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A proximal–distal difference in bat wing muscle thermal sensitivity parallels a difference in operating temperatures along the wing

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Flight is a demanding form of locomotion, requiring fast activation and relaxation in wing muscles to produce the necessary wingbeat frequencies. Bats maintain high body temperatures during flight, but their wing muscles cool under typical environmental conditions. Because distal wing muscles are colder during flight than proximal muscles, we hypothesized that they would be less temperature sensitive to compensate for temperature effects, resulting in proximal–distal differences in temperature sensitivity that match differences in muscle operating temperature. We measured contractile rates across temperatures in the proximal pectoralis muscle and an interosseous in the handwing of *Carollia perspicillata*, a small neotropical fruit bat, and compared their thermal dependence with that of a forearm muscle measured in a previous study. We found that the contractile properties of the pectoralis were significantly more temperature sensitive than those of the distal muscles. This suggests that cooling of the distal wing muscles imposes a selective pressure on muscle contractile function which has led to shifts in temperature sensitivity. This study is the first to demonstrate differences in temperature sensitivity along the length of a single limb in an endotherm and suggests that temperature variation may be underappreciated as a determinant of locomotor performance in endotherms generally.

1. Introduction

Endotherms, such as mammals and birds, maintain relatively high and constant body temperatures. It has been proposed that this may enable the specialization of physiological processes to higher temperatures at which biochemical rates are faster [1]. Body temperature is neither always high nor constant in endotherms, however. Variation in temperature among body regions, known as regional heterothermy, is both a consequence of the exchange of heat between a warm body and cooler environment and, in some cases, an adaptation to conserve energy. In this latter case, peripheral regions of the body are allowed to cool to insulate the core and to reduce metabolic energy expenditures directed towards heating [2]. Limbs have high surface-area-to-volume ratios that make them vulnerable to excessive heat loss and useful for dumping heat, and their mechanical function is integral to locomotion. Some locomotor muscles are thus situated in parts of the body susceptible to temperature variation due to regional heterothermy, though this temperature variation is frequently overlooked when considering the effects of temperature on physiological function in endotherms [3].

Muscles are powered by a series of biochemical reactions that are under the kinetic constraints common to physiological systems: reaction rates are dependent on temperature. In muscle, faster reaction kinetics at warmer temperatures results in faster force development, activation, relaxation and greater shortening velocity [4]. For muscles in which temperatures are maintained at an endothermic core body temperature, one might expect that performance is specialized

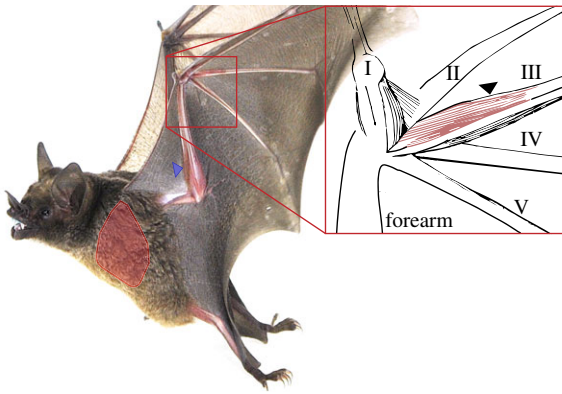


Figure 1. *Carollia perspicillata* in flight. The muscles of the forearm and hand are clearly visible through the skin, including the ECRL (blue arrow). The location of the pectoralis is indicated by the overlay. Inset: a schematic of the hand showing the two interosseous muscles on digit III. The muscle used in this study is in pink and is indicated by the black arrow. (Online version in colour.)

for operation at this temperature, declining at both cooler and warmer temperatures [3]. If high and constant body temperatures in endotherms enable the specialization of their biochemical processes to high temperatures, what happens at the periphery, where the temperature is not necessarily high nor constant? Does muscle contractile performance remain optimal at body temperature, or can this trait be optimized to cooler temperatures in muscles of endotherms which may operate at cooler temperatures during critical behaviours?

Bats are endotherms with remarkable appendicular anatomy that makes them ideally suited to address questions of muscle performance as it relates to operating temperature (figure 1). Their wings exhibit a proximal-to-distal temperature gradient during rest and activity. Continuous measurements of internal muscle temperature during wind tunnel flights indicate that the distal wing muscles of *Carollia perspicillata* operate at up to 12°C below core body temperature (T_b), while the more proximal pectoralis muscle remains near T_b during flight [5]. As nocturnal fliers, bats must maintain a high wingbeat frequency in the face of thermal conditions that likely result in net heat loss from their poorly insulated wings. Thus, during normal flight, different muscles of the flight apparatus may operate at vastly different temperatures, but because rate-related processes in muscle slow at cooler temperatures, temperature effects in the wing muscles have the potential to impair flight performance.

