RESEARCH ARTICLE

Temperature gradients in the flight muscles of Manduca sexta imply a spatial gradient in muscle force and energy output

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SUMMARY

There is a significant dorso-ventral temperature gradient in the dominant flight muscles [dorsolongitudinal muscles (DLM1)] of the hawkmoth Manduca sexta during tethered flight. The mean temperature difference was 5.6°C (range=3.8–6.9°C) between the warmer, ventral-most subunits and the cooler, dorsal-most subunits. As force generation in muscle depends on temperature, the mechanical energy output of more dorsal subunits will differ from that of deeper and warmer muscle subunits. To test this hypothesis, we isolated the dorsal subunits and the ventral subunits and recorded both single and 25 Hz (wingbeat frequency) isometric contractions at a range of temperatures. Our data show that the contractile dynamics of the various regions of the DLM1 are similarly affected by temperature, with higher temperatures leading to reduced contraction times. Furthermore, using standard electromyography, we showed that the different regions are activated nearly simultaneously (mean time difference=0.22 ms). These observations suggest that the existence of a temperature gradient will necessarily produce a mechanical energy gradient in the DLM1 in M. sexta.

Key words: muscle temperature, force, insect flight, temperature gradient.

INTRODUCTION

As with other biological rate processes, muscle function is strongly influenced by temperature. Specifically, muscle contraction rates (the rates of both force development and relaxation) are accelerated by an increase in temperature in both invertebrates and vertebrates (Josephson, 1984; Bennett, 1985). Work-loop studies, which measure the force produced by muscle while lengthening and shortening, have demonstrated that the mechanical power, or work output, of muscle increases as temperature increases (Stevenson and Josephson, 1991). Biologically relevant ranges of in vivo temperature can therefore have significant impacts on locomotor performance.

Many animals achieve elevated muscle temperatures via endogenous heat production during muscle contraction (Heinrich, 1995). This heat production follows from the inherent inefficiency of muscle, with only ~5–9% of the chemical energy appearing as mechanical work and the rest released as heat (Ellington, 1984; Josephson and Stevenson, 1991). In several large insects, this heat byproduct leads to elevated thoracic temperatures that are well aboveambient temperatures (Heinrich, 1974). Because the contractile rates of these muscles are temperature dependent, increased muscle temperature due to endogenous heat production allows these insects to increase their wingbeat frequency and thus produce greater mechanical power output (McCrea and Heath, 1971).

Previous studies of insect thermoregulation assumed that temperature is spatially uniform throughout the thoracic flight muscles, and therefore assessed temperature at only one spatial location. Although such methods provide valuable insight into operating thoracic temperatures in freely flying insects, they do not reveal any spatial variation in temperature (Heinrich, 1971; Heinrich and Casey, 1972; Janiszewski, 1984; Coelho, 1991). Such variation, however, is a probable consequence of metabolic heat production paired with dorsal convective cooling and ventral insulation surrounding the flight musculature. Given the strong thermal dependence of muscle function and the increased core temperature that insects generate, we chose to examine the possibility of thermal inhomogeneity within flight muscle. Unless compensated for, any spatial gradient in temperature that arises because of endogenous heat production necessarily creates spatial gradients in mechanical and energetic performance. This potential effect is particularly relevant to animals with large muscles that span a significant percentage of the body area and are aligned such that some sub-regions are located near the surface and others near the central axis. Consequently, cooler muscles near the surface could have dramatically different contraction dynamics than hotter, more centrally located muscles.

