabled muscle work to be determined as the area underneath this line (Josephson 1985). Absolute muscle power output was calculated as the mean rate of work during shortening.

A set of control length change and stimulation parameters was imposed on the muscle every four to five runs to monitor variation in the muscle's ability to generate power. Any variation in power output was assumed to correspond to a change in the ability of the muscle to produce force. This enabled muscle power output in between the control runs to be corrected by assuming a linear decline in performance over time (James et al. 1995). Muscle preparations usually decreased by less than 5% in their ability to produce power over the time course of the experiment. Muscle power output was also expressed relative to plantaris wet muscle mass (determined to the nearest 0.1 mg at the end of the experiment using an electronic balance [FA1604, Shapping], the muscle having first been blotted on absorbent paper to remove excess Ringer solution).

Biochemical Analysis

Biochemical analyses were performed to determine the overall metabolic profile of the hindlimb muscles of each frog. Interindividual variation in the overall metabolic profile of muscle (including aerobic metabolism) may be linked to interindividual variation in performance due to the functional tradeoff within skeletal muscle between endurance capacity and speed (Wilson et al. 2002, 2004; Wilson and James 2004). Frozen total hindlimb muscle samples ($N = 37$ individuals) were homogenised using a Teflon-glass homogenator (Marconi, Piracicaba, Brazil) in ice-cold Imidazol 20 mM (pH 7.4) buffer with EDTA 2 mM, NaF 20 mM, PMSF 1 mM, and Triton X 100%–0.1%. The homogenates were then submitted to sonication using a U-200S control unit (IKA-Labor Technik, Staufen, Germany) for three 10-s intervals and were directly used in the assays.

Pyruvate kinase (PK), lactate dehydrogenase (LDH), and citrate synthase (CS) activities were measured to indicate the relative glycolytic, lactate production, and oxidative capacities, respectively, of the hindlimb muscles. Activities of these enzymes were measured at 25°C by following the changes in NADH absorbance at 340 nm, or that of DTNB at 412 nm, under substrate saturation and no inhibitory conditions (V_{max}) on a spectrophotometer (DU-70, Beckman, Fullerton, CA). All reactions were performed in duplicate, initiated by addition of substrate, and the results were expressed in micromoles per minute per gram of wet muscle mass.

Enzyme protocols followed those of Bergmeyer (1983), with minor modifications as follows: pyruvate kinase (E.C. 2.7.1.40): Imidazol 100 mM (pH 7.0), $MgCl_2$ 10 mM; KCl 100 mM; ADP 2.5 mM; $F_{1,6}P_2$ 0.02 mM; NADH 0.15 mM; LDH 12 U mL^{-1}, muscle sample homogenated and phospho(enol)pyruvate −2.5 mM (omitted for control). Lactate dehydrogenase (E.C. 1.1.1.27): Imidazol 100 mM (pH 7.0); DTT 5 mM; NADH 0.15 mM, muscle sample homogenated and pyruvate 1 mM (omitted for control). Citrate synthase (E.C. 4.1.3.7): Tris 50 mM (pH 8.0); DTNB 0.1 mM; acetyl-CoA 0.2 mM, muscle sample homogenated and oxalacetate 0.9 mM (omitted for control). The ratios of enzyme activity PK/LDH and CS/LDH were calculated to indicate glycolytic and oxidative flux for the species.

Statistical Analysis

Where appropriate, results are presented as mean ± SD. All data were then log transformed for subsequent statistical analysis.

The regression relationship between log body mass and log snout-vent length for the 37 individuals used in this study yielded an r^2 value of 0.87, $P < 0.001$. Therefore, to simplify subsequent analyses, only snout-vent length was used to calculate body size residuals. The regression relationship between log-transformed snout-vent length and log-transformed independent variables was assessed. When a significant relationship existed between the log-transformed variables and snout-vent length, then residuals of snout-vent length were calculated. This data was subsequently used in discriminant function analysis, Pearson product moment correlations, and stepwise regression analysis. When there was no significant relationship between log-transformed variables and snout-vent length (for snout-vent length, there was no relationship with absolute plantaris maximum muscle power output, plantaris muscle LDH activity, or plantaris muscle PK activity), then the log-transformed data for that variable were used in the analysis with the snout-vent length residuals data of the other variables.