We hypothesized that the proximal–distal gradient in operating temperature of the wing muscles [5] would correspond with proximal–distal differences in muscle temperature sensitivity, predicting that the warm pectoralis muscle would be highly temperature sensitive, and that the cooler distal muscles would be less temperature sensitive. We previously reported that a forearm muscle, the extensor carpi radialis longus (hereafter ECRL), in *C. perspicillata*, a small neotropical fruit bat, has lower temperature dependence than expected based on values from other mammalian skeletal muscle [6]. Here, we report values for the *C. perspicillata* pectoralis and interosseous muscles, which are more proximal and more distal along the wing, respectively, than the ECRL muscle. We compare temperature effects on rate-dependent characteristics of the three muscles to demonstrate a difference in temperature sensitivity between the proximal pectoralis and the two more distal muscles.

2. Methods

(a) Animals and muscle dissection

Captive-bred *C. perspicillata* were housed in the Center for Animal Resources and Education at Brown University under a reversed 12 h:12 h dark:light cycle and provided with food and nectar *ad libitum*. These animals were adults, male and female (body mass 17.7 ± 0.5 g, $n=8$ for pectoralis preparations; 17.4 ± 0.8 g, $n=7$ for interosseous preparations). All experiments were conducted in accordance with a protocol approved by the Brown University IACUC and with USDA regulations. Bats were weighed and then anaesthetized with isoflurane to a surgical plane of anaesthesia, then euthanized by cervical dislocation.

(i) Pectoralis

The initial portions of the dissection were done under deep anaesthesia to maintain blood flow to the muscle to minimize muscle damage due to anoxia during the dissection. The skin was removed from the ventral thorax to expose the pectoralis muscle and its sternal and humeral attachments. The muscle was moistened regularly with oxygenated Ringer's solution. Two parallel scalpel incisions were made in-line with the direction of fascicles on one side, running from sternum to humerus. Using scalpel and scissors, the sternum was cut on either side of the midline, preserving the pectoralis attachment on the dissected side. After euthanasia, the strip of pectoralis was freed from the ribcage up to the shoulder, the humerus was disarticulated from the trunk, and the muscle strip placed into a dish with chilled, oxygenated Ringers solution. The muscle strip was then trimmed to an approximately 2 mm by 1 mm cross-section, such that it would be thin enough to allow for oxygen diffusion to its interior *in vitro* [7]. Care was taken to remove damaged or cut fibres from the exterior of the strip. A yoke, fashioned from an insect pin, was then tied to the sternum on either side of the muscle strip with surgical silk, to which a lightweight silver chain was then tied with surgical silk. The Ringer's solution was consistent with the composition of major ions in bat plasma [8] and contained 135 mmol l^{-1} NaCl, 5 mmol l^{-1} KCl, 2.0 mmol l^{-1} $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2 mmol l^{-1} MgSO_4 , 10 mmol l^{-1} glucose, 1.0 mmol l^{-1} NaH_2PO_4 and 10 mmol l^{-1} Hepes. The pH was adjusted to 7.4 after oxygenation.

(ii) Interosseous

Following euthanasia, both wings were dissected from the body and placed in dishes with chilled, oxygenated Ringer's solution (composition as above). The skin was carefully removed from the ventral side of the wrist and along digit III to expose four distinct tendons. The most superficial tendons, those of the palmaris longus and flexor digitorum profundus, were reflected, leaving two tendons of interosseous muscles (figure 1). The architecture of the more laterally situated muscle is complex. Its tendon is separable into two parts, each the termination of closely associated pennate muscle bellies originating at the base of metacarpal II. Only the smaller of these was used in the preparation. The tendons were separated from distal to proximal, and the tendon of the muscle belly of interest was tied with surgical silk, and then to a lightweight silver chain. The other tendon was cut, but the belly was left mostly intact until after the experiment to avoid damage to fibres in the muscle of interest.

(b) Muscle experimental setup and contractile protocol

Contractile properties were measured with methods similar to those described in [6]. For the pectoralis preparations, the humerus was clamped to a Plexiglas base and for the interosseous preparations, the thumb and proximal portion of the proximal phalanx of digit II were clamped. A cylindrical Plexiglas 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 hook and silver chain to the arm of a Cambridge Instruments Model 300 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 the temperature was monitored by a Keithley 871 digital thermometer with a calibrated thermocouple probe inserted into the solution surrounding the muscle. Supra-maximal stimuli of 0.2 ms duration were delivered from a Grass S48 stimulator via a Universal Isolated Stimulator Output (Hugo Sachs Elektronik–Harvard Apparatus) through two platinum plate electrodes placed on opposite sides of the muscle.

Series of isotonic contractions were performed at 22, 27, 32, 37 and 42°C; interosseous preparations were not viable at 42°C. The stimulation frequency used during tetanic contractions was high enough to maintain fused tetani at the current experimental temperature, which required a range from 100 to 450 Hz. Stimulation durations for isometric tetani were set such that the contraction was long enough to reach a plateau in force, 100–250 ms depending on temperature, and stimulation durations were reduced at low forces during the isotonic series. A rest period of three to four minutes was interposed between successive tetanic contractions. Force, length and velocity were recorded using 16-bit PowerLab/16SP and PowerLab 16/35 data acquisition systems for the pectoralis and interosseous preparations, respectively (ADInstruments, Sydney, NSW, Australia).

