Low thermal dependence of the contractile properties of a wing muscle in the bat *Carollia perspicillata*

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Summary Statement: The contractile properties of a bat wing muscle are less thermally dependent than those of other studied animals, which may help maintain muscle function during flight in thermally variable environments.

ABSTRACT

Temperature affects contractile rate properties in muscle, which may affect locomotor performance. Endotherms are known to maintain high core body temperatures, but temperatures in the periphery of the body can fluctuate. Such a phenomenon occurs in bats, whose wing musculature is relatively poorly insulated, resulting in substantially depressed temperatures in the distal wing. We examined a wing muscle in the small-bodied tropical bat *Carollia perspicillata* and a hindlimb muscle in the laboratory mouse at 5ºC intervals from 22 to 42ºC to determine the thermal dependence of the contractile properties of both muscles. We found that the bat ECRL had low thermal dependence from near body temperature to 10ºC lower, with Q_{10} values of less than 1.5 for relaxation from contraction and shortening velocities in that interval, and with no significant difference in some rate properties in the interval between 32 and 37ºC. In contrast, for all temperature intervals below 37ºC, Q_{10} values for the mouse EDL were 1.5 or higher, and rate properties differed significantly across successive temperature intervals from 37 to 22ºC. An ANCOVA analysis found that the thermal dependencies of all measured isometric and isotonic rate processes were significantly different between the bat and mouse muscles. The relatively low thermal dependence of the bat muscle likely represents a downward shift of its optimal temperature and may be functionally significant in light of the variable operating temperatures of bat wing muscles.

INTRODUCTION

Temperature has major effects on muscle performance (Bennett, 1984; James, 2013). These effects are clearly ecologically important in ectotherms that experience substantial variation in muscle temperature during normal activities (Bennett, 1990). Endotherms may also experience variation in muscle temperature resulting from either variation in regulated body temperature, e.g., torpor, or from regional variation in temperature. However, most of the studies of the influence of temperature on the contractile properties of endotherm muscle have emphasized basic muscle properties rather than the importance of thermal dependence to *in vivo* function (e.g., Close and Hoh, 1968; Ranatunga, 1980, 1982, 1984; Barclay et al., 2010; Baylor and Hollingworth, 2010). Exceptions include work on muscles of hamsters that undergo torpor (South, 1961) and *in vivo* studies of human forearm and hand muscles (e.g., Ranatunga et al., 1987; De Ruiter and De Haan, 2000). The current status of work on the thermal dependence of

muscle function in mammals leaves open the question of the extent of adaptive variation among muscles that routinely operate at temperatures different from the normal core temperature.

In this study, we examine the effect of temperature on the contractile properties of a wing muscle in the bat *Carollia perspicillata* that likely regularly operates at temperatures substantially below core body temperature during its nocturnal flights. Flying exposes bats to significant energetic and thermal challenges during routine locomotion. During typical night flights, bats are subject to convective and radiative heat loss (Reichard et al., 2010), but must maintain muscular performance sufficient to generate a minimum wingbeat frequency conducive to flight (Norberg and Norberg, 2012). Thermal imaging of flying *Tadarida brasiliensis* and *Rousettus aegyptiacus* indicates that while a high core T_b is maintained, distal wing temperature approaches that of the environment at air temperatures in the mid-20ºC range (Lancaster et al., 1997; Reichard et al., 2010). The variation in temperature along the wing, i.e., higher proximally and lower distally, almost certainly results in variable temperature within the locomotor muscles of the wing in these species.

Striking morphological differences between body and wing (Fig. 1) result in thermally distinct trunk and wing regions, and contribute to cooler tissue temperatures and greater temperature variability in the wings than in the trunk. Bats have slender bones and thin skin in the wing and wing membrane, which reduce distal wing mass and thereby diminish the inertial cost of flapping the wings (Swartz et al., 1996; Riskin et al., 2012). This reduced framework contains little fat and connective tissue and musculoskeletal structure is often covered with little to no fur on the arms and forearms, leaving the muscles of the wing separated from the environment by only a very thin layer of skin (Fig. 1) (Swartz et al., 1996). We have preliminary data showing that muscle temperatures in the forearm of *C. perspicillata* are substantially below core body temperature during wind tunnel flights at room temperature. Although the flight stroke is powered by large proximal muscles in the trunk, particularly the pectoralis, the more distal muscles in the wing are also important during flight to maintain and modulate three-dimensional wing conformation (Vaughan, 1959; Vaughan and Bateman, 1970; Konow et al., 2015). The extent to which bats compensate for a low operating temperature of the wing muscles is currently unknown. Compensation could occur by alterations of the thermal dependence of the contractile properties of the muscles, the biomechanics of the muscle-tendon unit, and/or alterations in recruitment.

Here, we address the possibility of compensation at the level of muscle tissue by examining the thermal dependence of the contractile properties of a distal wing muscle, the extensor carpi radialis longus (ECRL) in the bat *Carollia perspicillata* over a broad temperature range, from 22ºC to 37ºC. *C. perspicillata* is common throughout Central and South America up to altitudes of 2150 m (Cloutier and Thomas, 1992). Over this geographic range they will regularly encounter nighttime air temperatures of approximately 20ºC (e.g., on Barro Colorado Island: dataset provided by the Physical Monitoring Program of the Smithsonian Tropical Research Institute; Thies et al., 2006). For comparison, we present similar data from the extensor digitorum longus (EDL) of the laboratory mouse using the same methods, allowing for direct comparison over the temperature range used for the ECRL. *C. perspicillata* and the laboratory mouse are roughly comparable in size (approximately 18g and 25g, respectively) and maintain similar normothermic core body temperatures of approximately 36–39ºC (Hudson and Scott, 1979; Audet and Thomas, 1997). The ECRL and EDL are located distally in the limb and both are active during locomotion. Thicker layers of skin, subcutaneous fat, overlying muscle, and fur make the mouse EDL more thermally protected relative to the bat ECRL, and it likely operates nearer to core body temperature than does the bat ECRL. We hypothesized that the bat ECRL has lower thermal dependence than the mouse EDL.

METHODS

Animals

Captive-bred *C. perspicillata* were housed in the Animal Care Facility at Brown University under a reversed 12:12h dark:light cycle and provided with food and nectar *ad libitum.* The bats were adults of mixed sex with a mean body mass of 17.8 ± 0.29 g (n = 14). Outbred CD1 mice (Charles River), aged 4 to 8 weeks, were housed in the Animal Care Facility at Brown University and provided with food and water *ad libitum*. The mice were sub-adult females with a mean body mass of 25.5 ± 3.7 g (n = 8). All experiments were conducted in accordance with a protocol approved by the Brown University IACUC and in accordance with USDA regulations.