Discriminant function analysis was performed via Statistica (Statsoft, Tulsa, OK) using all independent variables expressed as body length residuals where appropriate (expressed as log-transformed data if no significant relationship existed between body length and the independent variable) to see whether the data from the two populations could be pooled. The subsequent pooling of data from the two populations was justified because the discriminant function analysis failed to correctly classify individuals as being from significantly different populations ($P = 0.23$; only 60% of the Anhanguera individuals were correctly assigned to their original population).

Pearson product moment correlation coefficients were determined to analyse the relationship between (i) the log of each independent variable and the log of maximal jump distance and (ii) the snout-vent length residuals of the log of jump distance and the log-independent variable (or snout-vent length residuals of log-independent variable as applicable). The truncated product method (Zaykin et al. 2002) was used to combine a set of P values (those for the Pearson product moment correlations for absolute data and for length-independent data were analysed as two separate sets) to determine whether it was

likely that all significant results in the set were truly significant or were due to chance.

ANOVA was used to determine whether there was a significant difference in mean frog jump performance between trials. Because there was no significant difference in mean frog jump performance between trials ($P = 0.23$), the repeatability of jump performance was calculated using the intraclass correlation coefficient as detailed by Lessels and Boag (1987).

Stepwise analysis of independent variables using multiple linear regression was used to determine possible predictors of maximal jump performance. Independent variables were included in the model only if they were judged to have a significant effect on the model (when the probability of the F value for the model was less than 0.05). Models were constructed with log maximum jump distance as the dependent variable. Dry and wet muscle mass measurements yielded similar relationships; therefore, total hindlimb wet muscle mass (excluding plantaris) and plantaris wet muscle mass were used as independent variables in the models along with total leg bone length, absolute maximum isometric plantaris muscle force, rate of plantaris tetanus activation, absolute maximum plantaris muscle power output, LDH activity, PK activity, and CS activity. The r^2 value (adjusted as a population estimate) of each model was used to indicate the percentage of variation in jumping performance explained by the model. Initially, log-transformed absolute values were used in the model. The stepwise multiple regression analysis was then repeated using body length residuals of log-transformed data in an attempt to remove the confounding effects of body size. The significance of each model was assessed using a two-tailed ANOVA.

Results

Jumping Performance and Physiology of Hyla multilineata

The overall mean value for maximum jump distance in *Hyla multilineata* was 0.775 ± 0.084 m. The interclass correlation coefficient value of 0.696 ($F_{36,74} = 7.86$) indicated that the maximum jump performance of each individual was repeatable, with most of the variation in jump performance being a result of differences between individuals.

Maximal contractile performance of plantaris muscle for this species was 0.973 ± 0.188 N for isometric force and 265 ± 109 W kg^{-1} for normalised power output (Table 1). Enzyme activity values for the species were 206 ± 49.4 U g^{-1} of muscle mass for LDH, 99.1 ± 23.2 U g^{-1} for PK, and 5.36 ± 1.33 U g^{-1} for CS (Table 1).

Relationships between Jump Distance and Absolute Morphophysiological Variables

Allometric scaling relationships are reported in Table 2; however, it must be noted that there was less than a threefold range in body mass. Maximum jump distance was significantly correlated with snout-vent length and body mass (Fig. 1; Table 1), leg length, total hindlimb muscle mass (excluding plantaris), total plantaris muscle mass, tetanus activation rate, and PK activity ($r > 0.4$, $P < 0.01$ in each case; Table 1; truncated product method analysis suggested these were genuine significant results). There was significant scatter in the relationship between maximum jump distance and snout-vent length (Fig. 1); therefore, size-independent variables may be important in predicting jump performance.

