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POWER OUTPUT OF SOUND-PRODUCING MUSCLES IN THE TREE FROGS *HYLA VERSICOLOR* AND *HYLA CHRYSOSCELIS*

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Summary

Sound-producing muscles provide the opportunity of studying the limits of power production at high contractile frequencies. We used the work loop technique to determine the power available from the external oblique muscles in two related species of North American gray tree frog, *Hyla chrysoscelis* and *Hyla versicolor*. These trunk muscles contract cyclically, powering high-intensity sound production in anuran amphibians. The external oblique muscles in *H. chrysoscelis* have an *in vivo* operating frequency of 40–55 Hz at 20–25 °C, whereas in *H. versicolor* these muscles contract with a frequency of 20–25 Hz at these temperatures. *In vivo* investigations have shown that these muscles use an asymmetrical sawtooth length trajectory (with a longer shortening phase compared with the lengthening phase) during natural cycles. To study the influence of this particular length trajectory on power output, we subjected the muscles to both sinusoidal and sawtooth length trajectories. In both species, the sawtooth trajectory yielded a significantly higher power output than the sinusoidal length pattern. The maximum power output during sawtooth cycles was similar in both species (54 W kg^{-1} in *H. chrysoscelis* and 58 W kg^{-1} in *H. versicolor*). These values are impressive, particularly at the operating frequencies and temperatures of the muscle. The sinusoidal length trajectory yielded only 60 % of the total power output compared with the sawtooth trajectory (34 W kg^{-1} for *H. chrysoscelis* and 36 W kg^{-1} for *H. versicolor*). The optimum cycle frequencies maximizing the power output using a sawtooth length pattern were approximately 44 Hz for *H. chrysoscelis* and 21 Hz for *H. versicolor*. These frequencies are close to those used by the two species during calling. Operating at higher frequencies, *H. chrysoscelis* maximized power at a strain amplitude of only 8 % compared with a value of 12 % in *H. versicolor*. These strains match those used *in vivo* during calling. The stimulus timing observed *in vivo* during calling was also similar to that yielding maximum power at optimal frequency in both species (6 ms and 8 ms before the start of shortening in *H. chrysoscelis* and *H. versicolor*, respectively). As expected, twitch duration in *H. chrysoscelis* is much shorter than that in *H. versicolor* (23 ms and 37 ms, respectively). There was a less remarkable difference between their maximum shortening velocities (V_{max}) of 13.6 L_0 s^{-1} in *H. chrysoscelis* and 11.1 L_0 s^{-1} in *H. versicolor*, where L_0 is muscle length. The force–velocity curves are very flat, which increases power output. At the myofibrillar level, the flat force–velocity curves more than compensate for the lower peak isometric force found in these muscles. The data presented here emphasize the importance of incorporating *in vivo* variables in designing *in vitro* studies.

Key words: work loop, external oblique muscle, frequency, sawtooth length trajectory, power output, vocalization, force–velocity, twitch, contractile properties, tree frog, *Hyla chrysoscelis*, *Hyla versicolor*.

Introduction

Sound-producing muscles can provide a suitable model to study the limits of power production at high contractile frequencies. The contractile frequencies of sound-producing muscles are amongst the highest known in animals (Young and Josephson, 1983, 1985; Rome et al., 1996). Several studies have focused on these muscles in a range of species (Mendelson, 1969, lobster; Josephson and Young, 1981, cicadas; Josephson, 1984, katydids; Marsh and Taigen, 1987, tree frogs; Lindholm and Bass, 1993, mid-shipman fish; Schaeffer et al., 1996, rattlesnake; Rome et al., 1996, toadfish). Muscles that operate at high frequencies for sustained periods are limited in terms of power output by their intrinsic properties. These muscles produce relatively low forces, because a significant proportion of the muscle fiber is occupied by an elaborate sarcoplasmic reticulum and a large number of mitochondria (Josephson and Young, 1981; Marsh and Taigen, 1987; Schaeffer et al., 1996; Rome et al., 1996). Second, to operate at high frequencies, these muscles require high shortening velocities, which in turn limits force production (Josephson, 1993). During any cyclical contraction, power output (the product of cycle frequency and the net work done per cycle) can be compromised by several

factors at high operating frequencies (for a review, see Josephson, 1993).

For the present investigation, we chose the external oblique trunk muscle in two related species of North American gray tree frog, *H. chrysoscelis* and *H. versicolor*. These trunk muscles, along with the internal oblique muscles, contract cyclically to power sound production in anuran amphibians (Martin, 1971; Martin and Gans, 1972; Girgenrath and Marsh, 1997). Both species produce trilled calls in which each call is made up of a number of sound pulses. The sound pulse frequency within calls in *H. chrysoscelis* is twice that in *H. versicolor* (Ralin, 1977; Girgenrath and Marsh, 1997). At 25 °C, the frequency is approximately 40–55 Hz in *H. chrysoscelis* and 20–25 Hz in *H. versicolor*. In both species, sound pulse frequency is directly correlated with the cycling frequency of the trunk muscle (Girgenrath and Marsh, 1997). The calls produced are high in sound intensity, a property essential for communication to ensure successful mate selection (Martin and Gans, 1972; Gerhardt, 1994). Presumably, most of the power transduced into sound energy is generated by the trunk muscles, which are the principal muscles involved in vocalization. These muscles have a mass of up to 15 % of the animal's body mass (Marsh and Taigen, 1987). They consist of 100 % fast oxidative glycolytic fibers and have high citrate synthase activity, high mitochondrial and capillary densities and high ATPase activity (Marsh and Taigen, 1987; Ressel, 1996). The trunk muscles in these hylid frogs are thus interesting because they operate at relatively high frequencies and yet must also be capable of generating high power to produce loud calls. To compare the power output of muscles operating at different frequencies, one often has to consider disparate species and also muscles used for different functions. However, the trunk muscles of gray tree frogs are ideal because they also provide an opportunity to investigate two homologous muscles similar in function and yet operating at very different frequencies. Thus, these muscles are well suited for *in vitro* performance studies whose goal is to provide useful insights into the characteristics needed to generate high power output at high cycle frequencies.

Designing *in vitro* experiments to predict the limits of performance during natural movements requires knowledge of how a given muscle is used *in vivo*. Several studies have shown the importance of designing *in vitro* experiments based on *in vivo* information (Marsh et al., 1992; James et al., 1995; Full et al., 1998). Our recent studies of the *in vivo* performance of trunk muscles (external and internal oblique) during calling in these two species of hylid frog revealed that these muscles undergo an interesting length trajectory, with a significantly longer shortening phase than the lengthening phase (Girgenrath and Marsh, 1997). Knowledge of the sawtooth length trajectory followed by these muscles provides an important foundation for our present *in vitro* studies. Recent studies have shown that length trajectory can significantly influence the power output (Marsh and Olson, 1994; Askew and Marsh, 1997). We hypothesized that this asymmetrical sawtooth trajectory followed by the trunk muscles could have evolved as a means of enhancing power output. In the present study, we focused specifically on the influence of *in vivo* length trajectories on the power produced by these muscles while operating at their natural frequencies. In addition, our previous work on *in vivo* performance of these muscles allows us to investigate whether the *in vivo* patterns of muscle strain and stimulation match the strain and stimulation patterns that yield maximum *in vitro* power output.

Materials and methods

Similar-sized *Hyla chrysoscelis* (6.89±0.3 g, N=11) and *H. versicolor* (7.76±0.3 g, N=5) (means ± S.E.M.) were used for this study. *H. chrysoscelis* were collected in Wilson County, Tennessee, USA, by a commercial supplier (Charles D. Sullivan Co., Inc.). *H. versicolor* were collected in the vicinity of Boston, Massachusetts, USA. All the data reported in this study were collected during the active breeding periods for these animals (from mid-May to mid-July). Animals were housed in glass aquaria with beds of moist sphagnum moss and a water source. Frogs were maintained at 25 °C on an L:D cycle of 15 h:9 h and were fed crickets at least twice a week. The crickets were coated with powdered calcium carbonate and multi-vitamins. Contractile studies were completed within 2 weeks after the animals arrived in the laboratory.

The animals were killed by destroying the brain followed by spinal pithing. Marsh (1999) describes the methods used for isolating the muscle and preparing it for *in vitro* measurements. The muscle was kept moist with Ringer's solution (115 mmol l^{-1} NaCl, 2.5 mmol l^{-1} KCl, 1.0 mmol l^{-1} $MgSO_4$, 20 mmol l^{-1} imidazole, 1.8 mmol l^{-1} $CaCl_2$, 11 mmol l^{-1} pyruvic acid, pH 7.9). This solution was oxygenated for at least 1 h before use. The muscle was placed vertically within an acrylic chamber and bathed constantly with circulating oxygenated Ringer's solution maintained at 25 °C. A lightweight silver chain was tied to the ventral end of the muscle with silk thread. The chain was used to attach the muscle to the lever of a Cambridge Technology ergometer (model 300B). The other end of the muscle was secured with a silk thread to a stainless-steel hook attached to the bottom of the chamber. Once the muscle was secured in its position, it was allowed to recover from the dissection for approximately 20 min.