Muscle dissection

Animals were weighed and then anesthetized with isoflurane until unresponsive, about five minutes. Deeply anesthetized animals were sacrificed by cervical dislocation.

Carollia perspicillata: The extensor carpi radialis longus (ECRL) has tendinous attachments to the distal humerus and the first and second metacarpals. Proximally, the ECRL shares an aponeurotic tendon of origin on the humerus with the extensor carpi radialis brevis (ECRB). The skin was removed from the forearm and distal arm to expose the proximal, humeral attachment of the muscle. The distal tendon was tied with surgical silk. The distal tendon, humerus, and radius were cut, and the muscle was removed with its humeral attachment intact. The muscle was kept moist at all times during the dissection using chilled oxygenated Ringer's solution. We dissected away the bulk of the ECRB but left a small section of cut fibers to keep the aponeurosis intact. A lightweight silver chain was tied to the distal tendon using surgical silk. The ECRL muscles from both wings were dissected, one of which was used immediately. The second muscle was refrigerated at approximately 6ºC in oxygenated Ringer's solution for up to four hours before use. The Ringer's solution was consistent with the composition of major ions in bat plasma (Korine et al., 1999) and contained 135 mM NaCl, 5 mM KCl, 2.0 mM $CaCl_2 \cdot 2H_2O$, 2 mM $MgSO_4$, 10 mM glucose, 1.0 mM NaH_2PO_4, and 10 mM Hepes. The pH was adjusted to 7.4 after oxygenation.

Mouse: The mouse extensor digitorum longus (EDL) has tendinous attachments on the proximal fibula and distal phalanges II–V. The tibialis anterior muscle was reflected and the distal tendons of the EDL muscle were cut. The medial compartment of the muscle was removed, and the remaining three tendons were tied with surgical silk. The muscle was removed with a portion of the femur and tibia and fibula. The muscle was kept moist at all times during the dissection using chilled oxygenated Ringer's solution with pH 7.4 (composition after Daut and Elzinga, 1989). A lightweight silver chain was tied to the distal tendons using surgical silk. The EDL muscles from both hindlimbs were dissected, but generally only one was used for an experiment. The muscle not in use was refrigerated at approximately 6ºC in oxygenated Ringer's solution and was used in cases in which the first muscle preparation was not successful.

Experimental apparatus and contractile protocol

Carollia perspicillata: The humerus was clamped to a plexiglass base. A cylindrical plexiglass chamber with inflow and outflow holes for Ringer's solution was fitted into the base to create a watertight bathing chamber. The distal end of the muscle was attached via the silver chain to the arm of a Cambridge Instruments Model 305 B servo-controlled muscle ergometer. The transducer was mounted on a custom-built stand to allow for fine adjustment of muscle length. Muscle temperature was maintained by a recirculating flow of Ringer's solution from a 0.5–1.0 L reservoir in a water bath, and temperature was monitored by a Keithley 871 digital thermometer with a calibrated thermocouple probe inserted into the solution surrounding the muscle. Supra-maximal (5–12 V) stimuli of 0.2 ms duration were delivered from a Grass S48 stimulator via a Crown power amplifier through two platinum plate electrodes placed on opposite sides of the muscle. Stimulation frequency used during tetanic contractions ranged from 200 Hz at 22ºC to 300 Hz at 37ºC: high enough to maintain a fused tetanus but not so high as to fatigue the muscle. Stimulation durations during isometric tetani were set such that the contraction was long enough to reach a plateau in force, approximately 200–300 ms depending on temperature (see Fig. S1 for representative traces). Stimulation durations were reduced at lower forces during the isotonic series. A rest period of 4–6 minutes was interposed between successive tetanic contractions. Force, length, and velocity were recorded using a 16-bit PowerLab/16SP data acquisition system (ADInstruments). The muscle was adjusted to the length at which tetanic force was maximal at the first experimental temperature for each preparation. For most preparations, we performed measurements at two temperatures. Temperature changes took 15–30 minutes and experiments were initiated soon after the target temperature was reached. At each temperature, a series of after-loaded isotonic contractions was performed, each series consisting of approximately 10 contractions (see Fig. S2 for representative traces). Peak velocity was measured from the time derivative of the position trace. Maximum isometric force (P_0) was measured at the beginning, middle, and end of this series, and the predicted isometric force at the time of each isotonic contraction was estimated by linear extrapolation between the measured isometric values. The decline in P_0 over the course of an isotonic series averaged 23, 16, 2, and 1 % at 37, 32, 27, and 22ºC, respectively. Two attempts were made to record isotonic contractions at 40ºC, but isometric force declined more than 40% at this temperature. Once measurements were complete, the muscle length was measured in place. Bone and extraneous

tissue, including the cut fibers of the ECRB, were dissected away from the muscle and it was weighed to the nearest 0.1 mg.

   Mice: The setup for the mouse muscles was similar to that for the bat muscles except that the tibia and fibula were clamped to the plexiglass base, and a Cambridge Instruments Model 300B servo-controlled muscle ergometer was used for the smaller EDL muscle. Stimulation frequency during tetani ranged from 125 Hz at 22ºC to 500 Hz at 42ºC. A rest period of 3 minutes was interposed between successive tetanic contractions. Stimulation durations during isometric tetani were somewhat shorter than for the ECRL, typically 100–250 ms depending on temperature. For most individual mouse preparations, isometric force remained sufficiently high to allow us to perform measurements at all four experimental temperatures (37, 32, 27, and 22ºC), and for three preparations at 40ºC and four preparations at 42ºC. The decline in P_0 over an isotonic series averaged 28, 10, 8, 0.5, 2, and 3% at 42, 40, 37, 32, 27, and 22ºC, respectively. Once measurements were complete, the muscle length was measured in place. The EDL muscle was cut away from the fibular attachment, excess tendon was cut away from the muscle belly both proximally and distally, and it was weighed to the nearest 0.1 mg.

Measurements of fiber length:

The muscles were fixed in 10% phosphate-buffered formalin at the rest length determined in the contractile measurements, and the connective tissue was digested in 30% nitric acid for up to four days until the fascicles were easily separable. Groups of fibers were photographed using a dissection microscope. For calibration, we photographed segments of insect pins of known length or a scale micrometer at the same magnification used for the fiber photographs. Fiber length was measured on the photographs using ImageJ. Cross-sectional area was calculated by dividing the mass of the muscle by the fiber length. The angle of the fibers relative to the long axis of the muscle was small enough for the ECRL (approximately 7º), and for the EDL (3.5º) (Close, 1964), that we did not take it into account in calculating muscle stress from cross-sectional area for either muscle.

