

Individual correlation of morphology, muscle mechanics, and locomotion in a salamander

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BENNETT, ALBERT F., THEODORE GARLAND, JR., AND PAUL L. ELSE. *Individual correlation of morphology, muscle mechanics, and locomotion in a salamander*. *Am. J. Physiol.* 256 (Regulatory Integrative Comp. Physiol. 25): R1200–R1208, 1989.—Measurements were made on locomotor performance (burst run and swim speed, run and swim endurance), morphology (body, tail, and hindlimb length, body mass), and skeletal muscle mechanics (isometric: twitch and tetanic tension, rates of force development and relaxation; isotonic: maximal velocity of shortening and power output) in a size range of individual salamanders (*Ambystoma tigrinum nebulosum*) at 10 and 20°C. The size dependence of each factor was determined, and the interindividual correlations among factors were measured after removal of size effects. Locomotor performance is positively related to body size: larger animals are faster and have higher endurance. Isometric tensions (standardized for muscle cross-sectional area) and isotonic properties are mass independent; rates of isometric force development and relaxation are negatively related to body mass. Locomotor performance capacities are not intercorrelated among individuals. Isometric force and rates of force development are highly intercorrelated as are maximal shortening velocity and power output. Several statistical techniques failed to uncover correlations among sets of variables (performance, morphology, muscle mechanics): neither fast individuals nor individuals with high endurance necessarily have relatively fast muscles or long limbs or tails after the effects of body size have been removed.

allometry; *Ambystoma*; body size; burst speed; endurance; interindividual variability; isometric; isotonic; mass; maximal velocity of shortening; power; tetany; twitch

CORRELATIONAL ANALYSIS has been a powerful tool in comparative physiology to suggest functional linkage between two or more variables. Most often correlational analysis has been done interspecifically, using species from different habitats or with different functional capacities as, in effect, an experimental variable. Although such studies have been very useful, they are subject to criticism in regard to the phylogenetic relationships among the species investigated. On one hand, species may be so distantly related that extraneous functional associations may complicate correlational analysis (19); on the other hand, the species examined may not be distantly related enough to provide true statistical independence of observations (11, 23).

Another type of comparative study that avoids these phylogenetic problems is one that examines interindivid-

ual differences in physiological variables within a single population of animals (4, 14, 18). Such studies ask whether variables are functionally correlated among different individuals of a species rather than among species. The same interpretive logic applies as in interspecific studies. That is, a failure to demonstrate correlation implies that a controlling or causal connection does not exist between the variables in the animals studied; a correlation is suggestive of such an association, which must then be proven through experimental studies (24). Such intraspecific studies have been a relatively neglected means of analyzing physiological variability and its underlying functional basis (4).

This study was undertaken to examine correlations among the morphology, skeletal muscle mechanics, and locomotor performance among individuals in a population of salamanders (*Ambystoma tigrinum nebulosum*). Salamanders were chosen for this study because of their quadrupedal gait, the amphibious nature of their activity, ease of analysis of their muscle mechanics, and the thermal dependence of their locomotor activity and physiological function. We were interested in determining whether locomotor performance is reflected in underlying structure or functional capacity. Does, for instance, an animal that can run or swim fast also have particularly fast skeletal muscles, or might its speed be due to other factors, such as longer limbs or tail? We were also interested in examining functional associations among different parameters of skeletal muscle mechanics. Are, for instance, rates of isometric and isotonic contraction strongly correlated? First, the effect of body size on each experimental variable was determined. Second, the effect of any size dependence was removed before interindividual analysis by computing mass-corrected residuals for each variable (4, 14). The thermal dependence of locomotor performance and muscle mechanics for these animals was reported in a previous publication (10).

MATERIALS AND METHODS

Animals. Twenty-one tiger salamanders (*Ambystoma tigrinum nebulosum*) were collected from ponds in northern Arizona (Arizona Game and Fish Department permit number 85-5 to J. Collins) and transported to the University of California, Irvine. Animals were maintained on a 12:12-h light-dark photoperiod and had constant access to fresh water and *Tenebrio* larvae for food.

Metamorphosed animals were kept in moist aquaria at either 10 or 20°C for at least 3 wk before testing of locomotor performance. Acclimation temperature was found to have no effect on any of the locomotor or muscle contractile variables measured (10); consequently data from both groups are combined for the present analysis. Before experimentation, body mass, snout-vent length, tail length, and hindleg length were measured.

Locomotor performance. Burst speed and endurance capacities of each animal on land and in water were measured at 10 and 20°C (range $\pm 0.5^\circ\text{C}$) on alternate days. The experimental schedule was as follows: *day 1*, running burst at 10°C; *day 2*, running burst at 20°C; *day 3*, running endurance at 10°C; *day 4*, running endurance at 20°C; *day 5*, swimming burst at 10°C; *day 6*, swimming burst at 20°C; *day 7*, swimming endurance at 10°C; and *day 8*, swimming endurance at 20°C. Repeatability measurements of these variables were not done in the interest of minimizing exposure to nonacclimation temperatures. However, locomotor performance variables have been found to be highly repeatable on an interindividual basis in other amphibians examined (36) and other species of lower vertebrates (3, 4, 14, 16, 17, 18). Details of the experimental setups and protocols have been previously reported (10). In brief, burst speed was measured in a narrow plastic trough (7 cm wide by 6 cm high by 2 m long) lined with damp paper towels for running burst and filled with water for swimming burst measurements. Animals were chased at maximal speed down the trough while being videotaped. Each animal was chased in three trials, separated by at least 1 h. The fastest 20-cm interval of the three trials is reported here, along with simultaneous hindleg or tail cycling frequencies (running and swimming, respectively). Running endurance (time and distance traveled) was measured with a step test on a motor-driven treadmill, initially set at 0.10 km/h and incremented by 0.05 km/h at 5-min intervals until exhaustion (lack of righting response) occurred. Swimming endurance (time and distance traveled) was measured by another step test in a flow tank with initial water speeds of 0.20 km/h, incremented by 0.10 km/h every 5 min. Endurance tests were performed once for each animal at each temperature.