A series of isometric tetani was used to determine the length at which force was maximal (L_0). Post-tetanic twitches were also recorded. For isotonic contractions, the muscle length was adjusted to slightly above L_0 at 32°C for each pectoralis preparation, and at 22°C for each interosseous preparation. Passive tension in the pectoralis muscle was relatively high at the starting length during isotonic contractions. Thus, calculation of active force during shortening required knowledge of the passive length–tension relationship, which we measured at each temperature for all preparations except the first two. For these preparations, a passive length–tension curve averaged from the others was used. (See electronic supplementary material for the details of estimating the passive length–tension properties.) Active force for the length at which velocity was measured was calculated by subtracting the calculated passive force for a given contraction from the total force. Passive force in the interosseous preparations was low and this correction was not necessary.

Following contractile measurements, muscle length was measured in place. The pectoralis muscle was pinned to experimental length so that any damaged fibres that did not contribute to force generation could be identified and removed. Both muscles were cut away from extraneous bone and tissue, and their wet masses measured to the nearest 0.1 mg.

(c) Measurements of fibre length

Fibre length for the parallel-fibered pectoralis was taken to be the muscle length. For the interosseous, the muscles were fixed in 10% phosphate-buffered formalin at the rest length determined in the contractile experiments. The connective tissue was digested in 30% nitric acid for up to 3 days until the fascicles were easily separable, and groups of fibres were photographed under a dissecting scope. Fibre length was measured in ImageJ. Cross-sectional areas for both muscles were calculated by dividing the mass of the muscle by the mean fibre length. Mean fibre angle varied across preparations and proximodistally along the length of the muscle belly; we measured it from photographs of intact muscles *in situ* from two bats and multiple locations along the length of the belly. Interosseous specific tension and contractile velocity values are not corrected for fibre angle, but the corrections would be small because fibre angles were relatively low (mean fibre angle: $20 \pm 2^\circ$; specific tension error: approx. 6%).

(d) Data analysis and statistics

Data were recorded in LabChart 7 (pectoralis) and 8 (interosseous), and isometric parameters were calculated from the 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}) and time to half-relaxation for twitch ($t_{50\%,tw}$) was measured as the time from peak twitch force to the time at which 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 tetanic force (P_0) had decreased by half.

Velocity measurements were converted from mm s^{-1} to fibre lengths per second (l s^{-1}) by dividing by the mean fibre length from all preparations. Using the nonlinear curve fitting algorithm in IgorPro (Wavemetrics Inc.), force–velocity curves were fitted to the hyperbolic-linear equation of [9]:

$$V = \frac{B(1 - P/P_0)}{A + P/P_0} + C\left(1 - \frac{P}{P_0}\right), \quad (2.1)$$

where V is the 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. As a measure of the curvature of the force–velocity relationship, we calculated the dimensionless power ratio as

$$\text{power ratio} = \frac{\dot{W}_{\max}}{(V_{\max}P_0)}, \quad (2.2)$$

where \dot{W}_{\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 mean values we report for V_{\max} , V_{40} , and power ratio were calculated from means for each preparation, not by taking the value obtained from the mean fit to all pooled preparations. To estimate the overall force–velocity curve for each temperature, we averaged the fitted force–velocity curves for each preparation at a given temperature.

The temperature coefficient Q_{10} was calculated as

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2 - T_1)}, \quad (2.3)$$

where R_1 and R_2 are rates measured at temperatures T_1 and T_2 , respectively [4]. Q_{10} as a function of temperature was calculated from the derivative of the quadratic fits to the rate–temperature relationship as in [10] (see electronic supplementary material). The isometric force was affected by temperature (electronic supplementary material), but we calculated mean specific tension (force per mean fibre cross-sectional area) using the largest force measured in an isometric tetanic contraction for each preparation at any temperature.

We compared the thermal dependence of contractile properties of the pectoralis and interosseous (this work), and ECRL [6], using a regression analysis of log-transformed variables as a function of temperature, implemented with the base stats package in R [11]. Because the relationships of contractile rate properties with temperature were nonlinear after log transformation and were fit well by a quadratic function, the comparisons of muscle temperature sensitivity were done with a quadratic regression with muscle, temperature, and temperature squared as predictors, including interactions. Power ratio and P_{tw}/P_0 were fitted to a linear model with a muscle–temperature interaction term. Regression models were chosen by evaluating nested model comparisons using ANOVA and AIC scores. The thermal sensitivity of a contractile rate was deemed significant for a given muscle if it had a nonzero slope, and significantly different among muscles if there was a significant interaction of muscle and temperature. Planned contrasts among the levels of the muscle–temperature interaction were evaluated to determine which muscles differed significantly

from each other in their rate–temperature relationships, and individual slopes were tested for significance using the emmeans package in R [12]. Graphically, the muscle–temperature interaction is illustrated by differences in the slopes among the muscles' fitted curves, and a non-zero slope indicates an effect of temperature.