The muscle was supramaximally activated using two parallel platinum plate electrodes. Square-wave stimuli were produced by an audio power amplifier connected to a Grass S48 stimulator that generated the stimuli under computer control. After the recovery period, a series of twitches and tetani was used to determine the optimal length (L_0) of the muscle. The optimal length of the muscle was defined as the length at which it produced maximal tetanic force (P_0). At L_0, the latent period, time to peak force in the twitch ($t_{P,tw}$) and time to half-relaxation ($t_{50\%R}$) were determined. Maximal force and fusion frequencies were measured during isometric tetani. Rest periods of 1 and 3 min were allowed between twitches and tetani, respectively.

Force and length outputs were digitized by a MacAdios II, 12-bit A/D converter running in a Macintosh IIci computer. Length was controlled with a 16-bit D/A converter, and the stimulus timing was set by a digital output that controlled a TTL switch. Sampling frequency varied with cycling frequency from 12 kHz at 12 Hz to 50 kHz at 50 Hz. The work loop technique (Josephson, 1985) was used to determine power output at the optimal length. The muscle was subjected to cyclical length changes while being stimulated phasically with one or two stimuli during the length cycle. The forces produced by the muscle during each cycle were measured. The muscles were subjected to 14 cycles in each run. Superscope II software was used to collect and analyze the data. A rest period of 3 min was allowed for recovery between each run.

In our initial set of experiments, oblique muscles of *H. versicolor* were subjected to three different strain trajectories (Fig. 1). First, we used a strain trajectory derived directly from the strain cycle measured *in vivo* (Girgenrath and Marsh, 1997), which we called the natural cycle. To construct the natural cycle, the strain trajectory wave derived from *in vivo* measurements was smoothed using the application Igor from WaveMetrics. Five representative cycles from the smoothed *in vivo* wave were averaged to construct one cycle of the natural length trajectory. Once constructed, 14 of these cycles were joined together to simulate the natural length trajectory during a call. Second, we used a simplified version of the *in vivo* cycle, which we refer to as a sawtooth trajectory. This trajectory was produced by piecing together a ramp with a constant shortening velocity and a ramp with a constant lengthening velocity and smoothing the resulting wave to eliminate the sharp transitions. Third, we used a sinusoidal pattern.

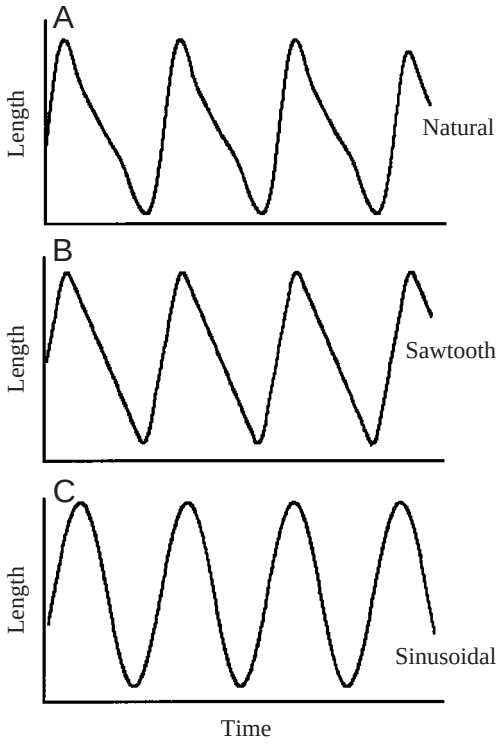

Fig. 1. The three length trajectories used in cyclical work measurements. (A) Natural, derived from the *in vivo* length cycle (Girgenrath and Marsh, 1997) as described in Materials and methods; (B) sawtooth, a simplified version of the natural cycle; and (C) sinusoidal.

The length recording was differentiated to calculate velocity. Instantaneous power was calculated as the product of force and velocity. Net work was calculated by integrating instantaneous power with respect to time, and net power was determined by dividing net work by cycle time (these calculations are equivalent to those of previous workers who calculated the area of work loops generated by plotting force *versus* length). Because the power obtained with natural cycles and the sawtooth cycle was very similar, we used only sawtooth and sinusoidal strain trajectories for most of the subsequent experiments (see Results and Discussion). The sawtooth trajectories used for the two species were different and based on their *in vivo* cycle patterns. *In vivo* measurements showed that the muscles in *H. chrysoscelis* at 25 °C spent approximately 60 % of the cycle shortening and 40 % lengthening. At similar temperatures, *H. versicolor* has sawtooth strain trajectory with approximately 75 % of the cycle duration spent shortening (Girgenrath and Marsh, 1997). Muscles were subjected to a range of cycle frequencies (20–50 Hz in *H. chrysoscelis* and 12–24 Hz in *H. versicolor*) to identify a frequency at which power was maximized. Both sinusoidal and sawtooth trajectories were used at all frequencies. At the optimal cycle frequency, the effects of strain amplitude, phase and number of stimuli on power output were studied systematically.

Isometric tetani were performed periodically to correct for any decline in force. A further correction was necessary to compensate for effects on the lever of operating at high frequencies using sawtooth cycles. Inertial effects were minimized by appropriate calibration of the lever, but could not be entirely eliminated. In addition, the lever showed slight hysteresis that resulted in a baseline shift between lengthening and shortening. These effects were measured by subjecting each preparation to cycles in which the muscle was inactive and to identical cycles with phasic stimulation (Fig. 2B). The force used in subsequent calculations was the difference between the force with phasic stimulation and that of the unstimulated muscle (Fig. 2C). The biggest artifact occurred at the turnaround at the shortest length, at which point the accelerations are greatest because of the asymmetrical sawtooth wave. A potential problem with this technique for correcting the force data is that it could remove a real passive force due to lengthening the parallel elastic elements in the muscle. As the data show, however, this effect was not significant. The apparent force produced by the unstimulated muscle does not increase with increasing length as would be expected from an effect of the parallel elastic elements (Fig. 2B). The reason for the lack of any significant parallel elastic effect is probably because the muscle operates at or below L_0 throughout the cycle. In fact, the stimulated muscle has considerably higher forces during lengthening because of

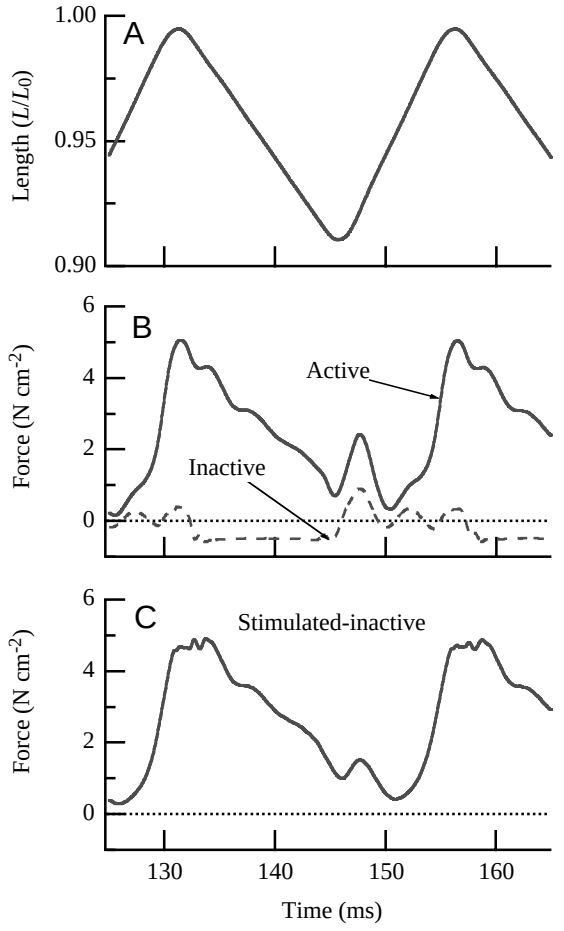

Fig. 2. Representative data from the external oblique of *Hyla chrysoscelis* at 40 Hz demonstrating the method of correcting the force measurements. (A) Muscle length L as a fraction of optimal length L_0. (B) Active force from a phasically stimulated muscle preparation and the force recorded from the same muscle in the inactive (relaxed) condition. (C) Calculated net force (active minus inactive).

the residual active force at the end of shortening and the beginning of stimulation during the later part of lengthening.

Muscles were also subjected to 10–12 after-loaded isotonic contractions to determine the force–velocity characteristics. At optimal length, the muscle was maximally stimulated and allowed to shorten. The maximum shortening velocity was recorded at stepwise decreasing forces. The data were described by fitting the three-parameter hyperbolic–linear equation (Marsh and Bennett, 1986):

$$V = [B(1 - P/P_0]/[A + P/P_0)] + C(1 - P/P_0)\,,$$

where V is the shortening velocity in muscle lengths s^{-1} ($L_0\,s^{-1}$), P/P_0 is the force P as a fraction of maximum isometric force P_0 and A, B and C are constants. These curves were fitted using a non-linear curve-fitting routine in the application Igor (WaveMetrics). Maximum shortening velocity (V_{max}) was estimated by extrapolating the curve to zero force. Maximum instantaneous power output was measured from these force–velocity relationships. Statistics for the force–velocity data were handled in a similar fashion as in Marsh (1999).