Table 1: Summary of absolute independent variables

	M_b (g)	L_{sv} (mm)	L_l (mm)	M_{lm} (g)	M_{pw} (g)	F (N)	T_{ta} (N s^{-1})	P_p (mW)	A_{ldh} (U g^{-1})	A_{pk} (U g^{-1})	A_{cs} (U g^{-1})
Mean ± SD[a]	3.20 ± .70	41.9 ± 3.11	52.7 ± 4.40	.45 ± .12	.067 ± .019	.97 ± .19	12.0 ± 2.34	8.94 ± 1.01	206 ± 49.5	99.1 ± 23.2	5.36 ± 1.33
Range	1.97–4.88	35.7–50.2	43.6–61.4	.26–.77	.035–.12	.68–1.39	7.19–16.1	3.14–17.2	116–315	59.1–167	2.89–8.50
r value for log absolute values[b]	.461	.569	.633	.544	.646	.199	.500	.210	.157	.474	.306
P	.003	.001	.001	.001	.001	.239	.002	.212	.355	.003	.066
r value for length residuals[c]	−.190	…	.340	.056	.381	−.069	.282	.045	.243	.472	.116
P	.260	…	.040	.743	.020	.687	.090	.791	.147	.003	.494

Note. $N = 37$. M_b = body mass, L_{sv} = snout-vent length, L_l = total leg bone length, M_{lm} = total limb (hind) muscle mass (excluding plantaris), M_{pw} = *plantaris* muscle mass, F = plantaris maximum absolute muscle force, T_{ta} = plantaris tetanus activation rate, P_p = plantaris absolute power output, A_{ldh} = lactate dehydrogenase activity, A_{pk} = pyruvate kinase activity, A_{cs} = citrate synthase activity. The truncated product method calculated P values for the repeated correlations of <0.0001 for r values for log absolute values and 0.004 for r values for length residuals.

[a] Absolute independent variables.

[b] Individual Pearson product moment correlation between log jump distance and log absolute independent variables.

[c] Individual Pearson product moment correlation between snout-vent length residuals of log jump distance and snout-vent length residuals of log independent variables (or log independent variables where applicable).

Table 2: Allometric equations for log independent variables

	D_j (m)	L_t (mm)	M_{lm} (g)	M_{pw} (g)	F (N)	T_{ta} (N s^{-1})	P_p (mW)	A_{ldh} (U g^{-1})	A_{pk} (U g^{-1})	A_{cs} (U g^{-1})
Body mass values:										
a	.48 ± .34	.13 ± .15	−5.7 ± .4	−6.8 ± .4	1.1 ± .7	−3.8 ± .6	NA	NA	NA	1.4 ± .8
b	.87 ± .21	.98 ± . 09	3.3 ± .2	3.5 ± .3	1.2 ± .4	1.5 ± .4	NA	NA	NA	1.3 ± .5
r^2	.31	.75	.86	.84	.17	.26	.07	.02	.01	.12
P	<.001	<.001	<.001	<.001	<.01	<.01	.07	.65	.37	<.05
Snout-vent length values:										
a	1.8 ± .04	1.6 ± .02	−.86 ± .03	−1.8 ± .04	2.8 ± .06	−1.6 ± .07	NA	NA	NA	.48 ± .09
b	.24 ± .08	.31 ± .04	1.1 ± .06	1.2 ± .08	.44 ± .13	.47 ± .13	NA	NA	NA	.47 ± .17
r^2	.20	.68	.90	.86	.23	.24	.06	.003	.03	.16
P	<.01	<.001	<.001	<.001	<.01	<.01	.07	.30	.81	<.01

Note. Equations were calculated as $y = ax^b$, where *a* represents the intercept at unity, *b* represents the slope of the line, and *x* represents log body mass or log snout-vent length. $N = 37$. D_j = maximum jump distance, L_t = total leg bone length, M_{lm} = total limb (hind) muscle mass (excluding plantaris), M_{pw} = plantaris muscle mass, F = plantaris maximum absolute muscle force, T_{ta} = plantaris tetanus activation rate, P_p = plantaris absolute power output, A_{ldh} = lactate dehydrogenase activity, A_{pk} = pyruvate kinase activity, A_{cs} = citrate synthase activity. NA = not applicable as $P > 0.05$.

When absolute data were used (i.e., when body length effects were not removed), the first model produced via stepwise multiple linear regression analysis suggested that 40% (i.e., $r^2 = 0.40$) of the interindividual variation in jump distance in this species could be explained by variation in total plantaris muscle mass ($P < 0.001$). However, the r^2 value rose to 0.54 when both plantaris muscle mass and PK activity were used to model jump distance ($P < 0.001$ for the model; partial correlation coefficients of 0.666 and 0.510 for plantaris muscle mass and PK activity, respectively). The r^2 value increased to 0.62 when plantaris muscle mass, PK activity, and total hindlimb muscle mass (excluding plantaris) were used to model jump distance ($P < 0.001$ for the model; partial correlation coefficients of 0.606, 0.577, and −0.437 for plantaris muscle mass, PK activity, and total hindlimb muscle mass [excluding plantaris mass], respectively).