3. Results

(a) Characteristics of the preparations

The mean dimensions of the pectoralis preparations were as follows: muscle mass: 41.5 ± 5.6 mg; length of the muscle preparation and muscle fibres: 28.13 ± 0.60 mm; cross-sectional area: 1.39 ± 0.18 mm². The mean specific tension of the pectoralis preparations was 8.93 ± 0.75 N cm⁻². The mean dimensions of the interosseous preparations were: muscle mass: 3.1 ± 0.5 mg ($n = 6$, one mass was not recorded); length of the muscle preparation and muscle fibres: 6.86 ± 0.50 mm and 0.85 ± 0.09 mm, respectively. The cross-sectional area was 3.78 mm² and the mean specific tension for the interosseous preparations was 6.08 ± 0.42 N cm⁻².

(b) Temperature sensitivity and between muscle comparisons

For all contractile rate properties, temperature squared and the interaction of muscle (including pectoralis, interosseous and ECRL) and temperature were significant (figures 2 and 3; quadratic regression, $p < 0.001$ for all models). Muscle \times temperature was also significant for power ratio and P_{tw}/P_0 (figure 4; electronic supplementary material, figure S4; linear regression, $p < 0.001$ for both models) (see electronic supplementary material, tables S1 and S2 for all regression models and coefficients). For both the pectoralis and interosseous muscles, all contractile properties were affected by temperature (i.e. significant nonzero slopes), except for power ratio and P_{tw}/P_0 of the interosseous (electronic supplementary material, table S3). The highest rates and lowest Q_{10} s occurred at the highest temperatures (figures 2 and 3 and insets).

Comparing the thermal dependences of the pectoralis and interosseous (this study) and the ECRL [6], revealed differences in the contractile rate–temperature relationship among the muscles. A review of the planned contrasts between levels of the muscle \times temperature interaction revealed that for time to peak twitch, relaxation from tetanus, and V_{40} , the temperature sensitivity of the pectoralis was significantly higher than that of both the ECRL and interosseous muscle, which did not differ from each other (i.e. the slope of the pectoralis curve was significantly greater than that of the ECRL or interosseous) (table 1; electronic supplementary material, tables S2 and S3, figures 2 and 3). For relaxation from twitch, the pectoralis was significantly more temperature sensitive than the ECRL, but neither differed from the interosseous muscle (table 1; electronic supplementary material, tables S2 and S3). For V_{max} , the ECRL had significantly lower temperature dependence than both the pectoralis and interosseous muscles, which did not differ from each other.

4. Discussion

(a) Proximal–distal differences in contractile properties

We hypothesized that the proximal–distal gradient in operating temperature of the wing muscles [5] would correspond

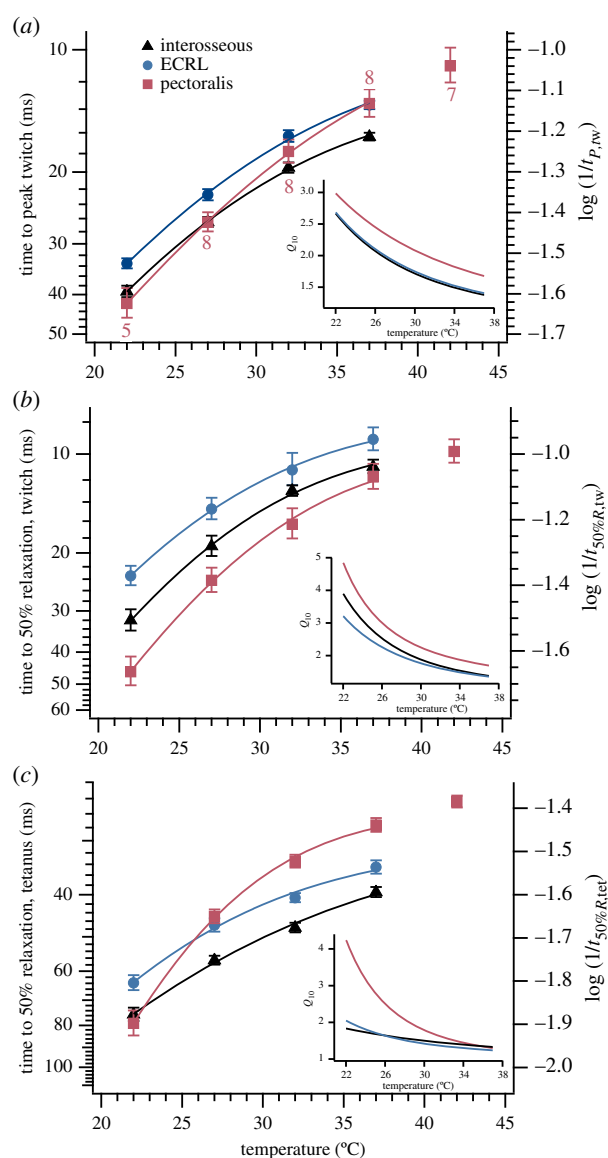


Figure 2. Contraction times as a function of temperature for the interosseous and pectoralis (this study) and the ECRL [6]. (a) Time to peak force in a twitch. (b) Time from peak force to 50% relaxation in a twitch. (c) Time from peak force to 50% relaxation in a tetanus. The points plotted are mean values (\pm s.e.m.) at each temperature. Sample sizes for the ECRL and interosseous are $n = 9$ and $n = 7$, respectively, at all temperatures. Sample sizes for the pectoralis are indicated in (a). The solid lines are quadratic regressions fitted using the log-transformed values (shown on right axis) at temperatures of 37°C and below. The left axes show the time on an inverted log scale. The right axes show the log of inverse contraction times ($m s^{-1}$). Insets: Q_{10} as a function of temperature calculated from the derivative of the fitted curves. (Online version in colour.)