At the end of the contractile measurements, muscle length was measured accurately. The muscle was removed from the experimental chamber, and the fragments of internal oblique muscle fibers and the damaged fibers of external oblique muscle were dissected away. The remaining strip of intact muscle was weighed. The cross-sectional area was calculated by dividing the muscle mass by the length of the muscle.

For our initial set of experiments, we used six *H. versicolor*. In subsequent studies, five *H. versicolor* and *H. chrysoscelis* were used. Results are presented as mean ± S.E.M. Mean values were compared using Student's *t*-test using the application Statview from Abascus concepts.

Results

Isometric contractile properties

Fusion of tetanic force during repetitive stimulation occurred at 210 Hz and 160 Hz at 25 °C for *H. chrysoscelis* and *H. versicolor*, respectively. The external oblique muscles in *H. chrysoscelis* and *H. versicolor* produced statistically indistinguishable isometric tetanic tensions at 25 °C, 10.4±1.19 N cm^{-2} and 9.2±0.46 N cm^{-2}, respectively (P=0.13, *t*-test). The latent periods measured for these two muscles, 2.15±0.07 ms in *H. chrysoscelis* and 2.2±0.05 ms in *H. versicolor*, were also similar (P=0.5). The time to peak twitch force in *H. chrysoscelis* (11.0±0.45 ms) was only 62 % of that found in *H. versicolor* (17.7±0.72 ms), and this difference was highly statistically significant (P<0.001, *t*-test). The oblique muscles in *H. chrysoscelis* also relaxed significantly faster than the muscles in *H. versicolor*. The times to 50 % relaxation were 12.4±0.86 ms and 18.9±1.84 ms, respectively (P<0.0035). The twitch:tetanus ratio in *H. chrysoscelis* was 0.31±0.01, whereas that for *H. versicolor* was 0.48±0.05 (P=0.0031). These twitch kinetics are similar to those reported by McLister et al. (1995) for the laryngeal muscle (tensor chordarum) at 25 °C in *H. chrysoscelis* and *H. versicolor*. As expected, the twitches were faster than those recorded for the external oblique by Marsh (1999) at 20 °C. Comparisons with Marsh's data yielded Q_{10} values of between 2.0 and 2.4 for most measures of twitch kinetics. However, the 50 % relaxation time in the muscles of *H. chrysoscelis* had a Q_{10} of only 1.2.

Influence of length trajectory on power output during work loop experiments

Similar power outputs were measured when the external oblique muscle of *H. versicolor* was subjected to natural and sawtooth cycles (55.39±5.14 W kg^{-1} and 55.07±5.47 W kg^{-1}, respectively) (P=0.9261, paired *t*-test). In the subsequent experiments with a separate set of muscles, only the sawtooth and sinusoidal length trajectories were used. In both species, the sawtooth trajectory yielded a significantly higher power output compared with the sinusoidal length pattern (P<0.0001, paired *t*-test) (Fig. 3). The maximum power output during sawtooth cycles was very similar in both species,

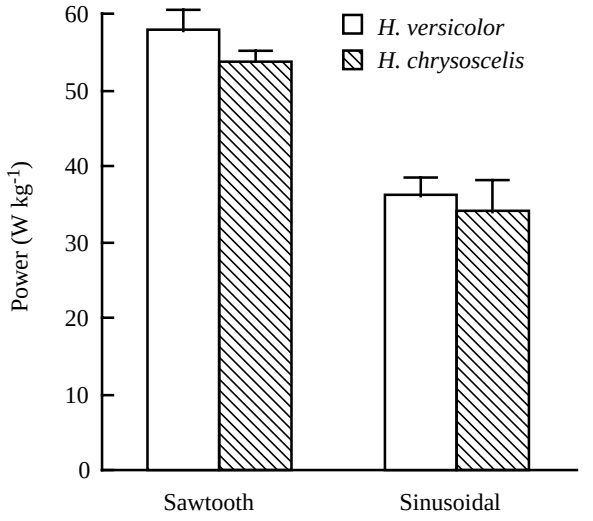

Fig. 3. Mean power output (in $W\,kg^{-1}$) using the sawtooth and sinusoidal length trajectories for *Hyla versicolor* (open columns) and *H. chrysoscelis* (hatched columns) at the optimum frequency for each preparation. The cycle frequencies used were 18–21 Hz and 40–44 Hz for muscles from *H. versicolor* and *H. chrysoscelis*, respectively. Values are means + S.E.M. (N=5).

53.9±1.35 $W\,kg^{-1}$ in *H. chrysoscelis* and 57.9±2.91 $W\,kg^{-1}$ in *H. versicolor* (P=0.78). With the sinusoidal length trajectory, the power outputs in both species were only 60 % of that available during sawtooth cycles (34.1±4.24 $W\,kg^{-1}$ and 36.2±2.36 $W\,kg^{-1}$ for *H. chrysoscelis* and *H. versicolor*, respectively).

The cycle frequency at which maximum net power was produced using a sawtooth length pattern was very different for the two species (40–44 Hz for *H. chrysoscelis* and 18–21 Hz for *H. versicolor*) (Fig. 4A,B). These frequencies are within the range of natural frequencies used by these muscles (Girgenrath and Marsh, 1997). For a given muscle, power output was maximized at a significantly lower frequency when the muscle was subjected to the sinusoidal length trajectory (25–40 Hz for *H. chrysoscelis* and 12–18 Hz for *H. versicolor*) (P=0.0004, paired t-test) (Fig. 4).

Examples of length and force recordings over two cycles of sawtooth and sinusoidal length trajectories for both species are shown in Fig. 5. The figure also illustrates the instantaneous power output during these cycles. Higher forces for a longer time were maintained during shortening with the sawtooth trajectory compared with the sinusoidal trajectory. A similar peak instantaneous power was produced during shortening (Fig. 5). However, the sawtooth trajectory yielded higher positive power over the complete cycle compared with the sinusoidal trajectory.

Anticlockwise work loops were generated when force produced by the muscle was plotted as a function of length during each cycle, as shown in Fig. 6. The shape and the area within the work loop were very different for the two length trajectories. In both species, the maximum force was developed at the peak length during sawtooth and sinusoidal cycles, but higher forces were maintained during shortening during the sawtooth trajectory than during the sinusoidal trajectory.

Using sawtooth cycles at the optimum frequency, the *in vitro* power output was maximized at 8 and 12 % strain amplitudes in *H. chrysoscelis* and *H. versicolor*, respectively (Fig. 7). Maximum net power output was produced with a stimulus 6 ms

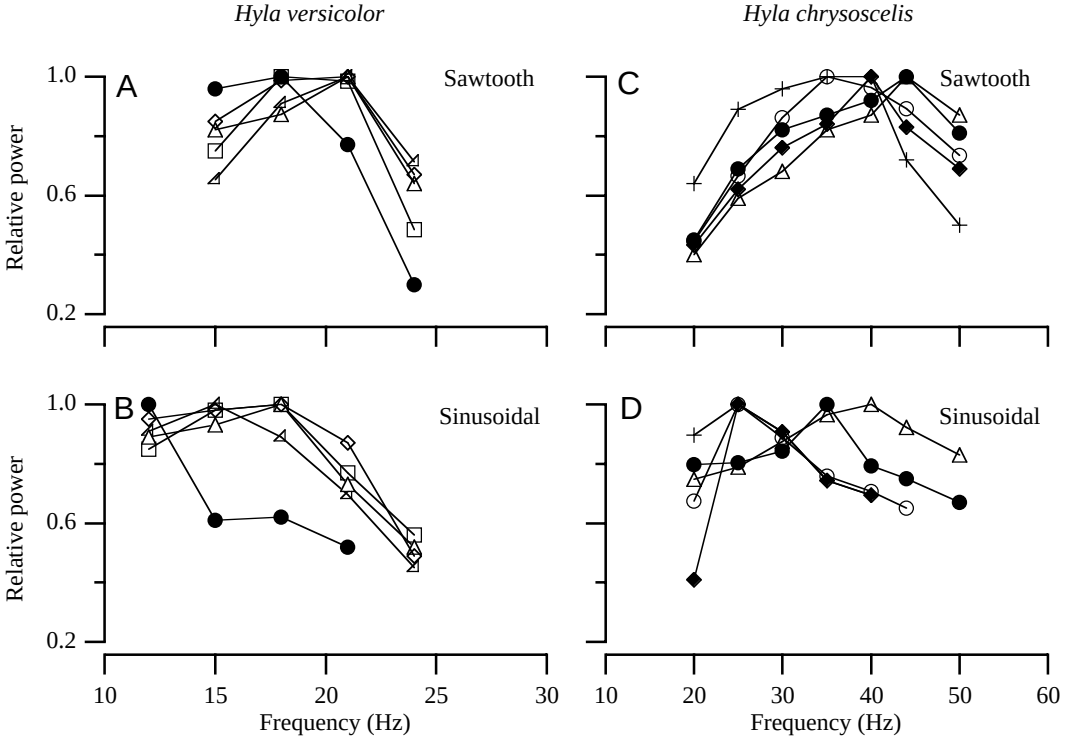

Fig. 4. Power output as a function of contraction frequency. Power is plotted as a fraction of the maximum power output at the optimum frequency. (A) Sawtooth cycles for muscles from *Hyla versicolor*. (B) Sinusoidal cycles for muscles from *H. versicolor*. (C) Sawtooth cycles for muscles from *H. chrysoscelis*. (D) Sinusoidal cycles for muscles from *H. chrysoscelis*. Note that the values on the *x*-axis are different for the two species. Different symbols are used for different individuals.