Here we document a temperature gradient and its possible functional consequences in the flight muscles of Manduca sexta, a large, active hawkmoth. Specifically, we address how the activation and contraction dynamics throughout an insect’s flight muscle vary due to regional temperature differences that arise from the spatial distribution of heat production and heat loss mechanisms. M. sexta generates a highly elevated thoracic temperature (~40–43°C maximum, ~15–25°C above ambient temperature) during free flight (Heinrich and Casey, 1972). The dominant flight muscles of M. sexta – the dorsolongitudinal muscles (DLM1) (sensu Kondoh and Obara, 1982) – power the down-stroke of the wings and occupy the majority of the mesothorax. The flight muscles of M. sexta are synchronous muscles, with a single muscle action potential eliciting each contraction, in contrast to the asynchronous flight muscles of Diptera and other insects (Kammer, 1968). Although activation is synchronous with wing beats, it is not known whether the timing of the activation of the subunits is simultaneous or phase-shifted.
If the DLM1 subunits have similar contractile dynamics and are simultaneously activated, a temperature gradient will likely result in warm, ventral subunits producing isolated Twitches whereas the cooler, more dorsal subunits, with their slower contraction dynamics, may remain in unfused tetany. These temperature-induced contractile differences could indicate an associated gradient in mechanical power output throughout the DLM1. Such a concept could have significant implications for muscle efficiency and overall animal locomotor performance. In this study, we address three related questions. First, does temperature vary spatially throughout the flight muscles? Second, are the DLM1 regionally specialized to compensate for the temperature-induced differences in contraction dynamics? Third, are muscle subunits activated independently in order to correct for a temperature-induced offset in the time of peak force?

MATERIALS AND METHODS

Moths

*Manduca sexta* (Linnaeus 1763) were obtained from a colony maintained by the Department of Biology at the University of Washington, Seattle, WA, USA. Moths were used within 5 days of eclosion. Prior to use, moths were maintained at 4°C for up to 1 day to immobilize them.

The DLM1 are composed of five subunits – DLM1a–e – that run longitudinally along the length of the mesothorax and attach to the cuticle at the first and second phragmata (Fig. 1A) (Tu and Daniel, 2004a). Each DLM1 subunit is ~1 mm thick and is separately innervated by neurons in the IN1c nerve (Kondoh and Obara, 1982; Eaton, 1988).

Temperature profiles

We chose to measure the temperature profiles of the DLM1 during tethered flight because our constraints of multi-site and continuous measurement made free flight recording problematic. Temperature of the DLM1 subunits was measured to the nearest 0.1°C using a copper–constantan thermocouple embedded into a 30 gauge hypodermic probe (HYP-1, Omega Engineering Inc., Stamford, CT, USA). The voltage output from this probe was relayed to a central processing unit. To verify the spatial resolution of the probe, we measured the temperature of well-mixed 49°C water within a beaker set in an air stream of 22°C. After detecting the initial temperature difference at the surface layer, the probe measured a constant 49°C throughout the water. This indicates that the thermocouple measures temperature at the tip of the probe rather than spatially averaging temperature along the distal needle. This probe was attached to a micromanipulator such that it could be positioned in any of the muscle subunits. 10-turn 5K Potentiometer (International Resistance Co., St Petersburg, FL, USA), attached to the pinion of the vertical drive of the micromanipulator, was used to monitor the thermocouple’s position within the DLM1. The probe was inserted through a small hole cut in the cuticle of the mesothorax to the right of the midline. Scales on the dorsal thoracic plates obscured the insertion point and so were removed. Moths were ventrally tethered to a brass rod that was set just posterior to the metathoracic legs. The rod was fixed in place with a mixture of cyanoacrylate and sodium bicarbonate powder. After a 10 min recovery period, moths were induced to fly with a constant airstream and occasional gentle probing. Individuals that did not exhibit continuous flight for at least 10 min were excluded from analysis. Upon initiation of flight, we lowered the probe through the DLM1 in ten 0.5 mm increments, roughly half the thickness of an individual subunit. Each ‘scan’ of the DLM1 took less than 20 s, and we repeated this scan every minute that the moth flew (N=10 moths).

To assure ourselves that removal of the dorsal thoracic scales did not significantly alter the temperature difference between the DLM1, we conducted two independent tethered-flight temperature measurements. We first tested moths with the dorsal scales in place. Then, after a 10 min rest period, we removed the scales on the same moth and repeated the temperature scan. Because these experiments were performed as a quick approximation to verify that dorsal scales did not significantly alter the temperature, we measured temperature only to the nearest degree using a digital multimeter (N=4 moths).

