The Ontogeny of Contractile Performance and Metabolic Capacity in a High-Frequency Muscle*

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ABSTRACT

High-performance muscles such as the shaker muscles in the tails of western diamond-backed rattlesnakes (*Crotalus atrox*) are excellent systems for studying the relationship between contractile performance and metabolic capacity. We observed that shaker muscle contraction frequency increases dramatically with growth in small individuals but then declines gradually in large individuals. We tested whether metabolic capacity changed with performance, using shaker muscle contraction frequency as an indicator of performance and maximal activities of citrate synthase and lactate dehydrogenase as indicators of aerobic and anaerobic capacities, respectively. Contraction frequency increased 20-fold in 20–100-g individuals but then declined by approximately 30% in individuals approaching 1,000 g. Mass-independent aerobic capacity was positively correlated with contractile performance, whereas mass-independent anaerobic capacity was slightly but negatively correlated with performance; body mass was not correlated with performance. Rattle mass increased faster than the ability to generate force. Early in ontogeny, shaker muscle performance appears to be limited by aerobic capacity, but later performance becomes limited equally by aerobic capacity and the mechanical constraint of moving a larger mass without proportionally thicker muscles. This high-performance muscle appears to shift during ontogeny from a metabolic constraint to combined metabolic and mechanical constraints.

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Introduction

In this study, we explore the scaling of contractile performance and metabolic capacity in a specialized muscle that sustains high levels of performance for long periods of time. We used shaker muscle contraction frequency as an indicator of performance and maximal activities of the metabolic enzymes citrate synthase (CS) and lactate dehydrogenase (LDH) as indicators of aerobic and anaerobic capacities, respectively. In general, there should be a positive relationship between aerobic capacity and maximal sustainable performance, because the ability of a muscle to sustain activity depends on the metabolic capacities to supply energy and avoid fatigue. However, the relationship between aerobic capacity and performance varies among species and even within species among individuals of different masses. For example, the aerobic capacity of muscle scales negatively with body size in many animals (e.g., Emmett and Hochachka 1981; Hochachka et al. 1988; Somero and Childress 1990), whereas in other species it is not related to size (e.g., Norton et al. 2000). Anaerobic capacity scales positively with body size in the white locomotor muscle of some fishes (Somero and Childress 1980, 1985, 1990; Childress and Somero 1990). These authors argued that this positive scaling, which opposes the general trend of an inverse relationship between body size and mass-specific metabolism, is associated with the power requirements for burst locomotion. In other fishes, such as striped bass (*Morone saxatilis*), anaerobic capacity scales positively with body size in small individuals but not in large individuals (Norton et al. 2000). If a clear relationship exists between metabolic capacity and performance independently of body mass, then this relationship should be most apparent in muscles that sustain high levels of performance.

The rattling system of rattlesnakes is an excellent system for studying the ontogeny of muscle contractile performance and metabolic capacity. Many rattlesnakes grow in mass more than 50-fold throughout life, and the phenomenal contractile performance of their shaker muscles varies with body size (Moon et al. 2002*b*). High-frequency contractions of the shaker muscles are necessary for generating rattling sounds, which deter potentially dangerous animals (Klauber 1972; Conley and Lindstedt 1996, 1998; Rome and Lindstedt 1998). In fact, rattling is one of the fastest sustainable vertebrate movements, and it is produced by specialized shaker muscles in the tail that have high aerobic and anaerobic metabolic rates and high endurance (Martin and Bagby 1972, 1973; Schaeffer et al. 1996; Kemper et al. 2001).

In western diamond-backed rattlesnakes (*Crotalus atrox*), we observed that the frequency of shaker muscle contractions and rattling increases dramatically from newborns to adults, which suggested that small individuals lacked the physiological capacity for sustaining high-frequency contractions. This observation appeared to be inconsistent with previous results indicating that shaker muscle contraction frequency decreases slightly from medium-sized to large adults (Moon et al. 2002*b*).

The reasons for this shift from radically increasing contractile performance to slightly decreasing performance are currently unknown, but evidence in the literature gives some clues about why the shift occurs. In the white locomotor muscles of some fishes, a transition from positive scaling to mass independence of anaerobic capacity (LDH activity) is probably associated with changing mechanical demands of locomotion in large individuals (e.g., Somero and Childress 1980, 1985; Childress and Somero 1990; Norton et al. 2000). A similar mechanical shift may occur in the rattling system of rattlesnakes, because the bony element (called the "style") onto which the shaker muscles insert develops from being lightly ossified in small individuals to being heavily ossified in large individuals (Zimmerman and Pope 1948). This pattern of bone growth suggests that the increasing mechanical demand to move a heavier appendage contributes to the decrease in performance in large individuals.

Finally, a clear relationship between muscle biochemistry and whole-animal performance independent of body mass has been difficult to demonstrate in other species (Gibb and Dickson 2002). Sound-producing muscles might be excellent systems for determining whether metabolic enzymes can be good indicators of performance independent of body mass because these muscles must achieve high levels of performance.

In this study, we tested the following hypotheses: (1) the dramatic increase in shaker muscle performance seen in small individuals is associated with increasing metabolic capacity; (2) metabolic enzymes in the shaker muscles are correlated with contractile performance independently of body mass; (3) shaker muscle develops higher capacities for aerobic and anaerobic metabolism than does locomotor muscle in the body; and (4) the gradual decrease in performance in large individuals is due to a mechanical constraint imposed by rattle mass increasing faster than muscle thickness.

