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Physiological properties of the dorsal longitudinal flight muscle and the tergal depressor of the trochanter muscle of *Drosophila melanogaster*

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Summary

A prerequisite for using muscle mutants to study contraction in *Drosophila melanogaster* is a description of the mechanics of wild-type muscles. Here we describe the mechanics of two different wild-type muscles; the dorsal longitudinal flight muscle which is asynchronous (nerve impulses are not synchronised with each contraction), and a leg muscle, the tergal depressor of the trochanter, which is synchronous. We have compared their mechanics to those of the asynchronous flight and the synchronous leg muscle from the giant waterbug *Lethocerus indicus*.

We found that the mechanics of the asynchronous flight muscles from the two species were similar. At rest length both muscles had a high relaxed stiffness, were partially activated by Ca^{2+} (low steady-state active tension) and, once activated, had a large delayed increase in tension, which was well maintained, in response to a rapid stretch. The rate constant for the delayed increase in tension was about 10 times greater for *D. melanogaster* than for *L. indicus* under the same conditions. The mechanics of the synchronous leg muscles from both species were different from those of the flight muscles and resembled those of other synchronous muscles such as vertebrate striated muscle. At rest length, both muscles had a lower relaxed stiffness than the flight muscles, were fully activated by Ca^{2+} (high steady-state active tension) and, once activated, had a small delayed increase in tension, which was less well maintained, in response to a rapid stretch. The rate constant for the delayed increase in tension was similar for the leg muscles of both species.

The different mechanical properties of the flight and leg muscles must arise from differences in their contractile proteins. The demonstration that satisfactory mechanical responses can be obtained from the small (less than 1 mm long) muscles of *D. melanogaster* will enable future responses from mutant muscles to be tested.

Introduction

Changes in protein structure, produced by genetic and protein engineering techniques, give information about the relationship between the structure and function of that protein. The application of this approach to muscle contraction requires a species whose genetics are well known, whose genes are readily manipulated, and whose muscles can be analysed by conventional structural and functional techniques, including mechanical recording. The two organisms in which numerous mutants affecting muscle structure and function have been produced are the nematode *Caenorhabditis elegans* and the fruitfly *Drosophila melanogaster*. Considerable progress has been made using genetic approaches in understanding the properties of the major proteins from the nematode (Karn *et al.*, 1983; Waterston & Francis, 1985). However, a limitation of this work is that the muscles are too small for mechanical experiments. In contrast, the muscles of the fruitfly, while considerably smaller than the muscles normally used in investigations of the mechanism of contraction in muscle, are large enough for mechanical studies (Molloy *et al.*, 1987).

To determine the effect of mutations on the mechanics of muscles from the fruitfly, the mechanics of the normal or wild-type muscle must first be determined. The aim of this paper is to describe the mechanical properties of two types of striated muscle found in *D. melanogaster*, the indirect flight muscle (dorsal longitudinal muscle, or DLM) and the leg (tergal depressor of the trochanter, or TDT) muscle. The flight muscle is asynchronous; its nerve input occurs at a much lower frequency than the wingbeat frequency of 200 Hz (Levine, 1973; Levine & Wyman, 1973). It is also fibrillar, implying a low sarcoplasmic reticulum content (Pak & Grabowski, 1978). Study of the structure at the electron microscope level (Shafiq, 1963 a,b; 1964) shows that the myofibrils are striated with a sarcomere length of about 3.4 μm and, in common with other asynchronous flight muscles including those of *Lethocerus indicus*, the I-band is extremely short. The leg

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muscle is probably synchronous: the timing of the mechanical contractions is determined by the nervous input, and non-fibrillar: the fibres have a dense sarcoplasmic reticulum.

The mechanics of the flight muscle from *D. melanogaster* are expected to be similar to those of other asynchronous flight muscles, of which the best studied is that from the genus *Lethocerus* (Pringle, 1957, 1978; White & Thorson, 1974; Tregear, 1977, 1983). Asynchronous muscles are only partially activated by calcium ions, in that both tension and ATPase activity are submaximal. Further activation (of tension and ATPase activity) is produced when the fibres are stretched by strains of the order of 3% above their rest length (stretch-activation). The muscles can also be fully activated, and produce mechanical work output, when the length of the activated fibres is sinusoidally oscillated over a restricted frequency range (Steiger & Rüegg, 1969; Lund *et al.*, 1988). Over this range of frequencies tension is delayed with respect to length; the rate constant of the delayed change in tension (r3) is related to the frequency (F) at which maximum work is produced during sinusoidal oscillations by the relationship $r3 = 2\pi F$. We have investigated the active tension responses of flight muscle from *D. melanogaster* in response to sinusoidal oscillations and to rapid changes of length and compared these responses to those from the flight muscle of *L. indicus*.

