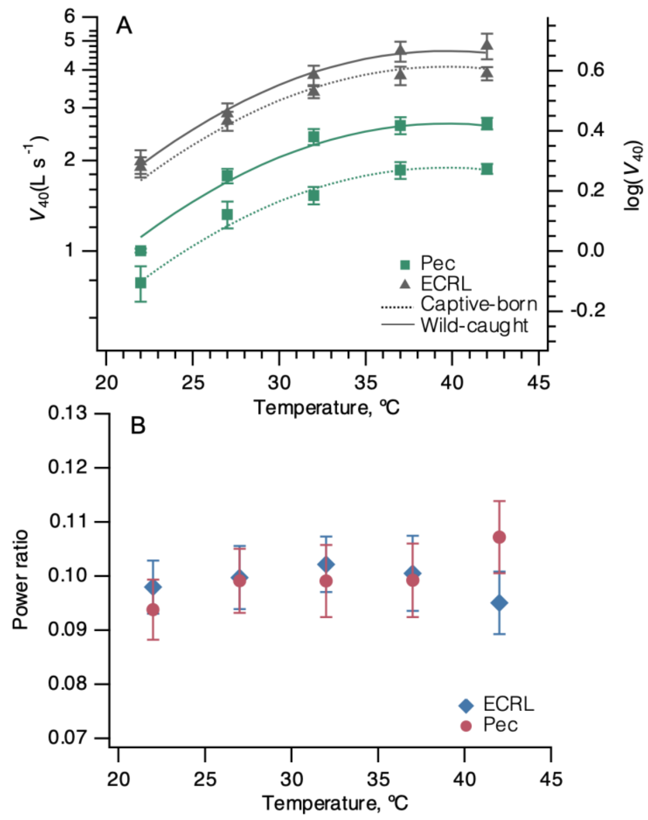

Fig. S1. (A) The effect of birth status on shortening velocity in *E. fuscus*. Shortening velocity as a function of temperature for the pectoralis and ECRL muscles from *E. fuscus* separated by birth status (captive-born *vs.* wild-caught). The points are mean values at each temperature (±s.e.m.); sample sizes for the pectoralis and ECRL were n = 4 for wild-caught and n = 3 for captive-born at all temperatures. The lines are quadratic regressions fitted using the log-transformed values. (B) Power ratio as a function of temperature for *E. fuscus* ECRL and pectoralis muscles. Power ratios were calculated from the individual force-velocity curves for each preparation, and points are mean values (±s.e.m.) at each temperature. N = 7 for each muscle at each temperature.

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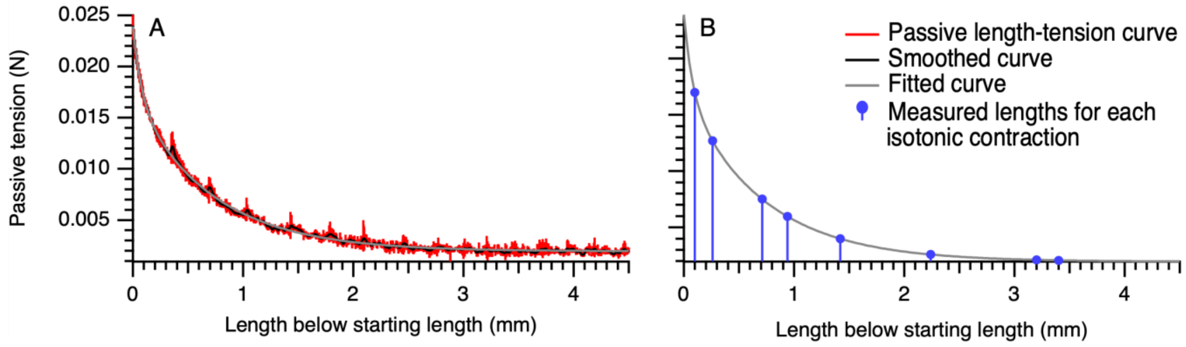
Supplemental Materials and Methods