Relationships between Jump Distance and Body-Length-Independent Morphophysiological Variables

When snout-vent length effects were removed (via calculation of snout-vent length residuals of log data), total leg bone length, total plantaris muscle mass, and PK activity were all significantly correlated with jump performance ($r > 0.33$, $P < 0.05$ in each case; Table 1; Fig. 2; truncated product method analysis suggested these were genuine significant results).

When body length effects were removed, stepwise multiple linear regression analysis suggested that 20% of the interindividual variation in jump distance was explained by variation in PK activity ($P < 0.01$). However, the r^2 value for this model increased to 0.33 when both PK activity and plantaris mass were used in the model ($P < 0.001$ for the model; partial correlation coefficients of 0.512 and 0.434 for PK activity and plantaris mass, respectively). The r^2 value increased to 0.43 when PK activity, plantaris muscle mass, and total hindlimb muscle mass (excluding plantaris) were used to model jump distance ($P < 0.001$ for the model; partial correlation coefficients of 0.571, 0.573, and −0.416 for PK activity, plantaris muscle mass, and total hindlimb muscle mass [excluding plantaris mass], respectively).

Discussion

Bases of Interindividual Variation in Jump Performance

This study suggests that interindividual variation in *Hyla multilineata* locomotor performance is partially accounted for by

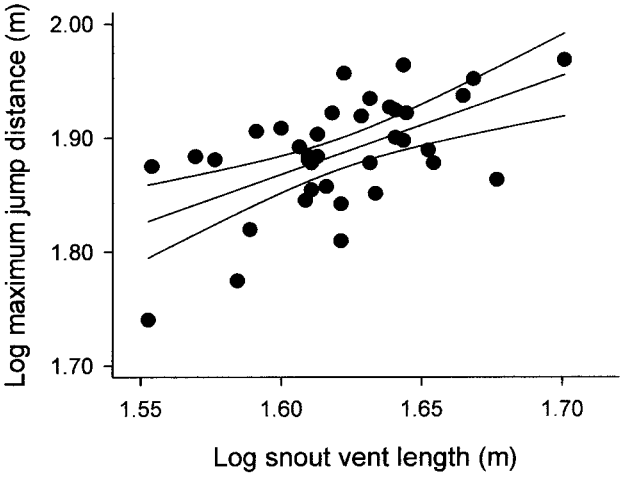

Figure 1. The relationship between log maximum jump distance and log snout-vent length at 25°C ($N = 37$). The lines represent the regression line for the allometric equation and 95% confidence intervals. Jump distance = $0.48 \times L_{sv}^{0.869}$ ($r^2 = 0.31$, $P < 0.001$).