Data Analysis and Statistics:

Isometric and isotonic parameters were calculated using stimulation timing, force, and length data recorded in LabChart 7 (ADInstruments), and reported as means ± s.e.m. Only post-tetanic twitches were used in descriptions of twitch kinetics. The onset of force production was quantified as the time following the stimulus at which measurable force rise began, which was determined by examining the derivative with respect to time of the force trace. Time to peak twitch ($t_{P,tw}$) was measured as the time from start of force production to time of peak twitch force. Time to half-relaxation for twitch ($t_{50\%R,tw}$) was measured as the time from peak twitch force to the point at which twitch force had declined by half. Time to half-relaxation for tetanus ($t_{50\%R,tet}$) was measured as the time from the last stimulus to the point at which tetanic force had declined by half.

Velocity measurements were converted from mm s^{-1} to fiber lengths per second (L s^{-1}) by dividing by the mean fiber length of all preparations. Force-velocity curves were fit to the data from individual preparations using the Levenberg-Marquardt algorithm as implemented in Igor Pro (Wavemetrics, Inc., 2017). The equation used was the modified hyperbolic equation of Marsh and Bennett (1986):

$$V = \frac{B\left(1 - \frac{P}{P_0}\right)}{A + \frac{P}{P_0}} + C\left(1 - \frac{P}{P_0}\right) \tag{1}$$

where V is velocity in lengths per second, P/P_0 is force as a fraction of measured maximum isometric tetanic force, B and C are constants with dimensions of velocity, and A is a dimensionless constant. To produce an estimate of the overall force–velocity curve for each temperature, we averaged the constants obtained by fitting equation 1 to each individual preparation at that temperature (Table S1). As a measure of the curvature of the force-velocity relationship, we used the dimensionless power ratio (Marsh and Bennett, 1986) calculated as:

$$\text{Power ratio} = \dot{W}_{\text{max}}/(V_{\text{max}}P_0) \tag{2}$$

where $\dot{W}_{\text{max}}$ is the maximum isotonic power and V_{max} is the maximum velocity predicted at zero force. In addition to V_{max}, which by necessity is extrapolated outside the range of measured values, we report the interpolated value at 40% of P_0 (V_{40}).

The temperature coefficient (Q_{10}) at 5°C intervals was calculated as:

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2-T_1}} \tag{3}$$

where R_1 and R_2 are rates measured at the temperatures T_1 and T_2, respectively. For velocity parameters, the Q_{10}'s were calculated using means of the values calculated from the individual fitted curves for each temperature. For duration measurements, the Q_{10}'s were calculated from the inverse of the times to express these variables as rates.

Because we found little effect of temperature on tetanic force (see Results), the mean specific tension (force per mean fiber cross-sectional area) was calculated using the largest force measured in an isometric tetanic contraction for each preparation at temperatures of 27ºC and above. R_{10}'s for specific tension were calculated by evaluating isometric tetanic contractions immediately before and after temperature changes to minimize the effects of force reduction resulting from fatigue (R_{10} is calculated as for Q_{10} for non-time dependent measures (Bennett, 1984)). We evaluated the statistical significance of the difference in force before and after a temperature changes using paired t-tests.

Statistical comparisons across temperatures within a given species were done using one-way ANOVA as implemented with the General Linear Model in SPSS Statistics (IBM, 2017). Given the *a priori* hypothesis that the rate functions would be affected by temperature, we used repeated contrasts to assess the significance of the difference in rate-related variables for each interval of temperature change. Because statistical analysis of ratios can be problematic, we approached comparison across temperatures of the twitch/tetanus ratio (P_{tw}/P_0) and power ratio in a step-wise manner. We first employed the Kruskal-Wallis non-parametric test to look for an overall effect of temperature. After confirming that the samples did not deviate from normality (Shapiro-Wilks test), we then used ANOVA with post-hoc multiple comparisons with Bonferroni corrections to pinpoint where significant differences existed.

Comparisons of the thermal dependence of contractile properties between the bat ECRL and the mouse EDL were performed using regression analyses of log-transformed variables as a function of temperature. The slopes these regressions are directly proportional to Q_{10}'s, and thus, a significant difference in the slopes for the two muscles indicates a significant difference in thermal dependence. The comparison of the regressions was done using analysis of covariance (ANCOVA) as implemented in the General Linear Model of SPSS. The relationships of contractile rate properties with temperature were non-linear after log transformation and were

well described by a quadratic equation. Therefore, the ANCOVA model was run with both temperature and temperature squared entered as covariates. The thermal dependence of the relationships of the variable to temperature for the two muscles was deemed significant if there was a significant interaction of muscle, temperature, and temperature squared.

RESULTS

Bat extensor carpi radialis longus

The mean dimensions of the ECRL preparations were as follows: mass, 42.6 ± 1.12 mg; length of the muscle belly, 24.28 ± 0.38 mm; fiber length, 3.68 ± 0.13 mm; and cross-sectional area 11.23 ± 0.46 mm^2.

The mean maximum isometric tetanic force for the preparations was 10.79 ± 0.83 N cm^{-2}. Our data indicate a modest change in P_0 with temperature because of a drop in force from 27 to 22°C. Comparing pairs of values before and after temperature changes of 5°C, the R_{10}'s were 1.28 for 22–27°C; 0.95 for 27–32°C; and 0.92 for 32–37°C. The only significant difference we found between pairs of forces was for the interval from 22 to 27°C (paired t-test, P < 0.001).

P_{tw}/P_0 varied with temperature, ranging from 0.11 at 37°C to 0.18 at 22°C (Fig. 2). Both the non-parametric Kruskal-Wallis test and ANOVA indicated that P_{tw}/P_0 was significantly affected by temperature (P = 0.008 and 0.006, respectively). Post-hoc comparisons indicated that the value at 22°C was significantly different from the values at both 32 and 37°C (P = 0.014 and P = 0.027 respectively).

ANOVA results indicated that during isometric contractions, all time-related variables were significantly affected by temperature (P < 0.001). Durations were shortest, and thus isometric rates fastest, at 37°C; e.g., relaxation to 50% P_0 took 34.5 ± 1.62 ms at 37°C and 63.8 ± 1.72 ms at 22°C (Fig. 3). Repeated contrasts tests indicate that the values changed significantly for each temperature interval across the entire experimental range for $t_{P,tw}$ and $t_{50\%R,tet}$, and for the intervals from 22–27°C and 27-32°C for $t_{50\%R,tw}$ (Table 1). Q_{10}'s for isometric parameters (Fig. 3) were lowest between 32 and 37°C and generally increased at the lower temperature intervals. Q_{10}'s were particularly low for $t_{50\%R,tw}$ with values below 1.4 from 27 to 37°C (Fig. 3).