Force measurements

We assessed the effect of temperature on the isometric contractile dynamics to determine whether there was regional specialization for temperature sensitivity on force production in each of the subunits comprising the DLM1. We adapted the protocol established by Tu and Daniel (Tu and Daniel, 2004a) for DLM1 isolation and force measurement. To facilitate access to the thoracic muscles, we removed the head, wings, legs and scales covering the dorsal and ventral surface. The DLM1 were isolated between two grips attached to micromanipulators: a posterior fixed grip inserted into the groove beside the second phragma and an anterior grip attached to the first phragma. The anterior grip was connected to a rigid lever force transducer (FORT250, WPI, Sarasota, FL, USA). The force signal was passed through a bridge amplifier (Measurements Group, Chapel Hill, NC, USA).

A strip of acetate transparency film was glued across the two grips and then cut down the middle. This method serves as a positioning guide to prevent any change in length or shape of the thorax (Tu and Daniel, 2004a). We then excised a thin strip of cuticle near the anterior grip to mechanically isolate the thoracic muscles. Using the micromanipulators, we repositioned the apparatus such that the acetate strips were aligned, returning the thorax to its original position.

To assess any regional specialization for contractile performance, we measured isometric force in three different preparations: (1) intact DLM1 comprising all five subunits; (2) DLM1 in which the ventral subunits (DLM1a,b) were removed, leaving only the dorsal DLM1 (DLM1c–e; Fig. 1B); and (3) DLM1 in which the dorsal subunits (DLM1c–e) were removed, leaving the ventral subunits intact (DLM1a,b).

To regulate the temperature of the muscle, we immersed the entire thorax in a temperature-controlled bath of *M. sexta* saline (Lei et al., 2004). An immersion circulator was used to heat water piped through an aluminum stage on which a Petri dish of this saline rested (Haake DC3, DM Scientific, Houston, TX, USA). The solution was then heated to 25, 30, 35 and 40°C to encompass the possible range of free-flight temperatures (Heinrich, 1974). To deliver supramaximal stimuli, a homemade stimulator was connected to two minuten pins that were inserted through the posterior and anterior notum along the same longitudinal transect of the DLM1. Stimuli were either square pulses (0.2 ms long, for single contractions) or a train of pulses at 25 Hz to elicit contractions at wingbeat frequency. We recorded the evoked potential with a differential electrode placed in the DLM1 near the posterior grip (N=5 moths per DLM1 group, with five single isometric contractions or 10 25 Hz contractions per moth). Rise time is defined as the time required for tension to develop from 10% of peak tension to peak tension, and fall time is the duration of time required to return to 10% of peak tension.
to isolate the DLM1 between the two grips. The DLM1 subunits were either posterior grip and a flexible anterior grip attached to a force transducer. A wings, legs and scales, we secured the mesothorax between a rigid wings, legs and scales, we secured the mesothorax between a rigid

Fig. 1. Manduca sexta preparation for force and electromyographic (EMG) measurement. (A) We recorded simultaneous EMGs during tethered flight from a stationary electrode in DLM1 and an adjustable electrode in all other DLM1 subunits. The temperature of each subunit was measured with a thermocouple probe. The dark gray outer layer represents the insulating fur covering the body of M. sexta. (B) After removing the moth’s head, wings, legs and scales, we secured the mesothorax between a rigid posterior grip and a flexible anterior grip attached to a force transducer. A thin circumferential cut was then made around the mid-mesothoracic cuticle to isolate the DLM1 between the two grips. The DLM1 subunits were either left intact or the dorsal (DLM1c–e) or ventral subunits (DLM1a,b) were isolated. Muscles were stimulated with supramaximal stimuli to induce either single isometric twitches or 25 Hz contractions. Figure adapted from Tu and Daniel (Tu and Daniel, 2004a).