Material and Methods

Study Animals

We measured rattling frequency and enzyme activities in 18 western diamond-backed rattlesnakes (*Crotalus atrox*) from southern Arizona. Animals ranged in snout to vent length (SVL) from 260 to 1,160 mm and in mass from 11.1 to 911 g. Five of these specimens were neonates born in the laboratory by two wild-caught females; incomplete absorption of the yolk sac indicated that two of these neonates were born prematurely. Five additional specimens from the wild were probably only 2 wk old on the basis of their body size and time of collection. We measured contractile performance of the recently born snakes within 1 wk of birth or capture.

To determine the allometric relationship between the mass of the proximal rattle segment and the cross-sectional area of shaker muscle, we used 22 additional preserved specimens ranging in size from 330 to 1,240 mm SVL and in mass from approximately 20 to 900 g. We collected all animals under permits from the Arizona Game and Fish Department and with Institutional Animal Care and Use Committees approval at the University of Washington (2230-05) and University of Louisiana at Lafayette (2003-8717-036 and 2004-8717-019).

Contractile Performance

As an indicator of performance, we measured shaker muscle contraction frequency by noninvasively measuring rattling frequency. This was possible because the muscles on each side of the tail contract once per cycle of rattle motion (Chadwick and Rahn 1954; Martin and Bagby 1973; Moon et al. 2003). We measured the rattling frequency of some individuals from videos recorded at 1,000 frames per second using a Redlake MotionScope PCI 2000s high-speed digital video camera, and we measured the frequency of other individuals using a position-sensing photodiode (following Moon et al. 2002*a*). Tests comparing these two recording methods showed that they gave identical results. Furthermore, because of the variable conditions available at the time, we measured contraction frequency of some individuals at 25°C and of others at 30°C. To be consistent with the enzyme assays, we converted all contraction frequencies measured at 30°C to values at 25°C using a Q_{10} of 1.65 (Schaeffer et al. 1996).

Metabolic Capacity

Tissue Sampling. After measuring the muscle contraction frequencies, we killed the snakes with pentobarbital and removed samples of epaxial locomotor muscle (Musculi semispinalis, longissimus dorsi, and iliocostalis) from the midtrunk and 3–5-mm sections of shaker muscle from the tail. We sampled shaker muscles from the distal part of the tail because we had previously determined by palpation and magnetic resonance imaging that only the distal part of the tail contains the thick vibratory muscles (Moon et al. 2002*a*). We froze the muscle samples in isopentane (2-methylbutane) that had been chilled in liquid nitrogen, and then we stored the samples at −70°C until they were needed for the enzyme assays.

Overview of Enzyme Assays. We measured the activities of two key enzymes in locomotor and shaker muscles following established protocols (Sidell et al. 1987; Seebacher et al. 2003; Southwood et al. 2003). Specifically, we assayed CS as an indicator of aerobic capacity and LDH as an indicator of anaer-

Table 1: Shaker muscle contractile performance and metabolic capacities in western diamond-backed rattlesnakes *Crotalus atrox*

	Body Mass (g)	Contraction Frequency (Hz)	CS Activity (U/g Muscle)	LDH Activity (U/g Muscle)
Locomotor muscle:				
Small individuals	11.1–22.7	<1	4.9–10.6	67.9–187.3
Large individuals	65–911	<1	2.9–8.8	126.7–369.4
Shaker muscle:				
Small individuals	11.1–22.7	4–79	55.9–124.8	240.3–492.3
Large individuals	65–911	51–54	135.9–239.5	289.2–530.2

Note. CS indicates citrate synthase, and LDH indicates lactate dehydrogenase. Typical contraction frequencies of locomotor muscle are unknown, but our observations of crawling speeds and axial-undulation frequencies suggest that the locomotor muscles typically contract at very low frequencies (<1 Hz). All values are for 25°C.

obic capacity. To prepare muscle samples for the enzyme assays, we thawed small pieces of each muscle on ice and removed all nonmuscle tissue by careful dissection under a microscope. We took care to ensure that shaker muscle samples contained only the large vibratory muscles and not the thin nonvibratory muscles present in the anterior tail and along the neural spines (Schultz et al. 1980). We then minced the muscle samples (of approximately 0.03–0.3 g) on an ice-cold stage and used a ground-glass homogenizer to homogenize them in 5% or 10% dilutions of ice-cold extraction medium (75 mM Tris-HCl, 1 mM EDTA, 2 mM $MgCl_2$, pH 7.4 at 25°C). To disrupt the cell membranes and release the enzymes into the solution, we froze the homogenates in liquid nitrogen and then thawed them again for use in the assays. We diluted homogenates whenever necessary to ensure that substrate concentration did not limit reaction rates (i.e., to ensure that reaction rates were directly proportional to amount of homogenate added); we found that the rates of change of absorbance for both assays were routinely less than 0.1 absorbance unit per minute and that reaction rates were linear for the duration of each assay. We measured the enzyme activities in duplicate or triplicate at 25°C with a Hitachi U2000 UV/VIS spectrophotometer equipped with a water-jacketed cell changer.

All enzyme activities were expressed in units of activity (micromoles of substrate converted to product per minute) per gram wet mass of muscle (U/g muscle). Our focus was on maximal metabolic capacities and muscle performance; therefore, we reported the results for the sample with the highest values of CS activity whenever we found variation in enzyme activity in samples from slightly different positions along the tail. In almost all cases, the samples that exhibited the highest CS activity also showed the highest LDH activity.