The mechanics of the leg muscles of *D. melanogaster* are expected to be different to those of the asynchronous flight muscles and more like those of other synchronous muscles, including vertebrate striated muscle. Such muscles are fully activated by calcium, and have isometric tension and ATPase activity whose dependence upon muscle length depends solely on filament overlap (that is, does not show strain activation in the way described above for fibrillar muscle), nor do they show increase in ATPase activity with length oscillation (Kawai *et al.*, 1987). Although delayed changes in tension and oscillatory work in response to changes of length are seen in synchronous muscles, the delayed tension is of smaller magnitude relative to isometric tension, and the power output obtainable from the muscles is smaller than with fibrillar muscle. We do not know of any mechanical studies on insect leg muscles of the type described here, so we also investigated the mechanical properties of the leg muscle fibres from *L. indicus*.

Materials and methods

Fibre Preparation

Flight and leg muscles were used from either the fruit fly (*Drosophila melanogaster*) or the giant water bug (*Lethocerus indicus*). *D. melanogaster* were of an inbred wild-type strain, *Oregon*, cultured at 25 °C on a yeast-agar-sucrose medium (Carpenter, 1950) in 3 × 1 inch glass vials. *L. indicus* were obtained from Dr R. Sanit from Thailand, kept in water tanks and fed with unlimited supplies of goldfish.

The flight muscle used from *D. melanogaster* was the dorsal longitudinal muscle (DLM) and the leg muscle used was the tergal depressor of the trochanter (TDT). There are twelve dorsal longitudinal flight muscle fibres, arranged in two columns of six fibres, one column in each half thorax (Fig. 1A; Williams & Williams, 1943; Levine & Hughes, 1973). The DLM fibres nearest the ventral surface of the fly are the longest (about 1 mm long). All the DLM fibres are about 100 μm in diameter. There are two TDT muscles, one on each side of the thorax, which are about 1 mm in length (Fig. 1B; O'Donnell *et al.*, 1989).

To prepare the muscles from *D. melanogaster*, the flies were first anaesthetised either with diethyl-ether or with CO_2, and placed in a narrow Plasticine (modelling clay) channel with the dorsal thorax uppermost. Each fly was fixed in place by embedding the wings in the Plasticine. The head and abdomen were removed and a longitudinal slit was made along the dorsal midline of the thorax using an electrolytically sharpened tungsten needle. The thorax was cut free from the wings with microdissection scissors, transferred to a small dissection dish containing a 50% glycerol solution (see below) at 0 °C, and