Passive force calculations for pectoralis preparations

To estimate active force during isotonic contractions, passive length-tension curves (LT curves) were used to estimate passive tension at the length at which muscle shortening velocity was highest. These calculated passive tension measurements were then subtracted from total force measurements to yield active force. For four of seven preparations, LT curves were recorded at each temperature after completion of a series of isotonic contractions. Curves were recorded by shortening the muscle using the length controller of the muscle lever and recording length and force continuously in LabChart. The passive tension was plotted as a function of the shortening below the starting length, minimally smoothed (smoothing spline in Igor Pro, Wavemetrics), and fitted with a double exponential function.

$$y = y_0 + A_1 e^{-\frac{x-x_0}{\tau_1}} + A_2 e^{-\frac{x-x_0}{\tau_2}}$$

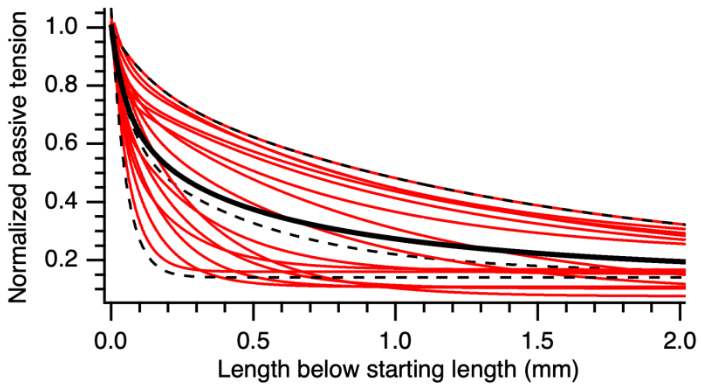
Using this curve we predicted the passive force at the time of peak shortening velocity during the isotonic contractions.



(A) A representative example of a passive length-tension curve, recorded at 32ºC, with both the smoothed curve and fitted curve superimposed on the original data. (B) Measured lengths at which peak shortening velocity occurred in each isotonic contraction, drawn up to the fit line. The corresponding y-values are the passive forces for each isotonic contraction and were used to calculated active force.

Calculation of average passive length-tension curve:

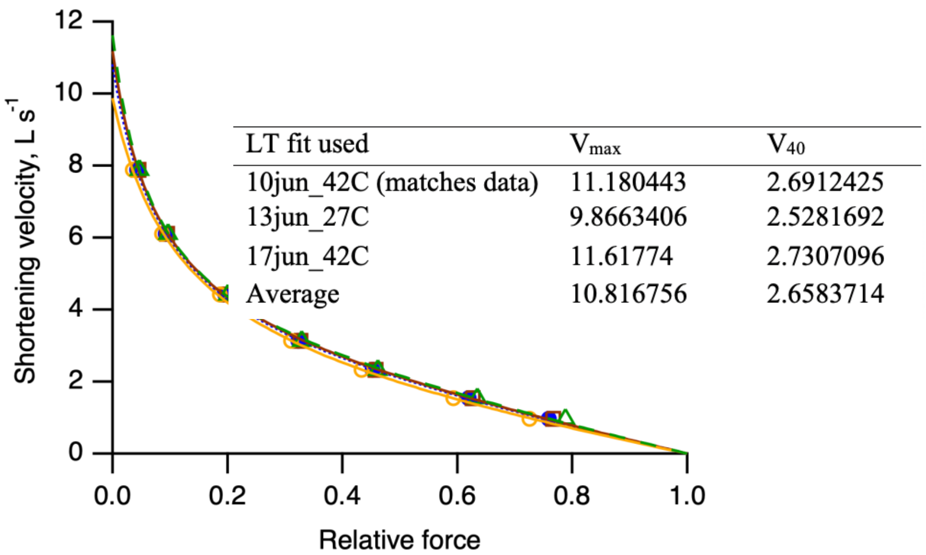
For the three preparations for which we did not have an individual LT curve, we constructed an average curve. This average LT curve was calculated from the LT curves from all preparations and temperatures normalized to the starting passive tension value (Fig. S4). The fitted exponential curves from all preparations and temperatures were averaged using the Waves Average function in IgorPro resulting in one average curve normalized to resting passive tension (tension at the starting length).