Citrate Synthase (Enzyme Commission Number: EC 4.1.3.7). We determined CS activity by measuring the reduction of DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] by free coenzyme A at 412 nm. The reaction mixture consisted of 0.5 mM oxaloacetic acid, 0.25 mM DTNB, 0.4 mM acetyl-CoA, 75 mM Tris-HCl, pH 7.83 at 25°C. We first assayed control solutions (before initiating the reaction with the oxaloacetic acid) to determine background deacylase activity, which was less than approximately 1% for shaker muscle; thus, background deacylase activity in shaker muscle homogenates was trivial compared with the CS activity following initiation of the reaction with oxaloacetic acid. Whenever background deacylase activity was greater than 5% of the initiated rate, we subtracted it from the activity recorded after initiation.

Lactate Dehydrogenase (Enzyme Commission Number: 1.1.1.27). We determined LDH activity by measuring the oxidation rate of NADH at 340 nm during the reduction of pyruvate to lactate. The reaction mixture consisted of 0.15 mM NADH, 1 mM KCN, 50 mM imidizole, and 1.0 mM pyruvate, pH 7.33 at 25°C. Preliminary tests revealed that 1.0 mM pyruvate elicited maximal LDH activity in both shaker and locomotor muscle.

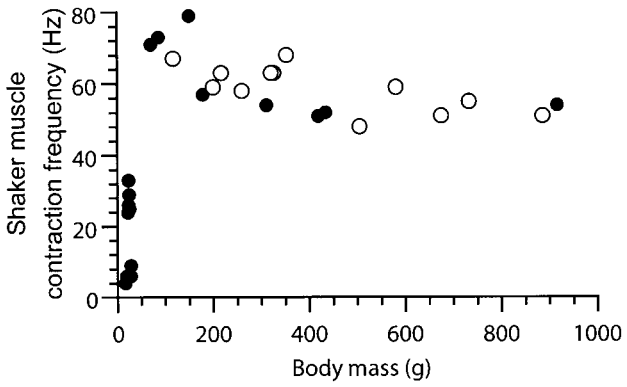

Figure 1. Relationship between shaker muscle contraction frequency at 25°C and body mass in western diamond-backed rattlesnakes *Crotalus atrox*. Solid symbols indicate individuals used in this study ($N = 18$); open symbols indicate data from previous studies ($N = 13$; Moon et al. 2002*a*, 2003). Contraction frequency increased 20-fold in small individuals but then declined by approximately 30% in the largest individuals.

We also ran control reactions that lacked pyruvate. In all cases, control rates were insignificant compared with rates after initiation of the reaction with pyruvate.

Morphology and Mechanics

To test the hypothesis that the decline in shaker muscle contraction frequency at larger sizes is due to an increasing mechanical limitation, it was necessary to measure the mass of the first rattle segment and determine the physiological cross-sectional area (pCSA) of shaker muscle. Shaker muscles insert onto the style inside the first rattle segment (Zimmerman and Pope 1948). The style develops from the fusion of approximately six to 10 vertebrae at the tip of the tail and makes up most of the mass of the rattle (Zimmerman and Pope 1948). Shaker muscle contractions pull on the style to shake the rattle. Throughout the life of a rattlesnake, the style continues to ossify and enlarge (Zimmerman and Pope 1948). To measure how style mass changes with body mass, we removed and weighed the first rattle segment (which contains the style) from 22 *C. atrox* encompassing an approximately 45-fold range in body mass.

We determined the pCSA of shaker muscle by first determining the height of the proximal rattle segment and using this value as a measure of tail diameter (Fitch and Pisani 1993). This approach enabled us to determine cross sections in specimens whose soft tissues were distorted by body condition and preservation. We then calculated the total cross-sectional area of the tail using the relationship πr^2, where r = tail radius. We subtracted 20% from the total cross-sectional area to account for the area occupied by nonmuscle tissue (as in Moon et al. 2002*a*). Next, we multiplied the total cross-sectional area by the cosine of the average fiber angle (0.35 radians; the average of the values for the three shaker muscles reported by Moon et al. 2002*a*) to estimate the pCSA, which is proportional to a muscle's capacity to generate force. Finally, we subtracted half of the total pCSA to obtain the pCSA of one side of the tail.

To assess whether changes in contraction frequency were related to changes in a mechanical constraint, we tested scaling predictions derived from linear and angular mechanics. Muscle force is proportional to pCSA. Therefore, mass × acceleration should be proportional to pCSA. For rattle acceleration (and hence oscillation frequency) to remain constant as body size increases, rattle mass should scale isometrically with shaker muscle pCSA. If rattle mass increases faster than muscle pCSA, then acceleration must decrease. The decreased acceleration could lead to smaller displacement per unit time or to a decreased frequency for the same displacement. Because rattle displacement does not decrease with increasing body size (based on data from Moon et al. 2002*b*), a decrease in acceleration must lead to a decrease in frequency. We tested whether rattle mass scales isometrically with shaker muscle pCSA.