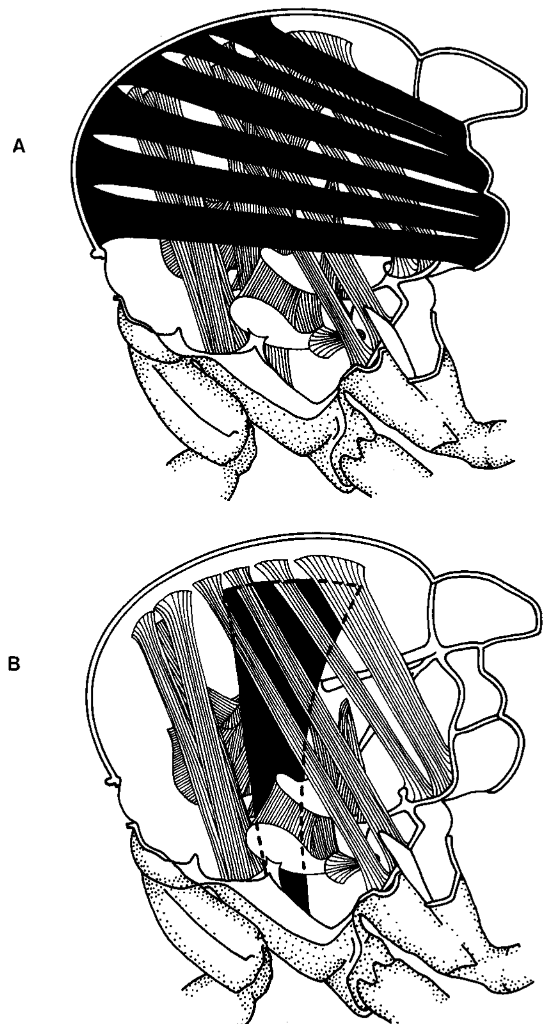

Fig. 1. Positions of (A) the dorsal longitudinal (DLM) muscles and (B) the tergal depressor of the trochanter muscle (TDT) in the thorax of *Drosophila melanogaster*.

completely cut in half longitudinally by cutting through the ventral surface of the thorax with the scissors. The muscles of the thorax were then glycerol extracted, still attached to the thorax.

The glycerol extraction solution contained: glycerol, 50% (v/v); potassium phosphate buffer, 20 mM; sodium azide, 1 mM; dithiothreitol (DTT), 1 mM; $MgCl_2$, 2 mM; pH 7.0. It was similar to that used previously (White & Thorson, 1972) except that DTT was added to reduce oxidation of sulfhydryl groups, magnesium ions were added to reduce enzymatic degradation of the myosin light chains (Weeds & Pope, 1977) and sodium azide was added to reduce bacterial contamination.

The dissected thoraces were kept immersed in the glycerol extraction solution at 0°C. The solution was stirred continuously and changed after 1 and 6 h. After 24 h, the thoraces were transferred to fresh solution and stored at − 20°C. The fibres were used after 2 days and within 2 weeks of extraction.

Fibres from the DLM were cut out of the thorax just before the experiment, as close to the cuticle as possible, and were pared down to a diameter of about 70 μm, using electrolytically sharpened needles. The TDT muscle was exposed after removing both the dorsal longitudinal and dorsal ventral flight muscles. It was removed from the thorax still attached to a piece of cuticle from the dorsal surface of the thorax at one end, and to the apodeme of the leg at the other end. Either the whole muscle was used, or the muscle on one side of the apodeme was removed and the remaining half was used.

Two different muscles from *L. indicus* were also used: the dorsal longitudinal muscle (DLM) and a metathoracic leg muscle, the trochanter levator. The thorax was prepared as previously described for the dorsal longitudinal muscles (Jewell & Rüegg, 1966; Barber & Pringle, 1966) except that the legs were not removed so that the leg muscle could also be used. The glycerol extraction procedure and solution were as described above. Single fibres were removed from the preparation by cutting close to the cuticle with scissors. The fibres were used after 5 days and within 2 months of extraction.

Following dissection, both ends of the fibres were crimped in aluminium T-clips (Goldman & Simmons, 1984) which had been made individually from aluminium kitchen foil rather than being photoetched. T-clips which had been made this way are stronger and do not tend to have the rough edges of photoetched T-clips, so are less likely to damage the fibre at the point where it emerges from the T-clip (during the crimping procedure). A sharp scalpel blade was used to make a sequence of cuts (Fig. 2) into foil stuck to a microscope slide with Araldite or cellulose acetate and the finished T-clips were removed by sliding the tip of the scalpel blade under the foil.

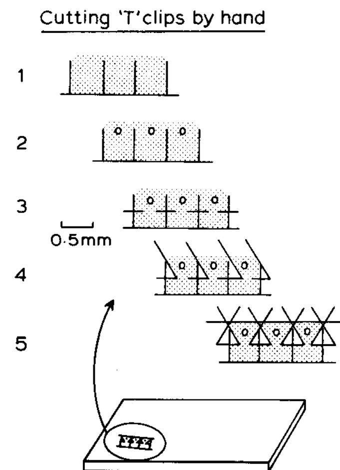

Fig. 2. Method of cutting T-clips from aluminium foil glued to a glass surface. Numbers indicate sequence of cuts into the foil.

Experimental Solutions

Fibres were transferred from the glycerol skinning solution to the filled experimental muscle bath on a small (1 mm × 2 mm) spatula. Bathing solutions were changed by means of two narrow gauge tubes (feed and drain), allowing the fibres to be completely immersed at all times. The compositions of the experimental solutions, based on those used previously (White & Thorson, 1972), are shown in Table 1.