with proximal–distal differences in muscle temperature sensitivity, predicting that the warm pectoralis muscle would be more temperature sensitive than the distal muscles, which cool during flight. We found a clear proximal–distal trend in the temperature dependence of the contractile properties of the wing muscles in *C. perspicillata*, supporting our hypothesis. Specifically, the rate-related contractile properties of the pectoralis were more temperature sensitive than those of the two more distal muscles. The Q_{10} s were generally higher in the pectoralis at every temperature than in either distal muscle, (figures 2 and 3, insets). This difference in muscle temperature sensitivity mirrors the gradient in temperature of these muscles during flight [5]. Although temperatures of the muscles of the

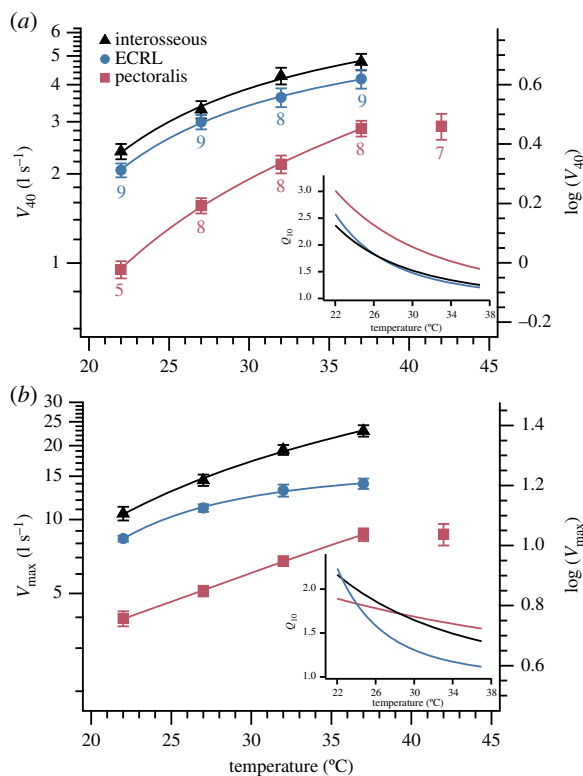


Figure 3. Shortening velocity as a function of temperature for the interosseous and pectoralis (this study) and the ECRL muscle [6]. (a) Shortening velocity at 40% of maximum force (V_{40} , $l s^{-1}$). (b) Predicted maximum shortening velocity at zero force (V_{max} , $l s^{-1}$). The points plotted are mean values at each temperature (\pm s.e.m.); sample sizes for the pectoralis and ECRL are indicated in (a); sample size was $n = 7$ for the interosseous at all temperatures. The solid lines are quadratic regressions fitted using the log-transformed values (shown on right axis) at temperatures of 37°C and below. Insets: Q_{10} as a function of temperature calculated from the derivative of the fitted curves. (Online version in colour.)

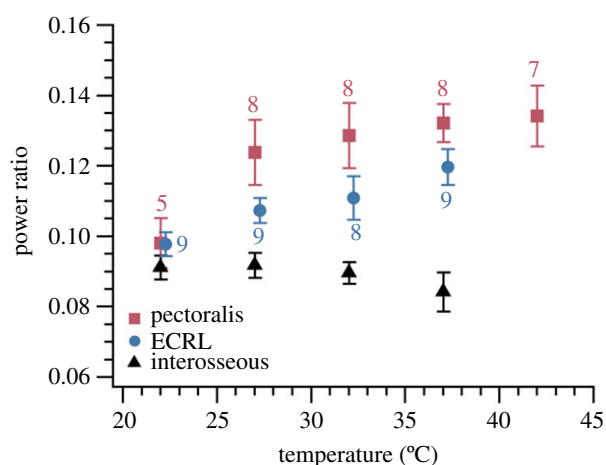


Figure 4. Power ratio as a function of temperature for the interosseous and pectoralis (this study) and the ECRL muscle [6]. Power ratios were calculated from the individual force-velocity curves for each preparation, and points are mean values at each temperature (\pm s.e.m.). Sample sizes for the pectoralis and ECRL are indicated for each point. $n = 7$ for the interosseous at each temperature. (Online version in colour.)

handwing have yet to be recorded, they are both smaller and more distal than the ECRL, which suggests that they are likely colder during flight than more proximal muscles. Thermal imaging of bats in flight shows that temperatures of the