Rattle oscillation involves rotational motion (Moon et al. 2002*a*, 2002*b*). Therefore, we also tested scaling predictions based on angular mechanics. The shaker muscles generate a torque that rotates the rattle. The muscle torque is equal to force × moment arm length (McMahon 1975; Ozkaya and Nordin 1999); because force is proportional to cross-sectional area (length^2), the torque is proportional to length^3. Rattle torque is equal to its mass moment of inertia × angular ac-

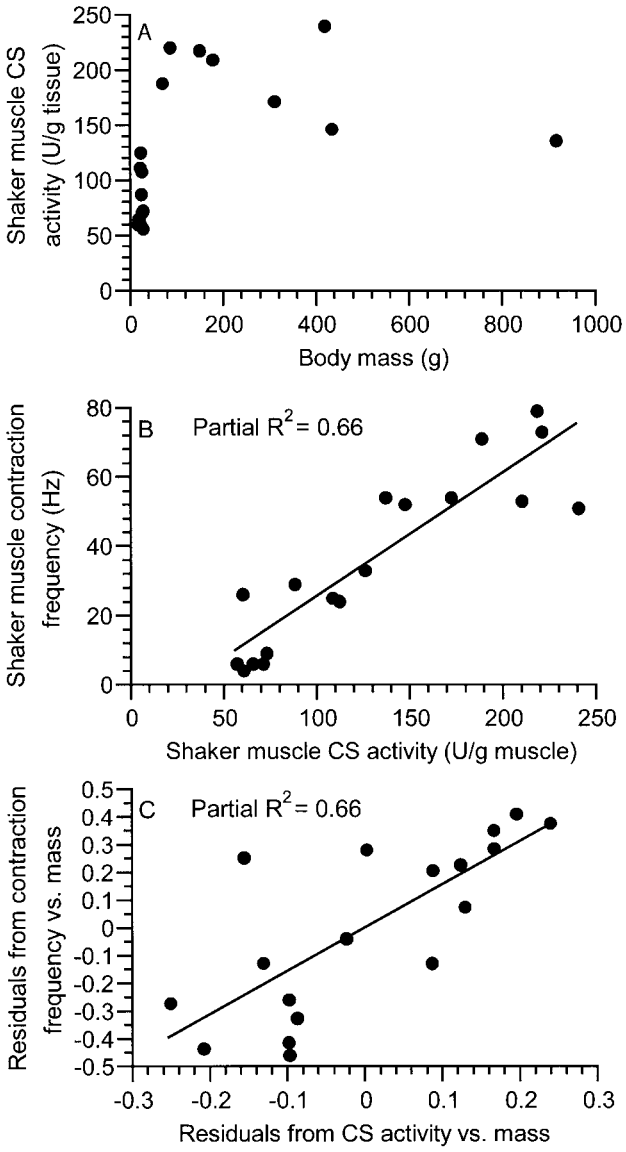

Figure 2. Aerobic enzyme activity in the shaker muscle of western diamond-backed rattlesnakes *Crotalus atrox* at 25°C. *A*, Relationship between citrate synthase (CS) activity and body mass. *B*, Relationship between contraction frequency and CS activity. *C*, Relationship between mass-independent contraction frequency and mass-independent CS activity. CS activity changed significantly with body mass and was significantly correlated with contraction frequency. Multiple regression results are provided in Table 2. $N = 18$ for all plots.

Table 2: Regression results for muscle contractile performance and metabolic capacity in western diamond-backed rattlesnakes *Crotalus atrox*

Dependent Variable	Regression Equation	R^2
Frequency	(.13 × mass) + (1.56 × CS*) − (1.26 × LDH*) + 1.17	.85*
S-CS	(.36 × mass*) + 1.43	.55*
S-LDH	(.15 × mass) + 2.30	.06
L-CS	(−.28 × mass) + 1.28	.13
L-LDH	(.29 × mass*) + 1.69	.30*
Residual frequency	(1.56 × residual CS*) − (1.26 × residual LDH*)	.71*

Note. The first and last rows indicate least squares multiple regression results; all other rows indicate reduced major axis bivariate regression results. Values are given for analyses of log transformed data. Frequency indicates shaker muscle contraction frequency (Hz); S = shaker muscle, L = locomotor muscle; CS indicates citrate synthase activity, and LDH indicates lactate dehydrogenase activity (U/g muscle); residual indicates that mass-independent residuals were used as independent variables. $N = 18$ individuals.

* Indicates significant terms ($P < 0.05$).

celeration (McMahon 1975; Ozkaya and Nordin 1999). Mass moment of inertia is equal to $\sum (ml^2)$, where m = mass (proportional to length^3) and l = length from the mass to the center of rotation (Alexander 1983). Therefore, rattle torque is proportional to length^5. For the muscle and rattle torques to balance, angular acceleration (which increases with frequency) should decrease in proportion to length^{-2}, which is equivalent to pCSA^{-1}. We tested whether rattling frequency decreases at larger body sizes in proportion to pCSA^{-1}.

Statistical Analyses

We first $\log_{10}$ transformed the data to improve linearity and reduce heteroscedasticity and then used SPSS software, version 8.0.2, for all analyses. The analyses explained below correspond to the hypotheses described in the introduction.

1. We used multiple regression analysis to test whether increasing contraction frequency in shaker muscles is correlated with increasing aerobic and anaerobic enzyme activities. This approach allowed us to determine the effect of each independent variable on the dependent variable while every other independent variable was held constant at its mean. This way of controlling for the effects of multiple independent variables is important because muscles typically cannot optimize both aerobic and anaerobic capacities simultaneously but instead tend to show a trade-off between these capacities (Lindstedt et al. 1998). In this analysis, the dependent variable was contraction frequency, and the independent variables were shaker muscle CS and LDH activities and body mass. For all analyses, we inferred a significant relationship whenever $\alpha < 0.05$.