Mechanical Apparatus

The mechanical apparatus was similar in concept to that used previously in single-fibre experiments (Ford, *et al.*, 1977; White, 1983). The fibre was mounted between two small tungsten hooks which had been electrolytically etched to a fine point. One of the hooks was attached to a tension tranducer (Akers AE801, SensoNor, Norway) via a short length of glass capillary, and the other to the moving arm of a servo-controlled motor (the galvanometer motor of Ford, *et al.*, 1977). The hooks were inserted into a temperature-controlled muscle bath through slits in the side of the bath.

The sensitivity of the transducer was 8 μN/mV with a noise level of about 2 μN. The natural resonant frequency of the undamped tension transducer, with a muscle fibre attached, was 8.3 kHz. The mechanical damping was adjusted to give critical damping. The sensitivity to movement of the motor arm was 100 μm/V with a noise level of 0.14 μm.

Data Acquisition

Both transient and sinusoidal analysis were used to determine the mechanical properties of the fibres.

i. Transient analysis

For the transient analysis motor arm position was driven with a ramp change of length of 200 μs duration, generated by means

Table 1. Solution Compositions

Solution	*Relaxing*	*Rigor*	*Activating*	*Activating + Pi*
Constituent: (mM)				
KCl	12	50	12	—
$MgCl_2$	14	12	14	14
EGTA	5	5	—	—
CaEGTA	—	—	5	5
ATP	15	—	15	15
Histidine	20	20	20	—
PO_4	—	—	—	10

All had a pH of 7.0

of an IBM PC microcomputer. The resulting output from the motor arm is shown in the inset to Fig. 5; the ramp is sigmoidal in shape with a central linear region. The length and tension output signals were input to a digital oscilloscope (Tektronix, Type 5223), data from which was collected, stored and analysed by an IBM PC. An IEEE-488 bus (Central Equipment Corporation, GPIB board) enabled parallel data transfer between the oscilloscope and the IBM PC, which was also fitted with an interface card (Tecmar, Labmaster) containing the necessary timers and digital-to-analogue converter to drive the motor and trigger the oscilloscope.

The control programs for the IBM PC were written in BASIC, with machine code routines for driving the apparatus and transfering, storing and plotting the data rapidly (Drew, 1984, with many additions).

The response of tension to a step change in length was analysed as a sum of up to four exponentials, using the curve-fitting program DISCRETE (Provencher, 1976 a,b).

The instantaneous stiffness of the fibre in any given solution was found by plotting tension against length during the ramp, and determining the slope at the steepest part of the curve. By choosing a suitably fast time-base on the digital oscilloscope the sampling rate during the ramp can be set to any desired value; typically about 100 points were sampled. The value of stiffness is taken from the linear part of the applied length change.

ii. Sinusoidal analysis

For sinusoidal analysis the length signal was derived from, and analysis of the resulting tension performed by, a Transfer Function Analyser (Solatron Type JM 1600). The tension signals were corrected for the performance of the motor. The data is presented in the form of Nyquist plots. The peak-to-peak amplitude of the length changes used in these experiments was 0.5%.

The sinusoidal length and tension traces could also be fed into the digital oscilloscope, and from there to the IBM PC for further analysis. This procedure was used to obtain traces of tension versus length, in order to determine the power output of the fibres, from the area of the length-tension loops.

Fibre Area and estimation of thick-filament numbers

Force measurements were normalised to enable comparison between different muscles by dividing the force by an estimate of the number of thick (A, or myosin-containing) filaments per fibre cross-section. The way in which this estimate was made was different for each type of muscle.

For the DLM from *L. indicus*, whole fibres were always used. Counts from transverse sections from five bugs gave values of 175 ± 7 myofibrils per fibre and 3359 ± 126 A-filaments per myofibril (means $\pm$ SEM), making an average of 5.89×10^5 A-filaments per fibre. These figures are similar to those obtained by Chaplain and Tregear (1966) for a related similar sized waterbug (*L. cordofanus*), vis: approximately 180 myofibrills per fibre and 3520 A-filaments per myofibril (6.34×10^5 A-filaments per fibre).