Shortening velocities during isotonic contractions were also influenced significantly by temperature (ANOVA, P < 0.001) (Figs. 4A and 5). Velocities were highest at 37ºC, at which V_{max} was 14.0 ± 0.54 L s^{-1}and V_{40} was 4.2 ± 0.22 L s^{-1}. Repeated contrasts tests indicated that V_{max} changed significantly from 22–27ºC and 27–32ºC, but not from 32–37ºC (Table 1). V_{40} increased significantly from 22–27ºC, but did not differ significantly across the other temperature intervals (Table 1). The lack of significant changes at the higher temperature intervals is indicated by the significant overlap of the values (Fig. 4A).

As indicated by the power ratio, the curvature of the force-velocity relation was significantly affected by temperature (Kruskal-Wallis, P = 0.015; ANOVA, P = 0.017) (Fig. 6). The curvature of the force-velocity curve increases with decreasing temperature, and post-hoc comparisons indicate that the power-ratio at 22ºC was significantly different from that at 37ºC (P = 0.011).

Mouse extensor digitorum longus

The mean dimensions of the EDL preparations were as follows: mass, 7.78 ± 0.52 mg; length of the muscle belly, 11.71 ± 0.31 mm; fiber length, 5.11 ± 0.25 mm; and cross-sectional area 1.44 ± 0.08 mm^2.

The mean maximum isometric force for the preparations was 13.84 ± 1.16 N cm^{-2}. As with the bat muscle, we found modest changes in force with temperature. The force decreased significantly from 32–27ºC ($R_{10} = 1.10$; P <0.001, paired t-test) and from 27–22ºC ($R_{10} = 1.15$; P = 0.018, paired t-test). Force tended to decline above 37ºC ($R_{10} = 0.85$), but our sample sizes were small over this range.

P_{tw}/P_0 varied with temperature, ranging from 0.082 at 40ºC to 0.18 at 22ºC (Fig. 2). As with the bat muscle, the non-parametric Kruskal-Wallis test and ANOVA indicated that P_{tw}/P_0 was significantly affected by temperature (P < 0.001 for both tests). Post-hoc comparisons indicated that the significant change with temperature was determined by increased P_{tw}/P_0 at the two lowest temperatures tested compared to the values at higher temperatures. The value at 22ºC was significantly different from those at all other temperatures except 27ºC (P <0.004 for all comparisons). The value at 27ºC was significantly different from the values at 37º and 40ºC (P < 0.05 for both comparisons).

As with the ECRL preparations, ANOVA results indicated that during isometric contractions, all time-related variables were significantly affected by temperature (P < 0.001). Durations were shortest, and thus isometric rates fastest, at higher temperatures, with the shortest durations at 42ºC for $t_{P,\text{tw}}$, and $t_{50\%R,\text{tet}}$ and at 40ºC for $t_{50\%R,\text{tw}}$ (Fig. 3). Relaxation to 50% P_0 took 16.4 ± 1.66 ms at 42ºC and 55.2 ± 1.17 ms at 22ºC. Rates increased significantly (repeated contrasts) for each interval from 22–37ºC for $t_{P,\text{tw}}$, $t_{50\%R,\text{tw}}$, and $t_{50\%R,\text{tet}}$ (Table 1). Q_{10}'s were near 1 for all isometric parameters from 37–42ºC (Fig. 3). Below 37ºC, the Q_{10}'s were greater than the values for bat muscle for each temperature interval.

The isotonic shortening velocities for the EDL were significantly affected by temperature (ANOVA, P < 0.001). Shortening velocities were highest ($V_{\text{max}} = 26.9 \pm 1.12$ L s^{-1}, $V_{40} = 8.0 \pm 0.18$ L s^{-1}) at 40ºC (Fig. 4B and 5). Rates increased significantly (repeated contrasts) for each temperature interval from 22–37ºC for V_{max} and V_{40} (Table 1). Velocities were lower at 42ºC but this difference was not significant for V_{max} or V_{40} and the Q_{10}'s over this range were close to 1 (Fig. 5). As we found for the isometric time-related values, the Q_{10}'s for shortening velocity were larger than those for the bat muscle over the range of temperatures from 22-37ºC (Fig. 5).

As in analysis of the bat ECRL data, the power ratio (Fig. 6) indicated a significant change in the curvature of the force-velocity relation at lower temperatures relative to higher temperatures (Kruskal-Wallis, P = 0.001; ANOVA, P < 0.001). Post-hoc comparisons indicated that the change with temperature was largely determined by the decreased power ratio at 22ºC (P < 0.005 for all paired comparisons to the values at temperatures of 32ºC and above).

Comparison of the thermal dependence of the ECRL and EDL

The thermal dependencies of the time dependent contractile properties were compared using ANCOVA based on quadratic regressions of the log-transformed variables as a function of temperature (Figs. 3 and 5). The ANCOVA models contained muscle (ECRL vs EDL) as a fixed factor and temperature and temperature squared as covariates. For all dependent variables, we found significant interactions among muscle, temperature, and temperature squared (P < 0.001) indicating the thermal dependencies for the ECRL and the EDL differed significantly.

DISCUSSION

The rate processes governing muscle contraction in the bat ECRL exhibit unusually low thermal dependence as temperature is reduced below normothermic T_b. Along with the muscle's overall capacity for force production, these rate processes determine muscle performance *in vivo*. Both bat and mouse muscles examined here had low thermal dependence of maximum isometric force production over a broad range of temperatures below normothermic T_b, but rate processes were more influenced by temperature, in agreement with previous studies (Bennett 1984, James 2013). Q_{10}'s in the bat ECRL are lower from 27 to 37°C for both isometric ($t_{50\%R,tw}$, $t_{50\%R,tet}$) and isotonic properties than has been observed previously in mammalian muscles (Fig. 7) (South, 1961; Ranatunga, 1980, 1982, 1984; Faulkner et al., 1990). The thermal dependence of bat muscle is also lower than has been observed in muscles from many ectotherms, such as lizards and frogs (Fig. 7) (Marsh and Bennett, 1985, 1986; Peplowski and Marsh, 1997).

Our data show that the ECRL muscle in *Carollia perspicillata* demonstrates significantly lower thermal dependence at temperatures below T_b for all rate-related properties of the muscle than does the mouse EDL (Figs. 3 and 5). The effect of the lower thermal dependence is substantial. For example, as a result of the lower Q_{10}'s, the V_{max} of the bat ECRL drops by only 20% between 37 and 27°C, compared to an almost 40% drop in V_{max} for the mouse EDL for the same temperature change. Similarly, shortening velocity at 40% of maximum force decreases by approximately 30% for the bat muscle and 50% for the mouse muscle over the same change in temperature.