Table 1. Results of contrasts between muscle \times temperature interaction terms. p -values less than 0.05 are shown in bold.

contractile property	p -value		
	pectoralis-ECRL	pectoralis-interosseous	ECRL-interosseous
$t_{P,tw}$	0.005	0.003	0.75
$t_{50\%R,tw}$	0.012	0.086	0.44
$t_{50\%R,tet}$	<0.001	<0.001	0.559
V_{max}	0.002	0.88	0.003
V_{40}	0.003	0.006	0.089
power ratio	0.42	0.002	0.010
P_{tw}/P_0	0.25	<0.001	0.011

distal wing likely fall to near environmental temperature during flight, supporting this idea [13,14].

For time to peak twitch, relaxation from tetanus, and V_{40} , the pectoralis muscle was significantly more temperature sensitive than either the ECRL muscle or the interosseous muscle. The proximal-distal trend was less pronounced for twitch relaxation, for which the interosseous temperature sensitivity is intermediate between that of the other two muscles (figure 2b). For V_{max} , the trend is less clear because thermal sensitivity in the interosseous was not significantly different from that found for the pectoralis (figure 3b). However, V_{40} values, which represent shortening under a load, are functionally more relevant as a measure of shortening speed than V_{max} , which is, in addition, an extrapolated value. Hence, differences in temperature sensitivity between V_{max} and V_{40} could arise from uncertainty in the fits near zero load (figure 5b). Differences among the muscles in the curvature of the force-velocity curves with increasing temperature (figures 4 and 5) may also account for the discrepancy in the temperature sensitivity between V_{max} and V_{40} .

(b) Constraints on thermal adaptation in muscle

The bat pectoralis major and mouse extensor digitorum longus (EDL), studied between 22 and 42°C in 5°C increments (this study and ref. [6]), likely have similar thermal dependences because they are both well-insulated and presumably operate at or near T_b (figure 6a). Limited comparisons can be made with data from other taxa; most measurements of temperature effects on contractile properties in mammalian muscle are at temperatures below normothermic T_b , or make too few measurements to build a thermal performance curve. However, Q_{10} values between 1.75 and 3 have been reported for temperature ranges in the 10°C below T_b [15–18]. The similarity in temperature sensitivity among these mammalian muscles, which likely operate near core T_b , suggests that the pattern observed in the bat pectoralis and mouse EDL may represent a typical mammalian condition, in which muscle biochemistry is tuned for peak performance at or near T_b . Rummel *et al.* [6] hypothesized that the low-temperature sensitivity seen in the *C. perspicillata* ECRL relative to the EDL muscle of the mouse was a result of a downward shift in its thermal performance curve. Here, in muscles of a single species, within a single locomotor appendage, we

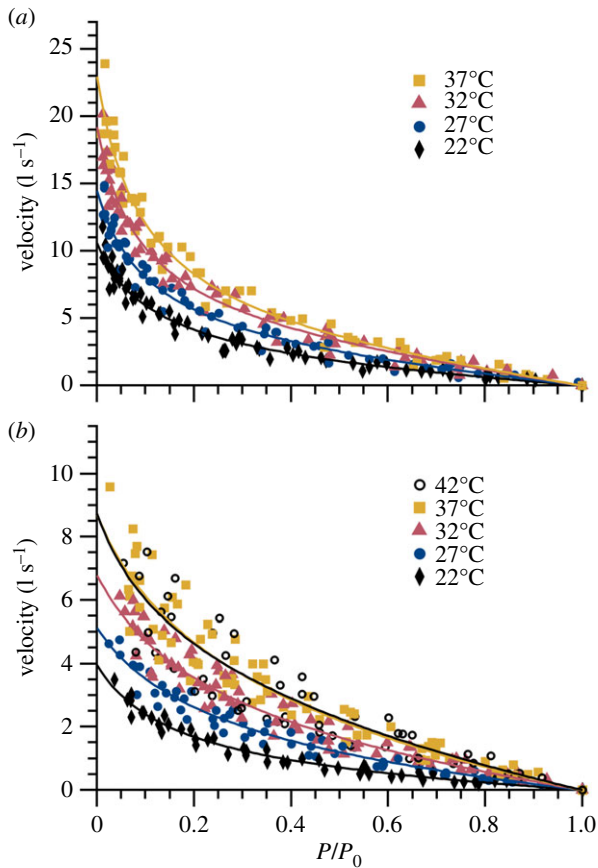


Figure 5. Force–velocity data from all preparations for the interosseous and pectoralis muscles. Points indicate experimentally obtained velocity values (lengths per second, $l\ s^{-1}$) and corresponding force values (P) normalized to maximum isometric force (P_0). The fit lines were constructed by averaging fitted curves for each preparation at each temperature. (a) Force–velocity curves for the interosseous muscle ($n=7$ preparations per temperature) and (b) pectoralis muscle ($n=5$ at 22°C, $n=8$ at 27, 32 and 37°C and $n=87$ at 42°C). (Online version in colour.)

observe a similar phenomenon (figure 6*b*). The ECRL and interosseous, in which operating temperatures drop to well below T_b , may take advantage of a functionally significant shift in thermal performance curves to compensate for cooler operating temperatures during flight.