For the DLM of *D. melanogaster*, each fibre was fixed and sectioned for electron microscopy. The mean number of thick filaments per myofibril estimated for a minimum of 3 myofibrils for each fibre (899 ± 11, $n = 10$; mean $\pm$ SEM, n is the number of fibres) was multiplied by the total number of myofibrils in the preparation to give an estimate of the number of thick filaments per fibre.

For the TDT muscle from *D. melanogaster*, either half or a whole muscle was used. This is a tubular muscle, which is pennate and has a maximum of 32 fibres at the point where the muscle branches away from the apodeme (Fig. 3a). Four fibres have an area of about 250 μm^2, and the remaining 28 fibres have a larger area of about 550 μm^2. Serial transverse sections made every 100 to 150 μm along the length of the muscle showed that the cross-sectional area of the muscle was roughly constant between the end of the apodeme and the dorsal cuticle (9.4×10^3 μm^2). This is the portion of the fibre which is generally held free between the T-clips. Since the myofibrillar size was somewhat variable, as was the number of myofibrils in different fibres (Fig. 3b, c), the total number of thick filaments was estimated differently from that of the flight muscle. Thick filament content was estimated from the number of thick filaments per square micron (827 ± 50, mean $\pm$ SEM, for 13 fibres, Fig. 3), and the fractional area of the muscle occupied by myofibrils (63% $\pm$ 6 SEM, for 7 fibres) which gave the total area of myofibrils as 5.9×10^3 μm^2. This gave an estimate of 4.9×10^6 thick filaments in a whole TDT muscle or 2.45×10^6 in each half. Note that estimates of the thick filament content of a few averaged-sized myofibrils from the TDT (rectangular in shape, with dimensions of 2×0.6 μm) were similar to those of the DLM.

Finally, for the leg muscle of *L. indicus*, all the fibres were fixed and sectioned for electron microscopy. This muscle is tubular like the TDT, and the size of the myofibrils is variable. The number of thick filaments per fibre was estimated by measuring the total area of the preparation from transverse sections, from the number of A-filaments per μm^2 (662 ± 104; mean $\pm$ SEM for 5 fibres) and the percentage myofibrillar content (65% $\pm$ 5, for 5 fibres). Separate estimates were used for each fibre in this case.

Analysis of protein composition

For 1–D SDS gel electrophoresis of myofibrillar proteins, skinned myofibrils were extracted with sample buffer containing 10% glycerol, 2.3% SDS, 0.0625M Tris pH 6.8, 5% B-mercaptoethanol, 0.02% Bromophenol Blue for 4 min at 95°C. 8 μl samples were loaded on a 12.5% SDS-ployacrylamide minigel prepared according to the method of Hames and Rickwood (1982) and run at 12 mA for 90 min. The gel was stained with Coomassie Blue (in 25% propan-2-ol: 10% acetic acid) and destained in 10% acetic acid.

Results

MECHANICAL PROPERTIES OF THE FIBRES

Steady-state Tensions and 'Instantaneous' Stiffness in the active and rigor states

The mechanics of the asynchronous flight muscle from *D. melanogaster* and *L. indicus* were similar in relaxing (ATP present, Ca^{2+} absent), rigor (ATP absent) and activating (ATP and Ca^{2+}) conditions (Fig. 4, Tables 2 & 3). The steady state tension in activating conditions in the two flight muscles was low; that in activating conditions in the absence of P_i was about the same magnitude as that in