Muscle mechanics. Contractile performance at 10 and 20°C (range $\pm 0.2^\circ\text{C}$) was determined on the musculus extensor iliotalibialis pars anterior, a cylindrical, parallel-fibered muscle that is an extensor of the hindleg (12). Detailed descriptions of the experimental apparatus and protocol have been previously reported (10). Both isometric and isotonic measurements were made on a calibrated servo-controlled ergometer (Cambridge Instruments model 300H) connected to a storage oscilloscope. The following isometric measurements were made on the muscle from the left hindleg, first at 20°C, then at 10°C: maximal tetanic force (P_o in kN/m^2), maximal rate of tetanic tension development (dP_o/dt in $\text{kN}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), maximal twitch force (P_t in kN/m^2), maximal rate of twitch force development (dP_t/dt in $\text{kN}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), time to peak twitch force (TPT in ms), and time from peak to half peak twitch force (half-relaxation time) ($RT_{1/2}$ in ms). Maximal velocity of shortening (V_{\max} in muscle

lengths/s) and maximal power output (W_{\max}) were measured on the muscle from the right hindleg, first at 20°C, then at 10°C. Force-velocity data were fit with a hyperbolic-linear equation (28) to estimate V_{\max} and to permit calculation of W_{\max} .

Statistical analyses. All statistical analyses were performed using routines in SPSS-PC+ (30) on IBM-PC compatible computers. All characters were \log_{10} transformed before statistical analyses: this procedure satisfactorily achieved linearity and homoscedasticity for regressions of each character on body mass. Linear regression was used to generate allometric equations of the form

$$\log_{10} Y = \log_{10} a + b \log_{10} M$$

or in arithmetic form

$$Y = aM^b$$

where M is body mass in grams and Y is any character. For the morphological variables, we also computed reduced major axis slopes (= regression slope divided by correlation coefficient) and used these values to test for deviations from geometric similarity using Clarke's *t* test (6).

Interindividual correlations among variables were examined with Pearson product-moment correlation coefficients on mass-corrected residuals. Correlations among two uncorrected variables may be inflated if the variables examined are each correlated with a third variable. This is a particular problem if the third variable has pervasive effects on many characters examined, as does body size (4, 14). Nearly all the variables examined here were found to be size dependent, and therefore two uncorrected characters often showed significant intercorrelations primarily or entirely because both were significantly correlated with body mass. To remove these confounding effects of overall body size, we computed residuals from allometric equations and examined correlations among these residual values (see Refs. 4, 14, 15 for discussions of these procedures). Body masses used for allometric equations were measured once during locomotor performance measurements and again when animals were killed for morphometric and muscle measurements.

We used several statistical approaches to test for relationships among residuals of the characters measured (for general discussions and examples of previous applications of these methods see Refs. 14, 15, 17, 30). All statistical analyses were performed separately on measurements made at either 10 or 20°C. First, we computed Pearson product-moment correlations between all possible pairs of residual characters. We examined the resulting correlation matrices (10 and 20°C), concentrating on values that were statistically significant at $P < 0.01$ (thus <1% of correlations would be expected to appear significant due to chance alone). Next, we conducted principal components analysis of these correlation matrices. To determine whether residual (= size-corrected individual) variation in the measures of locomotor performance could be explained (predicted) by the morphological or muscle mechanical characters measured, we then conducted a canonical correlation analysis ($n = 19$

individuals with complete data sets). Canonical correlation tests for an overall relationship between a set of "dependent" variables (the 4 measures of locomotor performance plus leg and tail cycling frequencies) and a set of "predictor" variables (the 3 morphological residuals plus the 8 muscle mechanical residuals). Finally, we used multiple-regression analysis ($n = 19$) to compute the best predictive equations for each of the six organismal performance measures considered separately.

RESULTS

Allometric scaling of morphological, locomotor, and muscle mechanical variables. Allometric equations for each variable at both 10 and 20°C are reported in Table 1. Analysis of covariance revealed no significant differences in slope for 10 vs. 20°C for any character measured. Body masses ranged over nearly an order of magnitude, from 3.17 to 23.8 g (mean mass = 8.16 g). Body proportions were consistent with geometric similarity ($b = 0.333$ for length measurements), except for tail length. The reduced major axis slope for tail length (0.594) is significantly >0.333 (Clarke's $t = 4.81$, approximate $df = 16.75$, $P < 0.001$); thus, tails are relatively longer in larger animals.

Interindividual variability in locomotor performance was great (Fig. 1). Running and swimming speeds varied four- and twofold, respectively, at each temperature among the individuals examined here. Total distance traveled in running and swimming endurance trials varied 20- and 5-fold, respectively, among individuals. Some (18–42%) of this variation is related to individual differ-

ences in body size (Table 1). At 10°C, these performance variables all increase significantly ($P < 0.05$) with body size. At 20°C, only running speed is significantly related to body mass at $P < 0.05$, but swimming speed and both terrestrial and aquatic endurance are correlated with mass at $P < 0.10$. Consequently, larger animals generally can run and swim faster and have more endurance both on land and in water than do smaller animals. Limb cycling frequency in running and tail cycling frequency during swimming do not vary significantly with body size over the mass range studied.