Electromyographic measurements
We used electromyographic (EMG) measurements to quantify the timing and any phase delays in activation of the five subunits. This allowed us to examine whether there are any clear neural correlates in the timing of muscle contraction peaks at different temperatures. EMGs were recorded from tethered moths that had sustained wing beating for at least 10 min. Regional EMG timing measurements were accomplished with one electrode fixed in the dorsal-most subunit, DLM1c, and a second electrode attached to a micromanipulator such that it could be lowered through the DLM1. This allowed us to evaluate the variation in activation timing between simultaneous signals from any DLM1 subunit and from DLM1c (N=10 moths, ~20 spikes per moth). In addition, a thermocouple was attached to the adjustable electrode to measure concurrent temperature profiles.

The electrodes were made of insulated insect pins soldered to 0.051 mm diameter stainless steel wires, insulated with Teflon® to a diameter of 0.114 mm (A-M Systems, Sequim, WA, USA). The stationary electrode was placed in DLM1c to the right of the midline. The adjustable electrode was then inserted posterior to the stationary electrode. A common reference wire was inserted into the abdomen (Fig. 1A). The signals were amplified (×1000) with a differential AC amplifier (model 1800, A-M Systems) and band-pass filtered (300–20kHz).

EMGs were analyzed using custom peak detection software in MATLAB (The MathWorks, Natick, MA, USA) developed by M. S. Tu (University of Washington, Seattle, WA, USA) to determine the relative time difference of paired muscle subunits for which there were concurrent recordings.

Data acquisition
Muscle temperature, probe position, force measurements and extracellular evoked potentials were sampled at 5000 Hz with a data acquisition system (USB-1408FS, Measurement Computing, Norton, MA, USA).

Statistical analysis
Variables, such as rise and fall time, were first averaged across trials to give means for each individual. Results and statistical tests for each experimental condition are reported as means across individuals. To evaluate the temperature-dependent response of these variables and the differences among subunit groups, we used ANOVA and Tukey–Kramer honestly significant difference (HSD) tests. Because we cannot assume the normality of our data, we confirmed our ANOVA with nonparametric Wilcoxon tests. Results from these tests did not lead to conflicting statistical conclusions. Data are presented as means ± s.e.m. unless otherwise indicated.

RESULTS
Temperature profiles
The spatial patterns of temperature throughout the DLM1 were recorded every minute that a moth flew, starting with the initiation of low-amplitude wing movement to encompass the warm-up period. At least 10 min of flight were required for analyses. Mean ambient temperature during flight trials was 21.3±0.6°C (s.d.). All trials showed a significant temperature gradient in the dorso-ventral direction (Fig. 2A,B). When calculating the mean temperature of each subunit across all individuals, we excluded the first 5 min of warm-up flight. DLM1e (the dorsal-most subunit) was the coolest; on average only 2.4±0.6°C above ambient temperature. Each subunit was progressively warmer in the ventral direction, with DLM1c 8.0±0.7°C above ambient temperature (ANOVA, P<0.0001; Fig. 2B). Though each neighboring subunit did not differ statistically, DLM1c was statistically different from DLM1d (Tukey–Kramer HSD, P<0.05). The two most extreme subunits, DLM1a and DLM1e, had a mean temperature difference of 5.6±0.3°C, with a maximum temperature difference of 8.3°C (t-test, P<0.0001).

Similar to the trials without dorsal scales, moths with intact scales maintained a significant mean temperature difference of 6±1°C between the outer DLM1 after a 5 min warm-up period (t-test, P<0.01). However, the mean temperature of each subunit was elevated in comparison to the scale-removed trial. The mean temperature difference of DLM1e and DLM1a from ambient temperature was 8±1°C and 15±1°C, respectively. The dorsal scales were then removed and the moths were induced to fly again. In these trials, moths maintained a slightly lower temperature difference between the outermost DLM1 subunits of 5±1°C (t-test, P<0.05).

Contractile rates
The temperature dependence of muscle contractile rates was analyzed for the intact DLM1 and two groups of subunits: DLM1a,b
and DLM1c–e. Muscles were electrically stimulated to induce both single contractions and 25 Hz contractions.