The low thermal dependence of the contractile properties of bat wing muscle over a broad temperature range below normothermic T_b is likely functionally significant for flight performance. The ECRL is cyclically active during the wingbeat cycle, with its activity occurring primarily during downstroke, and relaxation occuring during most of upstroke (Hedberg, 2014). Therefore, the timing of its contraction and relaxation is constrained by the bat's wingbeat frequency *in vivo*. Though the ECRL has a long, thin, and likely elastic tendon that may act to influence the behavior of the muscle-tendon unit during flight, the flight stroke remains dependent on timely force development and relaxation in the flight muscles. Cooler muscle temperatures will delay the rise in force and lengthen relaxation times. If contractile rates slow enough, the changes will disrupt muscle function during the wingbeat cycle. For most eutherian mammals, core T_b is held constant at a mean temperature of 37–39°C (Morrison and

Ryser, 1952), and the bellies of locomotor muscles are located close to the core or insulated under fur or blubber. As a result, the concern of a number of previous studies has been the influence of increases in muscle temperature with exercise rather than decreases in temperature (e.g., Brooks et al., 1971; Place et al., 2009). In contrast, the aerial ecology of bats subjects the poorly insulated wings to large convective heat loss resulting from low air temperatures, and bats' nocturnal flight habits also make them susceptible on clear nights to radiative heat loss to the cold night sky (Reichard et al., 2010). Because bats generate substantial heat from the pectoralis and other major flight muscles in the trunk during flight, the large surface area of the wings has been suggested to facilitate heat loss to prevent hyperthermia (Kluger and Heath, 1970; Carpenter, 1986; Thomas et al., 1991). However, though dumping heat from the wings to maintain a constant core temperature can be advantageous in high environmental temperatures, surface temperature of the wing has been found to drop to below air temperature at cooler temperatures because of the combined effects of convective, radiative, and, perhaps, evaporative heat loss (Reichard et al., 2010). The thermal environment of the wing musculature, taking into account radiative, convective, and evaporative heat loss is highly variable both temporally and spatially. Wing muscles with low sensitivity to temperature will lower the sensitivity to temperature of the flight stroke as a whole, making flight performance more resilient to low or fluctuating temperatures.

Our comparison of the bat ECRL and mouse EDL suggests the possibility that the bat muscle has adapted to routine operation below T_b by shifting its maximal performance to a lower temperature. In other words, the breadth of the performance curve for the ECRL with varying temperature has not changed, but the curve has been shifted to the left on the temperature axis (Fig. 8). Though it is difficult to be certain that the maximum temperatures at which muscle preparations are stable *in vitro* reflects *in vivo* function, and not the limitations of a muscle removed from its normal milieu, the bat ECRL was not stable enough to record reliable data above 37ºC, whereas we successfully collected data at 42ºC from the mouse EDL. At temperatures above normal T_b, 37ºC, the mouse EDL muscle has Q_{10}'s quite close to or less than 1.0, with no significant differences in contractile rates, indicating peak performance at these temperatures. Superimposing the mouse EDL contractile velocity versus temperature relation on that for bat ECRL, by shifting it to the left by 5ºC, highlights that the two curves correspond closely in shape (Fig. 8). Examining the data in this way demonstrates that for a given

temperature interval, the *C. perspicillata* ECRL loses a smaller percentage of its maximal performance than the mouse EDL, but the ECRL and EDL undergo the same performance loss relative to their respective maximum experimental temperatures as temperature declines.

Whether the low thermal sensitivity of the *C. perspicillata* ECRL over a range of temperature below body temperature is a feature of all bat muscles remains unknown. A previous report examined the effects of temperature on the contractile properties of a muscle from small (~7 g) bats living in temperature climates (Choi et al., 1998). They found very low temperature sensitivity for isometric and isotonic properties; however, methodological issues likely affecting the time resolution of their data (the use of a mechanical lever and an amplifier designed for use with a polygraph) make interpreting their data problematic. Their results, if valid, would raise the intriguing possibility that the muscles from temperate climate bats that use torpor may be even less temperature sensitive than the muscles in *C. perspicillata*.

Most experimental *in vitro* studies of vertebrate skeletal muscle have been conducted at or below normothermic body temperatures in mammals or preferred body temperature in ectotherms) (Ranatunga, 1982, 1984; Marsh and Bennett, 1986; Peplowski and Marsh, 1997). Marsh and Bennett (1985) examined contractile properties of a lizard muscle up to 44ºC (above the lizard's preferred temperature of 40ºC) and did not find a peak in the performance curves, perhaps indicating that the lizard muscle is normally operating well below its upper thermal maximum. The data in the literature on temperature effects in mammalian muscle do not include measurements at or above typical body temperatures for mammals (Ranatunga, 1980, 1982, 1984) and sometimes include too few temperatures to define the thermal performance curves (Vyskocil and Gutmann, 1977; Faulkner et al., 1990). If the *C. perspicillata* ECRL reaches peak performance around body temperature, as suggested by our data, it may operate closer to an upper thermal limit than other vertebrate muscles, and may risk impairment at even slightly elevated temperatures.

Shifting the temperature optimum of intrinsic contractile properties may result in tradeoffs in absolute muscle performance, especially at temperatures above the optimum. In most animals studied to date, Q_{10}'s for physiological processes are around 2 or higher near normal body or preferred environmental temperatures; as temperature increases, Q_{10}'s approach 1, a turning point, above which performance quickly declines. Since physiological systems are functional over a finite range of temperatures, shifting this range down may impair function at

high temperatures (Hochachka and Somero, 2002). These limits lead to important consequences for animals that encounter a range of environmental temperatures, or those that experience a range of body and muscle temperatures; their muscles may periodically be required to perform far from optimal conditions.

The focus of the present study is not the absolute performance of the bat and mouse muscles, but the thermal dependence of the muscles as temperature drops below body temperature. In terms of absolute performance, the mouse EDL attains higher shortening velocities and shorter relaxation times. However, different locomotor demands are imposed on these muscles in mice and bats, and they are only distantly related phylogenetically within mammals. No other comparable data are available on bat muscle with which to compare our data on the ECRL. Our values for mouse EDL shortening velocities are similar to some previous reports (Close, 1965; Luff, 1981) but are higher than some other studies (Brooks and Faulkner, 1988; Faulkner et al., 1990; Askew and Marsh, 1997). The reasons for variation in values are not clear, but fiber type differences among mouse strains may contribute (Askew and Marsh, 1997).

The low thermal dependence of contractile performance in *C. perspicillata* ECRL is particularly striking because it contrasts with data on locomotor muscles from ectotherms that routinely experience variation in body temperature and thus variation in the temperature of the locomotor muscles. For example, the contractile properties of locomotor muscles from lizards and frogs have been shown to have a strong thermal dependence over a range temperatures that the animals experience in their natural environment (Marsh and Bennett, 1985, 1986; Peplowski and Marsh, 1997). In these ectothermic animals, locomotor performance is less influenced by temperature than muscle performance because of other factors, including elastic mechanisms. More information is needed on the *in vivo* mechanical function of the ECRL before we can judge whether elastic mechanisms could also play a role in modifying its *in vivo* function. One intriguing difference between the bat ECRL and the locomotor muscles of ectotherms is that the bat muscle operates in the context of a local decrease in temperature, whereas in ectotherms the temperature of the entire locomotor apparatus varies in concert. Perhaps the selection pressures are different on the function of a muscle like the ECRL that is operating at a lower temperature than other muscles in the trunk that are responsible for driving the locomotor cycle. Also, the option of shifting the thermal performance curve to the left may not be selectively advantageous

in ectotherms that benefit from retaining the capacity to withstand temperatures above their preferred body temperature.