Some aspects of muscle mechanics are influenced by body mass (Fig. 2), even after standardization for muscle cross-sectional area (see Table 1 for units). Standardized isometric twitch and tetanic tensions are independent of body size. Isometric rate processes, however, all decrease with increasing body size: muscle from larger animals is slower than that from smaller animals. Isotonic properties measured are mass independent.

Interindividual correlations among locomotor performance and muscle mechanical variables. Correlations among mass-corrected residuals (see statistical section of the MATERIALS AND METHODS) at 10 and 20°C are given in Table 2. These correlations indicate associations among variables, independent of body size effects.

Locomotor performance capacities (terrestrial and aquatic speed and endurance) are generally uncorrelated with each other (except for a positive association between running and swimming endurance at 20°C). Thus individuals that are good at one type of locomotor performance are not necessarily either good or poor at others.

TABLE 1. Allometric equations for characters measured in *Ambystoma tigrinum*

Character	10°C					20°C				
	$a \pm 95\%CI$	$b \pm 95\%CI$	$r^2, \%$	P	CV^1	$a \pm 95\%CI$	$b \pm 95\%CI$	$r^2, \%$	P	CV^1
Locomotor performance										
Running burst speed, cm/s	5.67÷/×1.72	0.412±0.261	36.6	0.0037	27.5	8.12÷/×1.78	0.382±0.277	30.6	0.0093	29.1
Leg cycling frequency, Hz	1.78÷/×1.50	0.110±0.196	6.8	0.2552	20.6	2.49÷/×1.53	0.080±0.206	3.3	0.4289	21.7
Swimming burst speed, cm/s	16.5÷/×1.41	0.193±0.164	24.3	0.0233	17.3	18.4÷/×1.39	0.137±0.160	14.5	0.0887	16.8
Tail cycling frequency, Hz	5.53÷/×1.55	-0.104±0.210	5.3	0.3157	22.2	7.32÷/×1.49	-0.188±0.191	18.3	0.0528	20.1
Running endurance, m	9.39÷/×3.15	0.608±0.551	21.9	0.0324	58.1	13.6÷/×3.61	0.617±0.617	18.7	0.0501	65.0
Swimming endurance, m	23.0÷/×2.00	0.586±0.333	41.6	0.0016	35.1	60.0÷/×2.15	0.368±0.368	18.7	0.0503	38.8
Morphology										
Snout-vent length, cm	3.39÷/×1.13	0.308±0.061	85.5	<0.0001	5.4					
Tail length, cm	1.67÷/×1.37	0.501±0.153	71.1	<0.0001	13.6					
Hindleg length, cm	1.45÷/×1.15	0.267±0.070	77.0	<0.0001	6.2					
Isometric muscle contractile properties										
Tetanic force, kN/m ²	493÷/×2.06	-0.266±0.355	12.1	0.1328	32.6	515÷/×2.01	-0.233±0.341	10.2	0.1691	29.4
Rate of tetanic force development, kN·m ⁻² ·ms ⁻¹	14.9÷/×2.21	-0.746±0.388	47.5	0.0008	33.7	30.3÷/×2.55	-0.802±0.458	43.0	0.0017	39.4
Twitch force, kN/m ²	376÷/×2.26	-0.304±0.405	12.9	0.1317	32.6	321÷/×2.49	-0.298±0.454	10.2	0.1836	36.5
Rate of twitch force development, kN·m ⁻² ·ms ⁻¹	10.9÷/×2.32	-0.606±0.419	35.4	0.0072	33.7	20.1÷/×2.64	-0.590±0.484	28.0	0.0198	38.9
Time to peak twitch, ms	97.5÷/×1.28	0.175±0.125	34.2	0.0085	10.0	43.7÷/×1.27	0.155±0.117	31.4	0.0126	9.4
Time to twitch half-relaxation, ms	138÷/×1.78	0.150±0.287	6.7	0.2845	23.1	39.7÷/×1.79	0.310±0.290	23.1	0.0372	23.3
Isotonic muscle contractile properties										
Maximal contractile velocity, muscle lengths/s	1.58÷/×1.61	-0.034±0.233	0.5	0.7606	20.1	3.68÷/×1.54	-0.109±0.212	6.0	0.2967	18.3
Maximal power output, W/kg	73.4÷/×2.97	-0.205±0.533	3.5	0.4291	46.0	155÷/×2.32	-0.143±0.411	2.9	0.4739	35.5

Equations in the form character = $a(\text{body mass})^b$. $n = 21$ for performance, morphology; 20 for tetanic force, rate of tetanic force development, maximal contractile velocity, maximal power output; 19 for twitch force, rate of twitch force development, time to peak twitch, and time to twitch half-relaxation. a , Antilog of estimated y -intercept (value at body mass = 1 g) from log-log regressions. Entry for 95% confidence interval (CI) denotes, using running burst speed as an example, from $5.67 \div 1.72$ to 5.67×1.72 ; hence, on an arithmetic scale CIs are asymmetrical about the mean (a). ¹Approximate coefficient of variation, computed as $230.26 \times \text{SD}$ of residuals from allometric equation (see Ref. 14).