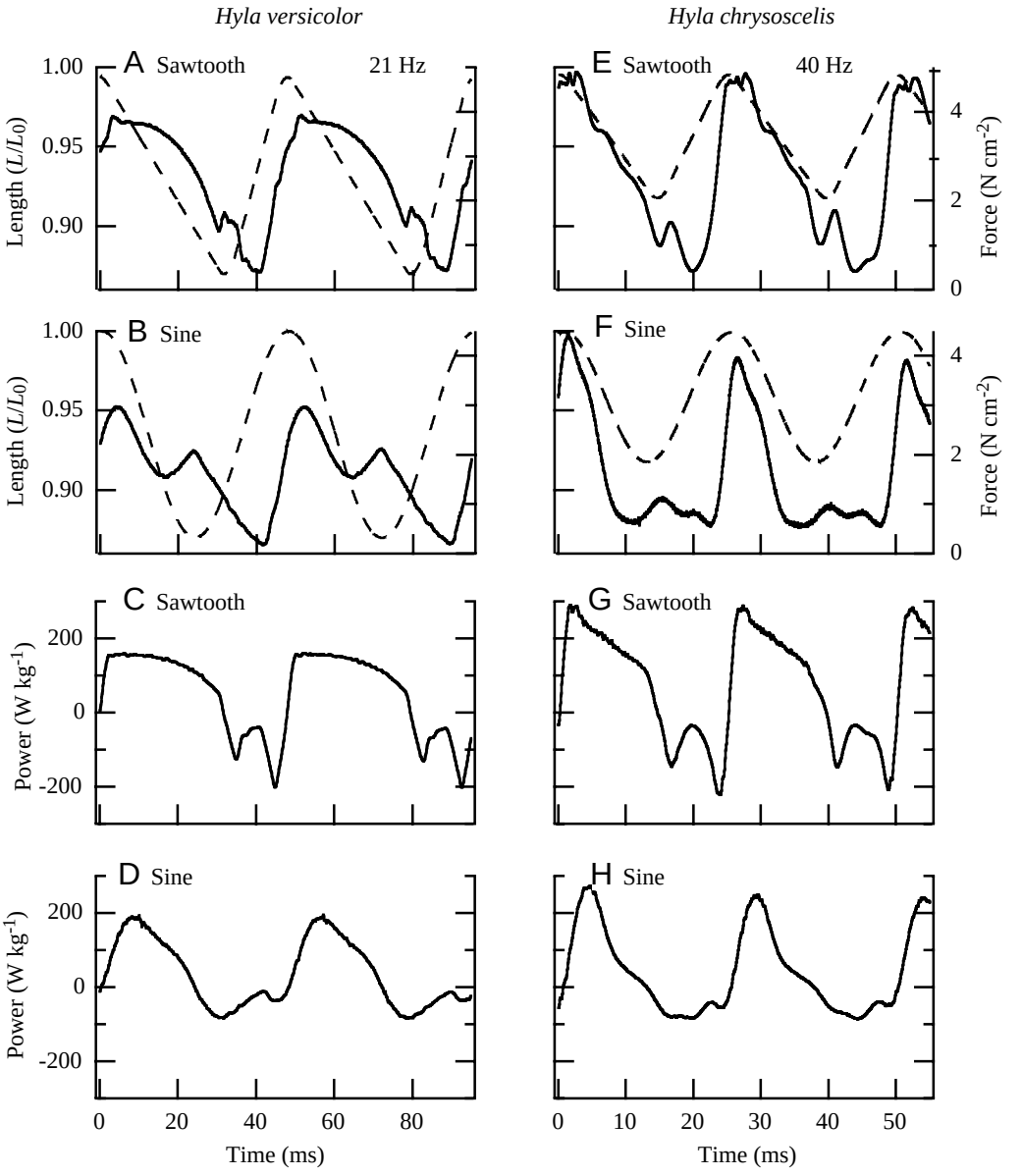

Fig. 5. Representative data from cyclical work measurements of the external oblique of *Hyla versicolor* (A–D) and *H. chrysoscelis* (E–H). A, B, E and F show force (solid lines) and length (dashed lines) using sawtooth (A,E) and sinusoidal (B,F) length trajectories. C, D, G and H show power outputs calculated for the cycles shown in A, B, E and F, respectively. The frequencies used were 21 Hz and 40 Hz for *H. versicolor* and *H. chrysoscelis*, respectively. Muscle length L is expressed as a fraction of optimal length L_0.

before the start of shortening in *H. chrysoscelis* and 8 ms before the start of shortening in *H. versicolor* (Fig. 8). These times of stimulation were optimal at all the cycle frequencies investigated.

In the data reported above, the muscles of *H. chrysoscelis* were stimulated once per cycle and those of *H. versicolor* were activated with two stimuli in each cycle. These parameters were set on the basis of our *in vivo* measurements (Girgenrath and Marsh, 1997). In *H. chrysoscelis*, increasing the number of stimuli to two at the optimum cycle frequency resulted in a decline in power to 86 % of the maximum net power produced with one stimulus. Decreasing the number of stimuli to one for *H. versicolor* at the optimum cycle frequency reduced the power output to 90 % of that produced with two stimuli. At lower frequencies (20–25 Hz), the oblique muscles of *H. chrysoscelis* produced 28 % higher power with two stimuli compared with the power produced with one stimulus.

Force–velocity relationships

Isotonic force and velocities were measured for all muscle preparations, and composite plots of force and velocity for *H. chrysoscelis* and *H. versicolor* are shown in Fig. 9. At 25 °C, the external oblique muscles of *H. chrysoscelis* had a maximum shortening velocity of $13.6\pm0.54\,L_0\,s^{-1}$. At the same temperature, this muscle from *H. versicolor* shortened with a maximum velocity of $11.1\pm0.40\,L_0\,s^{-1}$. The difference is statistically significant (P=0.0078, t-test). Power ratios, R_P ($W_{max}/V_{max}P_0$, where W_{max} is maximum power) were 0.158±0.002 and 0.146±0.008 (P=0.20) for *H. chrysoscelis* and *H. versicolor*, respectively. Instantaneous maximum powers were estimated on the basis of the force–velocity curve. For *H. chrysoscelis* and *H. versicolor*, the instantaneous powers calculated from P_0, V_{max} and R_P were $223.5\,W\,kg^{-1}$ and $150.11\,W\,kg^{-1}$, respectively (P<0.001). Maximal power was produced at a P/P_0 of 0.44±0.02 for *H.*

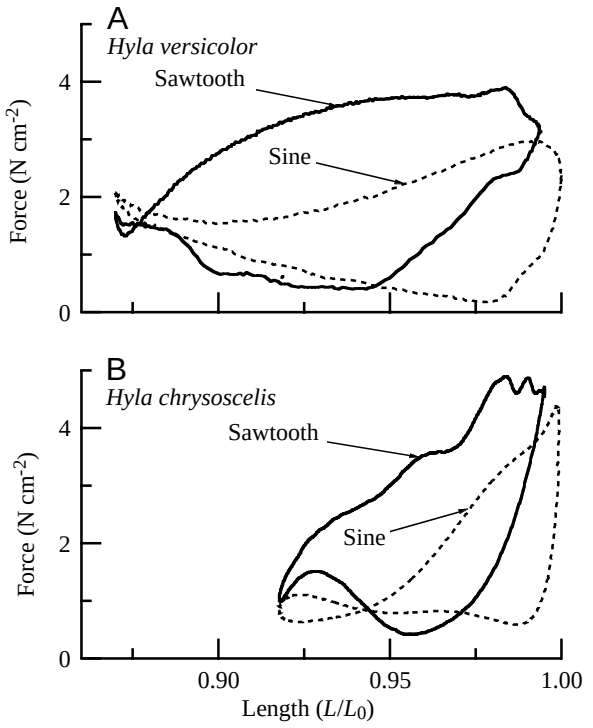

Fig. 6. Work loops for the external oblique muscles. Force is plotted as a function of length and generates an anticlockwise work loop. Data are shown for a single cycle using sawtooth (solid line) and sinusoidal (dashed line) trajectories for *Hyla versicolor* (A) and *H. chrysoscelis* (B). Frequencies used are 21 Hz and 40 Hz for *H. versicolor* and *H. chrysoscelis*, respectively. Muscle length L is expressed as a fraction of optimal length L_0.

chrysoscelis and 0.45±0.01 for *H. versicolor*. The values of V/V_{max} for maximal power were 0.36±0.01 and 0.38±0.01 for *H. chrysoscelis* and *H. versicolor*, respectively. Comparisons with the V_{max} values recorded by Marsh (1999) at 20 °C yielded Q_{10} values of 1.52 and 1.39 for *H. chrysoscelis* and *H. versicolor*, respectively.