CONCLUSIONS

The intrinsic properties of locomotor muscles are influenced by temperature, which is in turn influenced by both thermoregulatory and environmental factors. The remarkable flight ability of bats requires high performance from the flight muscles in a variable thermal environment. Therefore, the effect of temperature on the time-dependent contractile properties of these muscles will impact crucial functions of the locomotor muscles. We report here on the contractile properties of a bat wing muscle, the *C. perspicillata* ECRL, in comparison to a muscle from a non-flying mammal, the mouse EDL, at a range of temperatures from 22 to 42ºC. We show that the ECRL muscle has significantly lower thermal dependence than the EDL of the laboratory mouse at temperatures below T_b. We conclude that the low thermal dependence is likely functionally important in light of the thermal challenges associated with nocturnal flight and the morphology of the wing.

List of symbols and abbreviations

ECRL	Extensor carpi radialis longus muscle
EDL	Extensor digitorum longus muscle
P_0	Maximum isometric force
P_{tw}/P_0	Twitch/tetanus ratio
$t_{P,tw}$	Time to peak twitch
$t_{50\%R,tw}$	Time to half-relaxation, twitch
$t_{50\%R,tet}$	Time to half-relaxation, tetanus
V_{max}	Peak contractile velocity
V_{40}	Velocity at 40% P_0
$\dot{W}_{max}$	Maximum power
Q_{10}	Temperature coefficient

Supplementary information

Supplementary information is available at **.

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Table 1. Results of the ANOVA analyses of the effects of temperature on time related contractile properties of the extensor carpi radialis longus muscle (ECRL) of the bat *Carollia perspicillata* and the extensor digitorum longus muscle (EDL) of the laboratory mouse. The P-values shown are the results of repeated contrasts comparing the values at successive temperature intervals. P-values <0.05 are shown in bold.

Temperature Interval	P-value									
	Bat, ECRL					Mouse, EDL				
	$t_{P,tw}$	$t_{50\%R,tw}$	$t_{50\%R,tet}$	V_{max}	V_{40}	$t_{P,tw}$	$t_{50\%R,tw}$	$t_{50\%R,tet}$	V_{max}	V_{40}
22 *vs*. 27	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**	**<0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**
27 *vs*. 32	**<0.001**	**0.041**	**0.009**	**0.016**	0.060	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
32 *vs*. 37	**0.007**	0.202	**0.012**	0.282	0.088	**<0.001**	**0.011**	**0.012**	**<0.001**	**<0.001**
37 *vs*. 42	--	--	--	--	--	0.143	0.442	0.059	0.223	0.066