To obtain accurate scaling exponents for all bivariate scaling relationships, we computed reduced major axis (RMA) regressions using PAST software, version 1.26 (Rayner 1985; Hammer et al. 2001). For the scaling of contractile performance, we used RMA regression of contraction frequency against body mass. We computed the RMA slopes separately for small individuals (<50 g, which showed the most rapid increase in performance) and large individuals (>50 g, which showed the gradual decline in performance with increasing size).

2. To test whether CS and LDH activities in shaker muscle are correlated with contraction frequency independently of body mass, we analyzed residuals that represented variation in contractile performance and enzymatic capacity independent of mass. To do so, we obtained residuals of contraction frequency and of each enzyme activity after a linear regression of each variable against body mass. RMA residuals are inappropriate for this purpose because they are not fully independent of the x variable (Harvey and Pagel 1991). We then computed a multiple regression of residual frequency against residual CS and residual LDH activities.

3. To test for higher enzyme activities in shaker muscle than in locomotor muscle, we used a one-way ANOVA with CS or LDH activity of each muscle type as a dependent variable, muscle type as a fixed factor, and size class (<50 g or >50 g on the basis of the rationale described above) as a covariate.

4. To test whether decreasing contraction frequency in large individuals is associated with rattle mass increasing faster than muscle thickness, we computed an RMA regression using PAST software, version 1.26 (Hammer et al. 2001). We first regressed rattle mass against shaker muscle pCSA and then used the method of Zar (1984) to test for a scaling exponent significantly greater than 1. Specifically, we used a one-tailed t-test because we hypothesized that the increasing style mass reported by Zimmerman and Pope (1948) would lead to rattle mass increasing faster than muscle pCSA.

Results

Contractile Performance

All individuals rattled readily at the slightest disturbance, with contraction frequencies ranging from 4 to 79 Hz at 25°C in individuals of 11–911 g body mass (Table 1; Fig. 1). Although

the neonates rattled within hours of birth, the frequency of shaker muscle contractions and rattling was very low. For small individuals (<50 g), RMA regression results showed that contraction frequency scaled in proportion to mass$^{3.65}$. In individuals larger than 50 g body mass, contraction frequency scaled in proportion to mass$^{-0.20}$ and declined by 32% from 79 Hz at 145 g to only 54 Hz at 911 g.

Metabolic Capacity

Shaker muscle ranged from light red in the smallest individuals to deep red in the largest snakes. At all body sizes, the reddish color of shaker muscle contrasted markedly with the pale locomotor muscle in the body.

Shaker muscle CS activity and contraction frequency varied with body mass in a similar way (Fig. 2). CS activity increased fourfold from birth to a mass of 145 g and then declined gradually by up to 38% in the largest individual (911 g). Therefore, contraction frequency was significantly correlated with aerobic capacity (Table 2; Fig. 2). LDH activity varied with body mass somewhat differently than did contraction frequency (Fig. 3). Some of the smallest individuals had nearly the highest LDH activities, which obscured a possible trend of rapidly increasing LDH activity in small individuals. Because of this variation, contraction frequency was slightly but significantly correlated with anaerobic capacity (Table 2; Fig. 3).

After the effect of mass was removed from the data using an analysis of residuals, contractile performance remained significantly correlated with both CS and LDH activity (Table 2; Figs. 2, 3). Mass-independent CS activity had a large positive effect on performance (partial $R^2 = 0.66$, $P = 0.00$), but LDH activity had only a small negative effect (partial $R^2 = 0.30$, $P = 0.02$).

Finally, shaker muscle had significantly higher CS and LDH activities than did locomotor muscle (Table 1; Fig. 4). CS activity differed significantly between muscle types ($F_{1,33} = 390.63$, $P < 0.05$) but did not differ significantly between small and large individuals ($F_{1,33} = 3.61$, $P = 0.07$). LDH activity differed significantly between muscle types ($F_{1,33} = 79.66$, $P < 0.05$) and between small and large individuals ($F_{1,33} = 13.12$, $P < 0.05$). Regardless of snake size, shaker muscle aerobic capacity was typically an order of magnitude greater than that of locomotor muscle; the average glycolytic capacity of shaker muscle was 2.4 times higher than that of locomotor muscle in small individuals and 1.8 times as high as that of large individuals (Table 1; Fig. 4).