Interpolated passive length-tension curves for all preparations at all temperatures normalized to the passive tension at the starting length. The solid black line is the double exponential fit using all of the data. The curves drawn as dashed lines are the ones used in the sensitivity analysis.

Sensitivity analysis

Because the asymptotes of the individual normalized curves varied, a sensitivity analysis was performed to evaluate whether using the overall average curve might result in substantial errors in the calculated active force values. We used the average curve and three curves with asymptotic passive forces above, below and near that of the average curve. We used these curves to predict the active force in one of the preparations for which we had an individual curve. We found that the variation in the asymptotic passive force had a negligible effect on the resulting active force values and on the resulting force-velocity curve.



A sensitivity analysis of varying predictions of passive force, with V_{max} and V_{40} values calculated from each passive LT curve. The individual symbols represent predictions using the overall average curve and the individual curves dashed in the previous figure. The force and velocity data used were from 10jun_42C.

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Continuous Q_{10} calculations

Calculating and plotting Q_{10} as a function of temperature provides a visualization of the temperature sensitivity of a rate, and was calculated for each contractile property and each muscle by differentiating the log-transformed quadratic fits, multiplying by 10, and taking the antilog:

$$Q_{10} = 10^{10\times[\frac{d}{dT}logR]}$$

after (1). This equation can be derived by log-transforming the Q_{10} equation, $Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2-T_1}}$,

$$logQ_{10} = \frac{10}{T_2 - T_1}(logR_2 - logR_1)$$

which gives:

$$logQ_{10} = \frac{10}{dT}(dlogR)$$

1. Lighton JRB. Individual and Whole-Colony Respiration in an African Formicine Ant. Functional Ecology. 1989;3(5):523–30.

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Table S1. Effect of birth status and muscle on the relationship of contractile properties to temperature in *E. fuscus*. Results of quadratic regressions including birth and muscle as covariates.

	$t_{P,tw}$			$t_{50\%R,tw}$			$t_{50\%R,tet}$			V_{40}		
	R^2	F-statistic	p-value	R^2	F-statistic	p-value	R^2	F-statistic	p-value	R^2	F-statistic	p-value
	0.9304	231.6 (4, 65 DF)	<0.001	0.7472	51.98 (4, 65 DF)	<0.001	0.9006	157.3 (4, 65 DF)	<0.001	0.9291	181.9 (5, 64 DF)	<0.001
Coefficients	**Estimate**	**t value**	**p value**	**Estimate**	**t value**	**p value**	**Estimate**	**t value**	**p value**	**Estimate**	**t value**	**p value**
Intercept	-3.077	-20.264	<0.001	-2.6978	-8.958	<0.001	-3.0976	-20.985	<0.001	-1.2968	-7.949	<0.001
Temp	0.0824	8.511	<0.001	0.07123	3.708	<0.001	0.0685	7.276	<0.001	0.0963	9.148	<0.001
Musclepec	-0.2027	-3.484	<0.001	-0.5670	-4.913	<0.001	-0.2345	-4.145	<0.001	-0.3355	-16.032	<0.001
Temp^2	-0.0009	-6.109	<0.001	-0.0009	-2.953	0.004	-0.0009	-5.924	<0.001	-0.0012	-7.409	<0.001
Temp:Musclepec	0.0047	2.635	0.01	0.0166	4.713	<0.001	0.0109	6.315	<0.001	--	--	--
Birthwild	--	--	--	--	--	--	--	--	--	0.0519	2.654	0.01003
Musclepec:Birthwild	--	--	--	--	--	--	--	--	--	0.0954	3.446	0.00101

Table S2. Simple slopes of *E. fuscus* contractile property–temperature relationships. A significant p-value indicates that the slope is significantly different from zero and that there is an effect of temperature. Power ratio and twitch/tetanus ratio were not significantly affected by temperature for either muscle.