For both rise times and fall times from 25 to 35°C, we found no statistical difference between our two subunit groups, DLM1c–e and DLM1a,b (ANOVA, P>0.05; Fig. 3 and Table 1). There was, however, a significant difference in the rate of relaxation between these two groups at 40°C (ANOVA, P<0.05).

Consistent with prior studies (Bennett, 1984; Josephson, 1984; Langfeld et al., 1989; Johnson and Johnston, 1990; Swoap et al., 1993), an increase in temperature led to significantly reduced contraction rise and fall times (ANOVA, P<0.05; Fig. 3 and Table 2). Although none of the increases in temperature led to a significant pairwise difference in rise and fall times, we did find statistically significant differences across trials separated by 10°C or more (Tukey–Kramer HSD, P<0.05). From 25 to 35°C, the mean rise times of DLM1c–e and DLM1a,b combined decreased by 31.9% whereas mean fall times decreased by 36.6%.

In all three experimental groups, muscles subject to the wingbeat stimulation frequency of 25 Hz exhibited full relaxation between subsequent contractions at the warmer temperatures. In contrast, cooler muscles, with their reduced contraction rates, contracted with subsequent contractions at the warmer temperatures. In contrast, stimulation frequency of 25 Hz exhibited full relaxation between subsequent contractions at the warmer temperatures. In contrast, stimulation frequency of 25 Hz exhibited full relaxation between subsequent contractions at the warmer temperatures.

**DISCUSSION**

Several important results emerge from our study on temperature gradients and their functional consequences. First, metabolic heat production paired with heat-loss mechanisms necessarily leads to a substantial temperature gradient in the flight muscles of *M. sexta*. Second, given our measurements of force and electrical activity, the DLM1 do not appear to be regionally specialized. Third,
contractile performance of the DLM1 subunits showed a temperature-dependent response consistent with prior studies (Bennett, 1984; Johnson and Johnston, 1990; Rall and Woldedge, 1990). Combined, these results indicate that a temperature gradient will yield a functional gradient in the time course of force output of flight muscle, suggesting that a mechanical energy gradient is a direct consequence of a thermal energy gradient. Below, we elaborate on the consequences of this temperature gradient and its implications in the production and storage of energy in the musculoskeletal system.

**Significant in vivo temperature gradient**

Results from our spatial and temporal temperature measurements during tethered flight show a strong temperature gradient in the dorso-ventral direction. Regardless of the amount of scales covering the thorax, we saw a mean temperature difference of ~6°C across a mere 5 mm of muscle. This surprisingly large gradient across such a small spatial scale should occur in both tethered and free flight because metabolic heat production and convective and radiative heat loss are processes that would occur in both flight regimes. Convective heat loss in tethered and free-flying animals should be similar, as wing motions in tethered flight induce a significant local flow that is close to those associated with free flight (Sane and Jacobson, 2006). Moreover, because free-flight thoracic temperatures have been recorded at ~41°C, compared with tethered flight temperatures at 30–35°C, we might expect an even larger, more functionally significant gradient to occur in natural flight (Heinrich, 1971). Thus, temperature gradients throughout the dominant flight muscles may be common occurrence for a wide range of large insects able to elevate their core temperature, spanning numerous orders (e.g. Lepidoptera, Orthoptera, Hymenoptera and Coleoptera). Indeed, as we discuss below, temperature gradients in muscle may be more ubiquitous than previously thought.