Figures

Fig. 1. *Carollia perspicillata* **in flight. The arrow indicates the location of the ECRL muscle belly.** The head and trunk are covered in fur, and the muscles of the forearm, hand, and most of the hindlimb are visible through the skin. The whole image was adjusted in Photoshop (Adobe) to enhance clarity.
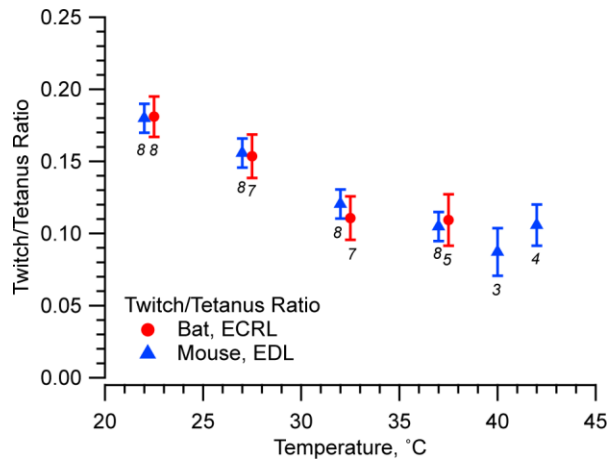
Fig. 2. The ratio of twitch force to tetanic force as a function of temperature for the extensor carpi radialis longus muscle (ECRL) of the bat *C. perspicillata* and the extensor digitorum longus muscle (EDL) of the laboratory mouse. The points plotted are the mean values at each temperature ± s.e.m.; sample sizes for the means are indicated in italics below each point.
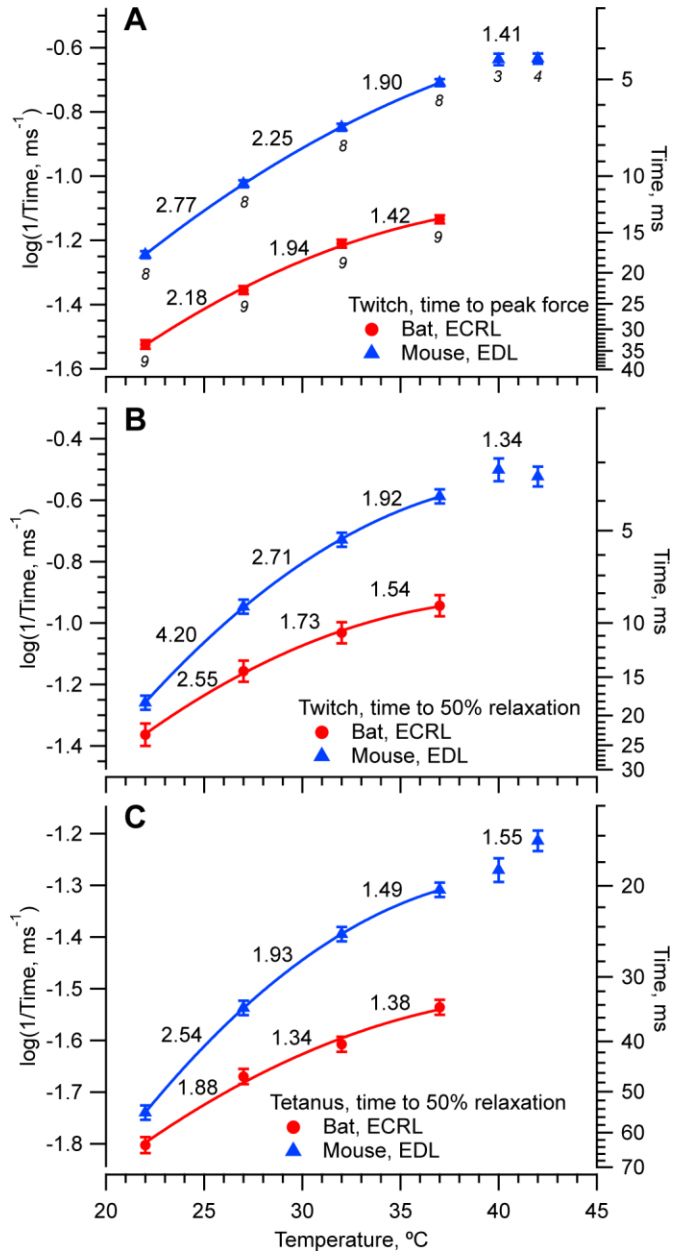
Fig. 3. Log of inverse contraction times as a function of temperature for the ECRL of the bat *C. perspicillata* and the EDL of the laboratory mouse. (A) Time to peak force in a twitch. (B) Time from peak force to 50% relaxation in a twitch. (C) Time from peak force to 50% relaxation in a tetanus. The points plotted are the mean values at each temperature ± s.e.m.; sample sizes for the means are indicated in italics below each point in panel A. The solid lines are quadratic regressions fitted using the individual log-transformed values at temperatures of 37ºC and below (n = the sum of the sample sizes at each temperature). The numbers above the curves indicate the Q_{10}'s calculated for 5ºC intervals. The right axes show the times on an inverted log scale.
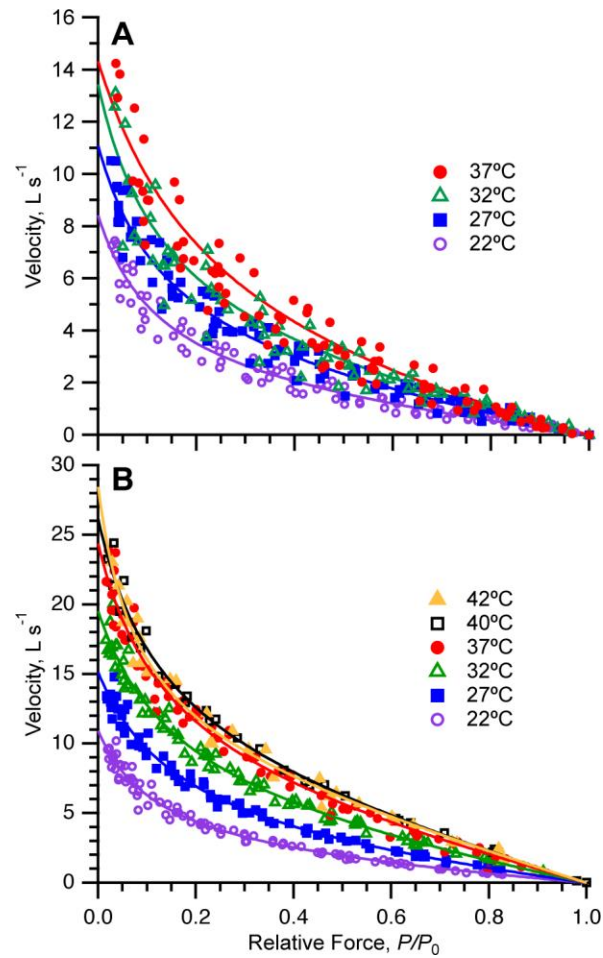
Fig. 4. Pooled force-velocity data from all preparations. Points indicate experimentally obtained velocity values, and corresponding force values normalized to P_0. The fitted lines were constructed using the averaged coefficients from fitted curves for each individual preparation (see Table S1). (A) Force-velocity curves for the ECRL of the bat *C. perspicillata* (n = 9 preparations per temperature). (B) Force-velocity curves for the EDL of the laboratory mouse (n = 4 at 42ºC, n = 3 at 40ºC, and n = 8 for all other temperatures).
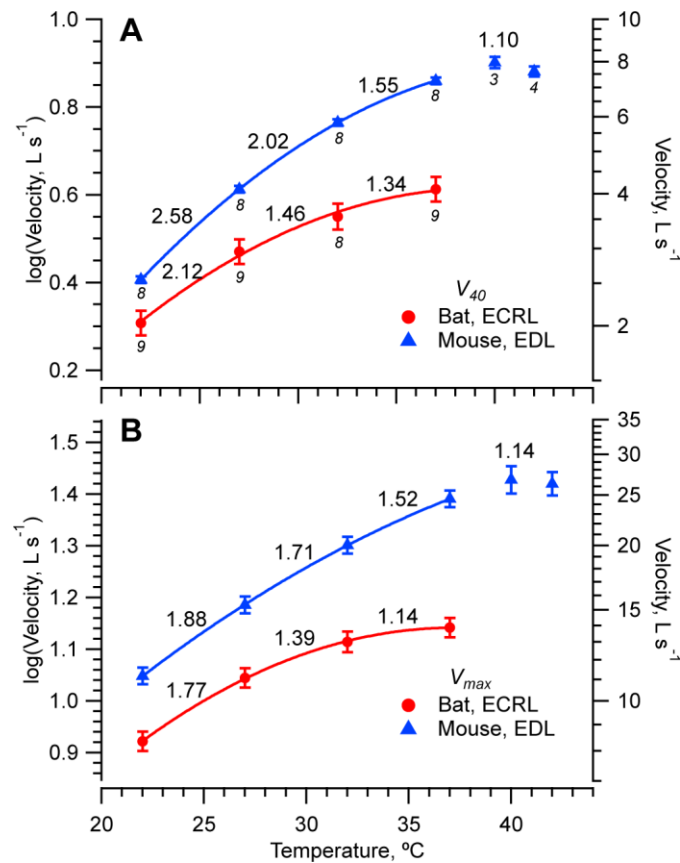
Fig. 5. Log-transformed shortening velocity as a function of temperature for the ECRL of the bat *C. perspicillata* and the EDL of the laboratory mouse. (A) Shortening velocity at 40% of maximum force. (B) Predicted maximum shortening velocity at zero force. The points plotted are the mean values at each temperature ± s.e.m.; sample sizes for the means are indicated in italics below each point in panel A. The solid lines are quadratic regressions fitted using the individual log-transformed values at temperatures of 37°C and below (n = the sum of the sample sizes at each temperature). The numbers above the curves indicate the Q_{10}'s calculated for 5°C intervals. The right axes show the velocities on a log scale.
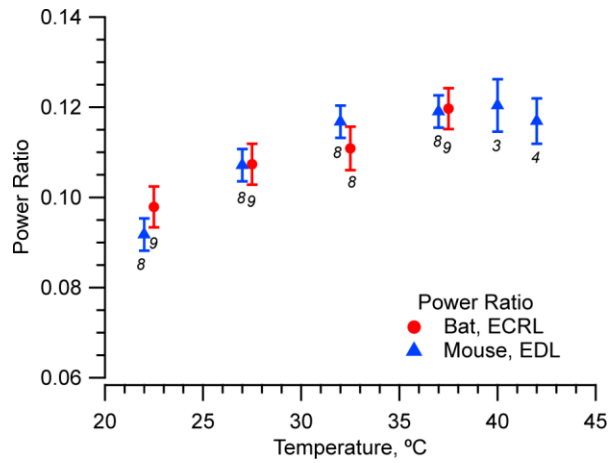
Fig. 6. The power ratio as a function of temperature. Power ratios were calculated from the individual force-velocity curves for each preparation of the ECRL of the bat *C. perspicillata* and the EDL of the laboratory mouse. The points plotted are the mean values at each temperature ± s.e.m.; sample sizes for the means are indicated in italics below each point.
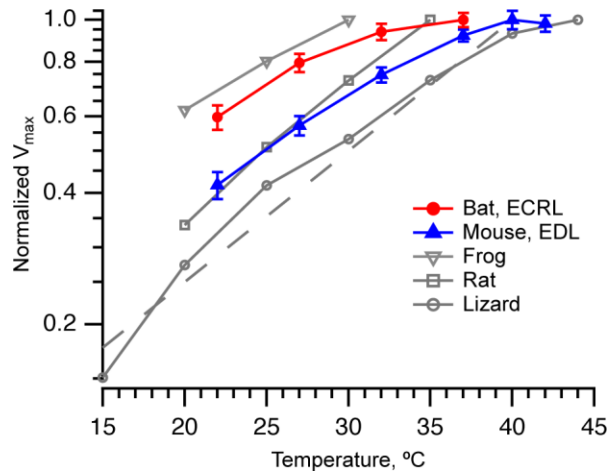
Fig. 7. The dependence of shortening velocities on muscle temperature in semi-log plot. For each dataset, velocities have been normalized by the maximum velocity recorded in that dataset. V_{max} data for mouse and bat muscles from this study (error bars: s.e.m.); frogs from Peplowski and Marsh, 1997; rats from Ranatunga, 1984, and lizards from Marsh and Bennett, 1985. Dashed line: $Q_{10} = 2$.
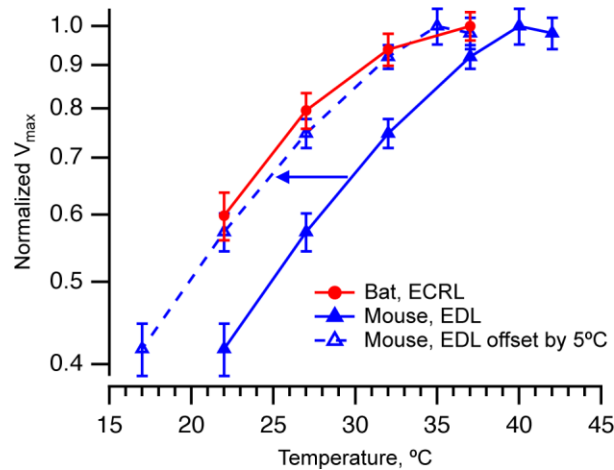
Fig. 8. The thermal dependence of shortening velocity in the ECRL of the bat *C. perspicillata* and the EDL of the laboratory mouse. The mouse data are plotted twice with the solid blue lines connecting the mean values recorded and the dashed lines connecting the same mean values shifted to the left by 5°C on the temperature axis. The shifted curve illustrates that the shape of the relationship of velocity to temperature for the two muscles is similar, but the data from the bat ECRL is shifted to a lower temperature. This indicates that though for a given temperature interval, the bat ECRL loses a lower percentage of its maximal performance than the mouse EDL, they both undergo the same performance loss relative to their respective maximum experimental temperatures.