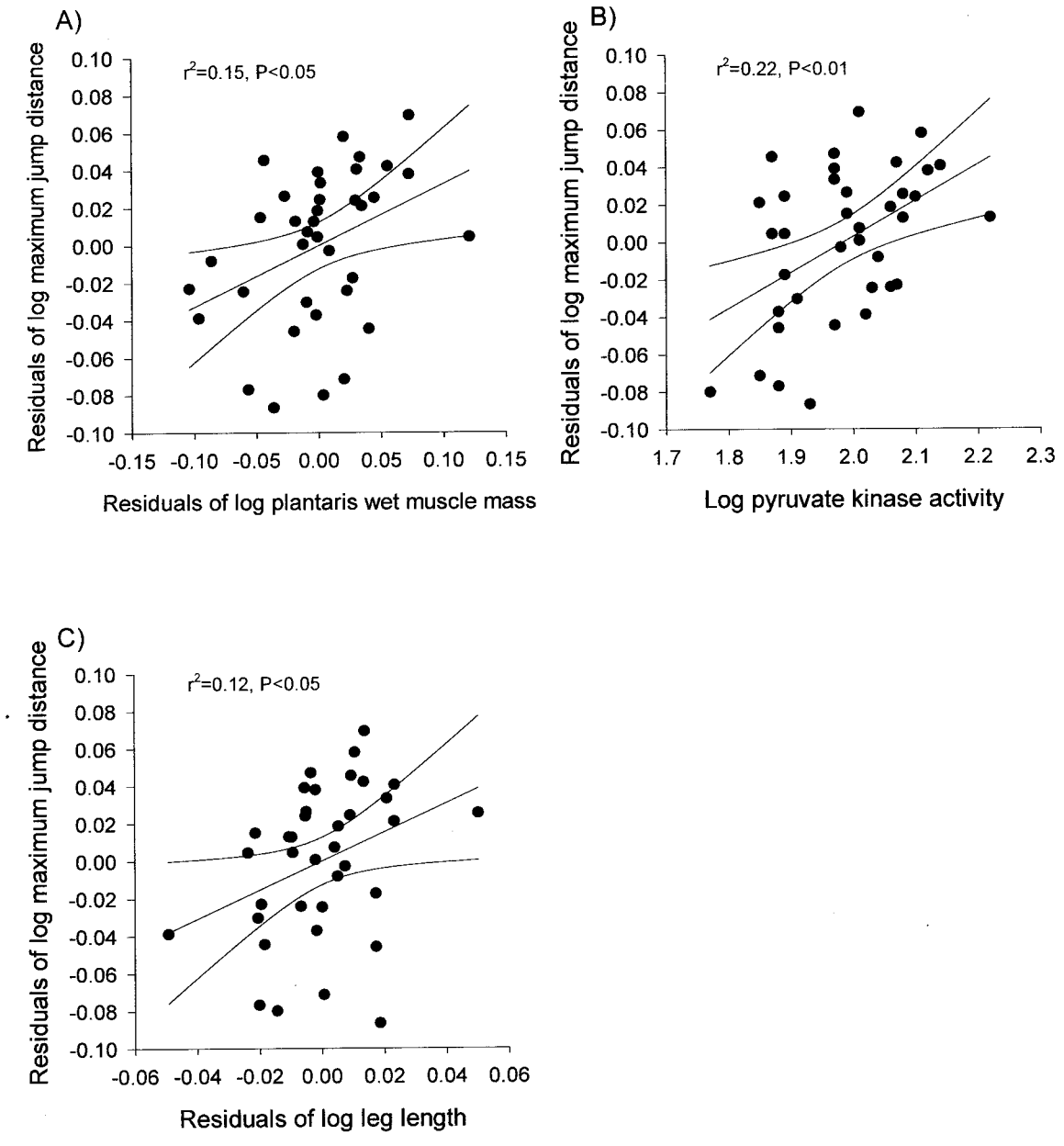

Figure 2. The effect of (*A*) plantaris muscle mass, (*B*) pyruvate kinase activity, and (*C*) leg length on maximum jump distance at 25°C. Data are snout-vent length residuals of log-transformed data or log-transformed data as applicable ($N = 37$). The lines on each graph represent a regression line and 95% confidence intervals. The r^2 and P values are given for each regression relationship.

interindividual variation in a combination of morphological and biochemical parameters. When absolute data were analysed by stepwise multiple regression analysis, the model suggested that 40% of the interindividual variation in maximum jump distance could be explained by variation in plantaris muscle mass alone. Increased mass of muscle used for jumping may increase muscle power and/or force produced during the jump, depending on the composition or structure of the muscle and its operating conditions. In contrast, Tejedo and coworkers (2000) found that absolute leg length was responsible for 77% of interindividual variation in absolute maximal absolute jump distance when analysing pooled data for hemiclones of metamorphosed *Rana esculenta*. It may be that the morphological variables that most affect jumping performance vary between species. Much of the relationship between jump distance and plantaris muscle mass in this study may be explained by the fact that jump performance was significantly related to body size (body mass and snout-vent length) and that plantaris mus-

cle mass was also significantly related to body size. It should be noted that any differences in scaling exponents between the present and previous studies (Marsh 1994) likely arise either from the relatively small size range used in this study (as analysis of scaling effects was not a prime consideration in this study) or that differences are due to variation in the extent of coevolution of morphology and locomotor performance between different taxa caused by differing selection pressures (Blomberg et al. 2003).