If the interosseous muscle operates at lower temperatures than the ECRL, why is its sensitivity to temperature not lower? Temperature sensitivity in muscle arises from the temperature dependence of reaction kinetics and enzyme stability, which constrain the function of biochemical systems [1]. These fundamental constraints on biochemical systems result in a limited operational temperature range. The high, relatively constant body temperatures of endotherms are thought to allow for specialization of rate processes to temperature optima to produce fast reaction kinetics coupled with enzyme stability [1]. The distal wing muscles in bats likely encounter a wider range of temperatures because of their minimal insulation and the resulting variable thermal environment of the distal wing. Compared to muscles whose temperatures are dictated by T_b , distal muscles may approach core T_b in warm environments, but will be substantially colder during flight under cooler environmental conditions. If one considers the pectoralis muscle as a thermal ‘specialist’, it is tempting to ascribe ‘generalist’ characteristics to muscles whose thermal milieu is more variable, such as the ECRL and interosseous.

However, our contractile data suggest that rather than a broadening of the thermal performance curve, which would indicate the maintenance of functional contractile rates over a wider range of temperatures, the thermal performance curve is shifted leftwards, such that its peak occurs at a lower temperature. The lack of a further leftward shift in the rate versus temperature curves may relate to the need to balance requirements for high performance at low temperature with the maintenance of warm temperature performance given fundamental biochemical constraints.

(c) Wing muscle function

The thermal dependence of the muscles of the wing probably influences their function during flight. Flight is a demanding form of locomotion, requiring ample power production to generate both lift and thrust while overcoming drag [19]. The pectoralis muscle is active cyclically during flight, beginning in late upstroke and continuing through downstroke, which occupies approximately half the wingbeat cycle (of an approx. 12 Hz wingbeat frequency in *C. perspicillata*) [20,21]. The power ratio of the bat pectoralis is higher at every temperature than that of either the ECRL or the interosseous (figure 4). Additionally, although there are no published *in vivo* measurements of pectoralis muscle strain trajectory or force generation in bats, unpublished data indicate that the pectoralis in *C. perspicillata* shortens by 30% or more during flight (Nicolai Konow 2020, personal communication). Together, relatively high power ratios and fascicle strains suggest that selection may have targeted maximizing power output in the bat pectoralis. The highest power ratio (0.13) occurs at 42°C in the bat pectoralis, which is substantially lower than values reported from zebra finch and budgerigar pectoralis muscle (0.22 and 0.17, respectively) [22]. However, bat pectoralis strains are generally higher than those measured in the pectoralis of zebra finches and budgerigars, and quail (12–16% and up to 23%, respectively) [23,24].

The function of the interosseous muscle is more speculative. The presence of handwing muscles is highly variable among species of bats, and the function of these muscles has been inferred from anatomy only [25–27]. The primary function of the interosseous group is thought to be to resist aerodynamic extension of the digits during downstroke [26,28], and to aid in the folding of the wing during upstroke [25]. The short belly, long tendon and pennate architecture of the preparation used in this study suggest that muscle activation does not result in substantial length changes of the muscle–tendon unit, and that the muscle may resist passive deflection rather than effecting movement about a joint.

The interosseous displays a low-temperature dependence relative to the pectoralis and muscles from other mammalian species, which suggests that its function could be time-dependent. Even if the muscle acts isometrically during flight, it must develop force and relax during the time course of the wingbeat cycle to limit interference with force and/or shortening produced by antagonist muscles. Rapid force development in muscle depends, among other factors, on a rapid cross-bridge cycle as indicated by high shortening velocity. If the interosseous is active during the downstroke and into early upstroke to contribute to wing folding, continued activity during late upstroke could impede the extension of wing joints in preparation for the onset of the next downstroke. We conclude that the time course of force