Muscle		**Contractile Property**			
Pectoralis		$t_{P,tw}$	$t_{50\%R,tw}$	$t_{50\%R,tet}$	V_{40}
	Slope	0.0284	0.0316	0.0241	0.0186
	p-value	<.001	<.001	<.001	<.001
ECRL		$t_{P,tw}$	$t_{50\%R,tw}$	$t_{50\%R,tet}$	V_{40}
	Slope	0.0237	0.015	0.0132	0.0186
	p-value	<.001	<.001	<.001	<.001

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Table S3. Results of quadratic regressions comparing the temperature sensitivity of contractile properties for pectoralis and ECRL in *E. fuscus* and *C. perspicillata.*

	$t_{P,tw}$			$t_{50\%R,tw}$			$t_{50\%R,tet}$			V_{40}		
	R^2	F-statistic	p-value	R^2	F-statistic	p-value	R^2	F-statistic	p-value	R^2	F-statistic	p-value
	0.9213	326.4 (5, 134 DF)	<0.001	0.8104	100 (6, 133 DF)	<0.001	0.9276	298 (6, 133 DF)	<0.001	0.8363	89.76 (8, 131 DF)	<0.001
Coefficients	**Estimate**	**t value**	**p value**	**Estimate**	**t value**	**p value**	**Estimate**	**t value**	**p value**	**Estimate**	**t value**	**p value**
Intercept	-2.805	-22.903	<0.001	-2.7572	-12.711	<0.001	-2.9450	-29.424	<0.001	-1.1172	-6.601	<0.001
Temp	0.0763	9.643	<0.001	0.0899	6.422	<0.001	0.0719	11.129	<0.001	0.0913	8.3	<0.001
Musclepec	-0.2234	-4.539	<0.001	-0.6247	-7.136	<0.001	-0.3005	-7.438	<0.001	-0.5590	-5.335	<0.001
Temp^2	-0.0008	-6.581	<0.001	-0.0011	-5.063	<0.001	-0.0009	-8.946	<0.001	-0.0012	-6.662	<0.001
Speciesept	-0.1782	-17.531	<0.001	-0.2694	-10.521	<0.001	-0.2004	-16.963	<0.001	-0.1205	-1.202	0.23137
temp:Musclepec	0.0057	3.732	<0.001	0.0146	5.374	<0.001	0.0112	8.921	<0.001	0.0103	3.081	0.00252
Musclepec:Speciesept	--	--	--	0.1229	3.451	<0.001	0.0579	3.522	<0.001	0.2248	1.624	0.10685
Speciesept:temp	--	--	--	--	--	--	--	--	--	0.0039	1.209	0.22877
Musclepec:Speciesept:temp	--	--	--	--	--	--	--	--	--	-0.0087	-1.997	0.04788

	Power Ratio			*Twitch-tetanus*		
	R^2	F-statistic	p-value	R^2	F-statistic	p-value
	0.3266	17.85 (4, 135 DF)	<0.001	0.2063	9.515 (4, 127 DF)	<0.001
Coefficients	**Estimate**	**t value**	**p value**	**Estimate**	**t value**	**p value**
Intercept	0.0657	6.561	<0.001	0.3116	7.56	<0.001
Temp	0.0016	4.854	<0.001	-0.0061	-4.57	<0.001
Musclepec	0.0059	2.042	0.04311	0.0437	3.681	<0.001
Temp^2	--	--	--	--	--	--
Speciesept	0.0237	1.718	0.08813	-0.1102	-1.976	0.050348
temp:Musclepec	--	--	--	--	--	--
Musclepec:Speciesept	--	--	--	--	--	--
Speciesept:temp	-0.0013	-3.12	0.00221	0.0048	2.733	0.0072
Musclepec:Speciesept:temp	--	--	--	--	--	--

Table S4. Simple slopes of the regression lines calculated with the emmeans package in R, with p-values indicating a significant effect of temperature; and contrasts between regression curves, the significance of which indicates a significant difference in the temperature relationship.