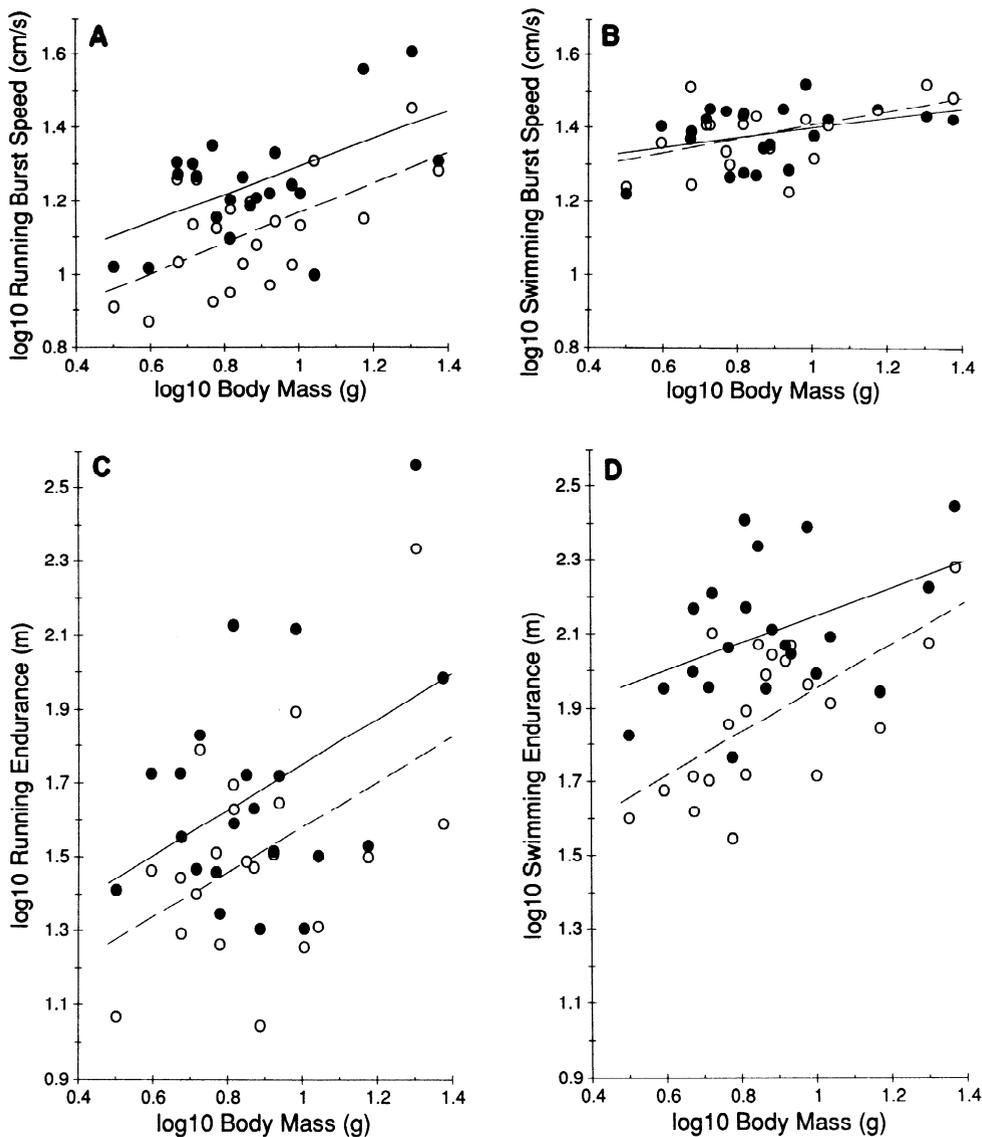


FIG. 1. Log-log plots of locomotor performance vs. body mass in *Ambystoma tigrinum*. Regression lines are allometric equations as presented in Table 1. Closed circles and solid lines are at 20°C; open circles and dashed lines are at 10°C.

Not surprisingly, running and swimming speeds are highly positively correlated with limb and tail cycling frequencies, respectively: the relatively faster the movement of the propulsive organ, the faster the locomotor speed of the animal.

Among muscle mechanical variables, isometric forces and rates of force development are very highly and positively intercorrelated: the faster the maximal rate of force development, the greater the maximal force in both twitch and tetany. Relaxation time is generally uncorrelated with other isometric or with isotonic variables measured, except for a positive association with TPT ($0.05 > P > 0.01$). Maximal shortening velocity and maximal power output are highly correlated: the faster the intrinsic speed of muscle contraction, the greater the maximal capacity to generate power. These isotonic variables, however, are generally uncorrelated with isometric variables (except for P_0 and V_{max} at 20°C).

No significant ($P < 0.01$) intercorrelations were found between sets of variables (morphology, performance, muscle mechanics). In other words, fast animals do not necessarily have relatively fast muscles or long limbs or

tails after body size has been removed as a confounding variable.

The associations, or lack of them, suggested by these product-moment correlations are generally supported by other statistical approaches to these data. First, principal components analysis (Table 3; based on the correlation matrices presented in Table 2) indicates an overall pattern of association within groups of variables (e.g., isometric variables, isotonic variables, locomotor performance, and cycling frequency) but relative independence among groups. That is, muscle mechanical variables do not generally load heavily on the same factors as do locomotor performance variables. At both temperatures, the first factor reflects intercorrelations among isometric muscle mechanical variables. Factor four at 10°C and factor one at 20°C reflect much of the variation in isotonic muscle variables.