Discussion

The work loop studies used to estimate the mechanical power outputs available during *in vivo* activity can be improved in their predictive power if the variables used are close to those actually measured during *in vivo* activity. Even though some of these variables are difficult to measure directly with precision, researchers have attempted to design *in vitro* experiments relying on information obtained from *in vivo* measurements (Marsh and Olson, 1994; Josephson and Stokes, 1989; Full et al., 1998). The present study was based on information on the *in vivo* function of the external oblique muscles (Girgenrath and Marsh, 1997) and allowed us to compare the optimal values for variables yielding maximum power output measured *in vitro* with those seen *in vivo*.

Within certain limits, we have replicated many features of the *in vivo* function of the external oblique. We based our work here on the cycles produced by the muscle in the major sound-producing part of the call (Girgenrath and Marsh, 1997). During the first few cycles, the muscle shortens progressively. During these first pulses, the activity of the muscles as judged by electromyograms (EMGs) is lower, as is the intensity of the sound produced. These initial pulses are difficult to model accurately *in vitro* because we do not know whether the increasing intensity of the EMGs is due to increasing fiber recruitment or is due to synaptic facilitation, as has been seen in the laryngeal muscles of *Xenopus laevis* (Tobias and Kelly, 1988). We also kept the timing of the stimulus constant, whereas *in vivo* the timing may drift late in the call (Girgenrath and Marsh, 1997).

Because of spatial and temporal limits to the accuracy of the strain cycle recorded using high-speed video by Girgenrath and Marsh (1997), we were uncertain about some of the fine details of the shape of the length trajectory. Therefore, we first compared an averaged and smoothed version of the natural length trajectory of *H. versicolor* with a simplified sawtooth trajectory that eliminates changes in velocity during shortening and lengthening, but retains the strain and relative duration of lengthening and shortening seen *in vivo*. The power output was essentially the same during these two types of cycle. This comparison appears to indicate that replicating the major features of the length trajectory using sawtooth cycles is adequate to describe the performance of these muscles. As the

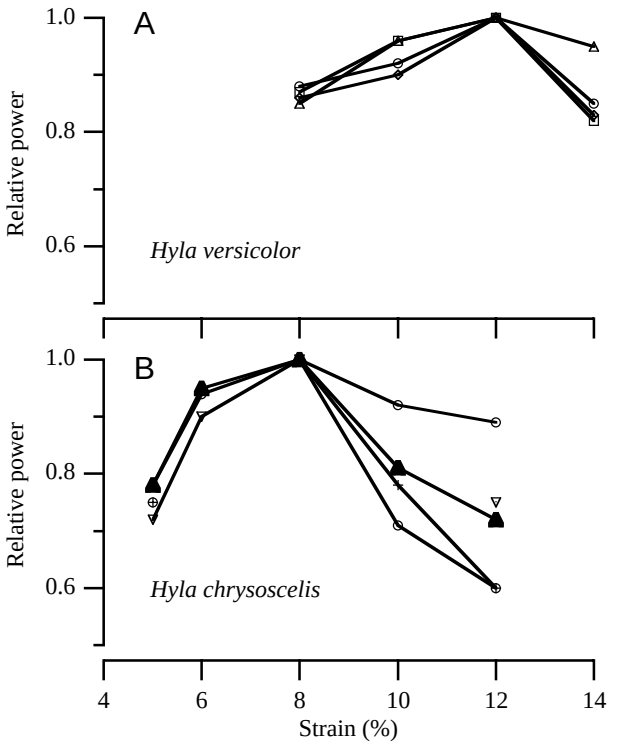

Fig. 7. Power output during sawtooth trajectories as a function of percentage strain. Power is plotted as a fraction of the maximum power output at the optimum strain. (A) *Hyla versicolor*; (B) *H. chrysoscelis.* The frequency used for *H. versicolor* was 21 Hz and that for *H. chrysoscelis* was 40 Hz. Different symbols are used for different individuals.

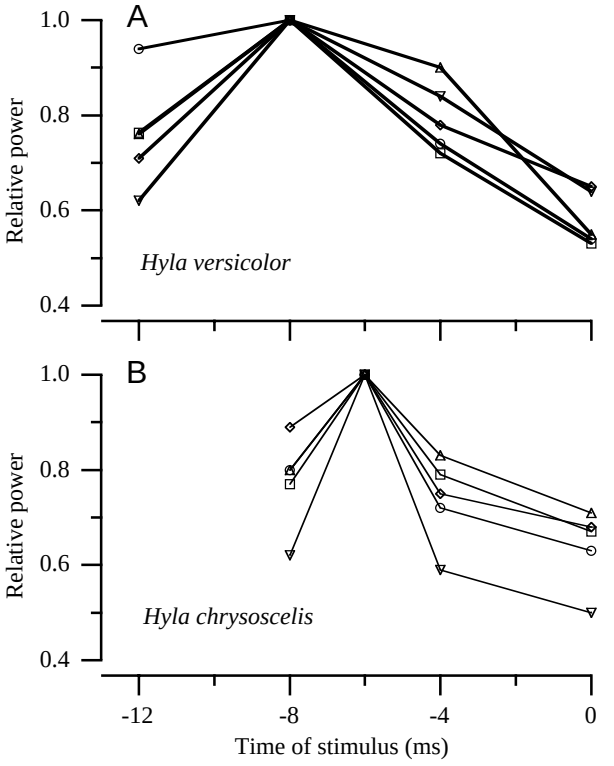

Fig. 8. Effect of stimulus timing relative to the start of shortening on power output using a sawtooth trajectory for *Hyla versicolor* (A) and *H. chrysoscelis* (B). The frequencies used were 21 Hz for *H. versicolor* and 40 Hz *H. chrysoscelis*. Power output is presented as a fraction of the maximum power at optimum stimulus timing. Zero time indicates the beginning of the shortening phase. Different symbols are used for different individuals.

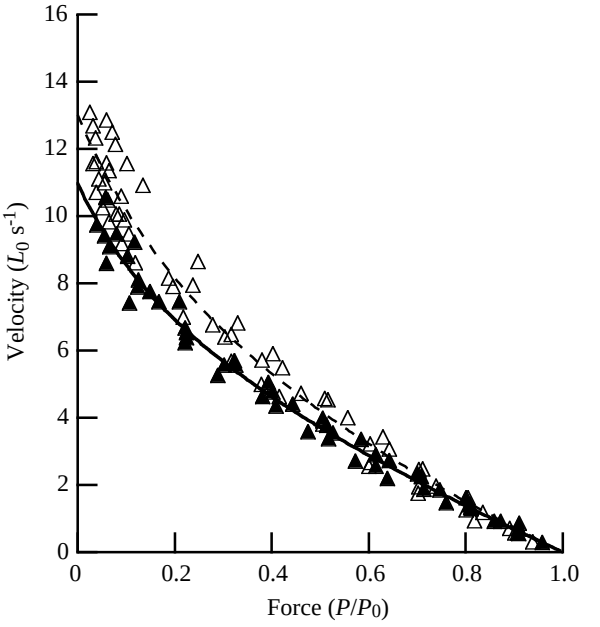

Fig. 9. Composite force–velocity data for the external oblique muscle of *Hyla versicolor* (filled symbols) and *H. chrysoscelis* (open symbols). The constants from the hyperbolic–linear equation (see Materials and methods) were as follows: A=0.37±0.04, B=2.98±0.36 L_0 s^{-1}, C=5.53±1.4 L_0 s^{-1} for *H. chrysoscelis*; A=0.91±0.021, B=0.5078±0.031 L_0 s^{-1}, C=5.53±1.07 L_0 s^{-1}) for *H. versicolor*, where L_0 is optimal length. Force P is expressed as a fraction of maxmum isometric force P_0.

discussion below emphasizes, the major features of the cycle, including the sawtooth shape, the strain and the timing of the stimulus, are crucial for high power output.