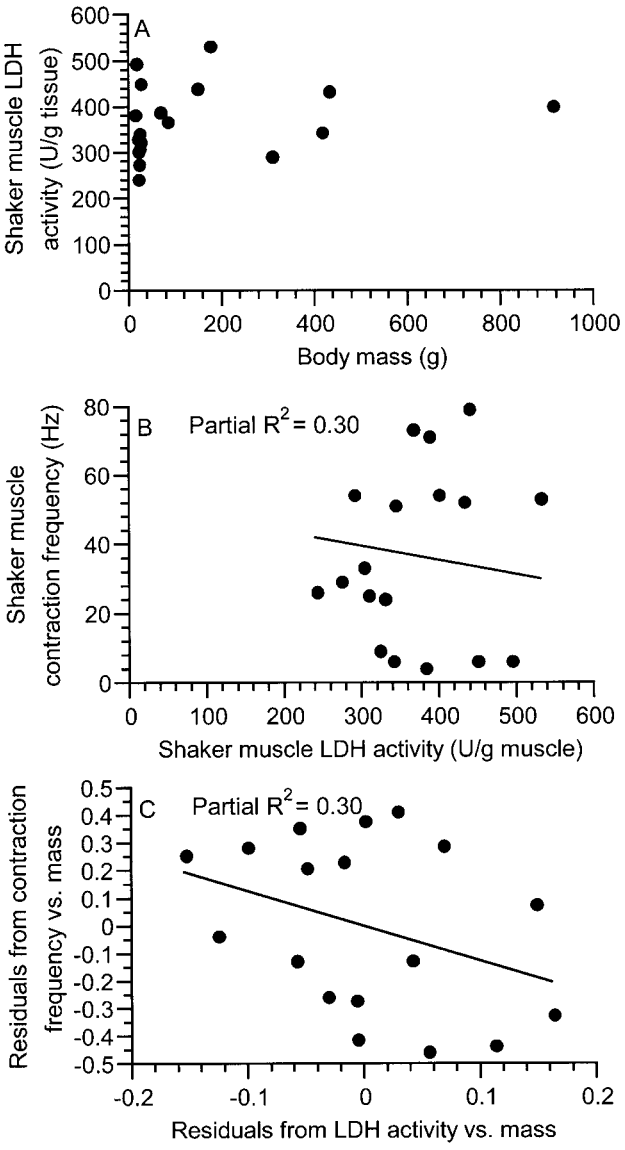

Figure 3. Anaerobic enzyme activity in the shaker muscle of western diamond-backed rattlesnakes *Crotalus atrox* at 25°C. *A*, Relationship between lactate dehydrogenase (LDH) activity and body mass. *B*, Relationship between contraction frequency and LDH activity. *C*, Relationship between contraction frequency and LDH activity after the effect of mass is removed with an analysis of residuals. LDH activity was not significantly correlated with body mass. Contraction frequency was weakly and negatively correlated with LDH activity. Multiple regression results are provided in Table 2. $N = 18$ for all plots.

Morphology and Mechanics

Rattle mass, as measured by mass of the first rattle segment containing the style, increased significantly faster than the pCSA of shaker muscle (RMA scaling exponent significantly >1; $t_{0.05(1),20} = 6.38$, $P < 0.001$). The allometric equation for this relationship was $y = -2.62x^{1.36}$, where y = style mass and x = shaker muscle pCSA (Fig. 5). Thus, rattle mass increased faster than the force-producing ability of the shaker muscles.

Rattling frequency decreased approximately in proportion to pCSA^{-1}, as expected on the basis of angular mechanics. In our sample, individuals from 145–911 g in mass showed a 39%

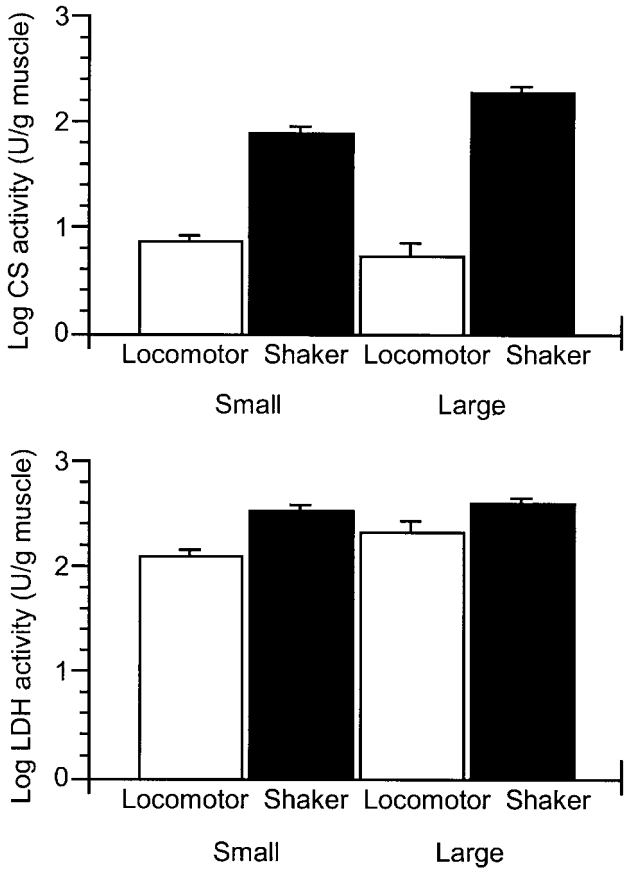

Figure 4. Differences in citrate synthase (CS) and lactate dehydrogenase (LDH) activities at 25°C in the locomotor muscle (*open bars*) and shaker muscle (*solid bars*) of small (<50 g) and large (>50 g) western diamond-backed rattlesnakes *Crotalus atrox*. Error bars indicate SEs. Both CS and LDH activity were significantly higher in shaker muscle than in locomotor muscle. ANOVA results are given in the text. $N = 10$ small and 8 large snakes.

decline in $pCSA^{-1}$. This decrease in force-producing ability closely matched the 32% decline in actual performance.

Discussion

Our results for *Crotalus atrox* show for the first time the rapid development of contractile performance in shaker muscle. The results have shown that the rapid increase in shaker muscle performance with growth in small individuals is associated with a parallel increase in aerobic capacity but not anaerobic capacity.