Canonical correlation analysis (results not shown) yielded no significant canonical variates at either 10 or 20°C ($P > 0.15$). Thus the overall association between the six measures of organismal performance on the one hand and the three morphological plus eight muscle

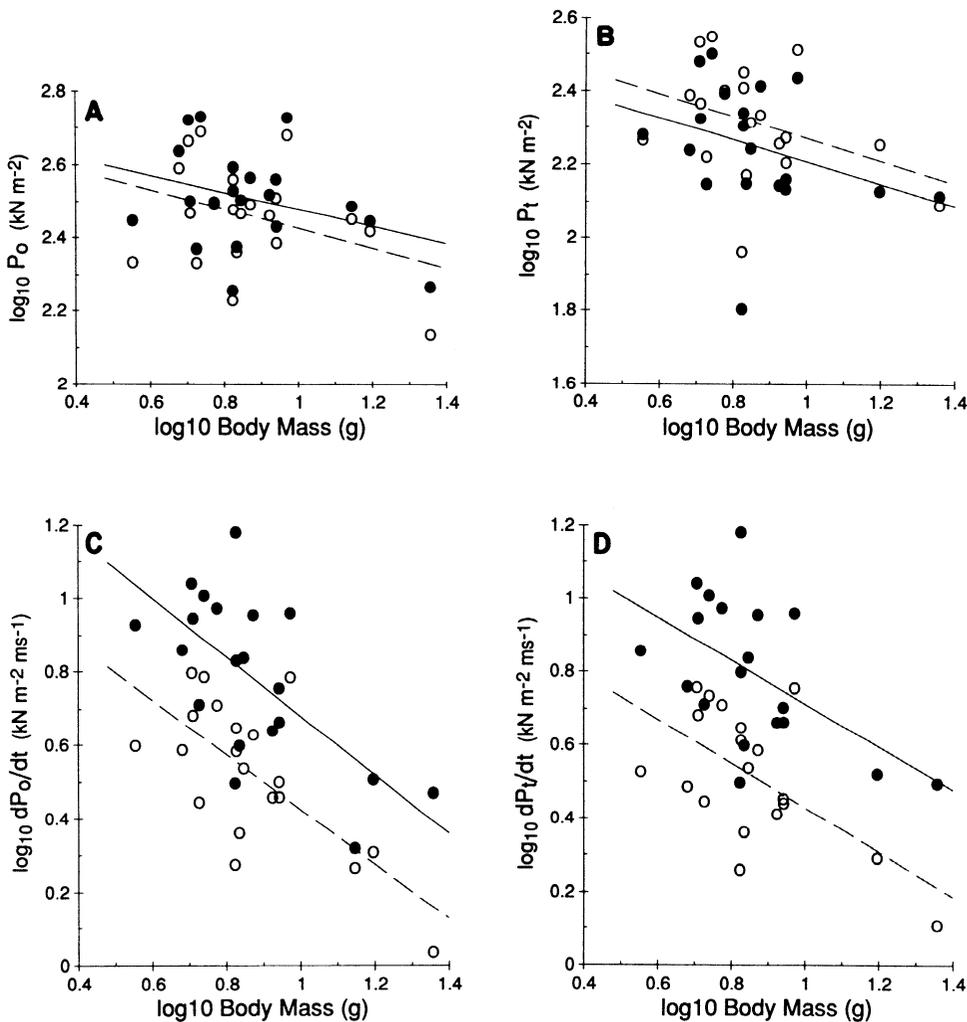


FIG. 2. Log-log plots of muscle mechanical variables versus body mass in *Ambystoma tigrinum*. Symbols as in Fig. 1. See text for definition of abbreviations.

mechanical variables on the other is weak.

Multiple regressions of locomotor performance on morphological and muscle mechanical characters (Table 4) indicate that 70–82% of the individual (residual) variation in running speed is statistically explainable by differences among individuals in maximal hindleg cycling frequency. Thus relatively little of the individual variation in running burst speed is attributable to differences in hindleg length (see Table 4), which is a presumed correlate of stride length (but see discussion in Ref. 15). About one-half of the variation in swimming speed is explainable by individual differences in tail cycling frequency. At 10°C, an additional 16% of the variation is attributable to differences in tail length: individuals with relatively long tails for their body size attain higher speeds.

Our measures of muscle mechanics explained relatively little of the individual variation in organismal performance (Table 4). Moreover, the partial regressions that are significant are not consistent across measurement temperatures. No muscle contractile properties are consistently associated with either limb or tail cycling frequencies, the most likely candidates to be directly associated with muscle mechanical variables.

In summary, the consistent pattern demonstrated by all of these statistical analyses is that variation in loco-

motor performance is relatively imprecisely predicted by individual differences in the muscle mechanical properties or morphological factors measured herein. However, certain measures of muscle mechanical performance are fairly tightly intercorrelated among themselves.

DISCUSSION

Allometry of locomotor performance. Among the salamanders studied here, locomotor performance capacity is greater in larger animals: bigger individuals are absolutely faster and have greater endurance, in both terrestrial and aquatic environments. This improvement is probably the result of ontogenetic changes in locomotor capacity, but without longitudinal studies on individual animals through time, the influence of directional selection on this allometric pattern cannot be excluded (5). In other words, it is possible that natural selection simply eliminates salamanders with relatively poor performance before they attain large size (and old age); hence, field-collected adults represent a biased sample. Moreover, body size and age are related and confounding variables in this and most other ontogenetic allometric studies, so the effects of size per se vs. age cannot be separated.

A similar increment in locomotor capacity with increasing body size has also been reported in most other ectothermic vertebrates examined. Burst speed is faster