Optimal length (L_0) determined *in vitro* was very similar to the peak length at which these muscles operate *in vivo* during the major sound-producing part of a call, ($L_{p,max}$ of Girgenrath and Marsh, 1997). Operating near L_0 should allow these muscles to maximize the force production and thus the power output during each pulse. Power output using the sawtooth trajectory is maximized at frequencies of 40–44 Hz for *H. chrysoscelis* and 18–21 Hz for *H. versicolor* (Fig. 4). These frequencies are similar to previous *in vivo* measurements of contractile frequency recorded by us and close to those predicted by others (40–55 Hz and 20–25 Hz at 25 °C for *H. chrysoscelis* and *H. versicolor*, respectively) (Ralin, 1977; Bogart and Jaslow, 1979; Girgenrath and Marsh, 1997). Above and below the optimum frequencies, power declines for both species, but power output is relatively high throughout the range of pulse frequencies used during natural calling. Several other studies have reported

Table 1. In vitro *power output of several aerobic muscles*

	Frequency (Hz)	Power output at T_{exp} (W kg^{-1})	Power output at 25 °C (W kg^{-1})	Reference
Schistocerca nitens (flight muscle)	25	72 (30 °C)	50.9	1
Schistocerca americana (flight muscle)	20	75 (30 °C)	53.0	1
Neoconocephalus riope (flight muscle)	20	76 (30 °C)	53.7	2
Manduca sexta (flight muscle)	28	90 (40 °C)	31.8	3
Hyla versicolor (external oblique)	25	–	57.9	4
Hyla chrysoscelis (external oblique)	50	–	53.9	4

T_{exp}, experimental temperature, given in parentheses.
Values shown here are those reported at various temperatures.
For comparison, we have corrected these values to 25 °C (the temperature of our experiments) using a Q_{10} of 2.
[1]Mizisin and Josephson (1987); [2]Josephson (1985); [3]Stevenson and Josephson (1990); [4]this study.

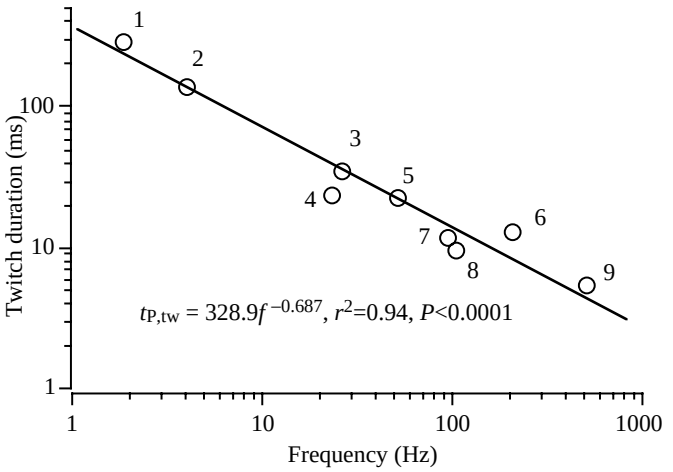

Fig. 10. Relationship between operating frequency (f) and twitch duration ($t_{P,tw}$) in various power-producing synchronous muscles. Twitch duration is defined as the sum of the time to peak force and the 50 % relaxation time. The numbers beside each point indicate the muscles studied as follows: (1) adductor muscle in the scallop *Argopecten irradians* (Olson and Marsh, 1993); (2) adductor muscle in the scallop *Chlamys hastata* (R. L. Marsh and J. M. Olson, unpublished data); (3) external oblique muscle in the gray tree frog *Hyla versicolor* (this study); (4) pectoralis muscle in the quail *Coturnix chinensis* (G. N. Askew and R. L. Marsh, unpublished results); (5) external oblique muscle in the gray tree frog *H. chrysoscelis* (this study); (6) swim bladder muscle in the toadfish *Opsanus tau* (Rome et al., 1996); (7) shaker muscle in the rattlesnake *Crotalus atrox* (Rome et al., 1996); (8) remotor muscle in the lobster *Homarus americanus* (Mendelson, 1969); and (9) tymbal muscle in the cicada *Okanagana vanduzeei* (Josephson and Young, 1985).

similar relationships between frequency and power output *in vitro* (Altringham and Johnston, 1990; James et al., 1995; Stevenson and Josephson, 1990; Askew and Marsh, 1997).

The more rapid twitch kinetics of the external oblique in *H. chrysoscelis* compared with that of *H. versicolor* is consistent with its higher pulse frequency during calling. However, the difference in twitch time is not completely matched by the pulse frequency difference observed in these two species. The muscle of *H. chrysoscelis* is only 1.6 times faster than the same muscle in *H. versicolor*. Thus, the twitch duration is a smaller fraction of the cycle time in *H. versicolor*. Our *in vivo* data show that the external obliques in *H. versicolor* are stimulated with two twitches in each pulse compared with only one in *H. chrysoscelis*. Thus, even though the twitch duration is a smaller fraction of the shortening duration, the muscle produces high force for a longer fraction of the cycle (Fig. 5). In *H. chrysoscelis*, which operates at a higher frequency as well as with a smaller fraction of the cycle spent shortening, one stimulus is adequate to activate the muscle sufficiently and allows deactivation to occur largely before the beginning of lengthening. In *H. chrysoscelis* preparations, having two stimuli during *in vitro* experiments decreased the power output by 14 % because of the higher residual force during lengthening.

At the optimum cycle frequency, maximum power was produced when the muscles were stimulated before the start of the shortening period (6 ms in *H. chrysoscelis* and 8 ms in *H. versicolor*) (Fig. 8). Electrical activity measured *in vivo* also occurs at an identical phase in relation to shortening during the first part of the call in both species (Girgenrath and Marsh, 1997). This allows the force to rise during the lengthening phase of the cycle, with maximum force developed at the beginning of shortening. It has been shown by Askew and Marsh (1997) that the rate of rise in force is enhanced if the lengthening velocities are increased. Thus, having a shorter lengthening phase in the cycle, the muscle lengthens with a higher velocity ($8\,L_0\,s^{-1}$) (Girgenrath and Marsh, 1997), allowing it to develop force more rapidly. The consequences of the drift in stimulus phase and the increase in strain that occur late in the call (Girgenrath and Marsh, 1997) remain to be investigated.

Power output was maximized for both species at their respective *in vivo* strain amplitudes (approximately 8 % of L_0 in *H. chrysoscelis* and approximately 12 % in *H. versicolor*) (Girgenrath and Marsh, 1997). Relative to the optimum strain amplitude, power output declined at longer and shorter strains for both species (Fig. 7). The influence of strain appears to be greater in *H. chrysoscelis*, probably because at higher frequencies a similar change in absolute strain amplitude will cause a larger increase in V/V_{max}. Several other studies have reported a similar influence of strain amplitude (James et al., 1995; Askew and Marsh, 1997).

Even though the external obliques of *H. chrysoscelis* operate with a reduced strain, the *in vivo* shortening velocity of external oblique muscles in *H. chrysoscelis* is 1.35 times higher than that in *H. versicolor* (Girgenrath and Marsh, 1997). Consequently, to operate at similar V/V_{max}, the muscles of *H. chrysoscelis* need to have a higher V_{max}. The V_{max} of the oblique muscles in *H. chrysoscelis* was found to be 1.2 times that of *H. versicolor*. Thus, during shortening *in vivo*, these muscles operate at $0.39V/V_{max}$ and $0.36V/V_{max}$ in *H. chrysoscelis* and *H. versicolor*, respectively. These values are very close to those predicted to yield peak power during isotonic contractions.

Maximum isometric forces were similar (approximately $10\,N\,cm^{-2}$) in both species. However, compared with the sartorius, a locomotor muscle, these forces are much lower. The maximum peak tension in the sartorius is approximately $25\,N\,cm^{-1}$ in both species (McLister et al., 1995; Marsh, 1999). As has been pointed out previously, the lower forces in these muscles can be explained at least in part by the reduced space allocated to the contractile machinery (Josephson and Young, 1985; Schaeffer et al., 1996; Block, 1994). Fractional volume density for myofibrils is estimated to be approximately 0.5 in *H. versicolor* (Marsh and Taigen, 1987). Thus, if corrected for force per myofibrillar cross-sectional area, the force produced will be twice as high (approximately $20\,N\,cm^{-2}$). This value is still lower than that found for the sartorius, which would be expected to have a value of $30\,N\,cm^{-2}$ of myofibrils (Marsh, 1999; Marsh and Taigen, 1987). Force per myofibrillar area

produced by the mesothoracic muscle of a tettigoniid insect is also reported to be much lower than that of the metathoracic muscles ($10\,N\,cm^{-2}$ myofibrils for mesothoracic and $24\,N\,cm^{-2}$ myofibrils for metathoracic muscle) (Josephson, 1984). Mesothoracic muscles are used for stridulation and flight with a maximum operating frequency of 200 Hz at 35 °C, whereas metathoracic muscles have an operating frequencies of 20 Hz at 25 °C and are used for flight only. Other muscles with very brief twitches are also reported to produce very low tension (Mendelson, 1969; Close and Luff, 1974). Thus, the possibility exists that specialization for power output at high frequencies compromises the ability to produce force (Josephson, 1984; Rome et al., 1996). A recent study of super-fast toadfish muscles, which are used to produce sound, suggests that in these muscles the ratio of rates of cross-bridge detachment to attachment is much higher than the same ratio in low-frequency locomotor muscles (Rome et al., 1999). Thus, Rome et al. (1999) suggest that the low force production of these muscles is due to a lower number of myosin heads attached in the active muscle rather than to low force produced per myosin head.