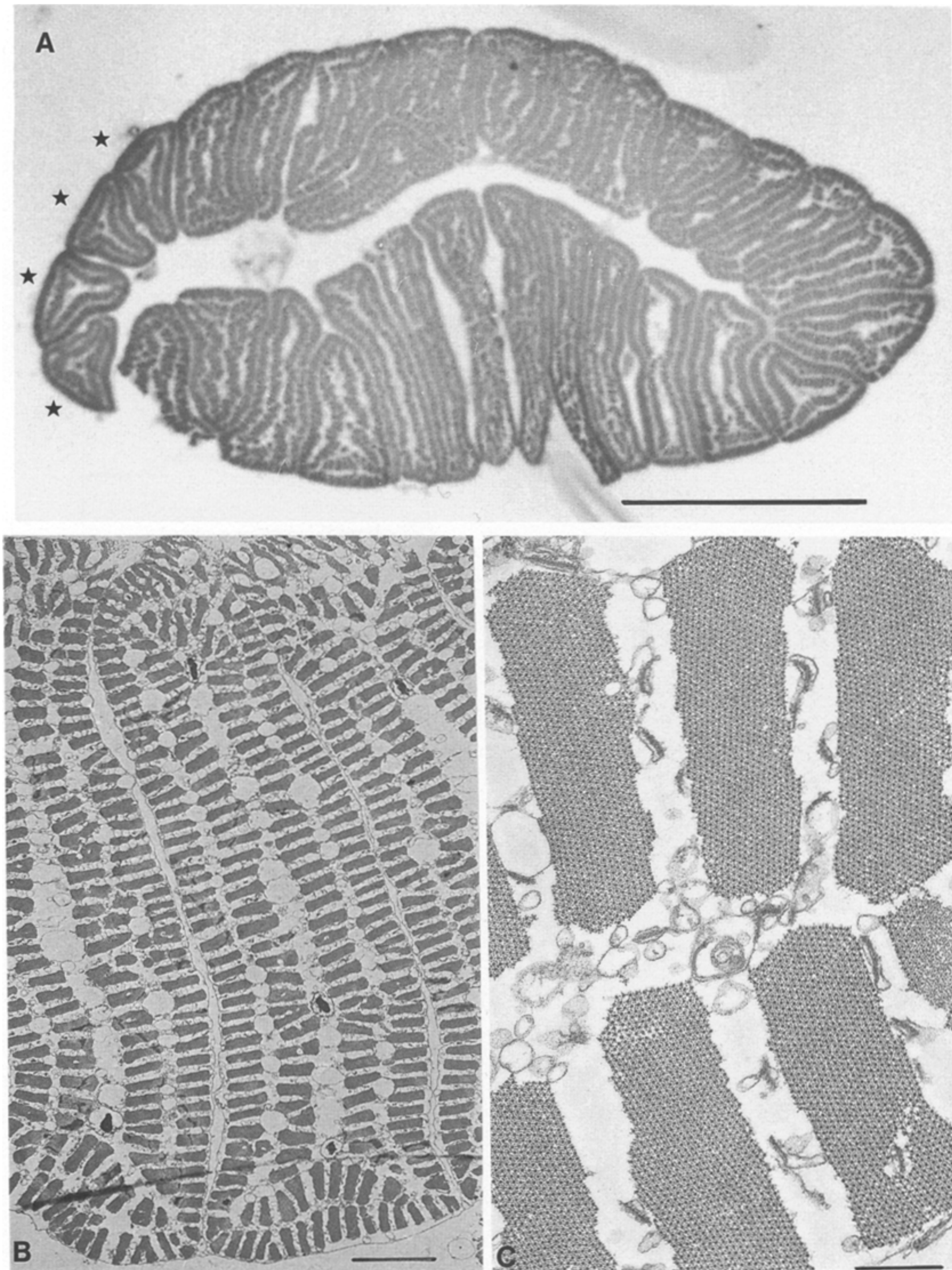

Fig. 3. Structure of skinned TDT muscle: (A) Light micrograph of a cross-section of a whole TDT muscle sectioned about half way along its length. The 4 smaller muscle fibres are marked with an asterisk. Scale bar represents 50 μm. (B) Low power electron micrograph of a TDT muscle to show the arrangement of myofibrils in a few of the 28 larger muscle fibres. Scale bar represents 5 μm. (C) High power electron micrograph of a TDT muscle to show the arrangement of myosin-containing filaments in a few myofibrils from one of the 28 larger muscle fibres. Scale bar represents 500 nm.

rigor conditions in both flight muscles. The active tension of the flight muscles was too low to determine its dependence on calcium concentration satisfactorily.