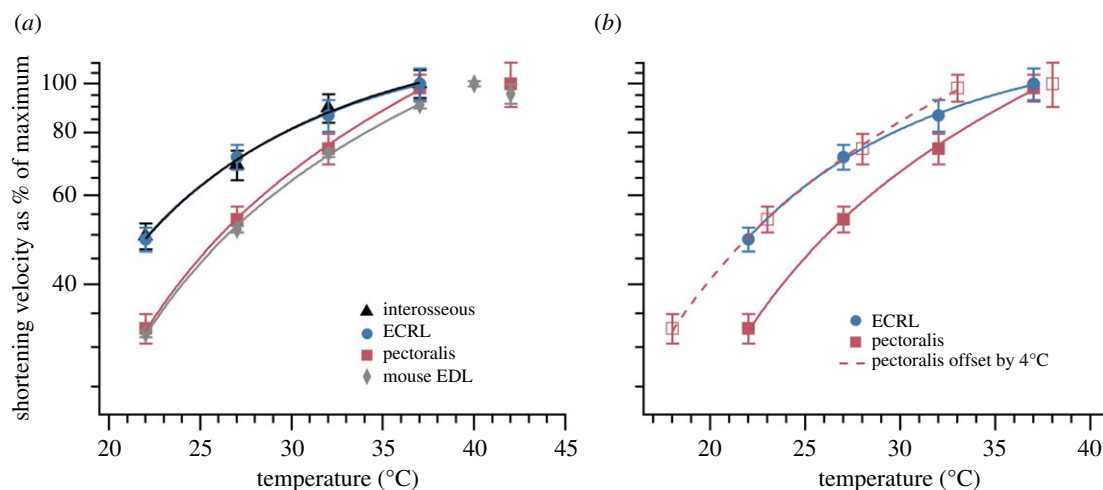


Figure 6. Thermal dependence of wing muscles from *C. perspicillata*. (a) Shortening velocity of the pectoralis and interosseoous (this study) and the ECRL and mouse EDL [6], normalized to each muscle's maximum. The bat pectoralis and mouse EDL have a similar and relatively high thermal dependence, while the ECRL and interosseoous muscles have virtually the same lower thermal dependence. (b) When the pectoralis curve is offset by 4°C, the offset curve and ECRL curve are more closely aligned, which suggests that the ECRL is tuned to a lower optimum temperature than the pectoralis. (Online version in colour.)

development and deactivation of the interosseoous muscle studied here, as well as that of other muscles of the hand-wing, is likely important in maintaining proper wing conformation over the course of the wingbeat cycle.

Another role for higher shortening velocities of the ECRL and interosseoous relative to the pectoralis may be to compensate for the lower contractile rates that would otherwise arise from the low temperatures of the distal wing muscles. At a given temperature, intrinsically faster muscles will generate higher shortening velocities and develop force faster than intrinsically slower muscles, even if temperature sensitivities are the same. Fibre velocities of the pectoralis at 37°C (T_b) roughly match those of the ECRL and interosseoous at approximately 10°C below T_b , which may indicate that there is a minimum rate of force development that these distal colder muscles must achieve to preserve the wingbeat cycle.

(d) Regional differences in temperature sensitivity in other species

Few studies explore regional variation in the temperature sensitivity of muscle contractile properties, either in endotherms or ectotherms. Tuna, which are regional endotherms, show significant variation in temperature sensitivity in muscles from different anatomical locations along the length and depth of the body [29]. Other studies have not yielded robust evidence of similar regional variation in muscle physiological properties. A study of the thermal dependence of contractile properties in the mouse diaphragm and soleus reported conflicting results for time-dependent isometric properties and power output [30]. They found that force development and relaxation were more temperature dependent in the soleus, the limb muscle, compared to the values for the centrally located diaphragm, but the reverse was true for power output [30]. The comparisons in [30] used non-log-transformed values, and thus examined the absolute changes with temperature rather than relative changes, as we have done. Thus, their results may reflect differences in intrinsic contractile rates between the two muscles (see electronic supplementary material, figure S5).

The contractile properties of a mouse superficial foot muscle, the flexor brevis, showed slightly higher temperature sensitivity

in comparison to other rat and mouse muscles (Q_{10} values greater than 3) [31]. The lack of low-temperature sensitivity in these mouse and rat appendicular muscles might suggest that their limb muscles operate close enough to T_b that there is little need for fast contractile rates below T_b ; that these muscles play a role in locomotion that is not time-dependent; or that there are other physiological, biomechanical or behavioural compensatory mechanisms for temperature effects.

5. Conclusion

This study is the first to demonstrate a proximal-to-distal difference in muscle temperature sensitivity along the span of a single limb, corresponding to differences in muscle temperature during locomotion. This pattern supports our prediction that proximal, thermally insulated muscles would be highly temperature sensitive, and that distal, thermally variable muscles would be less temperature sensitive. In this case, regional variation in temperature is extreme enough to result in physiological compensation for temperature effects in the distal limb muscles, in the form of a significant decrease in temperature sensitivity. Locomotor behaviour, physiology, appendicular anatomy and thermoregulation likely result in spatial as well as temporal temperature gradients that may impose selective pressure on the thermal sensitivity of muscle physiology. The distinctive and specialized case of bat wings highlights regional heterothermy and associated physiological specialization, but these and similar phenomena may well be widespread. The potential effects of temperature on locomotor function in endotherms have so far been underappreciated, and thermal effects could profoundly influence locomotor performance in many species and habitats.

Ethics. This work was approved under Brown University IACUC nos 1603000199 and 19-01-0012. We followed USDA standards for animal care.

Data accessibility. The datasets supporting this article are provided in the electronic supplementary material [32]. ECRL data can be found at <https://repository.library.brown.edu/studio/item/bdr:814948/>.

Authors' contributions. A.D.R.: Conceptualization, formal analysis, investigation, methodology, writing-original draft, writing-review and editing; S.M.S.: funding acquisition, resources, supervision, writing-

review and editing; R.L.M.: conceptualization, methodology, supervision, writing-review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

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