**No detectable regional specialization in contractile dynamics and neural activation**

We found no evidence that the DLM1 subunits employ varying regional twitch characteristics. Our isometric contraction tests did not reveal any significant difference between the outermost DLM1 in their temperature dependence of twitch timing and contraction dynamics. The temperature dependence we observed was comparable to that in previous studies; both the rise and fall time decreased as temperature was increased (Bennett, 1984; Josephson, 1984; Rall and Woldedge, 1990; Marden, 1995). The rise and fall times of the intact DLM1 group had 40% values of 1.41 and 1.74, respectively, across a temperature range of 25–35°C (Bennett, 1984). It is also important to note that although the greatest temperature difference of ~6°C occurred between DLM1e and DLM1g, there was still a ~4°C difference between DLM1g and DLM1. Though our studies did not show statistical differences for trials separated by 5°C, Josephson (Josephson, 1984) found that the rise and fall times of the mesothoracic first tergocoxal muscle of *Neoconecephalus robustus* decreased by ~30 and 20%, respectively, from 25 to 30°C. In addition, our data show that the mean time of peak force for contractions at 25 Hz occurred ~21% earlier in the cycle when temperature increased from 25 to 30°C. This indicates that temperature differences of ~5°C could have significant functional consequences. Combined with our observation of decreasing temperature associated with progressively more fused contractions at 25 Hz, it is likely that the cooler muscles undergo reduced cross-bridge cycling and thus have diminished mechanical work output. At the highest temperature, 40°C, we did observe a modest difference between the ventral and dorsal subunits; however, these subunit differences were much smaller than the effect of varying temperature regimes (Fig. 3 and Fig. 4B). In addition, our measurement of separate DLM1 subunit evoked potentials showed only a minor difference, 0.22 ms, in activation times. Although this difference was statistically different from zero, the greater delays in the time of peak force during 25 Hz contractions associated with decreased temperatures indicate that it will not result in a functional difference (Fig. 4).

Given the constraints of our experimental preparation, isolation and stimulation of individual muscle subunits was not easily achieved. Instead we chose to resolve the regional twitch dynamics at the spatial scale of two to three subunits. It is possible that individual subunit differences could be masked by more dominant muscles. However, given our data, we can claim that the more dorsal and the more ventral DLM1, which have different mean operating temperatures, exhibit similar temperature-dependent responses in terms of contractile dynamics.

**Temperature gradients induce mechanical gradients**

Because there was no significant difference in twitch timing between muscle subunits, any spatial variation in contractile dynamics will largely be a consequence of spatial variation in temperature. Decreased contraction rates and unfused tetany at cooler temperatures suggests that dorsal subunits will undergo a

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**Table 2.** Mean rise and fall times recorded from single isometric contractions at different temperatures for three *M. sexta* muscle preparations: (1) intact DLM1 comprised of all five subunits, (2) the dorsal-most subunits (DLM1c–e) and (3) the ventral-most subunits (DLM1a,b)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Rise time (ms)</th>
<th>Fall time (ms)</th>
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<tbody>
<tr>
<td></td>
<td>Intact DLM1</td>
<td>DLM1c–e</td>
</tr>
<tr>
<td>25</td>
<td>16.49±0.17</td>
<td>19.26±0.80</td>
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<tr>
<td>30</td>
<td>13.44±1.01</td>
<td>13.90±0.83</td>
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<tr>
<td>35</td>
<td>11.68±0.39</td>
<td>12.50±0.97</td>
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<tr>
<td>40</td>
<td>10.15±0.35</td>
<td>10.93±0.66</td>
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Values are reported as means ± s.e.m. (N=5 moths per group).
substantially smaller length change than the warmer, ventral subunits. Therefore, the dorsal-most subunits of the DLM1 may produce significantly less mechanical power, and thus could serve a primary function other than that associated with direct wing movement. Interestingly, a previous study has shown that body temperature in *M. sexta* must be ~32°C for take-off and ~29°C for horizontal flight (McCrea and Heath, 1971). This observation is consistent with our findings that unfused tetany occurred at temperatures of ~25°C, indicating that there may be insufficient power output at these temperatures for the required locomotor performance. Stevenson and Josephson have already demonstrated a strong relationship between muscle temperature and power output in *M. sexta* (Stevenson and Josephson, 1990): from 40 to 20°C, mean maximal power output decreased by ~70 W kg⁻¹. In that study, an optimal phase of activation was used to produce maximal power output; therefore, all temperatures led to positive power output. However, Tu and Daniel found that surprisingly few phases of activation generate positive mechanical power output, with the *in vivo* phase and length change generating only 40–67% of the maximal realizable power output (Tu and Daniel, 2004b). Therefore, it is highly possible that cooler, dorsal subunits may produce close to zero or negative power output.

