Mechanical trade-offs explain how performance increases without increasing cost in rattlesnake tailshaker muscle

Brad R. Moon[1],*, J. Johanna Hopp[2] and Kevin E. Conley[1,2]

[1]*Department of Radiology, Box 357115* and [2]*Department of Physiology and Biophysics, Box 357290, University of Washington Medical Center, Seattle, WA 98195, USA*

*Present address: Department of Biology, PO Box 42451, University of Louisiana at Lafayette, Lafayette, LA 70504-2451, USA
(e-mail: BradMoon@louisiana.edu)

Summary

Rattling by rattlesnakes is one of the fastest vertebrate movements and involves some of the highest contraction frequencies sustained by vertebrate muscle. Rattling requires higher accelerations at higher twitch frequencies, yet a previous study showed that the cost per twitch of rattling is independent of twitch frequency. We used force and video recordings over a range of temperatures to examine how western diamondback rattlesnakes (*Crotalus atrox*) achieve faster movements without increases in metabolic cost. The key findings are (i) that increasing muscle twitch tension trades off with decreasing twitch duration to keep the tension–time integral per twitch nearly constant over a wide range of temperatures and twitch frequencies and (ii) that decreasing lateral displacement of the rattle joint moderates the mechanical work and power required to shake the rattle at higher frequencies. These mechanical trade-offs between twitch tension and duration and between joint force and displacement explain how force, work and power increase without an increase in metabolic cost.

Key words: western diamondback rattlesnake, *Crotalus atrox*, muscle, force, work, power, contraction frequency, rattling.

Introduction

High-frequency muscle contractions, such as those required for sound production, are energetically expensive (Conley and Lindstedt, 1996; Taigen and Wells, 1985). To sustain high-frequency contractions, muscles must reduce the metabolic energy cost per twitch (Conley and Lindstedt, 1996; Rome et al., 1999), perhaps by exerting low forces, producing only small joint displacements and doing little work.

Rattlesnake tailshaker muscles may show these features particularly clearly because they can sustain high contraction frequencies of 20 to nearly 100 Hz for up to several hours (Martin and Bagby, 1972). Faster rattling at higher temperatures requires faster accelerations of the rattle. Rattling force must increase to accelerate the rattle faster, yet the cost per twitch is independent of twitch frequency (Conley and Lindstedt, 1996). The constant cost per twitch is surprising because the costs of muscle contraction and animal movement are proportional to force exertion (Kram and Taylor, 1990; Kushmerick and Paul, 1976b; Taylor et al., 1980). How does muscle force increase without increasing cost?

In this study, we used force and high-speed video recordings of rattling in western diamondback rattlesnakes (*Crotalus atrox*) to test how muscle twitch frequency and tension increase without increasing cost and how changes in joint motion reduce the mechanical work required from the muscles to shake the rattle. Specifically, we tested whether twitch tension and duration trade off to keep the tension–time integral per twitch constant over a wide range of temperatures, which would explain the constant cost per twitch, and whether force and joint displacement trade off to keep the work and power of rattling low.

Materials and methods

Animals and handling

We used nine western diamondback rattlesnakes, *Crotalus atrox* Baird and Girard, from central and southern Arizona for force recordings. To handle a snake, we first drew the snake up into a clear acrylic tube and then transferred it into a Tupperware container. The tail and rattle passed out horizontally through a hole in the side of the container and were able to move freely. The tail was secured in place by Velcro tape wrapped around the body just anterior to the cloaca; the tape around the body bonded to complementary tape lining the hole in the container, which prevented the tail from being pulled into the container during the experiments.

Rattling frequency is temperature-dependent (Chadwick and Rahn, 1954; Martin and Bagby, 1972). We controlled the animal's body temperature over the range 5–35 °C by circulating temperature-controlled water through copper tubing in the bottom of the snake container. We varied the

starting temperature and direction of temperature change (heating or cooling by approximately $5\,°C\,h^{-1}$) for each animal, and collected data at intervals of $2.5–5\,°C$.

Anatomy

We determined tailshaker muscle masses *in vivo* from magnetic resonance images. For imaging, each snake was placed in a Tupperware container with the tail placed in an imaging coil. The container, snake and coil were then placed in a Signa 1.5 T scanner (General Electric Medical Systems, Milwaukee, WI, USA). Cross-sectional images were obtained from three-dimensional spin echo images taken along the entire tail. The imaging parameters were: repetition time=27.4, echo time=5.6, 1 mm slice with no interval between slices, 512×224 pixels per slice, number of excitations=2. We used NIH Image 1.62 to measure the cross-sectional area of tailshaker muscle in each slice, and then summed the areas from all (29–55) slices to determine muscle volume. To calculate muscle mass, we multiplied muscle volume by density ($1.06\,g\,ml^{-1}$). Total tail volumes measured with the magnetic resonance images agree with volumes determined from measurements made by hand on the tails. Tailshaker muscle accounts for 89 % of the total tail volume in females and 66 % in males; non-muscle tissue includes skin, vertebrae, major blood vessels, scent glands and (in males) hemipenes. To determine the physiological cross-sectional area, we multiplied the cross-sectional area of muscle anterior to the rattle by the cosine of the aveage muscle fibre angle measured from dissection of two specimens; the cross section was taken from the region of maximum diameter nearest the rattle to avoid inaccuracy due to the short zone of tapering diameter where the tail joins the rattle. The muscle fibres immediately anterior to the rattle span the distal-most tail vertebrae, including the terminal rattle joint where the last individual vertebra articulates with the rattle shaker element, and insert directly onto the base of the rattle; these muscle fibres shake the rattle during rattling.

Rattling frequency and force

To record the forces exerted *in vivo* by the tailshaker muscles during rattling, we made an optical force transducer based on the design used by Tu and Daniel (1999). The transducer consisted of a 0.8 mm×12.7 mm brass beam clamped at one end and forming a 50 mm cantilever at the free end. A cotter pin soldered to the beam projected vertically downwards into the space between a light-emitting diode and a position-sensing photodiode. Displacement of the beam, cotter pin and its shadow on the photodiode were proportional to the applied force.

We used a two-step procedure to calibrate the force transducer. First, to calibrate transducer output to newtons of force, we turned the transducer on its side and placed known weights on the brass beam where the rattle was attached during experiments. Second, we tested the transducer dynamics by oscillating it with a muscle lever (Cambridge Technology, model 305, dual-mode servo system) driven by a sine-wave function of constant peak-to-peak amplitude and at frequencies of 10–100 Hz. The optical transducer and muscle lever gave independent measures of force that we used to calibrate the transducer output over the wide range of rattling frequencies. The results of the transducer plus muscle lever calibration were consistent with the physics of forced vibrations (Giancoli, 2000) (see p. 379). In addition, the forces measured with the transducer agreed with the forces determined from our kinematic measurements of rattle displacement and work (described below).

For the force recordings, we attached the rattle to the free end of the transducer beam with thin wire tied around the rattle in the dorsoventral groove formed by the articulation between the first and second segments. The proximal groove of the rattle is immediately distal to the insertions of the terminal shaker muscles and very closely approximates the centre of mass of the rattle. To induce the snakes to rattle vigorously, we tapped the container and stimulated the body musculature electrically using the method of Conley and Lindstedt (1996). Our stimulation regime involved 0.5 ms pulses of 10–50 V, given in 1–3 trains of five pulses each.

The force signals were digitised with LabView using an A/D sampling rate of 2000 Hz per channel, which was more than 20 times faster than the maximum rattling frequency we observed. After acquisition, the signals were digitally filtered below 20 Hz (the lower limit of rattling frequency) to remove baseline fluctuations. From the filtered data, we analysed five consecutive cycles of the highest amplitudes of rattling from a sequence of at least 10–20 cycles of stable amplitude and frequency. From the digitised data, we measured rattling frequency and peak force over the range of experimental temperatures.

We calculated peak muscle force by dividing the peak rattling force by the cosine of the muscle angle relative to the force transducer (in the plane of transducer motion) and then dividing this value by the cosine of the mean fibre angle relative to horizontal. To calculate the specific tension, we then divided peak muscle force by the physiological cross-sectional area of the muscles on one side of the rattle joint.

Rattling work and power

The complex morphology and motion of the rattle required several analytical steps, described in detail below, to yield a simple measure of rattle work per cycle of motion and inertial power. The analysis involved determining the mechanical work and inertial power of rattling from the changes in rattle kinetic energy, which required measuring rattle motion from high-speed video recordings, determining the moments of inertia and angular velocities for each component of motion and accounting for mechanical energy transfer along the rattle. We then tested the results against the work and power required to oscillate a pendulum of similar geometry and motion to a real rattle.

Video recordings

We used a high-speed digital video camera (Redlake *Motion*Scope 2000S) to record rattle motion. Film speed was

500 frames s^{-1} for rattling frequencies lower than 50 Hz and 1000 frames s^{-1} for frequencies higher than 50 Hz. These film speeds of over 10 times the rattling frequency were sufficient to capture the motion accurately (Blickhan and Full, 1992; Harper and Blake, 1989). The camera recorded a dorsal image of the rattle directly and lateral and transverse images *via* mirrors placed at 45 ° along the side and at the end of the rattle. We then used NIH Image 1.62 to measure rattling frequency and the angular displacements of each rattle segment.

Work

We determined the mechanical work of rattling by measuring the changes in kinetic energy of the rattle throughout each cycle of motion. It was unnecessary to measure gravitational potential energy because the rattle was held nearly horizontal and did not change in vertical position during our recordings.

The kinetic energy of a rotating object depends on its moment of inertia and angular velocity. Because rattle motion involves lateral rotation and longitudinal twisting (i.e. torsion), we measured the moments of inertia and angular velocities separately for lateral displacement and torsion of the rattle. To simplify the analysis of rattle motion, we treated the rattle as a segmented pendulum that twists as it swings from side to side, and calculated the total kinetic energy by summing the kinetic energies for lateral and torsional motion of each rattle segment.

Moment of inertia. The moment of inertia of an object depends on the distribution of its mass. To determine the mass of each rattle segment, we measured three rattles, cut them into segments and determined the density of each segment by dividing its mass by its volume determined from measurements of the segment length, width and height. This measure of density did not reflect the actual density of the rattle keratin, but did allow accurate determination of segment masses from their dimensions. We then used the density and measurements of intact rattles in the remaining snakes to determine the mass of each segment and of the whole rattle. This approach allowed us to determine the mass and moment of inertia of each rattle without having to remove and destroy the rattle from every snake.

We then used the mass of each rattle segment and its distance from the centre of rotation to calculate its moment of inertia, following the strip method of Van Den Berg and Rayner (1995). To simplify this calculation, we treated each rattle segment as a bar of uniform density and ignored the effect of air being accelerated by the rattle (the added mass). For lateral rotation of the rattle, the moment of inertia (I) is:

$$I = \sum_{i=1}^{n} [(m_i r_i^2 + m_i w^2)/12] \,, \tag{1}$$

where m_i is the mass of segment i (in kg), r_i is the distance from centre of segment i to the point of rotation (in m), w is the width of each segment (in m) and n is the number of segments. Long rattles often vibrated with a node near the

distal end of the rattle at which the rattle did not oscillate from side to side. Nodes in these long rattles occurred at all temperatures and varied in the rattle segment at which they occurred. Whenever a node was present, we calculated the moment of inertia of each segment relative to the nearest node. A larger sample would be required to analyze node position in detail because of its variation with rattle length and rattling frequency.

The twisting motion of each rattle segment can be viewed as a rectangular bead swinging from a string that passes through one of its ends; the entire rattle twists because the rattle segments are loosely interlocked. To calculate the moment of inertia for rattle torsion, we treated each segment as a rectangular beam rotating about an axis parallel to the long axis of the rattle at one-quarter of the rattle's dorsoventral depth. The moment of inertia for this geometry and motion is:

$$I = (m_i d_i^2/12) + [m_i(d_i/4)^2] \,, \tag{2}$$

where d_i is the dorsoventral length (i.e. depth) of the segment i (in m).

Angular velocity. The angular velocity, ω, of the rattle is a function of time and displacement. We calculated the maximum angular velocity (ω_{max}) of the rattle as:

$$\omega_{max} = \pi\theta f, \tag{3}$$

where θ is the angular displacement (in rad) and f is the rattling frequency (Van Den Berg and Rayner, 1995), both measured from the video recordings. Whenever there was a node in the rattle, we calculated θ and the angular velocity of each segment relative to the nearest node. We determined ω_{max} separately for lateral and torsional motion.

In the video images, torsion was easily measured at the tip of each rattle, but was difficult to measure at the base of the rattle. Therefore, we set torsion at the first segment of the rattle (the base of the shaker element) to 10 % of the torsion at the tip and then interpolated values for the intermediate rattle segments. This approach gave values that were consistent with the few direct estimates we could make from the video images for torsion at the base of the rattle.

Energy transfer. The loose segments of the rattle are passive elements that are driven by the bony shaker element in the base of the rattle. Consequently, the kinetic energy of the rattle all derives from the shaker element, onto which the muscles insert, in the base of the rattle. We assumed 100 % energy transfer by taking the largest kinetic energy value for a segment (usually that of the shaker element, but occasionally of the distal-most segment) as a measure of the total kinetic energy of the rattle.

Mechanical work. We determined the mechanical work done by the rattle from its changes in kinetic energy in each cycle of motion. The kinetic energy of an object reflects the sum of external and internal kinetic energy. External kinetic energy results from translation of the object's centre of mass, and internal kinetic energy results from movement of parts relative to the centre of mass. Rattling involves a sinusoidal motion with no net translation of the rattle's centre of mass.

Therefore, we needed to measure only the internal kinetic energy (E_{kin}) as:

$$E_{kin} = 0.5I\omega_{max}^2 . \qquad (4)$$

To determine the total E_{kin}, we summed the E_{kin} values for lateral and torsional rotations for each rattle segment.

This measure of E_{kin} represents the maximum kinetic energy, which is reached at one-quarter of a cycle. In the second quarter of the cycle, the kinetic energy of rattle deceleration is equivalent to the kinetic energy of acceleration in the first quarter of the cycle; similarly, the second half of the cycle is symmetrical to the first half of the cycle. By the work/energy theorem, the change in kinetic energy from zero at the beginning of a cycle to its maximum value at one-quarter of a cycle is equivalent to the mechanical work done by the rattle for that portion of the cycle. Therefore, the total mechanical work per cycle of rattling (W) is:

$$W = 4E_{kin} . \qquad (5)$$

This approach gives equal weight to the work of acceleration and deceleration, which seems reasonable given the lack of data on how much, if any, negative work is done by the tailshaker muscles during rattling.

To determine whether our measure of rattle work was accurate and how changes in joint motion affect mechanical work, we used two models of inverted pendulums that were geometrically similar to large rattles. Both models involved a rigid pendulum of similar size and shape to the largest rattle in our sample. Pendulum motion included lateral and long-axis rotations (to approximate torsion) with similar amplitudes to those we measured for the real rattles. One of the models had a constant lateral excursion, whereas the other decreased in lateral excursion as oscillation frequency increased. Rattle work determined from kinetic energy changes was consistent with the pendulum model with decreasing amplitude.

Inertial power

Finally, we determined rattle inertial power and its changes with twitch frequency. To calculate rattle inertial power (P_{in}), we multiplied the work per cycle by the rattling frequency:

$$P_{in} = Wf . \qquad (6)$$

Statistical analyses

We used multiple regression analyses in SPSS for Windows, Release 8.0.2 (1998; Chicago) to test for the effects of twitch frequency and muscle mass on muscle and rattle function. In our sample, snake mass varied sevenfold and tailshaker muscle mass varied by more than fourfold. Preliminary bivariate plots indicated that the rattling frequency, the twitch tension and the muscle tension–time integral (TTI) per twitch were size-dependent. The multiple regression analyses allowed us to test for a relationship between twitch frequency and tension or TTI while size was held constant; it is not our goal in this paper to analyse the allometry of muscle and rattle function. This approach allowed us to remove the effects of size on muscle function and to avoid confounding size effects with noise, measurement error or treatment effects (Packard and Boardman, 1987, 1999). In these analyses, the dependent variable was muscle twitch tension or TTI and the independent variables were twitch frequency and muscle mass. The results from this analysis (Table 1) indicate the bivariate relationship between twitch frequency and each of the dependent variables when the other dependent variable was held constant.

In preliminary data plots, individuals varied in the slope of the relationship between twitch frequency and rattle work, independently of body size; much of this variation probably results from variation in rattle size, shape and condition. Therefore, in the kinematic analyses, we controlled for individual variation (Zar, 1984) (see p. 346), so that it was not confounded with noise or measurement error or incorrectly

Table 1. *Regression results for tailshaker muscle function in western diamondback rattlesnakes* (Crotalus atrox)

Dependent variable	Slope		Intercept	Adjusted r^2	$F_{d.f.}$	N
	Temperature	Muscle mass				
Twitch frequency (Hz)	2.6**	–1.7**	2.4	0.93	649_{93}**	94
	Frequency	Muscle mass				
Peak tension (Pa)	242**	1302**	–6430**	0.68	97_{93}**	94
TTI per twitch (Pa)	0.7**	16.4**	25.9	0.36	28_{93}**	94
	Frequency[†]					
Work	2.0×10^{-6}*		-6.7×10^{-5}	0.48	5.5_{29}*	30

Each slope indicates the bivariate relationship between the dependent variable and the particular independent variable when the other independent variable is held constant. For example, when muscle mass is held constant, temperature significantly affects twitch frequency with a slope of 2.6.

*Significant at $P<0.01$; **significant at $P<0.001$; [†]controlled for individual following Zar (1984, p. 346).

Slope values for independent variables are unstandardised partial regression coefficients.

TTI, tension–time integral.

attributed to temperature or frequency effects. The dependent variable in the regression was rattle mechanical work, and the independent variables were twitch frequency and individual. We initially included muscle mass as an independent variable, but it was rejected by SPSS because of significant multicollinearity with individual. The results from this analysis (Table 1) show the bivariate relationship between twitch frequency and rattle work when individual variation (i.e. individual slope) is held constant. We did not include a regression of rattle inertial power against twitch frequency because power was determined using twitch frequency.

Results

The nine western diamondback rattlesnakes used in this study measured 833 mm mean snout-to-vent length (range 610–1135 mm), 429 g mean body mass (range 112–810 g), 4.0 g mean tailshaker muscle mass (range 1.6–6.3 g) and 1.8 g mean rattle mass (range 0.8–2.5 g). There are three large tailshaker muscles on each side of the tail. The fibre angles relative to horizontal were 26 ° (anterodorsal–posteroventral) for the dorsal muscle, 20 ° (anteroventral–posterodorsal) for the lateral muscle and 15 ° (anteroventral–posterodorsal) for the ventral muscle, and the physiological cross-sectional area of all three muscles on one side was $0.45\pm0.12\,cm^2$ (mean ± S.D.).

Rattling frequency ranged from 18 to 100 Hz over temperatures of 8.5 to 35 °C; peak rattling frequency at each temperature was reached within the first few cycles of rattling. Rattling frequencies measured with the force transducer and those measured with the video recordings were not significantly different in slope ($t=0.38$, d.f.=277, $P>0.05$) or intercept ($t=0.28$, d.f.=278, $P>0.05$). The Q_{10} for rattling frequency was 1.7 for temperatures of 8.5–35 °C.

Muscle mechanics

The twitch duration of rattling is the inverse of rattling frequency and varied from 38 to 13 ms between 10 and 30 °C (Table 2). Rattling forces were approximately sinusoidal (Fig. 1) and involved mean twitch tensions of 4.8 kPa at 10 °C and 16.8 kPa at 30 °C (Fig. 2; Table 2).

Table 2. *Comparison of tailshaker muscle mechanics at 10 and 30 °C in western diamondback rattlesnakes*
(Crotalus atrox*)*

Variable	Temperature		Factorial change
	10 °C	30 °C	
Twitch frequency (Hz)	26±4	76±9.1	2.9
Twitch duration (ms)	38±5	13±1.6	0.34
Peak twitch tension (kPa)	4.8±2.4	16.8±4.7	3.5
Muscle TTI per twitch (kPa s)	0.11±0.05	0.14±0.05	1.3

Peak twitch tension and duration trade off to minimise change in the tension–time integral (TTI) per twitch.

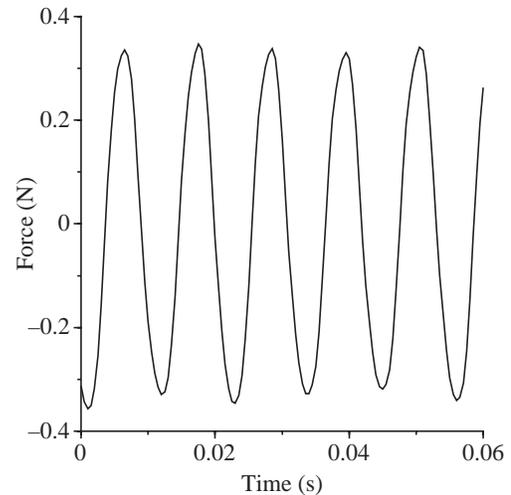Values are means ± S.D. for nine animals.

Fig. 1. The sinusoidal rattling force of a western diamondback rattlesnake (*Crotalus atrox*) rattling at 34.5 °C and 91 Hz. Snake mass 112 g, tailshaker muscle mass 1.6 g, rattle mass 0.78 g.

As temperature increased, peak muscle twitch tension increased dramatically to accelerate the rattle faster (Tables 1, 2). In contrast, the area under the tension–time curve (the muscle tension–time integral, or TTI) per twitch increased very little with temperature. For example, between 10 and 30 °C, mean peak twitch tension increased 3.5-fold whereas mean TTI increased by only 1.3-fold (Figs 3–5; Table 2). The slope shown in Fig. 4 for the normalised increase in tension is significantly greater than that for the increase in TTI ($t=10.7$,
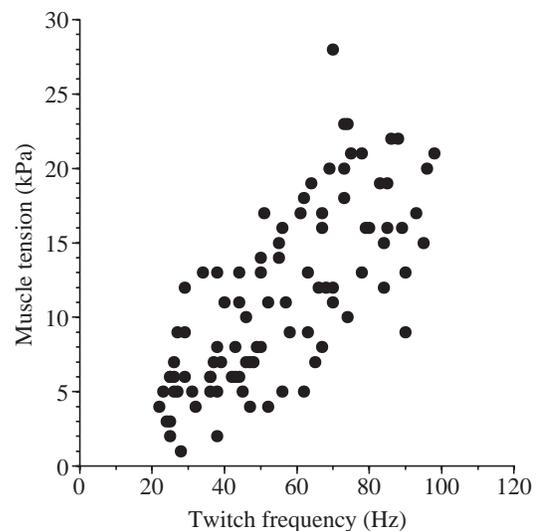

Fig. 2. Peak muscle tension increases dramatically with twitch frequency during rattling in western diamondback rattlesnakes (*Crotalus atrox*). These tension values are based on the forces recorded with the optical transducer. Each point represents the mean value of five continuous contractions from each of nine snakes. The multivariate regression results for twitch frequency, muscle mass and peak muscle tension are given in Table 1.
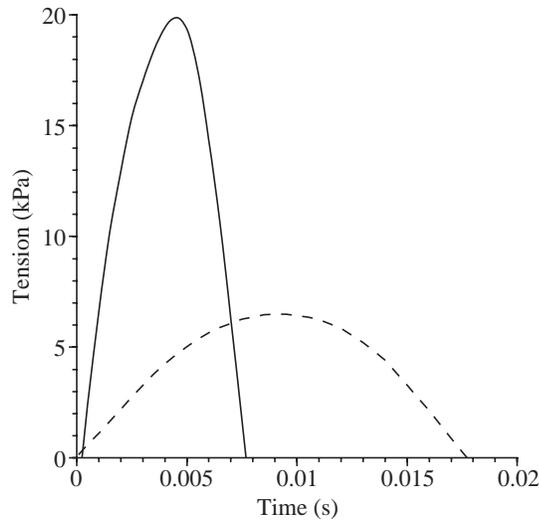
Fig. 3. Individual twitch curves from the tailshaker muscles of a western diamondback rattlesnake (*Crotalus atrox*) rattling at 10 °C (dashed line) and 30 °C (solid line). The area under each curve represents the tension–time integral (TTI) for the half-cycle. In this example, the peak tension at 30 °C is 3.1 times the value at 10 °C, whereas the TTI at 30 °C is only 1.5 times the value at 10 °C. Mean changes in tension and TTI are shown in Table 2 and Fig. 4. Snake mass 810 g, tailshaker muscle mass 6.2 g.
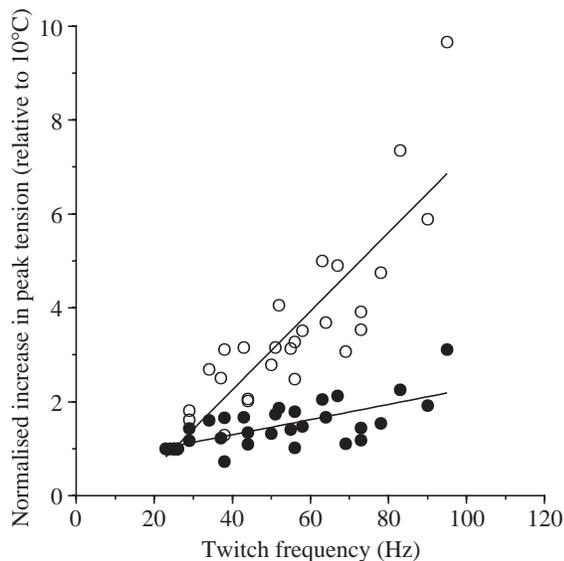

Fig. 4. Normalised increases in tailshaker muscle peak tension (open circles) and tension–time integral (TTI, filled circles) per twitch during rattling in western diamondback rattlesnakes (*Crotalus atrox*). Between 10 and 30 °C (26–77 Hz), muscle twitch tension increased an average of 3.5-fold whereas TTI increased by only 1.3-fold. The trend for tension increase is $y=0.084x–1.100$ ($r^2=0.79$, $P<0.001$), and that for TTI increase is $y=0.016x+0.660$ ($r^2=0.47$, $P<0.001$); $N=9$ snakes. The slopes are significantly different, as indicated in the text.

d.f.=60, $P<0.001$). Although the increase in TTI per twitch was also significant, it was much smaller than the increase in tension because of a trade-off between twitch tension and
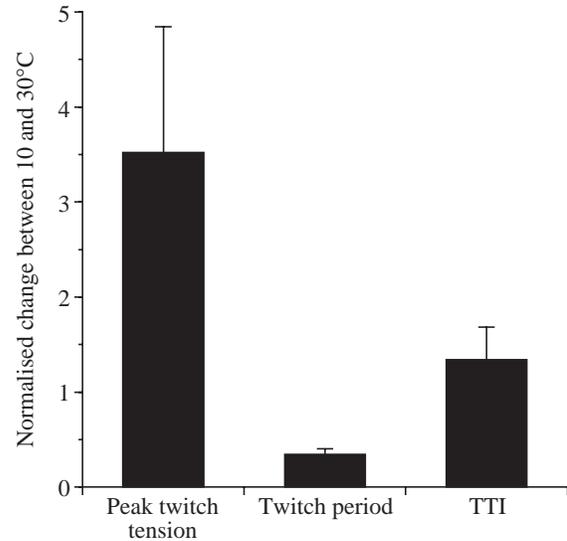

Fig. 5. The trade-off between twitch tension and duration in tailshaker muscle during rattling in western diamondback rattlesnakes (*Crotalus atrox*). The tension–time integral (TTI) is approximately equal to the product of tension and period, so the dramatic increase in twitch tension trades off with the decrease in twitch period to minimise change in TTI. A TTI value of 1 would indicate an exact trade-off between twitch tension and period. Columns indicate mean values and error bars indicate 1 S.D. in a sample of five snakes.

duration (Figs 4, 5; Table 2). Thus, large increases in peak muscle force and rattle acceleration were possible with increasing temperature in the absence of large increases in the TTI and energetic cost.

Joint mechanics

The muscle forces exerted on the rattle shaker element produce two motions. Lateral displacement of the rattle joint can be produced by at least two of the tailshaker muscles shortening simultaneously, and torsion can be produced by one of the muscles shortening slightly more or faster than the other muscles or partially out of phase with them. These two motions involve separate components of joint displacement and work.

As twitch frequency increased, rattle work was kept low (0.01–0.4 mJ cycle^{-1}) by a change in joint motion. Between 10 and 30 °C, lateral displacement of the rattle decreased threefold and torsion increased 1.3-fold (Table 3). At low twitch frequencies, the majority of rattling work and power was due to lateral displacement, whereas at higher twitch frequencies, a slight majority of the work and power was due to torsion (Table 3).

The pendulum models showed that the decrease in lateral displacement of the rattle joint was the major factor in keeping the work and power from increasing as much as they would if lateral displacement had remained constant as frequency increased (Figs 6, 7); this effect is large enough to be important even with slight changes in lateral displacement. In contrast, joint motion and sound production can be increased for little

Table 3. *Comparison of rattle mechanics at 10 and 30 °C in western diamondback rattlesnakes (*Crotalus atrox*)*

Variable	Temperature		Factorial change
	10 °C	30 °C	
Twitch frequency (Hz)	26±6.4	78±5.0	3.0
$I_{lateral}$ (kg m^2)	10.0×10^{-8}±6.2×10^{-8}	Same as left	–
$I_{torsion}$ (kg m^2)	5.4×10^{-8}±3.1×10^{-8}	Same as left	–
$\theta_{lateral}$ (rad)	0.28±0.20	0.09±0.09	0.3
$\theta_{torsion}$ (rad)	0.08±0.03	0.10±0.07	1.3
$\omega_{max,lateral}$ (rad s^{-1})	19.7±11.4	22.9±24.4	1.2
$\omega_{max,torsion}$ (rad s^{-1})	6.5±3.5	24.6±15.6	3.8
Total work (mJ cycle^{-1})	0.05±0.05	0.14±0.10	2.8
Percentage of work due to lateral displacement	82	44	0.2
Percentage of work due to torsion	18	56	1.3
Inertial power (mW)	1.28±0.29	10.94±7.67	8.5

As temperature and twitch frequency increase, rattle lateral displacement decreases and torsion increases, which keeps the increase in work proportional to the increase in tension.

Values are means ± S.D. for nine animals.

$I_{lateral}$, moment of inertia for lateral displacement of the rattle; $I_{torsion}$, moment of inertia for rattle torsion; $\theta_{lateral}$, angular displacement for lateral rotation of the rattle centre of mass; $\theta_{torsion}$, angular displacement for torsion; $\omega_{max,lateral}$, maximum lateral angular velocity of the rattle centre of mass; $\omega_{max,torsion}$, maximum angular velocity for torsion.

Total work=$\sum(2I\omega_{max}^2)$ for lateral and torsional motion.

Inertial power=total work × twitch frequency.

extra force, work and power by increasing torsion because the moment of inertia and angular velocity for torsion are much lower than for lateral displacement. Thus, higher muscle forces accelerate the rattle joint faster, but over smaller excursions. The change from mainly lateral to torsional motion of the joint allows rattle motion and sound production to be sustained at high twitch frequencies with very little work.

Muscle forces determined kinematically from mechanical work and joint displacement (peak tension 4.8 kPa at 10 °C and 16.8 kPa at 30 °C) independently confirmed those measured with the transducer (peak tension 2.8 kPa at 10 °C and 15.1 kPa at 30 °C). The tensions determined kinematically were typically slightly lower than those measured when the muscles were loaded by the transducer, although the range was the same for both analyses and the differences were not significant ($t=0.98$, d.f.=11, $P>0.05$ for tension at 10 °C, and $t=2.33$, d.f.=6, $P>0.05$ for tension at 30 °C). The close agreement between forces measured with the transducer and determined from rattle kinematics indicates that our results for mechanical work are reasonably accurate and are adequate for estimating mechanical efficiency (as mechanical work/ metabolic cost) using the cost reported by Conley and Lindstedt (1996). The mechanical efficiency of rattling was
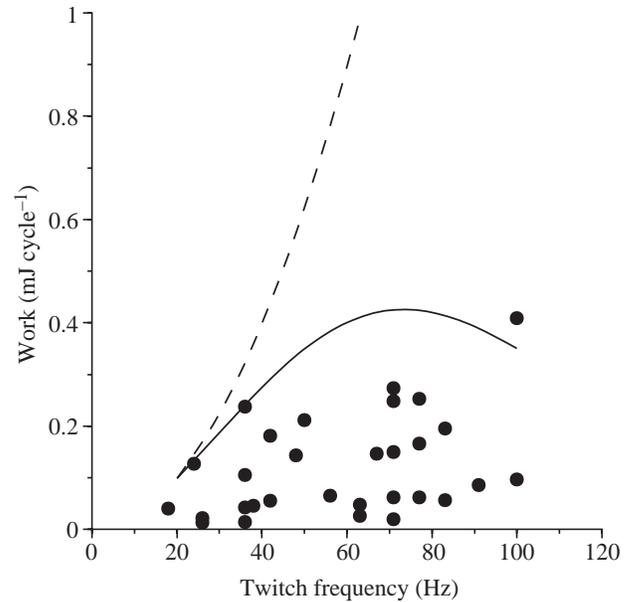

Fig. 6. The mechanical work of rattling in western diamondback rattlesnakes (*Crotalus atrox*). The circles indicate rattlesnake data; the lines indicate the work required to oscillate a pendulum of similar size, shape and mass to a large rattle when pendulum displacement is constant (dashed line) and when it decreases as oscillation frequency increases (solid line). The regression results for mechanical work are given in Table 1.
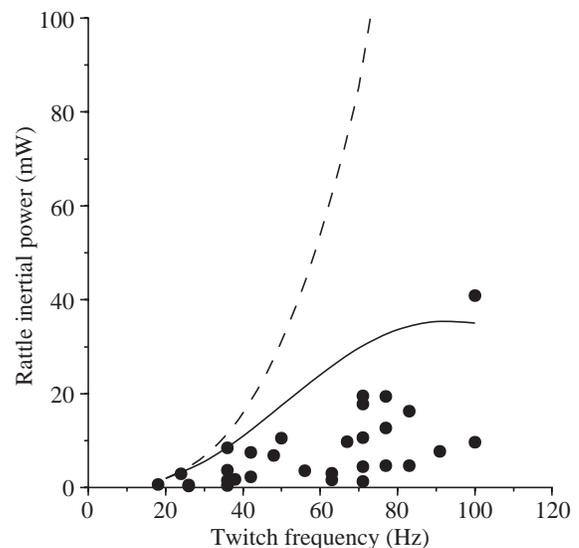

Fig. 7. Rattle inertial power in western diamondback rattlesnakes (*Crotalus atrox*). The circles indicate rattle data; the lines indicate the power required to oscillate a pendulum of similar size, shape and mass to a large rattle when pendulum displacement is constant (dashed line) and when it decreases as oscillation frequency increases (solid line).

low (0.3–11 %) but also temperature-dependent (1.9-fold change per 10 °C) because of the increasing work and constant cost per twitch.

Discussion

Rattling by rattlesnakes is one of the fastest vertebrate movements and involves some of the highest twitch frequencies sustained by vertebrate muscle (Rome and Lindstedt, 1998; Rome et al., 1996). Rattling requires higher forces and accelerations at higher twitch frequencies, yet the cost per twitch of rattling is independent of twitch frequency (Conley and Lindstedt, 1996). How does muscle force increase without increasing cost? Our recordings of *in vivo* forces and rattle motion show that mechanical trade-offs explain how force, work and power increase without increasing cost.

Muscle mechanics

In animal locomotion, the metabolic cost of movement is directly proportional to force exertion (Kram and Taylor, 1990; Taylor et al., 1980). The basis of this relationship is that the cost of muscle contraction is proportional to the force and duration of contraction (Kushmerick and Paul, 1976a,b). Therefore, the low tailshaker muscle forces explain the low energetic cost per twitch of rattling reported by Conley and Lindstedt (1996), and the nearly constant tension–time integral per twitch that results from the trade-off between twitch force and duration with changing frequency explains how the cost per twitch is kept constant.

The low twitch tensions of shaker muscle are consistent with a cross-sectional area of myofibrils that is only one-third of that of typical skeletal muscle (Schaeffer et al., 1996). The low twitch tension (17 kPa at 30 °C) is slightly less than one-third of the twitch tension of lizard leg muscle (75 kPa at 30 °C) reported by Bennett (1985) and is comparable with those of other sound-producing muscles such as katydid singing muscle (27 kPa at 25 °C) (Josephson, 1973) and toadfish swimbladder muscle (24 kPa at 15 °C) (Rome et al., 1999). Similarly, shaker muscle tetanic tension (approximately 62 kPa at 30 °C) (Martin and Bagby, 1973) is approximately one-third of the tetanic tension (187–200 kPa) reported for lizard leg muscle (Bennett, 1985; Marsh and Bennett, 1986). The increased twitch tension at higher temperatures is consistent with a shift in the force/velocity curve towards faster and stronger twitches at higher temperatures (Rome et al., 1996).

Joint mechanics

The lateral and torsional displacements of the rattle result directly from the forces exerted by the muscles on the rattle shaker element. Our analysis indicated how changes in rattle joint motion keep the mechanical work and inertial power of rattling low.

As twitch frequency increases, lateral displacement of the rattle joint decreases. The pendulum models show that a decrease in lateral displacement of the rattle joint is the major factor in keeping the work and power from increasing as much as they would if lateral displacement had remained constant as frequency increased (Figs 6, 7). This effect is dramatic enough to be important even with slight changes and considerable variability in rattle displacement. In contrast to lateral displacement, increases in joint torsion require little extra force, work and power because the moment of inertia and angular velocity are typically much lower for rattle torsion than for lateral displacement (Table 3). As temperature increases, higher muscle forces accelerate the rattle joint faster, but for shorter periods and over smaller lateral excursions. Thus, a decrease in lateral displacement of the rattle at higher twitch frequencies is the major factor that keeps the mechanical work and power of rattling low.

It is difficult to compare the work and power of rattling with values for muscles and appendages in other animals. Most work and power values are reported per kilogram of body mass, which would be less informative for rattlesnakes because of the small proportion of body mass represented by tailshaker muscle. Also, the appendages of most other animals oscillate at much lower frequencies than rattlesnake rattles. However, as a broad comparison, the power required to shake the rattle is roughly an order of magnitude lower than that required to flap the wings of small birds and bats (Van Den Berg and Rayner, 1995). Small rattle mass and the mechanical trade-offs used in rattling are the major factors the help keep the work and power of rattling very low. The low mechanical efficiency (0.3–11 %) that results from the low work of rattling is comparable with those of other high-frequency muscles such as insect singing and flight muscles (Dickinson and Lighton, 1995; Josephson, 1985; Josephson and Stevenson, 1991) and frog vocalisation muscles (McLister, 2000; Prestwitch et al., 1989).

Mechanical and sound energy output are sustained without fatigue because the mechanical trade-offs used in rattling function in concert with physiological specializations, such as high energy flux by the abundant mitochondria and high blood flow for oxygen delivery and lactate removal (Conley and Lindstedt, 1996; Kemper et al., 2001). Maximum work and nearly maximum power output are produced at twitch frequencies that correspond to the preferred activity temperature of western diamondbacks in the field (Beck, 1995).

Acoustic, behavioural and ecological significance of rattle mechanics

The role of the rattle is to produce sound that deters predators. The abruptness and loudness of rattling sound are important components of the startling effect of rattling (Fenton and Licht, 1990; Klauber, 1972) and are determined in part by tailshaker muscle mechanics. The muscles are extremely fast and can reach maximum contraction frequency within the first few cycles of rattling. Rattle loudness is determined in part by rattling frequency and force because higher frequencies and forces produce more mechanical power that is transduced into sound power; this relationship explains how rattle loudness increases with temperature, as reported by Rowe and Owings (1996). In addition, the vibrational nodes present in longer rattles may explain the finding of Rowe and Owings (1996) that the sound clicks are blurred in longer rattles, because the part of the rattle distal to the node moves out of phase with the proximal part.

In addition to deterring predators, rattling sounds provide important information to potential prey animals about snake size and warmth (Rowe and Owings, 1990, 1996). For

example, louder rattling indicates a larger snake, and faster rattling indicates a warmer snake, which can strike faster and more accurately (Rowe and Owings, 1990). Ground squirrels (*Spermophilus beecheyi*) induce approaching rattlesnakes (*Crotalus viridis*) to rattle by kicking dirt at them and even by biting them, and then use the rattling sounds to choose appropriate defensive behaviours (Rowe and Owings, 1978; Swaisgood et al., 1999).

At low temperatures, the shaker muscles produce little mechanical energy, which limits rattling sound energy. The quiet, low-frequency rattling produced by a cold snake can indicate to a potential predator or prey animal that the snake is in suboptimal condition for defence or feeding. These factors support the hypothesis of Rowe and Owings (1990, 1996) that hesitancy to rattle at lower temperatures is a behavioural tactic used to avoid revealing the snake's suboptimal condition to potential prey animals. These relationships demonstrate that muscle and rattle mechanics are important not just to physiology but also to diverse aspects of behaviour and ecology.

References

Beck, D. D. (1995). Ecology and energetics of three sympatric rattlesnake species in the Sonoran Desert. *J. Herpetol.* **29**, 211–223.

Bennett, A. F. (1985). Temperature and muscle. *J. Exp. Biol.* **115**, 333–344.

Blickhan, R. and Full, R. J. (1992). Mechanical work in terrestrial locomotion. In *Biomechanics: Structures and Systems* (ed. A. A. Biewener), pp. 75–96. Oxford: IRL Press.

Chadwick, L. E. and Rahn, H. E. (1954). Temperature dependence of rattling frequency in the rattlesnake, *Crotalus v. viridis. Science* **119**, 442–443.

Conley, K. E. and Lindstedt, S. L. (1996). Minimal cost per twitch in rattlesnake tail muscle. *Nature* **383**, 71–72.