Both flight muscles had a high 'instantaneous stiffness' in relaxing conditions (Fig. 5, Table 4), as previously reported for asynchronous flight muscle (Machin & Pringle, 1959; Jewell & Rüegg, 1966). Subtraction of the relaxed stiffness to obtain the crossbridge contribution (White, 1983), increased the difference between active and rigor stiffness from three to six times. In general, force and

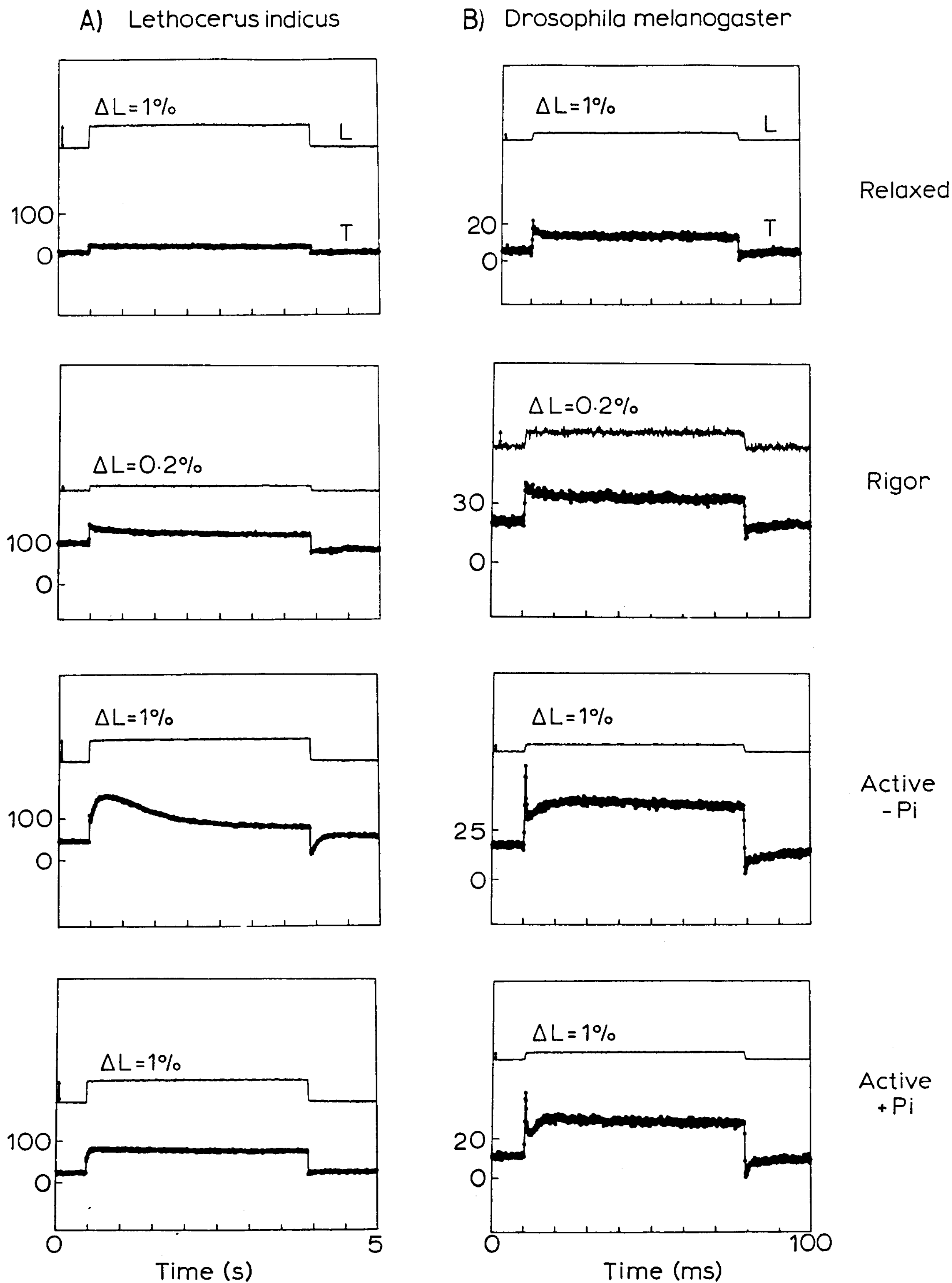

Fig. 4. Comparative responses of tension (lower traces, T) to a small rapid stretch (upper traces, L) in relaxing and rigor solutions and in activating solutions with and without added 10 mM phosphate. The amplitude of the stretch was 1% in relaxing and activating solutions, and 0.2% in the rigor solution. Responses are for the asynchronous flight muscles of (A) *L. indicus* and (B) *D. melanogaster*. Tension (T) units are pN/A-filament.

Table 2. