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RESEARCH ARTICLE

A comparison of thermal sensitivities of wing muscle contractile properties from a temperate and tropical bat species

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ABSTRACT

Endotherms experience temperature variation among body regions, or regional heterothermy, despite maintaining high core body temperatures. Bat forelimbs are elongated to function as wings, which makes them vulnerable to heat loss and exaggerates regional heterothermy. A tropical bat species, *Carollia perspicillata*, flies with distal wing muscles that are substantially (>10°C) cooler than proximal wing muscles and significantly less temperature sensitive. We hypothesized that the difference between proximal and distal wing muscles would be even more extreme in a temperate bat species that is capable of flight at variable environmental temperatures. We measured the contractile properties of the proximal pectoralis muscle and distal extensor carpi radialis muscle at a range of temperatures in the big brown bat, *Eptesicus fuscus*, and compared their thermal dependence with that of the same muscles in *C. perspicillata*. We found that, overall, temperature sensitivities between species were remarkably similar. The sole exception was the shortening velocity of the pectoralis muscle in *E. fuscus*, which was less temperature sensitive than in *C. perspicillata*. This decreased temperature sensitivity in a proximal muscle runs counter to our prediction. We suggest that the relative lability of body temperature in *E. fuscus* may make better pectoralis function at low temperatures advantageous.

KEY WORDS: Chiroptera, Contractile properties, Flight, Muscle physiology, Muscle temperature

INTRODUCTION

Temperature has a pervasive and unavoidable effect on muscle function. Changes in muscle temperature result in changes to contractile properties including activation, force development and relaxation rates. Changing muscle temperatures and associated changes in muscle contractile properties are known to be important factors in the locomotor performance of ectotherms (Bennett, 1990). Endotherms also experience regional variation in body temperature (T_b), also known as regional heterothermy, despite maintaining high core body temperatures. In some instances, regional heterothermy can be extreme enough, especially distally in the limbs, to induce changes in muscle contractile rates that could affect locomotor performance (Rummel et al., 2019).

Bats are flying, endothermic mammals. Flight is a demanding form of locomotion, which in bats is supported by unique forelimb anatomy that predisposes the wings to heat loss (Fig. 1). Bat wings cool substantially during flight, as convective and radiative heat loss induce a temperature gradient from the proximal core to the more distal portions of the wing (Lancaster et al., 1997; Reichard et al., 2010; Rummel et al., 2019). In the tropical fruit bat *Carollia perspicillata*, a regional heterothermy gradient corresponds to a gradient in muscle thermal sensitivity, which may have evolved to compensate for temperature effects. For example, the contractile properties of the proximal pectoralis muscle, which operates close to core body temperature, are more temperature sensitive in *C. perspicillata* compared with distal muscles, which operate at colder and/or more variable temperatures (Rummel et al., 2021, 2018).

The big brown bat, *Eptesicus fuscus*, is a small, temperate, insectivorous species that regularly flies at low environmental temperatures (Klüg-Baerwald et al., 2016, 2017). We hypothesized that regional heterothermy would be more variable and/or extreme in a temperate bat, and therefore differences between proximal and distal wing muscle temperature sensitivity would be even more pronounced in *E. fuscus* than in *C. perspicillata*. Alternatively, distal wing temperatures could be more influenced by wing anatomy and its role in modulating heat delivery and loss. *Eptesicus fuscus* (Family Vespertilionidae) is widely distributed latitudinally across North America and into South America (Kurta and Baker, 1990). Winter activity of bats from *E. fuscus* populations in the Canadian prairies has been observed at environmental temperatures as low as −10°C (Klüg-Baerwald et al., 2016, 2017; Lausen and Barclay, 2006). Individuals from desert *E. fuscus* populations have been observed in flight and captured in mist nets at environmental temperatures as low as 4.5°C, with T_b values ranging from 28.7 to 41°C in the field (O'Farrell and Bradley, 1977). Flight at reduced T_b (under 30°C) has also been observed in the vespertilionid *Pipistrellus hesperus* (Bradley and O'Farrell, 1969).

Although much is known about the ecology and thermoregulatory strategies of *E. fuscus*, the contractile properties of its muscles have never been described. We evaluated the thermal dependence of contractile properties of two wing muscles – the proximal pectoralis major and the more distal extensor carpi radialis longus (ECRL) – from a population of *E. fuscus* in Ontario, Canada. As temperate *E. fuscus* regularly fly at cooler environmental temperatures compared with tropical *C. persipicillata*, we predicted that the temperature sensitivity of the distal ECRL muscle would be lower in *E. fuscus* (Rummel et al., 2018).