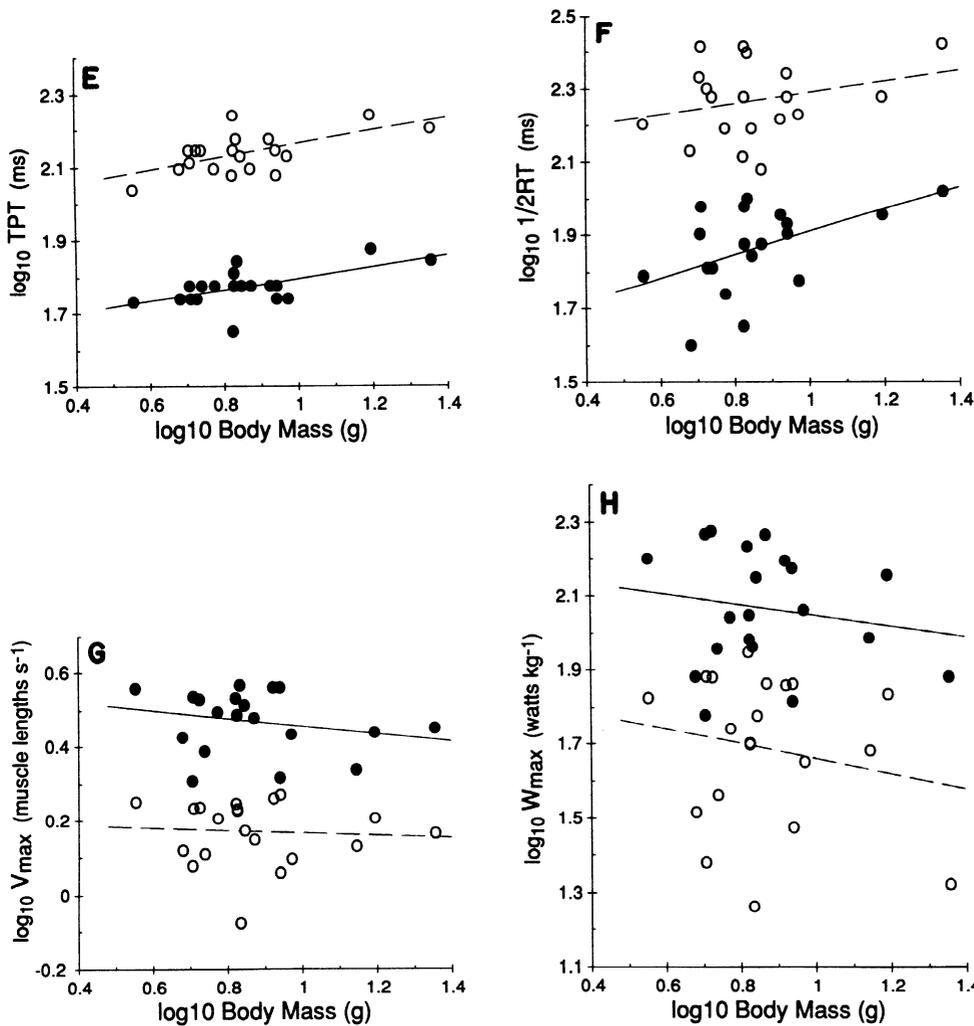


FIG. 2.—Continued.

TABLE 2. Interindividual Pearson product-moment correlations among mass-corrected residuals

	RBS	LCF	SBS	TCF	RE	SE	SV	Tail	Leg	P _o	dP _o /dt	P _t	dP _t /dt	TPT	RT _{1/2}	V _{max}	W _{max}
RBS	0.196	0.821*	0.126	-0.008	0.178	-0.097	-0.196	0.314	0.234	-0.265	-0.519	-0.383	-0.423	-0.001	-0.119	-0.052	0.307
LCF	0.910*	-0.090	0.191	-0.007	0.110	0.053	-0.024	0.079	-0.059	-0.307	-0.370	-0.340	-0.301	-0.146	0.001	-0.021	0.319
SBS	0.263	0.094	0.363	0.664*	0.278	0.298	-0.403	-0.098	-0.308	0.142	-0.108	-0.243	-0.092	-0.404	-0.108	-0.410	-0.289
TCF	0.179	0.008	0.709*	0.541	0.376	0.333	-0.274	-0.352	-0.286	0.420	0.281	0.216	0.243	0.096	0.064	-0.438	-0.449
RE	0.197	0.040	0.411	0.039	0.867*	0.587*	0.021	0.127	0.006	0.018	0.264	-0.004	0.152	0.136	0.126	-0.226	-0.116
SE	0.078	0.014	0.166	-0.158	0.249	0.460	-0.058	0.037	-0.424	-0.008	0.277	-0.171	0.099	-0.221	-0.070	-0.082	-0.181
SV	0.253	0.332	-0.187	-0.077	-0.083	0.507		-0.291	0.036	-0.256	0.102	0.123	-0.039	0.412	0.531	0.327	0.194
Tail	0.208	0.183	-0.032	-0.530	0.265	0.148		-0.291		0.403	-0.113	-0.240	-0.118	-0.205	-0.166	-0.205	0.241
Leg	0.470	0.483	-0.007	-0.070	-0.008	-0.158		0.403		-0.366	-0.348	-0.176	-0.330	0.002	0.020	0.238	0.276
P _o	0.062	0.183	0.061	-0.022	-0.003	-0.415		-0.262		-0.167	-0.402	0.975*	0.668*	0.788*	0.176	-0.002	-0.666*
dP _o /dt	0.140	0.261	0.100	0.069	-0.074	-0.313		-0.255		-0.332	0.880*	0.846*	0.846*	0.974*	0.178	0.086	-0.268
P _t	0.178	0.285	0.122	0.070	-0.094	-0.348		-0.267		-0.352	0.910*	0.924*	0.915*	0.856*	0.466	0.234	-0.434
dP _t /dt	0.156	0.252	0.178	0.172	-0.133	-0.286		0.048		-0.337	-0.347	0.832*	0.975*	0.911*	0.882*	0.188	0.132
TPT	0.290	0.309	0.151	0.195	0.016	0.080		0.325		-0.442	-0.213	0.245	0.089	0.336	0.151	0.599*	0.572
RT _{1/2}	0.212	0.270	0.571	0.519	-0.039	0.196		0.340		-0.391	0.023	-0.042	-0.016	0.144	0.101	0.571	0.746*
V _{max}	-0.084	-0.268	0.119	0.301	-0.047	-0.036		-0.153		-0.144	-0.074	-0.352	-0.150	-0.224	-0.125	-0.195	-0.217
W _{max}	0.101	-0.100	0.059	0.128	0.122	0.027		-0.071		0.055	0.030	-0.234	-0.078	-0.265	-0.072	-0.272	0.814*