Dickinson, M. H. and Lighton, J. R. B. (1995). Muscle efficiency and elastic storage in the flight motor of *Drosophila. Science* **268**, 87–90.

Fenton, M. B. and Licht, L. E. (1990). Why rattle snake? *J. Herpetol.* **24**, 274–279.

Giancoli, D. C. (2000). *Physics for Scientists and Engineers*, vol. 1. Upper Saddle River, NJ: Prentice Hall.

Harper, D. G. and Blake, R. W. (1989). A critical analysis of the use of high-speed film to determine maximum accelerations of fish. *J. Exp. Biol.* **142**, 465–471.

Josephson, R. K. (1973). Contraction kinetics of fast muscles used in singing by a katydid. *J. Exp. Biol.* **59**, 781–801.

Josephson, R. K. (1985). The mechanical power output of a tettigoniid wing muscle during singing and flight. *J. Exp. Biol.* **117**, 357–368.

Josephson, R. K. and Stevenson, R. D. (1991). The efficiency of a flight muscle from the locust *Schistocerca americana. J. Physiol., Lond.* **442**, 413–429.

Kemper, W. F., Lindstedt, S. L., Hartzler, L. K., Hicks, J. W. and Conley, K. E. (2001). Shaking up glycolysis: Sustained, high lactate flux during aerobic rattling. *Proc. Natl. Acad. Sci. USA* **98**, 723–728.

Klauber, L. M. (1972). *Rattlesnakes: Their Habits, Life Histories and Influence on Mankind.* Berkeley: University of California Press.

Kram, R. and Taylor, C. R. (1990). Energetics of running: a new perspective. *Nature* **346**, 265–267.

Kushmerick, M. J. and Paul, R. J. (1976a). Aerobic recovery metabolism following a single isometric tetanus in frog sartorius muscle at 0 °C. *J. Physiol., Lond.* **254**, 693–709.

Kushmerick, M. J. and Paul, R. J. (1976b). Relationship between initial chemical reactions and oxidative recovery metabolism for single isometric contractions of frog sartorius at 0 °C. *J. Physiol., Lond.* **254**, 711–727.

Marsh, R. L. and Bennett, A. F. (1986). Thermal dependence of contractile properties of skeletal muscle from the lizard *Sceloporus occidentalis* with comments on methods for fitting and comparing force–velocity curves. *J. Exp. Biol.* **126**, 63–77.

Martin, J. M. and Bagby, R. M. (1972). Temperature–frequency relationship of the rattlesnake rattle. *Copeia* **1972**, 482–485.

Martin, J. M. and Bagby, R. M. (1973). Properties of rattlesnake shaker muscle. *J. Exp. Zool.* **185**, 293–300.

McLister, J. D. (2000). Physical factors affecting the cost and efficiency of sound production in the treefrog *Hyla versicolor. J. Exp. Biol.* **204**, 69–80.

Packard, G. C. and Boardman, T. J. (1987). The misuse of ratios to scale physiological data that vary allometrically with body size. In *New Directions in Ecological Physiology* (ed. M. E. Feder, A. F. Bennett, W. Burggren and R. B. Huey), pp. 216–239. Cambridge: Cambridge University Press.

Packard, G. C. and Boardman, T. J. (1999). The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comp. Biochem. Physiol. A* **122**, 37–44.

Prestwitch, K. N., Brugger, K. E. and Topping, M. J. (1989). Energy and communication in three species of hylid frogs: power input, power output and efficiency. *J. Exp. Biol.* **144**, 53–80.

Rome, L. C., Cook, C., Syme, D. A., Connaughton, M. A., Ashley-Ross, M., Klimov, A., Tikunov, B. and Goldman, Y. E. (1999). Trading force for speed: why superfast crossbridge kinetics leads to superlow forces. *Proc. Natl. Acad. Sci. USA* **96**, 5826–5831.

Rome, L. C. and Lindstedt, S. L. (1998). The quest for speed: muscles built for high-frequency contractions. *News Physiol. Sci.* **13**, 261–268.

Rome, L. C., Syme, D. A., Hollingworth, S. H., Lindstedt, S. L. and Baylor, S. M. (1996). The whistle and the rattle: the design of sound producing muscles. *Proc. Natl. Acad. Sci. USA* **93**, 8095–8100.

Rowe, M. P. and Owings, D. H. (1978). The meaning of the sound of rattling by rattlesnakes to California ground squirrels. *Behaviour* **66**, 252–267.

Rowe, M. P. and Owings, D. H. (1990). Probing, assessment and management during interactions between ground squirrels and rattlesnakes. I. Risks related to rattlesnake size and body temperature. *Ethology* **86**, 237–249.

Rowe, M. P. and Owings, D. H. (1996). Probing, assessment and management during interactions between ground squirrels (Rodentia: Sciuridae) and rattlesnakes (Squamata: Viperidae). II. Cues afforded by rattlesnake rattling. *Ethology* **102**, 856–874.

Schaeffer, P. J., Conley, K. E. and Lindstedt, S. L. (1996). Structural correlates of speed and endurance in skeletal muscle: the rattlesnake tailshaker muscle. *J. Exp. Biol.* **199**, 351–358.

Swaisgood, R. R., Owings, D. H. and Rowe, M. P. (1999). Conflict assessment in a predator–prey system: ground squirrels *versus* rattlesnakes. *Anim. Behav.* **57**, 1033–1044.

Taigen, T. L. and Wells, K. D. (1985). Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). *J. Comp. Physiol.* **155**, 163–170.

Taylor, C. R., Heglund, N. C., McMahon, T. A. and Looney, T. R. (1980). Energetic cost of generating muscular force during running. *J. Exp. Biol.* **86**, 9–18.

Tu, M. S. and Daniel, T. L. (1999). Work and power output of the dorsolongitudinal muscles of the hawkmoth *Manduca sexta. Am. Zool.* **39**, 118A.

Van Den Berg, C. and Rayner, J. M. V. (1995). The moment of inertia of bird wings and the inertial power requirement for flapping flight. *J. Exp. Biol.* **198**, 1655–1664.

Zar, J. H. (1984). *Biostatistical Analysis.* Second edition. Englewood Cliffs, NJ: Prentice-Hall.