MATERIALS AND METHODS

Animals

We tested eight *E. fuscus* from a captive research colony at McMaster University (Hamilton, ON, Canada). Bats in the colony

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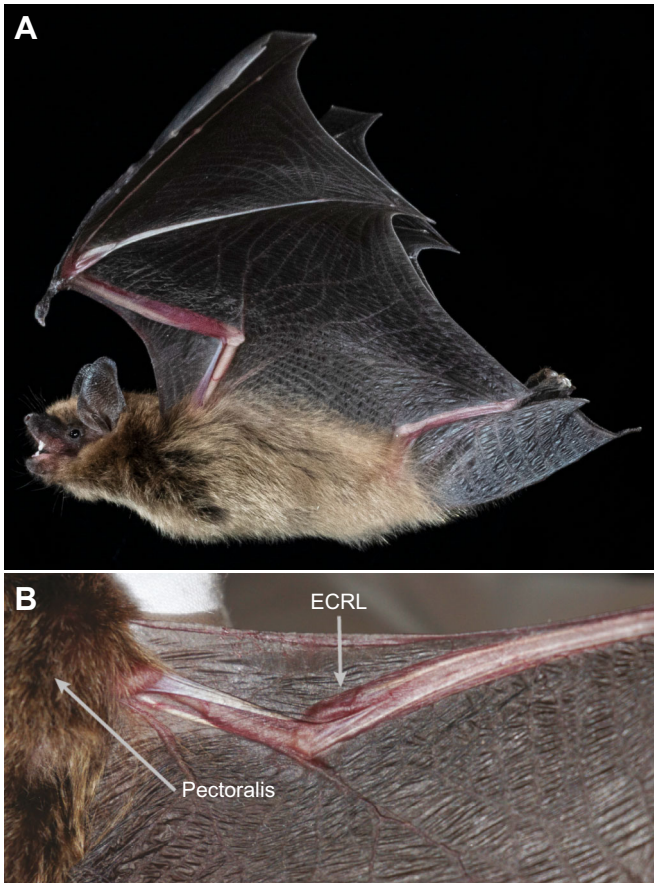

Fig. 1. The unique anatomy of bat wings. The muscles of the arm and forearm are clearly visible through the skin, with little to no fur, fat or connective tissue separating them from the environment, in contrast to the densely fur-covered pectoralis. (A) *Eptesicus fuscus* in flight, and (B) a close-up of the wing of *E. furinalis*, a congener of *E. fuscus*, showing the pectoralis and extensor carpi radialis longus (ECRL) muscles. Photos: M. Brock Fenton, Neysa Grider-Potter.

live in an indoor free-flight area (2.5×1.5×2.3 m) with year-round access to a larger (2.5×3.8×2.7 m) outdoor flight enclosure (Skrinyer et al., 2017). Colony temperature and lighting vary with ambient conditions, and bats have *ad libitum* access to water and food (yellow mealworms, *Tenebrio molitor*, Reptile Feeders, Norwood, ON). All experimental procedures met the Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and were approved by the Animal Research Ethics Board of McMaster University. Data were collected in June 2018 from four non-pregnant female *E. fuscus* that were born in captivity from wild-caught mothers in 2017, and from four non-pregnant wild-caught females introduced to the lab in May 2017.

Muscle dissection

Methods for dissecting the ECRL and pectoralis are similar to those described in Rummel et al. (2018) and Rummel et al. (2021), respectively. Differences in procedure are noted as follows. Animals were weighed, anesthetized with isoflurane until unresponsive, and then euthanized. Both humeri were cut mid-shaft so the wings could be placed into dishes with chilled, oxygenated Ringer's solution while the pectoralis dissection was completed. A strip of pectoralis, with its sternal and humeral attachments intact, was dissected from the thorax as described in Rummel et al. (2021), and then pinned to an approximately *in situ* length and immersed in cold, oxygenated Ringer's solution. The ECRL dissection was performed as described in Rummel et al. (2018).

Experimental apparatus and contractile protocol

Contractile properties were measured with methods similar to those previously described (Rummel et al., 2018, 2021), although both the ECRL and pectoralis preparations were run simultaneously. For both preparations, each muscle's attached humerus piece was clamped to identical plexiglass bases. Cylindrical plexiglass chambers with inflow and outflow holes for Ringer's solution were fitted into each base to create watertight chambers. Muscle temperature was maintained by a recirculating flow of Ringer's solution from a common reservoir in a water bath. Temperature was monitored throughout the experiments with a Keithley 871 digital thermometer with calibrated thermocouples inserted into the solution surrounding the muscle in each chamber. The proximal end of the pectoralis preparation was attached via the yoke, hook and silver chain to the arm of a Cambridge Instruments Model 300B servo-controlled muscle ergometer, and the transducer was mounted to a Digimatic Height Gauge (Mitutoyo) to allow for fine adjustment of muscle length. The distal end of the ECRL preparation was attached via hook and silver chain to the arm of a Cambridge Instruments Model 305B servo-controlled muscle ergometer, with the transducer mounted on a custom-built stand for adjustment of muscle length. Supra-maximal stimuli lasting 0.2 ms in duration were delivered through paired platinum plate electrodes placed on opposite sides of each muscle from a Grass S48 stimulator via a Universal Isolated Stimulator Output (Hugo Sachs Elektronik–Harvard Apparatus).

A series of isotonic contractions were performed for each muscle at 22, 27, 32, 37 and 42°C at stimulation frequencies ranging from 125 to 325 Hz. Stimulation durations for isometric tetani ranged from 125 to 475 ms, depending on temperature, so that muscle contraction was long enough to reach a plateau in force, but were reduced at low forces during the isotonic measurement series. Contractions were alternated between muscles, with a rest period of 3 to 4 min interposed between successive tetanic contractions for a given muscle. Muscle contractile force, length and velocity were measured on a PowerLab 16/S 16-bit data acquisition system (ADInstruments).