Contractile Performance

The rapid increase in contractile frequency from neonates to young snakes indicates that development of the rattling system is not complete before birth. This inference is further supported by the results of Zimmerman and Pope (1948) that showed incomplete ossification of the bony style in the base of the rattle at birth. The dramatic increase in performance in small individuals probably involves complex interactions among physiological and morphological development and may be related to the importance of rapid contractions and defensive sound production as early in growth as possible. The rapid increase in rattling performance is consistent with the hypothesis of Rowe and Owing (1996) that natural selection has favored the ability of rattlesnakes to "sound big" as quickly as possible during growth. The decreasing contraction frequency (which scales as $mass^{-0.20}$) in large individuals is consistent with limb cycling frequencies in other animals and matches the prediction for elastic similarity (McMahon 1975; Rayner 1988; Full 1997; Medler 2002).

Metabolic Capacity

Sound-producing muscles, including rattlesnake shaker muscles, typically contract at very high frequencies and hence require high rates of ATP production (Conley and Lindstedt 1996, 1998). Moreover, rattlesnakes can sustain high-frequency contractions for at least 3 h (Martin and Bagby 1972). As expected on the basis of those results, rattlesnake shaker muscle had an extraordinarily high capacity for oxidative metabolism compared with rattlesnake locomotor muscle. Shaker muscle CS activity was typically more than an order of magnitude greater than that of locomotor muscle (Table 1; Fig. 4), which is not surprising given shaker muscle's high volume density of mi-

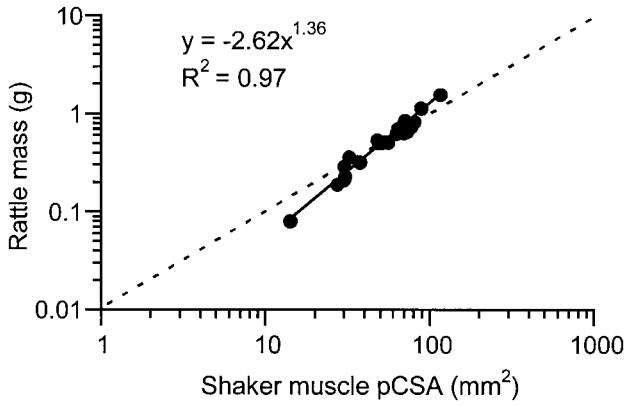

Figure 5. Relationship between the mass of the proximal segment of the rattle (which contains the bony style) and the physiological cross-sectional area (pCSA) of shaker muscle in western diamond-backed rattlesnakes *Crotalus atrox*. For rattle acceleration to remain constant as size increases, its mass should scale isometrically with respect to muscle pCSA (indicated by the dashed line with slope of 1). Our results indicated that rattle mass increases faster than muscle pCSA and, therefore, that rattle acceleration decreases as size increases. Because rattle displacement does not decrease at larger sizes, the reduced acceleration must lead to a reduced frequency. $N = 22$.

Table 3: Mitochondrial volumes and citrate synthase activities of highly aerobic muscles from various vertebrates

Animal	Tissue Type	Cell Volume Occupied by Mitochondria (%)	Cell Volume Occupied by Myofibrils (%)	CS Activity at 25°C (U/g Muscle)
Mediterranean swordfish (*Xiphias gladius*)	Heater muscle	68[a]	0[a]	193[b]
Frog (*Hyla versicolor*)[c]	Calling muscles	21	53	102
Tuna (*Katsuwonus pelamis*)[d]	Red axial muscle	32	…	80
Hummingbird (*Selasphorus rufus*)	Flight muscle	35[e]	45–50[f]	170[g]
Shrew (*Sorex vagrans*)	Gastrocnemius muscle	45–50[f]	45–50[f]	28[g]
Adult rattlesnake (*Crotalus atrox*)	Shaker muscle	26[h]	32[h]	136–240[i]

Note. Citrate synthase activities obtained at temperatures other than 25°C were corrected to this temperature using a Q_{10} of 2. Footnotes indicate sources.

[a] Block 1991.

[b] Tullis et al. 1991.

[c] Averages for internal and external oblique muscles and laryngeal muscles from Marsh and Taigen 1987.

[d] Moyes et al. 1992.

[e] Suarez et al. 1991.

[f] Estimates cited in Emmett and Hochachka 1981.

[g] Suarez et al. 1990.

[h] Schaeffer et al. 1996.

[i] This study.

tochondria and the low value in locomotor muscle (Schultz et al. 1980; Schaeffer et al. 1996). Furthermore, the aerobic capacity of rattlesnake shaker muscle exceeds the capacities of highly aerobic muscles from other vertebrates (Table 3). Shaker muscle aerobic capacity even rivals that of billfish heater muscle, which is particularly impressive because shaker muscles produce movement and hence must have some myofibrils, whereas heater muscle lacks contractile filaments entirely and is more fully packed with mitochondria (55%–70% of cell volume; Block 1991). Hochachka et al. (1988) hypothesized that the highest enzyme activities measured in homeotherms (e.g., hummingbirds and shrews) are near the upper aerobic limits for muscle, above which further increases must incur reductions in other cellular components such as myofibrils. This hypothesis is supported by evidence indicating that extremely high aerobic capacities are often associated with reductions in myofibrils and sarcoplasmic reticulum (Lindstedt et al. 1998). Rattlesnake shaker muscles are also consistent with this hypothesis in having an extremely high CS activity and volume density of mitochondria but a low volume density of myofibrils (Schaeffer et al. 1996). Consequently, although shaker muscle contracts at very high frequencies, it generates low forces and power (Rome et al. 1996; Moon et al. 2002*a*).