	$t_{P,tw}$		$t_{50\%R,tw}$		$t_{50\%R,tet}$		V_{40}		***Power Ratio***		***Twitch-tetanus***	
	Slope	**p-value**	**Slope**	**p-value**	**Slope**	**p-value**	**Slope**	**p-value**	**Slope**	**p-value**	**Slope**	**p-value**
C.p. ECRL	0.0239	<0.001	0.0185	<0.001	0.0137	<0.001	0.015	<0.001	0.001558	<0.001	-0.00609	<0.001
C.p. pec	0.0297	<0.001	0.0331	<0.001	0.0248	<0.001	0.0253	<0.001	0.001558	<0.001	-0.00609	<0.001
E.f. ECRL	0.0239	<0.001	0.0185	<0.001	0.0137	<0.001	0.0189	<0.001	0.000218	0.4474	-0.00133	0.2403
E.f. pec	0.0297	<0.001	0.0331	<0.001	0.0248	<0.001	0.0206	<0.001	0.000218	0.4474	-0.00133	0.2403
	Contrast	**p-value**	**Contrast**	**p-value**	**Contrast**	**p-value**	**Contrast**	**p-value**	**Contrast**	**p-value**	**Contrast**	**p-value**
C.p. ECRL - E.f. ECRL	–	–	–	–	–	–	-0.00393	0.2288	0.00134	0.0022	-0.00476	0.0072
C.p. ECRL - C.p pec	-0.00571	0.0003	-0.0146	<0.001	-0.0112	<0.001	-0.01035	0.0025	–	–	–	–
C.p. ECRL - E.f. pec	-0.00571	0.0003	-0.0146	<0.001	-0.0112	<0.001	-0.0056	0.0877	0.00134	0.0022	-0.00476	0.0072
E.f. ECRL - C.p. pec	0.00571	0.0003	0.0146	<0.001	0.0112	<0.001	-0.00641	0.026	-0.00134	0.0022	0.00476	0.0072
E.f. ECRL - E.f. pec	-0.00571	0.0003	-0.0146	–<0.001	-0.0112	<0.001	-0.00166	0.5481	–	–	–	–
C.p. pec - E.f. pec	–	–	–	–	–	–	0.00475	0.0978	0.00134	0.0022	-0.00476	0.0072

Table S5. Maximal shortening velocity in lengths per second for the pectoralis and ECRL muscles from *E. fuscus* separated by birth status (captive-born *vs.* wild-caught). The values, extrapolated from the force velocity curves in Figure 4, are means at each temperature (±s.e.m.); sample sizes for the pectoralis and ECRL were n = 4 for wild-caught and n = 3 for captive-born at all temperatures. The results and associated statistical analyses reported in the main text refer only to V_{40} values.

	Pectoralis		ECRL	
Temperature	Captive	Wild	Captive	Wild
42	6.38 ± 0.62	10.96 ± 0.40	16.15 ± 2.02	20.96 ± 0.46
37	7.53 ± 0.72	10.95 ± 0.64	14.95 ± 2.37	19.27 ± 0.47
32	7.01 ± 0.74	9.23 ± 0.41	13.19 ± 1.67	15.37 ± 0.37
27	5.59 ± 0.65	7.29 ± 0.40	10.87 ± 0.98	11.61 ± 0.36
22	3.38 ± 0.47	4.47 ± 0.16	7.70 ± 0.80	8.25 ± 0.36

Dataset 1. *Eptesicus fuscus* contractile properties data.

Click here to download Dataset 1

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