Table S1: Average coefficients of fit for the bat and mouse force-velocity curves, using the equation $V = \dfrac{B\left(1 - \frac{P}{P_0}\right)}{A + \frac{P}{P_0}} + C\left(1 - \frac{P}{P_0}\right)$ **(From Marsh, R.L., Bennett, A.F.,** *J. Exp. Biol.* **126,** 63 (1986)).

	Temperature	A	B	C	n
Bat	37	0.240 ± 0.041	2.723 ± 0.512	2.966 ± 0.691	9
	32	0.130 ± 0.019	1.260 ± 0.225	3.737 ± 0.352	9
	27	0.140 ± 0.009	1.150 ± 0.064	2.874 ± 0.299	9
	22	0.124 ± 0.007	0.811 ± 0.056	1.892 ± 0.170	9
Mouse	42	0.0976 ± 0.00774	1.636 ± 0.111	9.426 ± 0.374	4
	40	0.119 ± 0.0262	2.019 ± 0.312	9.445 ± 0.308	3
	37	0.175 ± 0.0446	3.172 ± 0.952	7.085 ± 1.01	8
	32	0.155 ± 0.0235	2.214 ± 0.350	5.832 ± 0.486	8
	27	0.154 ± 0.00855	1.828 ± 0.147	3.549 ± 0.193	8
	22	0.117 ± 0.0119	1.065 ± 0.137	2.221 ± 0.192	8

Fig. S1: Example isometric tetanic contractions. Bat ECRL at 32ºC (A) and mouse EDL at 27ºC (B).
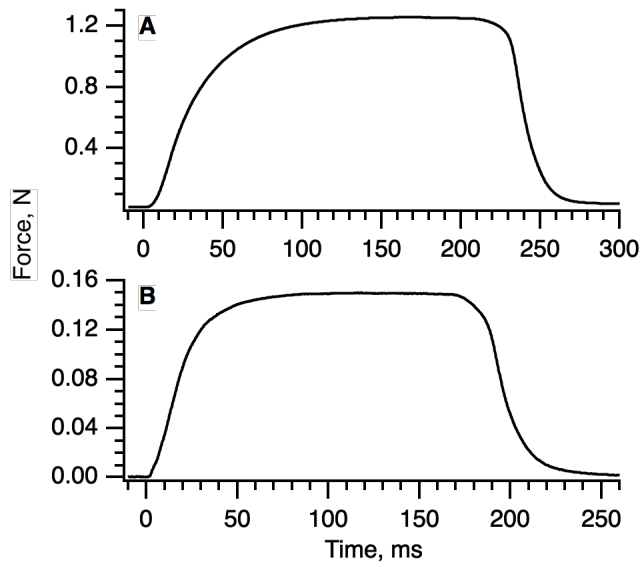
Fig. S2: Representative force and velocity traces for the bat ECRL muscle (A, B) at 32ºC and mouse EDL muscle (C, D) at 37ºC, both at approximately 40% maximum isometric force. The portion of the traces from which shortening velocity was measured is indicated in bold (B, D).