Values below diagonal are at 10°C; above are at 20°C; on diagonal are between 10 and 20°C. RBS, running burst speed; LCF, leg cycling frequency; SBS, swimming burst speed; TCF, tail cycling frequency; RE, running endurance; SE, swimming endurance; SV, snout-vent length; Tail, tail length; Leg, hindleg length. See text for other abbreviations. * Significant at $P < 0.01$. See Table 1 for sample sizes and allometric equations used to generate residuals.

in larger individuals of most species of snakes (16, 20; Jayne and Bennett, unpublished observations) and lizards (data reviewed in Refs. 15, 17). Terrestrial endurance is also enhanced by larger body size and/or age (14, 16, 17, 32, 33; Jayne and Bennett, unpublished observations). Swimming speed and endurance both tend to

increase ontogenetically in fishes (data reviewed in Ref. 2). Interspecific performance capacity generally also increases with increasing body size within different vertebrate classes (data reviewed in Refs. 13, 31). Apparently, one advantage of large size both within and among species is an increased capacity to escape, to chase, or to fight.

TABLE 3. Principal components analysis of correlation matrix presented in Table 2

	10°C						20°C					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI
Running burst speed	0.233	0.679*	-0.351	0.499*	0.201	0.029	-0.526*	-0.388	0.249	0.631*	0.058	0.244
Leg cycling frequency	0.349	0.633*	-0.521*	0.311	0.248	-0.051	-0.436	-0.380	0.388	0.375	0.027	0.552*
Swimming burst speed	0.230	0.453	0.494*	0.419	-0.496*	0.064	0.141	-0.821*	0.135	-0.129	-0.227	-0.048
Tail cycling frequency	0.252	0.335	0.739*	0.296	-0.076	-0.299	0.529*	-0.548*	0.314	0.012	-0.204	-0.112
Running endurance	-0.096	0.227	0.011	0.329	-0.529*	0.529*	0.205	-0.359	0.554*	0.082	0.549*	-0.318
Swimming endurance	-0.319	0.439*	0.067	-0.273	-0.016	0.691*	0.208	-0.473*	0.358	-0.466*	0.497*	-0.078
Snout-vent length	0.036	0.531*	-0.061	-0.416	0.550*	0.326	-0.047	0.647*	0.546*	-0.173	-0.027	0.052
Tail length	-0.422	-0.022	-0.639*	0.347	-0.353	0.157	-0.352	-0.106	-0.312	0.384	0.566*	-0.288
Hindleg length	-0.350	0.413	-0.456*	0.357	0.078	-0.480*	-0.475*	0.263	-0.165	0.388	0.137	-0.494*
P _o	0.882*	-0.334	-0.181	0.077	-0.156	0.081	0.845*	-0.085	-0.252	0.281	-0.034	0.114
dP _o /dt	0.891*	-0.270	-0.104	0.184	0.108	0.166	0.866*	0.197	-0.017	-0.092	0.392	0.180
P _t	0.950*	-0.173	-0.105	0.068	0.005	0.062	0.829*	0.375	-0.106	0.334	0.099	0.121
dP _t /dt	0.910*	-0.192	-0.001	0.156	0.143	0.132	0.854*	0.183	-0.104	0.078	0.299	0.257
TPT	0.452	0.493*	0.166	-0.327	0.091	0.030	0.292	0.521*	0.476*	0.390	-0.169	-0.200
RT _{1/2}	0.330	0.718*	0.354	-0.279	-0.166	-0.207	0.157	0.442	0.655*	0.122	-0.157	-0.119
V _{max}	-0.314	-0.200	0.573*	0.493*	0.420	0.074	-0.601*	0.481*	0.106	-0.403	0.181	0.083
W _{max}	-0.324	-0.188	0.305	0.646*	0.451	0.285	-0.622*	0.278	0.016	-0.035	0.379	0.421
Eigenvalue	4.53	2.96	2.44	2.13	1.54	1.40	4.94	3.12	1.94	1.64	1.47	1.18
% of variance explained	26.6	17.4	14.4	12.5	9.0	8.2	29.1	18.3	11.4	9.7	8.6	6.9
Cumulative % explained	26.6	44.0	58.4	70.9	80.0	88.2	29.1	47.4	58.8	68.5	77.1	84.0

Values are loadings (component correlations) of each residual variable on the first 6 factors. * Values >0.456 in magnitude may be considered significant at approximately $P < 0.05$.

TABLE 4. Results of multiple regression analyses

Dependent Variable	=	Independent Variables (partial r^2)	Multiple r^2 , %	Overall F	P
10°C					
Running burst speed	=	+ Leg cycling frequency (81.9)	81.9	77.2	<0.0001
Running burst speed (Leg cycling frequency omitted)	=	+ Hindleg length (23.6)	23.6	5.3	0.0350
Leg cycling frequency	=	+ Hindleg length (25.6) + P _t (24.5)	50.1	8.0	0.0038
Swimming burst speed	=	+ Tail cycling frequency (50.0) + tail length (15.8) + RT _{1/2} (9.1)	74.9	14.9	0.0001
Swimming burst speed (Tail cycling frequency omitted)	=	+ RT _{1/2} (32.6) + W _{max} (15.6) - Snout-vent length (13.5)	61.8	8.1	0.0019
Tail cycling frequency	=	- Tail length (29.7)	29.7	7.2	0.0158
Running endurance	=	none			NS
Swimming endurance	=	none			NS
20°C					
Running burst speed	=	+ Leg cycling frequency (69.6) + hindleg length (8.8)	78.4	29.0	<0.0001
Running burst speed (Leg cycling frequency omitted)	=	none			NS
Leg cycling frequency	=	none			NS
Swimming burst speed	=	+ Tail cycling frequency (41.1) - TPT (21.9)	63.0	13.6	0.0004
Swimming burst speed (Tail cycling frequency omitted)	=	- Snout-vent length (24.4)	24.4	5.5	0.0317
Tail cycling frequency	=	- V _{max} (22.5)	22.5	4.9	0.0401
Running endurance	=	none			NS
Swimming endurance	=	none			NS