The strong correlation between shaker muscle contractile performance and aerobic capacity is congruent with the finding that approximately two-thirds of the ATP used by active shaker muscle derives from oxidative metabolism (Kemper et al. 2001). We also expected to see a significant positive correlation between contractile performance and LDH activity because approximately one-third of ATP use during shaker muscle activity is derived from glycolysis and lactate production (Kemper et al. 2001). Interestingly, however, contractile performance was negatively correlated with LDH activity, although the correlation was weak (Table 2; Fig. 3). It is possible that the relative contributions of aerobic and anaerobic metabolism to shaker muscle activity change as snakes grow, but this hypothesis has not yet been tested.

Shaker muscle contractile performance was significantly correlated with CS and LDH activity even after the effects of body mass were removed statistically, although the relationship for LDH appeared to be relatively weak (Table 2; Fig. 3). A clear relationship between muscle biochemistry and whole-animal performance independent of body mass has been difficult to demonstrate in other species (Gibb and Dickson 2002). Our results add confidence to the inference that aerobic enzyme activity is a reasonable indicator of metabolic capacity and actual performance in shaker muscle and is not largely confounded by other correlates of animal size.

The results of the mechanical and metabolic portions of this study indicate that the dramatic ontogenetic change in shaker muscle performance in small snakes was associated with a parallel increase in aerobic, but not anaerobic, capacity. The 38% decline in aerobic capacity in large individuals is sufficient to explain the 32% decline in contractile performance in these individuals. However, the declining aerobic capacity may not be the only factor limiting contractile performance. The continuously increasing rattle mass shown by Zimmerman and Pope (1948) suggests that biomechanical demands increase as aerobic capacity declines in large individuals, which may further limit contractile performance. Our morphological and biomechanical results allowed us to test whether increasing mechanical demands are also important in limiting contractile performance in large individuals.

Morphology and Mechanics

The scaling of rattle mass and shaker muscle pCSA indicates that rattle mass increases faster than ability of shaker muscle to generate force and accelerate the rattle. Because rattle displacement does not decrease at larger body sizes (based on data from Moon et al. 2002*b*), the reduced acceleration must lead to a reduced frequency. Similarly, the scaling of angular mechanics indicates that rattle oscillation frequency, and hence shaker muscle contraction frequency, decreases in large individuals. Both the linear and angular mechanics indicate that the contractile performance of large individuals is limited by the need to accelerate a larger mass without a correspondingly larger cross-sectional area of shaker muscle. Therefore, although shaker muscle performance appears to be limited mainly by aerobic capacity in small individuals, it becomes equally limited by aerobic capacity and a mechanical constraint in large individuals.

Acoustic and Ecological Implications of Shaker Muscle Ontogeny

Newborn rattlesnakes are under parental care for approximately 10–14 d after birth, until they shed their skins for the first time (Price 1988; Greene et al. 2002). Subsequently, the young snakes disperse and the mother leaves to resume activity and feeding. After the first shedding cycle, the young rattlesnakes lose the "prebutton" covering the tip of the tail and gain the larger but still mute "button," which has no other loose rattle segments to click against (Zimmerman and Pope 1948; Klauber 1972). The rattles of newborn rattlesnakes remain mute until they shed their skins for the second time and develop a loose (dead) segment that is attached to and clicks against the living segment at the base of the rattle (Zimmerman and Pope 1948; Klauber 1972). Therefore, the onset of sound production likely occurs well after the young have dispersed away from their mother. The inability to generate defensive sounds may increase the risk of predation on young snakes until they shed a second time and develop audible rattles. If this is the case, then the small snakes face the conflicting demands of needing to reduce surface activity in order to reduce the risk of predation and needing to feed more frequently than large individuals. Furthermore, for some time after the development of audible rattles, the low contraction frequencies of the shaker muscles and the small rattles of young snakes generate relatively quiet sounds (Young and Brown 1993, 1995; Cook et al. 1994; Rowe et al. 2002). The period in which shaker muscle performance is submaximal remains poorly known and may last into the second activity season.

The decline in shaker muscle contraction frequency in large individuals may affect rattling sound output and hence the effectiveness of the rattling system during defense. The loudness and frequency content of rattling sounds are affected by rattle material, rattle size, and body temperature, which determines rattle vibration frequency (Young and Brown 1993, 1995; Cook et al. 1994; Rowe and Owings 1996). Furthermore, the loudness of rattling increases asymptotically with body mass and approaches the maximum in snakes of moderate body masses (Rowe and Owings 1996). Therefore, the gradual decline in rattle vibration frequency that occurs in large individuals may be associated with only a small reduction in the effectiveness of rattling as a defensive behavior. In addition, the reduced rattling performance of very large individuals may have advantages in other circumstances. The reduced rattling frequency (and click rate) in very large snakes may make them sound less dangerous to prey animals such as ground squirrels, which actively elicit rattling during encounters and use the click rate as an indicator of the snake's body temperature and hence its ability to strike quickly and accurately (Rowe and Owings 1990, 1996; Swaisgood et al. 1999). Thus, the slightly reduced rattling performance of very large snakes may enhance predation success. These factors indicate that the complex morphology, physiology, and ontogeny of the rattling system are associated with equally complex acoustics, behaviors, and ecology.

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