The functional consequences of a temperature-induced mechanical energy gradient could have significant effects on locomotor performance. Because temperature modulates function, a temperature gradient suggests that the DLM1 may have multiple functions. The consequences of such regional functional specialization for power output in the DLM1 are not yet known. We suggest that while ventral subunits are the main power generators, depressing the wings, more dorsal subunits exhibit progressively reduced power output following the decrease in temperature. Thus, cooler subunits could operate with: (1) a reduced but still positive power output; (2) near zero power output, allowing them to behave as springs; or (3) negative power output, thereby acting as dampers on the system. The notion that dorsal subunits could behave as springs that, at the end of the contraction, act in concert with the dorsoventral muscles to elevate the wings is consistent with a prior study on asynchronous muscles (Dickinson et al., 2005). Thus, although it is generally presumed that the rubber-like protein within the cuticle, resilin, is the main site of energy storage for insect flight (Gosline et al., 2002), recent research on asynchronous muscle in *Drosophila* suggests that a significant amount of energy storage resides in the myofilaments and cross-bridges themselves (Dickinson and Lighton, 1995; Dickinson et al., 2005). Cooler, dorsal subunits operating with zero power output suggests yet another method by which energy production and storage could be regulated. The dorsal

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**Fig. 4.** (A) Force plots of 25 Hz isometric contractions at 25, 30, 35 and 40°C. The sequences shown are from an individual moth with intact DLM1. At 40°C the muscles fully relaxed before the next nerve impulse. As muscle temperature decreased, contraction rates also decreased. As a result, DLM1 at 25°C were unable to completely relax between contractions, resulting in unfused tetany. To determine how the different subunits respond to temperature at 25°C, we compared the time at which peak force occurred in relation to the contraction cycle (a, peak of 40°C; b, peak of 25°C). (B) In all three DLM1 groups, peak force occurred significantly later in the contraction cycle as muscle temperature decreased (ANOVA, *P*<0.0001; Tukey-Kramer HSD, *P*<0.05). There was no statistical difference between the three groups from 25 to 35°C (ANOVA, *P*>0.15; Table 1). Values are reported as means ± s.e.m. (*N*=5 moths per group).
DLM₁ may serve as an elastic restoring force on the cuticle, with cross-bridges remaining, on average, more attached to the thin filaments and potentially serving a spring-like function. Potential mechanical energy stored in the cross-bridges from elastic deformation could be released as kinetic energy during the second phase of the wingbeat cycle. Thus, the cooler subunits would reduce the work required to elevate the wings and enhance the efficiency of flight. The extent to which the power output profile advantageously benefits from the induced temperature gradient warrants further examination. Ultimately, these temperature-induced functional differences propose a flight system that is capable of adjusting the energetic input and output on multiple levels.

Importantly, because muscles generate heat and experience convective and radiative cooling at the surface, muscle temperature gradients in a wide range of moving animals may be more prevalent than previously assumed. Though there are surprisingly few instances documenting temperature gradients in the literature, temperature differences of 3–5°C and 10°C have been reported in mammalian quadricep muscles (Jones et al., 2004) and throughout the body of big-eye tuna (Carey and Teal, 1966), respectively. Although regional contractile performance was not evaluated in these cases, mammals and fish are known to experience increased contractile rates with increases in temperature (Bennett, 1984). It is therefore reasonable to assume that a functional gradient could follow the temperature gradient in these organisms. Thus, the presence of an induced functional gradient could have profound implications for our understanding of energy storage and production in the musculoskeletal system.

LIST OF ABBREVIATIONS

DLM₁ dorsolongitudinal muscles
DLM₁a,b ventral-most subunits
DLM₁c-e dorsal-most subunits
EMG electromyographic

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