Based on mass-corrected residuals from allometric equations presented in Table 1 ($n = 19$). Only significant ($P < 0.05$) independent variables and signs of their partial regression coefficients are given. See text for definition of abbreviations.

Allometry of skeletal muscle mechanics. Force-generating capacity of a unit cross-sectional area of skeletal muscle is predicted to be constant (25). Thus a cross-sectional unit of muscle should produce similar force regardless of body size of the animal in which it occurs. Indeed, interspecific studies show a rather small range of P_o produced by skeletal muscle (29, 34), although very fast contracting muscle develops less force because of its lower myofibrillar density (25, 34). Intraspecifically, both twitch and tetanic tension (normalized for cross-sectional area) are independent of body mass in both this salamander (Table 1, Fig. 2) and in the lizard *Dipsosaurus dorsalis* (26).

Mechanical rate processes, however, are predicted to be inversely related to body mass, largely on the basis of considerations of limb cycling frequencies (22, 29). The few available data lend support to these predictions. Muscle shortening speed decreases interspecifically with increasing body mass for three species of mammals (9). Intraspecific examinations of body size dependence are few. Observations on mammalian and avian skeletal muscle often show a developmental shift from slow to fast contractile kinetics (see Ref. 1). However, such data are not directly comparable to those in this study, because earlier stages are taken from fetuses or altricial

neonates, not from functionally independent animals. Isometric contractile rates were negatively size dependent in the salamanders examined here; isotonic rates were size independent (Table 1, Fig. 2). In the lizard *Dipsosaurus dorsalis*, isometric twitch times (TPT and $RT_{1/2}$) increase (rates decreased) and maximal contractile velocity decreases with increasing body size (26). More empirical data on size effects on muscle functional capacity are badly needed, as body size per se as well as developmental processes may apparently influence a variety of muscle contractile functions.

In summary, results of the present experiments and those on *Dipsosaurus* (26) indicate that traditional methods of standardization do not necessarily remove the effects of body size on muscle mechanics, especially for rate processes. Thus body size (and possibly age, if known) should be analyzed as a covariate in studies that involve a size range of individuals.

Correlations among variables. Performance capacity in different locomotor modes is uncorrelated in these salamanders after removal of body size effects. An enduring swimmer is not necessarily a fast swimmer, nor is it either fast or enduring on land. These results suggest no evidence for specialization (a tradeoff, for instance, between speed and endurance) nor do they suggest the existence of animals that are superior in all types of activity. A similar lack of association between locomotor abilities has been found in lizards (3, 14, 17), but burst speed and endurance are positively but weakly correlated in garter snakes (16; Jayne and Bennett, unpublished observations) and toads (36).

Among mechanical variables of skeletal muscle, there is a suite of intercorrelated factors relating to isometric tension development, even after removal of body size effects. P_o , P_t , dP_o/dt , and dP_t/dt are all significantly associated at both temperatures examined. Under isometric conditions, a muscle that develops tetanic force rapidly will generally develop twitch force rapidly and produce a relatively great amount of force during both twitch and tetany. These correlations suggest a common suite of underlying factors (e.g., rate of calcium release to the myofibrils, cross-bridge cycling frequency) affecting the rate and level of force production. It is noteworthy that $RT_{1/2}$ time is not correlated with any of the foregoing factors, suggesting an independence of the mechanisms of relaxation (e.g., calcium removal by the sarcoplasmic reticulum) from those of activation. The isotonic properties (V_{max} , W_{max}) correlated strongly with each other, indicating that a muscle with a faster intrinsic velocity of shortening also has the highest capacity for power production. There is apparently no negative association between V_{max} and the shape of the force-velocity curve, measured as a/P_o (21) or $W_{max}/(V_{max}P_o)$ (27, 28).

Isometric and isotonic factors were not significantly intercorrelated (except for P_o and V_{max} at 20°C), indicating no necessary association between the intrinsic speed of shortening and isometric rates of tension development. Close (7, 8) proposed, for instance, that rates of isometric tension development are correlated with rates of muscle shortening and that these variables are governed by similar mechanistic factors. The intraspecific data re-

ported here do not support this proposed association, nor do other intraspecific studies (e.g., 35).

Analysis of correlations (Tables 2–4) does not indicate a close association among suites of morphological, locomotor performance, and skeletal muscle characters. Apparently, one suite does not exert a controlling influence over another. We do not believe that this lack of association is due to inadequate sample size: previous investigations (14, 18) on similar numbers of individuals have detected significant suborganismal correlates of locomotor performance. Different statistical approaches (correlation, principal components analysis, canonical correlation, multiple regression) all suggest a lack of strong interactions among groups of variables. Therefore these data provide little support for the view that such organismal functional variables as burst speed or endurance can be explained by the morphological or muscle mechanical factors measured here. In contrast, previous studies of lizards have been considerably more successful in explaining variation in locomotor performance and metabolism with individual differences in various morphological, physiological, and biochemical characters (14, 17, 18).

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