An important issue in this study is whether interindividual differences in performance are explained by size-independent differences in morphology, biochemistry, or physiology. For example, we need to determine whether the interindividual differences found in plantaris muscle mass (a major muscle used to power jumping) predict interindividual differences in jump performance because plantaris muscle mass is related to body size or because there is significant body-size-independent variation in plantaris muscle mass. When body length residuals were analysed (i.e., to remove the effects of body length), interindividual variation in jump performance could best be predicted using measurements of PK activity. In *H. multilineata*, interindividual variation in PK activity accounted for 20% of the body-length-independent interindividual variation in maximum jump distance. PK can be involved in either fast glycolytic or slow oxidative ATP resynthesis from carbohydrate utilisation. However, the short time span from removing an individual from its glass tank to completing the jumping sequence would indicate that the immediate energy sources of phosphocreatine and ATP would probably be sufficient to fuel most of the locomotor activity performed in this study. Therefore, the model probably indicates that high PK activity is indicative of frogs with metabolic profiles suitable for greater burst performance and/or is linked to some other factor that is important in maximal jump performance. However, a recent study demonstrated that interindividual variation in both length-specific and absolute sprint swimming performance in cod was best explained by variation in cytochrome *c* oxidase activity, an enzyme important in oxidative phosphorylation (Martínez et al. 2004). Martínez and coworkers (2004) postulated that high cytochrome *c* oxidase activity was important for aerobic preparation and recuperation of muscle ready for sprint swimming. In agreement with our study, previous work by Marker and Gatten (1993) found no relationship between interindividual variation in sprint performance in *Rana pipiens* and interindividual variation in plantaris muscle enzyme activity of either LDH or phosphofructokinase.

The body-length-independent model with the highest r^2 value suggested that a combination of interindividual variation in plantaris muscle mass, PK activity, and total leg muscle mass (excluding plantaris muscle mass) explained 43% of interindividual variation in maximal jump performance. It should be noted that the partial correlation coefficients in this model were positive for PK activity and plantaris muscle mass but negative for total leg muscle mass (excluding plantaris muscle mass). This suggests that individuals with greater PK activity and jumping muscle mass (indicated by plantaris mass, which plays a major role in powering jumping in frogs; Marsh 1994) than would be predicted for their body length jump farther than would be expected for their body length. Our total leg muscle mass measurement includes all leg muscles (except plantaris) to produce a combined mass for muscles regardless of whether they have a major, a minor, or no role in jumping. Therefore, the negative partial correlation between total leg muscle mass and jump distance could be interpreted as support for Alexander's (2000) suggested trade-off between maximising muscle size and speed to enhance jump performance and minimising muscle size and speed to reduce energetic cost and allow more energy to be allocated to other functions; that is, it appears that frogs who jumped farther than would be predicted for their size probably had relatively larger muscle used for jumping (e.g., plantaris) and probably had relatively smaller nonjumping muscles. However, further work analysing interindividual variation in size of all hindlimb muscles in *H. multilineata* would be required to confirm this interpretation of our data.

Interspecies Comparisons of Performance

The mean maximum jump distance achieved by *H. multilineata* used in this study (0.775 m) demonstrates that this species is relatively good at jumping (cf. data in Table VI in Marsh 1994) and is similar to previous findings for arboreal species of frogs and hylid species in general (Zug 1978). In addition, maximum jump distance of individual *H. mulitlineata* was significantly repeatable over the time frame of our experiments, suggesting that this trait is a real indicator of each individual's level of locomotor performance at time of collection. Our Pearson product moment correlation values for jump performance are somewhat lower than the *r* value of 0.97 reported for juvenile *Hyla regilla* reared in a laboratory (Watkins 1997), possibly because of greater variance in animal condition of wild-caught frogs.

The total hindlimb muscle mass of 16.1% ± 1.40% of body mass found in this study is very similar to that of 16.7% found for the Cuban tree frog *Osteopilus septentrionalis* (Peplowski and Marsh 1997) but on the low end of the range of 12%–24% reported for other species (for a review, see Marsh 1994). Despite these relatively low proportions of hindlimb muscle mass, both of these species of tree frogs produce comparatively high jump performance. It is possible that the relatively low hindlimb muscle mass of these tree frogs is a consequence of specialisation for jumping performance as the primary mode of locomotion resulting in relatively smaller nonjumping muscles. In contrast, many Ranid species routinely use both swimming and jumping and have a higher percentage of body mass devoted to locomotor muscle (Olson and Marsh 1998).