For both muscles, a series of isometric tetani were used to determine the length at which contractile force was maximal (L_0). Muscle length was adjusted to slightly above L_0 for isotonic contractions. Post-tetanic twitches were recorded for both muscles at each experimental temperature. Passive tension at the starting length was high for the pectoralis muscle, so we also measured a passive length–tension curve at each temperature from which we could calculate active force at the length at which each velocity was measured from each isotonic contraction (Supplementary Materials and Methods).

Following contractile measurements, muscle length was measured in place. The pectoralis muscle was pinned to *in vitro* length so that cut and damaged fibers could be removed. Both muscles were cut away from extraneous bone and tissue and weighed to the nearest 0.1 mg.

Measurements of fiber and fascicle length

The pinnate ECRL muscles were fixed in 10% phosphate-buffered formalin at the experimental length. The connective tissue was then digested in 30% nitric acid for up to 3 days, until the fascicles were easily separable. Fibers were photographed under a dissecting scope

and fiber length was measured in ImageJ. The pectoralis preparation consisted of parallel fascicles with essentially no tendon at either end; thus, we used the length of the fascicles to calculate cross-sectional area.

Data analysis and statistics

Data were recorded in LabChart 7. Isometric parameters were calculated from force, length and stimulation timing. Time to peak twitch ($t_{P,tw}$) was measured as the time from the start of force production to the time of peak twitch force (P_{tw}). Time to half-relaxation for twitch ($t_{50\%,tw}$) was measured as the time from peak twitch force to the time at which active force had decreased by half. Only post-tetanic twitches were used in descriptions of twitch kinetics. Time to half-relaxation for tetanus ($t_{50\%,tet}$) was measured as the time from the last stimulus to the time at which active tetanic force (P_0) had decreased 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 from all preparations. Using the non-linear curve fitting algorithm in IgorPro (Wavemetrics Inc.), length–tension curves were fit to an exponential equation and force–velocity curves were fit to the hyperbolic–linear equation of Marsh and Bennett (1986):

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

where V is velocity in L s^{-1}, P/P_0 is force as a fraction of measured maximum isometric force, and A, B and C are constants. To measure the curvature of the force–velocity relationship, we calculated the dimensionless power ratio as:

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

where $\dot{W}_{max}$ is the maximum isotonic power and V_{max} is the maximum velocity predicted at zero force. Because V_{max} is, by necessity, extrapolated outside the range of measured values, we instead report the interpolated value at 40% of P_0 (V_{40}) and have used this in our statistical analyses (see Fig. 4 and Table S5 for V_{max} values). The means reported for V_{40} and the power ratio were calculated from the mean values of each preparation. To estimate the overall force–velocity curve at each temperature, we averaged the fitted force–velocity curves for each preparation at a given temperature. Contractile properties data from each preparation are available in Dataset 1.

The temperature coefficient Q_{10} was calculated as:

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

where R_1 and R_2 are rates measured at temperatures T_1 and T_2, respectively (Bennett, 1984). The inverse of the duration measurements was used to calculate Q_{10} for $t_{P,tw}$, $t_{50\%,tw}$ and $t_{50\%,tet}$. We calculated Q_{10} as a function of temperature as in Lighton (1989) using the derivative of the quadratic fits to the rate–temperature relationship (Supplementary Materials and Methods).

Temperature had a measurable effect on maximum isometric tension. Because maximum tetanic force varied from preparation to preparation, we report the average rate coefficient, R_{10}, calculated from isometric tetanic contractions immediately before and after temperature changes of 5°C in individual preparations. The R_{10} value is calculated as for Q_{10} but with non-rate variables (Bennett, 1984). Mean specific tension (i.e. force per mean fiber cross-sectional area) was calculated by dividing the maximum force measured in an isometric tetanic contraction at any temperature for each preparation by the physiological cross-sectional area for each preparation, which in turn was calculated by dividing muscle mass by fiber or fascicle length and muscle density (1.06 g cm^{-3}) (Mendez and Keys, 1960).

We compared the thermal dependence of the contractile properties of the pectoralis and ECRL muscles with a regression analysis of log-transformed variables, using temperature and muscle as predictors, including interactions, implemented in R (https://www.r-project.org/). The contractile rate–temperature relationships were non-linear after log transformation and were well described by a quadratic equation, so temperature squared was included as a term in the regression. We also included birth status (whether the animal was wild-caught or born in captivity to a wild-caught mother) as a main effect or interaction (see Results). The power ratio was fit to a linear model with a muscle–temperature interaction term. The relationship between muscle contractile rate and temperature was deemed significant when the slope of the curve differed from zero, as determined using the emmeans package in R (https://CRAN.R-project.org/package=emmeans). The relationship was deemed different among muscles if there was a significant interaction of muscle and temperature. Graphically, a significant interaction of muscle and temperature is represented by differences in the apparent slopes of the curves, and a significant effect of temperature is indicated by a non-zero slope.

We also compared the pectoralis and ECRL contractile data measured from temperate *E. fuscus* with similar contractile data from the pectoralis and ECRL muscles of tropical *C. perspicillata* (Rummel et al., 2018, 2021) to determine whether differences in temperature sensitivity existed between the wing muscles of these species. Because birth status did not affect the temperature dependence of either the pectoralis or ECRL muscles of *E. fuscus* (see Results), and because our *C. perspicillata* data included only captive-born bats, we did not include birth as a covariate in the between-species comparison (see Tables S1, S3). We evaluated quadratic regression models including a three-way interaction of temperature, muscle and species with temperature squared as a covariate, given our hypothesis that the temperature sensitivity of only the distal muscle's contractile properties would differ among species. If the three-way interaction was not significant, two-way interactions were evaluated for significance and significant interactions were included in a contractile property's final model (Table S3).

RESULTS

Characteristics of the muscle preparations

Mean dimensions of the pectoralis preparation were as follows: mass of the dissected portion of the muscle, 51.2±7.1 mg; length of the muscle belly and fascicle length, 21.15±0.91 mm; and cross-sectional area 2.31±0.34 mm^2. Mean dimensions of the ECRL preparation were: mass, 23.5±1.4 mg; length of muscle belly, 22.0±1.9 mm; fiber length, 2.14±0.11 mm; and cross-sectional area, 10.5±0.84 mm^2. The mean specific tensions for the pectoralis and ECRL preparations were 14.4 and 13.1±1.6 N cm^{-2}, respectively.

Temperature had a significant effect on isometric tension in the pectoralis from 22 to 27°C and 27 to 32°C (paired *t*-test, P=0.001 and P=0.05, respectively). Comparing values before and after temperature changes of 5°C, the pectoralis R_{10} values were 1.34 for

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22–27°C, 1.07 for 27–32°C, 1.03 for 32–37°C and 0.80 for 37–42°C. Temperature also had a significant effect on the isometric tension in the ECRL from 27 to 32°C, 32 to 37°C and 37 to 42°C (paired *t*-test, P=0.002, P<0.001 and P=0.004, respectively). The ECRL R_{10} values were 1.01 for 22–27°C, 0.95 for 27–32°C, 0.91 for 32–37°C and 0.73 for 37–42°C.

Variation in muscle shortening velocity

After data collection, we noted that the shortening velocity appeared to be greater across all temperatures in the bats that were born in the wild compared with those born in captivity, despite both groups having spent similar amounts of time living in captivity. Birth status had a significant effect on V_{40} and there was a significant interaction between muscle and birth status (Fig. S1A, Table S1). The significant interaction term was due to the larger difference in V_{40} in the pectoralis compared with the ECRL; however, birth status did not affect the temperature dependence of V_{40} or any isometric property as indicated by non-significant birth×temperature interactions (Table S1). Therefore, we have pooled both groups of bats for the remainder of the results.

Comparisons between *E. fuscus* pectoralis and ECRL

All contractile rate variables for the pectoralis and ECRL muscles were affected by temperature (quadratic regressions, P<0.001 for all models, significant non-zero slopes, Figs 2, 3; Tables S1, S2). Isometric rates were fastest at 42°C and slowest at 22°C (Fig. 3). Similarly, shortening velocity was fastest at 37 and 42°C, and slowest at 22°C (Fig. 2). The curvature of the force–velocity curves, as indicated by the power ratio, did not vary significantly with temperature for either muscle (Fig. 4; Fig. S1B).

A muscle×temperature interaction was significant, indicating different temperature sensitivities between the *E. fuscus* muscles for all isometric properties (quadratic regression, $t_{P,tw}$: P=0.010; $t_{50\%,tw}$: P<0.001; $t_{50\%,tet}$: P<0.001; Fig. 3; see Table S1 for all regression models and coefficients). The pectoralis was more temperature sensitive than the ECRL for each isometric rate property, with higher Q_{10} values at every temperature (Fig. 5B–D). In contrast, there was no muscle×temperature interaction for V_{40} (Figs 2, 5A, quadratic regression, P=0.001; Table S1), indicating no difference in the thermal sensitivity of shortening velocity.

Comparisons of *E. fuscus* with *C. perspicillata*

Temperature effects for each contractile property were well described by quadratic regressions, including effects of species

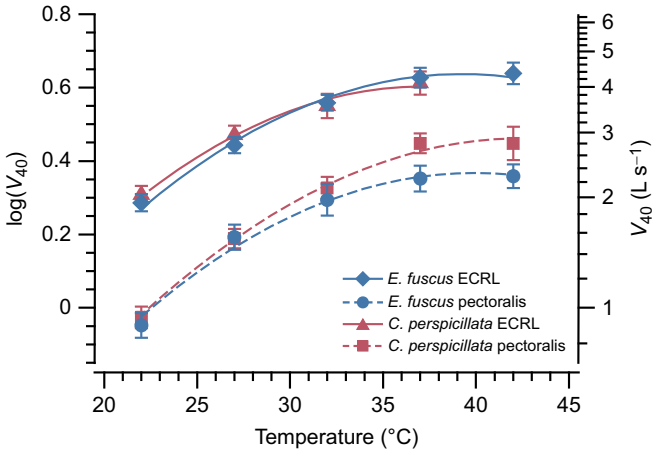

Fig. 2. Mean (±s.e.m.) shortening velocity as a function of temperature for the pectoralis and ECRL muscles from *E. fuscus* (this study) and *Carollia perspicillata* (Rummel et al., 2018, 2021). The points are mean (±s.e.m.) values at each temperature; sample sizes for the *E. fuscus* pectoralis and ECRL were n=7 at all temperatures. Sample sizes for *C. perspicillata*, from low to high temperature, were 5, 8, 8, 8 and 7 for the pectoralis, and 9, 9, 8 and 9 for the ECRL. The solid lines are quadratic regressions fitted using the log-transformed values.

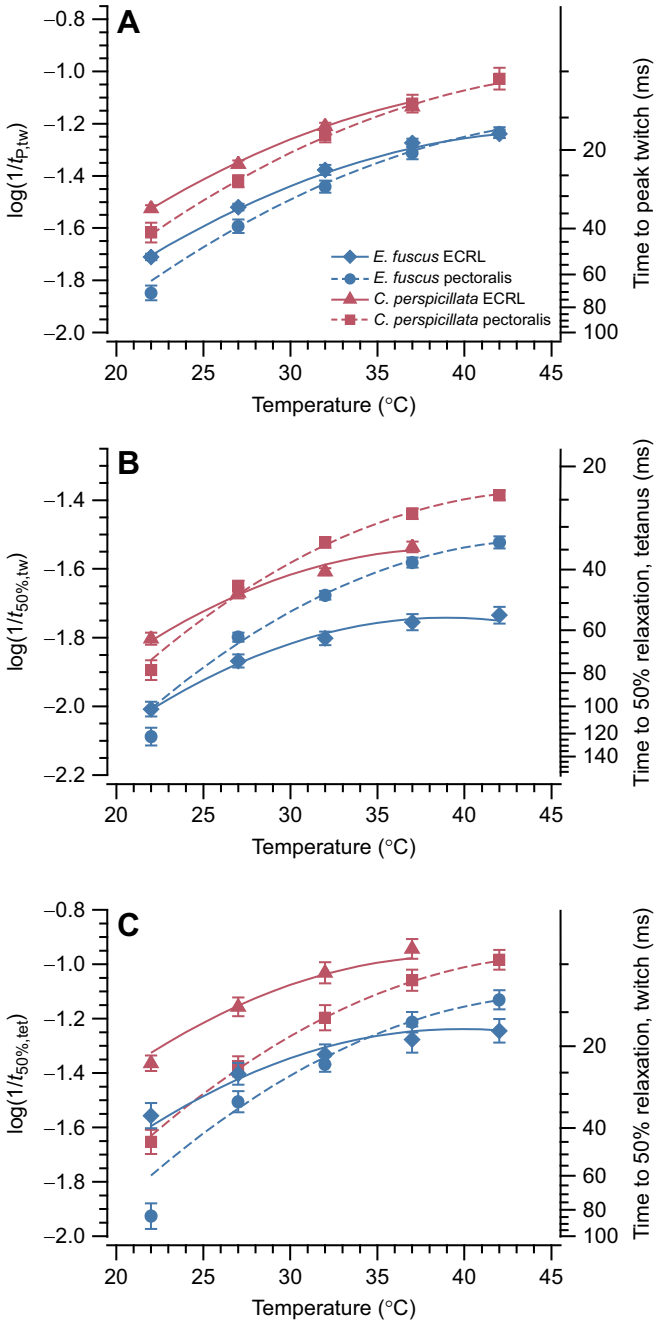

Fig. 3. Contraction times as a function of temperature for the *E. fuscus* pectoralis and ECRL muscles (this study) and the *C. perspicillata* pectoralis and ECRL muscles (Rummel et al., 2018, 2021). (A) Time to peak force in a twitch. (B) Time from peak force to 50% relaxation following a twitch. (C) Time from peak force to 50% relaxation in a tetanus. Sample sizes for the *E. fuscus* muscles were n=7 at all temperatures. Sample sizes for the *C. perspicillata* pectoralis as above, and n=9 at each temperature for the ECRL. The solid lines are quadratic regressions fitted using the log-transformed values. The left axes show the log of inverse contraction (ms^{-1}).

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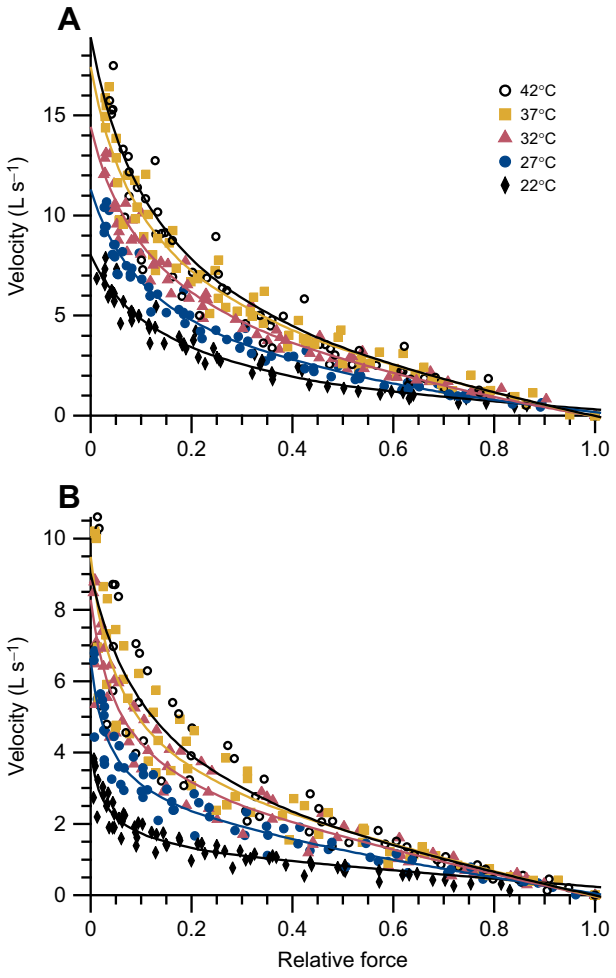

Fig. 4. Force–velocity curves from all preparations. Experimentally obtained velocities are reported in fiber lengths per second (L s^{-1}) and corresponding force values normalized to maximum isometric force. The fit lines were constructed by averaging fitted curves for each preparation at each temperature. *N*=7 preparations per temperature for both the ECRL (A) and pectoralis (B) muscles.

and muscle (P<0.001 for all models; see Table S3 for details of each model). A comparison of the isometric rate properties for *E. fuscus* and *C. perspicillata* (Rummel et al., 2018, 2021) revealed differences in absolute rates but not in temperature sensitivity between the two species. The temperature sensitivity of the pectoralis was higher than the ECRL for both species (significant muscle×temperature interaction), but for a given muscle there was no difference in temperature sensitivity between the two species (Figs 3, 5B–D). For $t_{50\%,tw}$ and $t_{50\%,tet}$, absolute rates were faster in *C. perspicillata* for both muscles, and for a given species, the difference in rates between muscles was greater for *E. fuscus* (significant muscle×species interaction; Tables S3, S4). A non-significant species×temperature interaction for these contractile properties indicates that the thermal sensitivities were similar across species for a given muscle (Figs 3, 5B–D). For shortening velocity, there was a significant three-way muscle×species×temperature interaction, indicating that the temperature–muscle relationship varied between species, with the *E. fuscus* pectoralis and ECRL velocities having similar relationships to temperature, and the *E. fuscus* pectoralis sensitivity matching that of the *C. perspicillata* ECRL muscle (Figs 2, 5A; Table S4). The absolute shortening velocities were similar in *E. fuscus* and *C. perspicillata*.

DISCUSSION

We hypothesized that there would be a proximal–distal gradient in wing muscle temperature sensitivity in *E. fuscus* and that it would be even more pronounced in this temperate species compared with the tropical species *C. perspicillata*. In support of this prediction, we found that the temperature sensitivity of the pectoralis was greater than that of the ECRL in *E. fuscus* for isometric rate properties. However, the temperature sensitivity of shortening velocity did not differ between the proximal pectoralis and distal ECRL. Significant differences between isometric force values were found across several temperatures in both *E. fuscus* muscles, and the R_{10} values were relatively close to 1, indicating a low temperature sensitivity. This finding is similar to the thermal sensitivity of isometric force reported in other species (Bennett, 1984). A species-level comparison with the *C. perspicillata* pectoralis and ECRL muscles revealed differences in the absolute rates of isometric properties but no differences in temperature sensitivities (Fig. 5B–D). The relationship of shortening velocity to temperature differed according to muscle and species, with similar temperature sensitivities between the pectoralis and ECRL muscles in *E. fuscus*, but greater temperature sensitivity in the pectoralis of *C. perspicillata*.

The temperature sensitivity of shortening velocity of the pectoralis muscle in *E. fuscus* is similar to that of its ECRL, in contrast to our initial hypothesis that this large flight muscle, which comprises the primary flight motor, would be more temperature sensitive than the ECRL as reported for *C. perspicillata* (Rummel et al., 2021). We made this prediction because the anatomical position of the pectoralis in the trunk and the covering of the muscle with relatively thick and densely furred skin likely keeps it at or near T_b during activity and at rest, in contrast to the condition for the more distal muscles of the wing, including the ECRL (Fig. 1). However, a lower thermal sensitivity in the pectoralis could be advantageous because *E. fuscus* is active over a larger range of body temperatures than is *C. perspicillata*, which is active at T_b>36°C (Audet and Thomas, 1997). In the laboratory, non-torpid *E. fuscus* maintain T_b at 32–36°C at ambient temperatures less than 30°C, and they are capable of flight with a T_b as low as 26°C in the laboratory and 28°C in the field (Herreid and Schmidt-Nielsen, 1966; O'Farrell and Bradley, 1977). Furthermore, operating T_b may change seasonally in this species, with winter-acclimated bats being active at lower T_b values than summer-acclimated bats (O'Farrell and Bradley, 1977).

Rewarming during seasonal hibernation and daily torpor could induce a need for better muscle function at lower body temperatures and could enhance the effectiveness of shivering thermogenesis, which may select for changes in muscle thermal sensitivity. However, Hayward and Lyman (1967) found that curarized *E. fuscus* rewarmed from torpor at the same rate as untreated bats, suggesting that brown adipose tissue catabolism may be more central to the rewarming process than muscular activity. Even if shivering thermogenesis is less important as a rewarming strategy for *E. fuscus*, their thermoregulatory flexibility may require muscle activity at temperatures below T_b. Chest muscles heat slightly faster than rectal temperature and blood flow is directed anteriorly when bats rewarm from torpor (Rauch and Beatty, 1975; Studier, 1974). Seasonal plasticity in the mass, metabolism and protein content of *E. fuscus* pectoralis muscles has been observed (Yacoe, 1983), but it is unclear whether these changes would result in a lower temperature sensitivity of the pectoralis muscle. Winter acclimation may also be accompanied by increases in body fat (insulation) and changes in blood flow, changes that decrease heat loss and help to buffer bats from the effects of low temperature on metabolism

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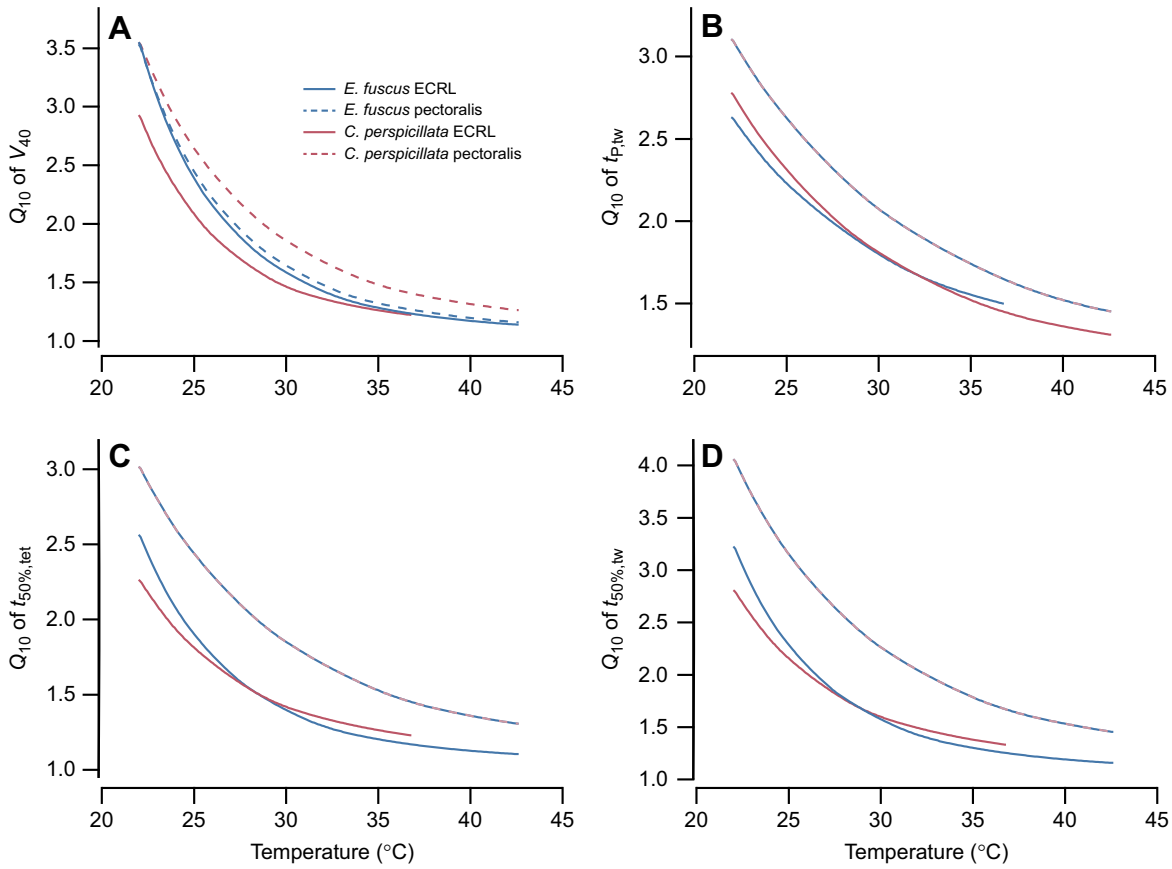

Fig. 5. Q_{10} as a function of temperature calculated from the derivatives of the fitted curves in Figs 1 and 2. Data are shown for (A) shortening velocity, (B) time to peak force in a twitch, (C) time from peak force to 50% relaxation in a twitch and (D) time from peak force to 50% relaxation in a tetanus. Higher Q_{10} values indicate greater temperature sensitivity. For the isometric properties (B–D), the pectoralis is more temperature sensitive than the ECRL, with no species differences. For shortening velocity, *E. fuscus* pectoralis and ECRL temperature sensitivity is the same, with Q_{10} values lower than in *C. perspicillata* across the range of temperatures.

(Rauch and Beatty, 1975; Shump and Shump, 1980). Although mechanisms that buffer T_b against seasonal environmental temperature fluctuations may not be adequate to eliminate core temperature fluctuations in flight muscles, particularly in species such as *E. fuscus* that can fly at low T_b values, the lower temperature sensitivity of the shortening velocity may still provide a functional advantage.

Our results indicate pectoralis shortening velocity is less temperature sensitive in *E. fuscus* than in the pectoralis of *C. perspicillata* and other mammalian limb muscles. This contrasts with our finding that isometric rate properties of the pectoralis in *E. fuscus* are as temperature sensitive as predicted. From a mechanistic perspective, shortening velocity is dictated by myosin ATPase activity in the cross-bridge cycle, while the whole-muscle isometric rates we measured – twitch times and relaxation from tetanus – relate to the rate of calcium flux in muscle cells, which is mediated by enzymes that pump or sequester Ca^{+2} ions (Bárány, 1967; Rall and Woledge, 1990). Both biochemical processes are affected by temperature, resulting in a temperature sensitivity of the whole-muscle contractile properties they mediate. The lower temperature dependence of shortening velocity, but not isometric rates, in the pectoralis suggests that the temperature sensitivity of myosin ATPase, but not enzymes involved in calcium flux, is under selective pressure.

The temperature sensitivities of the ECRL muscles from tropical *C. perspicillata* and temperate *E. fuscus* are similarly low. This may indicate that the operating temperatures of the muscles are similar. That *E. fuscus* can fly at lower body temperatures suggests that peripheral wing temperatures are also low. Kluger and Heath (1970) measured wing and body temperatures with forearm and dorsal midline thermocouples in restrained *E. fuscus*, and found that forearm temperature remained several degrees below T_b even with ambient temperatures of 25–32°C and when T_b was experimentally raised. Studies of *Rousettus egyptiacus*, *Tadarida brasiliensis* and *C. perspicillata* suggest that forearm temperature is substantially lower than T_b during flight, with wing temperature approaching environmental temperature under some conditions (Lancaster et al., 1997; Reichard et al., 2010; Rummel et al., 2019). This phenomenon is likely to be even more pronounced in temperate overwintering bat populations that hibernate but undergo periodic arousals when air temperature is well below 0°C (Geiser, 2004; Klüg-Baerwald et al., 2016). The winter hibernacula of *E. fuscus* are warmer than ambient temperature but can still be close to freezing (Klüg-Baerwald et al., 2017). Thus, environmental and body temperatures, in roosting and in flight, extend substantially lower for *E. fuscus* than *C. perspicillata* (Audet and Thomas, 1997), so their forearm muscles likely operate at lower temperatures.

Given these differences in thermal ecology, the similar temperature sensitivities of the ECRL muscle between the two species may relate to physiological limits on the range of temperatures over which muscle contractile rates can be maintained. Rummel et al. (2018, 2021) hypothesized that preservation of contractile performance in

distal muscles at low temperatures is constrained by the need to maintain performance at high temperatures. The biochemical systems underlying muscle contraction are mediated by enzyme kinetics and stability, both of which are intimately related to temperature and result in enzyme functionality over a limited range of temperatures (Hochachka and Somero, 2002). Thus, changes in temperature sensitivity may result from a shift in the temperature at which contractile rates peak, rather than an increase in the range of temperatures over which a given contractile rate can be attained (Rummel et al., 2018, 2021). If the *E. fuscus* ECRL achieved near-optimal contractile rates at cooler operating temperatures, it could experience heat damage when warmed to T_b. The low temperature sensitivity of the ECRL in both bat species is unusual compared with other mammalian muscles studied to date; however, few studies have explored the temperature sensitivity of mammalian limb muscles in detail and/or across a wide range of operating temperatures. Typically, muscle contractile rates in mammalian muscles peak a few degrees above typical T_b, with Q_{10} values near 2 at physiological temperatures (Faulkner et al., 1990; Rummel et al., 2018; Vyskočil and Gutmann, 1977). Decreases in temperature sensitivity of mammalian muscle contractile properties have not been reported, even in species that hibernate seasonally (South, 1961; Vyskočil and Gutmann, 1977), including in another heterothermic vespertilionid bat species, *Murina leucogaster* (Choi et al., 1998).

Conclusions

The remarkable anatomy of bat wings predisposes them to heat loss, which results in colder distal muscles relative to proximal core muscles. In *E. fuscus*, a small temperate bat, the distal ECRL muscle is generally less temperature sensitive than the proximal pectoralis muscle, a trend that matches results seen in *C. perspicillata*, a tropical species. Shortening velocity temperature sensitivity of the *E. fuscus* pectoralis, however, was the same as in its ECRL and less temperature sensitive than the *C. perspicillata* pectoralis. This decrease in thermal dependence may relate to differences in the thermoregulatory strategy between the two species. The ECRL muscles of the two species have similar temperature sensitivities for isometric properties and shortening velocity, suggesting that a selective trade-off may exist between enhancing performance when muscles are cool and maintaining function when muscles operate at higher temperatures and in warmer environmental conditions. Temperature effects in mammalian muscles are less well-studied compared with the muscles of ectotherms. Nevertheless, our results demonstrate that physiological compensation in muscle may accompany regional and temporal flexibility in body temperature.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.D.R., R.L.M.; Methodology: A.D.R., R.L.M.; Formal analysis: A.D.R.; Investigation: A.D.R.; Resources: P.A.F.; Writing - original draft: A.D.R.; Writing - review & editing: A.D.R., S.M.S., R.L.M., P.A.F.; Supervision: S.M.S., R.L.M.; Project administration: S.M.S.; Funding acquisition: A.D.R., P.A.F.

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