Force–velocity curves for the external obliques are flatter than those of typical locomotor muscles. The power ratio for sartorius is approximately 0.1 (Marsh, 1999) compared with 0.158 and 0.147 in *H. chrysoscelis* and *H. versicolor*, respectively. This allows the oblique muscles to produce 50 % more power for a given P_0 and V_{max}. Thus, at the myofibrillar level, the increase in power due to a flatter curve more than makes up for the 30 % lower P_0.

Sawtooth versus *sinusoidal length trajectory*

Power output was much higher with the sawtooth length trajectory than with sinusoidal cycles (1.8 times higher in *H. chrysoscelis* and 1.6 times higher in *H. versicolor*) (Fig. 3). At all the frequencies measured, there was a consistent difference in power for the two length trajectories. This observation confirms our prediction that the *in vivo* sawtooth length trajectory used by these muscles increases the power output at their respective frequencies (Girgenrath and Marsh, 1997). Interestingly, this result has recently been confirmed in a study with two mammalian skeletal muscles which showed that, at any given frequency, the power output can be increased by increasing the proportion of time spent shortening during a sawtooth cycle (Askew and Marsh, 1997). These data suggest that the effect of an asymmetrical sawtooth cycle is a general one and not simply a feature of these calling muscles.

Several factors contribute to the higher power output that occurs with a longer shortening phase compared with the lengthening phase. During cyclical contractions, muscles produce positive work only during the shortening phase of the cycle, whereas work is required to lengthen muscles (Josephson, 1993). Also, since force and velocity are inversely related, a muscle can operate at a velocity more favorable for force production when it spends more time shortening. This allows the muscle to produce higher positive work in a given cycle. The longer shortening phase further allows the muscle to accomplish a greater strain amplitude while maintaining a favorable V/V_{max} (Askew and Marsh, 1997). Thus, work being the product of force and distance, the sawtooth length trajectory enhances work per cycle because of increased strain amplitude, and hence increased power output is achieved.

The work loops generated during sawtooth trajectories are quite different from those produced by sinusoidal trajectories. Because of the constant velocity of shortening (Figs 5, 6), high forces are maintained over a longer period during a sawtooth length trajectory than during a sinusoidal length cycle, and this can explain the higher work output per cycle using a sawtooth length pattern. Power output increases to some extent when a linear strain trajectory is used with equal shortening and lengthening duration compared with a sinusoidal length trajectory (Askew and Marsh, 1997). However, increasing the shortening phase in a linear length trajectory greatly enhances power output (Askew and Marsh, 1997).

During cyclical contractions, maximum power output is possible if the muscle is fully activated during the shortening phase and relaxed during the lengthening phase. However, these processes are not instantaneous and may contribute to lower power ouputs as a result of incomplete activation and deactivation during shortening and lengthening, respectively (Josephson, 1985). Particularly at higher operating frequencies, these events will occupy a greater proportion of the cycle time and can significantly reduce the power output. Increasing the proportion of time spent shortening allows an increased allocation of time for these processes. A muscle can therefore be fully active in the shortening phase and also relax to a greater extent before the lengthening phase begins, resulting in enhanced power output.

The advantages of the asymmetrical sawtooth cycle suggest that the mass-specific power output of cyclically driven systems will be enhanced in those cases in which power is mainly produced by movement in one direction. For example, during calling, the external and internal oblique muscles shorten actively to produce sound power and to expand the vocal sac (Girgenrath and Marsh, 1997). The elastic recoil of the vocal sac drives lengthening of the oblique muscles. Similarly, in birds that have been shown to use asymmetric cycles during flight (Tobalske and Dial, 1996; Biewener et al., 1998; G. N. Askew and R. L. Marsh, unpublished results), the downstroke produces most of the power for flight and the upstroke is lightly loaded. Obviously, certain systems of movement are constrained to symmetrical cycles of power output, e.g. fish swimming, and thus cannot take advantage of the enhanced performance possible by increasing the duration of shortening.

Performance of external oblique muscle compared with other synchronous muscles

Twitch duration

The twitch durations (defined as the sum of the time to peak force and the 50 % relaxation time) reported for many invertebrate sound-producing muscles are shorter than those of the external obliques of *H. chrysoscelis* and *H. versicolor*

(Mendelson, 1969; Young and Josephson, 1983; Josephson, 1984; Josephson and Young, 1985). Compared with other vertebrate sound-producing muscles, the twitch kinetics of the external oblique muscles in *H. chrysoscelis* are similar to those reported for the shaker muscles of rattlesnakes, when the effects of temperature are considered. The twitch duration of rattlesnake shaker muscle is 12 ms at 35 °C (Rome et al., 1996) and that for the external oblique of *H. chrysoscelis* is 23 ms at 25 °C. However, the toadfish swim bladder muscle, which operates at 200 Hz at a comparable temperature, has a twitch duration of approximately 13 ms at 16 °C (Rome et al., 1996). Despite the variable twitch kinetics and operating frequencies, all these sound-producing muscles show similarities at the ultrastructural level at least. Unusually large proportions of the fibers are occupied by sarcoplasmic reticulum and, in most cases, mitochondria (Marsh and Taigen, 1987; Appelt et al., 1991; Rome et al., 1996; Schaeffer et al., 1996; Josephson and Young, 1985). Structural and functional similarities suggest a convergent evolution of these sound-producing muscles in disparate species.

A logarithmic plot (Fig. 10) of twitch duration against cycle frequency of various muscles involved in power production shows a very close correlation between the two variables. The frequencies of operation included in this correlation extend over a broad range from 1.8 Hz in scallops to approximately 500 Hz in one species of cicada. Young and Josephson (1984) also demonstrated a very strong correlation between twitch duration and cycle time in the sound-producing muscles of insects. A certain amount of caution is warranted in interpreting the relationship shown in Fig. 10. First, more data are needed at low frequencies to indicate the accuracy of this relationship for power-producing muscles in general. The scallop muscles shown on the plot, which operate at approximately 2 and 4 Hz, may be unusual in that they operate with only 1–2 stimuli per cycle despite their low operating frequency. Second, it is important to point out that, for some muscles under consideration, the logarithmic relationship plotted here can obscure large differences in the operating frequencies of muscles with similar twitch kinetics. For example, in the flight muscles of the painted quail *Coturnix chinensis*, the operating frequency is approximately 22 Hz (G. N. Askew and R. L. Marsh, unpublished results). However, the twitch duration in this muscle is similar to that found in the external oblique of *H. chrysoscelis*, which operates at twice the frequency. The relatively shorter twitch allows the quail to use multiple stimuli per cycle (G. N. Askew and R. L. Marsh, unpublished results), which keeps the muscle active for a longer time (to accommodate the longer cycle period) even when each twitch is short. As more data accumulate on twitch times of muscles with known *in vivo* functions, a more robust evaluation of the relationship shown in Fig. 10 will be possible.

Power output

The external oblique muscles of both species of tree frog produce similar power (approximately 55 W kg^{-1}) at their respective operating frequencies (Fig. 3). Because myofibrillar volume is only 0.5 of the total volume, power output will be twice as high when corrected for this ratio (110 W kg^{-1}). The muscles of *H. chrysoscelis* are capable of producing similar power at much higher frequencies because of their faster activation and relaxation times along with higher shortening velocities compared with those of *H. versicolor*. The power outputs from these muscles at their operating frequencies are quite impressive when compared with those of other aerobic vertebrate muscles (see Fig. 8 in James et al., 1995). Invertebrate aerobic muscles have higher power outputs when operating at similar frequencies to those of *H. versicolor*. However, when corrected for temperature, the power outputs are very similar to those produced by the external obliques of *H. versicolor* (Table 1). The external oblique muscles in *H. chrysoscelis* are even more impressive because they produce similar powers while operating at a much higher frequency (Table 1).

Concluding remarks

The trunk muscles of male hylid frogs have an unusual suite of characteristics compared with most anuran muscles. These characteristics include a very high aerobic capacity (Taigen et al., 1985; Marsh and Taigen, 1987), high contractile velocities and very flat force–velocity curves (Marsh, 1999; the present study). We have further demonstrated in the present study that the external and internal oblique muscles of two species of gray tree frog are capable of producing high power output matching their function in power generation during sound production. Using the *in vivo* strain trajectory, power output was optimized *in vitro* when the strain amplitude, the frequency and the stimulus timing were matched to *in vivo* values.

The characteristics of these muscles appear to have resulted from natural selection operating on a system crucial for mate choice. In reaching this conclusion, we are fortunate in having numerous studies of mate choice by female anurans in relation to the characteristics of the male's calls, including a number of studies of gray tree frogs (for a review, see Gerhardt, 1994). Field and laboratory studies that have shown that females prefer calls of a specific frequency and pulse shape. Females also prefer louder and longer calls. These studies directly demonstrate the importance of high power output at specific frequencies. Females also prefer calls produced at high rates, which may be related to selection for endurance.