The mean maximum normalised plantaris power output of

265 W kg^{-1} was slightly higher than values of 206 and 223 W kg^{-1} found in plantaris muscle fibre bundles from *Rana temporaria* (Navas et al. 1999) and sartorius muscle from *O. septentrionalis*, respectively (Peplowski and Marsh 1997).

CS activity was similar in *H. multilineata* leg muscle to previous values for *Rana pipiens* (Putnam and Bennett 1983), 5.36 compared with 5–7 U g^{-1}, respectively. However, these values are much lower than the 19–26 U g^{-1} previously found for *Bufo boreas* and *Xenopus laevis* muscles. *Hyla multilineata* leg muscle yielded much higher values for LDH activity than those previously found, 206 compared with 71–108 U g^{-1} (Putnam and Bennett 1983). The mean CS/LDH ratio of 0.0276 determined in this study on *H. multilineata* indicates a relatively high flux capacity from pyruvate to lactate production rather than to aerobic metabolism (Hochachka and Somero 2002). These enzymatic activities may be correlated with locomotor mode because *H. multilineata* primarily use burst jumping performance, whereas the other species mentioned above routinely rely on aquatic locomotion (*X. laevis*) or repeated short hops (*B. boreas*) that both require lower muscle power output (Zug 1978).

Conclusions

Our study on frog jumping has been relatively successful in explaining the factors affecting interindividual variation in jump distance when compared with previous studies on variation in lizard sprint performance. Multiple regression analysis using a range of morphological and physiological variables has explained 0%–29% of the variation in sprint speed in previous studies using lizards and salamanders (Gleeson and Harrison 1988; Bennett et al. 1989). Sprint speed in *Dipsosaurus dorsalis* lizards was inversely related to muscle fibre size, with 29% of variation in sprint speed explained by fast oxidative glycolytic fibre cross-sectional area (Gleeson and Harrison 1988) such that lizards with smaller muscle fibres had larger catabolic enzyme activities and greater sprint speed. In contrast, 24% of interindividual variation in sprint speed in *Ambystoma tigrinum nebulosum* salamanders was explained by hindlimb length (Bennett et al. 1989). It seems that the factors affecting sprint performance during running in lizards and salamanders are generally more complex than those affecting jumping in frogs. However, in both our present study on frog jumping and Bennett et al.'s (1989) study on running in salamanders, there were no correlations between interindividual variation in burst locomotor performance and interindividual variation in muscle mechanics. This seems surprising when there are significant interindividual trade-offs between contractile speed (or maximal power output) and fatigue resistance (or endurance) within anuran and mammalian skeletal muscles (Wilson et al. 2002, 2004; Wilson and James 2004). In humans, vertical jump performance has been found to be correlated with measures of maximum muscle force (Jameson et al. 1997), although multivariate analysis has not been performed. However, for many muscles in jumping animals, it has to be remembered that although a muscle might be important for power production during jumping, it may also act in conflicting ways during other behaviours (Kargo and Rome 2002), possibly causing a limit on how much the muscle can be optimised for jumping performance. Indeed, Losos (1990) suggested that evolution of sprinting and jumping are strongly associated in *Anolis* lizards, involving specialisation of shared morphophysiologies, which would limit specialisation in either performance and limit the ability to adapt to certain microhabitats.

Other unmeasured parameters might contribute to the interindividual differences in locomotor performance, including differences in the origin and insertion of muscles (for a review, see Emerson 1985), muscle fascicle length and pennation angles (previously shown to affect human sprint performance; Kumagai et al. 2000), age, state of health (although we have already removed individuals of particularly low body mass for their length and we collected only calling males), and physiological, morphological, motivational, or behavioural differences between individuals.

Although we have demonstrated a link between body-size-independent performance and body-size-independent morphological and biochemical traits, further studies would be needed to investigate the links between both fitness and performance and fitness and physiological or organismal traits in this species. Such studies would help to elucidate whether the traits linked to performance and maximal jump performance itself are selected for and the possible evolutionary consequences of these traits.

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