High power output is required of the trunk muscles for the animals to produce loud calls. Prestwich et al. (1989) suggest that the transduction of muscle power to sound energy is not very efficient in tree frogs, possibly because of a mismatch between the size of the resonator and the primary frequency of the sound. This limited transduction efficiency increases the power output required to produce a given volume of sound. We have found that the power outputs from the trunk muscles (operating at their given temperatures and frequencies) are high compared with those of other vertebrate synchronous muscles.

Obviously, power output in these muscles has to be matched to the supply of ATP to the myofibrillar- and Ca^{2+}-ATPases.

The trunk muscles function aerobically *in vivo* and have a considerable portion of their volume occupied by mitochondria and lipid droplets (Marsh and Taigen, 1987). The consequent reduction in myofibrillar volume reduces force and power output, representing the trade-off found when muscles are simultaneously selected for high power output and endurance.

For reasons not understood at present, the sound-producing muscles also produce lower force than the locomotor muscles of frogs when expressed per cross-sectional area of myofibrils. This lower force would tend to decrease myofibrillar-specific power output. However, the trunk muscles also have much flatter force–velocity curves than those found in locomotor muscles, which boosts power output and more than compensates for the low force output.

The data presented here clearly show that the sawtooth trajectory used *in vivo* allows the muscles to produce substantially higher power compared with a sinusoidal length trajectory. The use of the sawtooth trajectory allows the external oblique muscles to produce high power while operating at a high frequency. The initial data collected on these frog muscles provided the impetus for an investigation of the influence of sawtooth cycles in mouse muscles (Askew and Marsh, 1997). We conclude that asymmetrical cycles provide a distinct advantage in producing power during cyclical contractions. This conclusion has the corollary that systems designed for cyclical work have an advantage in power output if they are powered mostly during one phase of the cycle. Such is the case for tree frog calling in which the trunk muscles produce the power when air is forced from the lungs into the vocal sac. This power is used both to produce sound and to stretch the elastic vocal sac. The return of air to the lungs and the restretching of the trunk muscles are driven passively by the elastic energy stored in the vocal sac. The demonstrated importance of the asymmetrical sawtooth length trajectories used by these muscles once again emphasizes the importance of incorporating *in vivo* variables in understanding the true limits of muscle performance.

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References

Altringham, J. D. and Johnston, I. A. (1990). Modelling muscle power output in a swimming fish. *J. Exp. Biol.* **148**, 395–402.

Appelt, D., Shen, V. and Franzini-Armstrong, C. (1991). Quantification of Ca ATPase, feet and mitochondria in superfast muscle fibres from toadfish, *Opsanus tau*. *J. Muscle Res. Cell Motil.* **12**, 543–552.

Askew, G. N. and Marsh, R. L. (1997). The effect of length trajectory on the mechanical power output of mouse skeletal muscles. *J. Exp. Biol.* **200**, 3119–3131.

Biewener, A. A., Corning, W. R. and Tobalske, B. W. (1998). *In vivo* pectoralis muscle force–length behavior during level flight in pigeons (*Columba livia*). *J. Exp. Biol.* **201**, 3293–3307.

Block, B. A. (1994). Thermogenesis in muscle. *Annu. Rev. Physiol.* **56**, 535–577.

Bogart, J. P. and Jaslow, A. P. (1979). Distribution and call parameters of *Hyla chrysoscelis* and *Hyla versicolor* in Michigan. *Life Sci. Contrib. R. Ont. Mus.* **117**, 3–13.

Close, R. I. and Luff, A. R. (1974). Dynamic properties of inferior rectus muscle of the rat. *J. Physiol., Lond.* **236**, 259–270.

Full, R. J., Stokes, D. R., Ahn, A. N. and Josephson, R. K. (1998). Energy absorption during running by leg muscles in a cockroach. *J. Exp. Biol.* **201**, 997–1012.

Gerhardt, H. C. (1994). The evolution of vocalization in frogs and toad. *Annu. Rev. Ecol. Syst.* **25**, 293–324.

Girgenrath, M. and Marsh, R. L. (1997). *In vivo* performance of trunk muscles in tree frogs during calling. *J. Exp. Biol.* **200**, 3101–3108.

James, R. S., Altringham, J. D. and Goldspink, D. F. (1995). The mechanical properties of fast and slow skeletal muscles of the mouse in relation to their locomotory function. *J. Exp. Biol.* **198**, 491–502.

Josephson, R. K. (1984). Contraction dynamics of flight and stridulatory muscles of tettigonoid insects. *J. Exp. Biol.* **108**, 77–96.

Josephson, R. K. (1985). Mechanical power output from striated muscle during cyclical contraction. *J. Exp. Biol.* **114**, 493–512.

Josephson, R. K. (1993). Contraction dynamics and power output of skeletal muscle. *Annu. Rev. Physiol.* **55**, 527–546.

Josephson, R. K. and Stokes, D. R. (1989). Strain, muscle length and work output in a crab muscle. *J. Exp. Biol.* **145**, 45–61.

Josephson, R. K. and Young, D. (1981). Synchronous and asynchronous muscles in cicadas. *J. Exp. Biol.* **91**, 219–237.

Josephson, R. K. and Young, D. (1985). A synchronous insect muscle with an operating frequency greater than 500 Hz. *J. Exp. Biol.* **118**, 185–208.

Lindholm, M. M. and Bass, A. H. (1993). Early events in myofibrilogenesis and innervation of skeletal, sound-generating muscle in a teleost fish. *J. Morph.* **216**, 225–239.

Marsh, R. L. (1999). Contractile properties of muscles used in sound production and locomotion in two species of gray tree frog. *J. Exp. Biol.* **202**, 3215–3223.

Marsh, R. L. and Bennett, A. F. (1986). Thermal dependence of isotonic 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.

Marsh, R. L. and Olson, J. M. (1994). Power output of scallop adductor muscle during contractions replicating the *in vivo* mechanical cycle. *J. Exp. Biol.* **193**, 139–156.

Marsh, R. L., Olson, J. M. and Guzik, S. K. (1992). Mechanical performance of scallop adductor muscles during swimming. *Nature* **357**, 411–413.

Marsh, R. L. and Taigen, T. L. (1987). Properties enhancing aerobic capacity of calling muscles in gray tree frogs *Hyla versicolor*. *Am. J. Physiol.* **252**, R786–R793.

Martin, W. F. (1971). Mechanics of sound production in toads of genus *Bufo*: passive elements. *J. Exp. Zool.* **176**, 273–294.

Martin, W. F. and Gans, C. (1972). Muscular control of the vocal tract during release signaling in the toad *Bufo valliceps*. *J. Morph.* **137**, 1–28.

McLister, J. D., Steven, E. D. and Bogart, J. P. (1995). Comparative contractile dynamics of calling and locomotor muscles in three hylid frogs. *J. Exp. Biol.* **198**, 1527–1538.

Mendelson, M. (1969). Electrical and mechanical characteristics of a very fast lobster muscle. *J. Cell Biol.* **42**, 548–563.

Mizisin, A. D. and Josephson, R. K. (1987). Mechanical power output of locust flight muscles. *J. Comp. Physiol.* A **160**, 413–419.

Olson, J. M. and Marsh, R. L. (1993). Contractile properties of the striated adductor muscle in the bay scallop *Argopecten irradians* at several temperatures. *J. Exp. Biol*. **176**, 175–193.

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

Ralin, D. B. (1977). Evolutionary aspects of mating call variation in a diploid–tetraploid species complex of tree-frogs (Anura). *Evolution* **31**, 721–736.

Ressel, S. J. (1996). Ultrastructural properties of muscles used for call production in neotropical frogs. *Physiol. Zool.* **69**, 952–973.

Rome, L. C., Syme, D. A., Hollingworth, S., 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.

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.

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.

Stevenson, R. D. and Josephson, R. K. (1990). Effect of operating frequency and temperature on mechanical power output from moth flight muscle. *J. Exp. Biol*. **149**, 61–78.

Taigen, T. L., Wells, K. D. and Marsh, R. L. (1985). The enzymatic basis of high metabolic rates in calling frogs. *Physiol. Zool.* **58**, 719–726.

Tobalske, B. W. and Dial, K. P. (1996). Flight kinematics of black billed magpies and pigeons over a wide range of speeds. *J. Exp. Biol*. **198**, 263–280.

Tobias, M. L. and Kelly, D. B. (1988). Electrophysiology and dye-coupling are sexually dimorphic characteristics of individual laryngeal muscle fibers in *Xenopus laevis*. *J. Neurosci*. **8**, 2422–2429.

Young, D. and Josephson, R. K. (1983). Mechanisms of sound-production and muscle contraction kinetics in cicadas. *J. Comp. Physiol*. **152**, 183–195.

Young, D. and Josephson, R. K. (1984). 100 Hz is not the upper limit of synchronous muscle contraction. *Nature* **309**, 286–287.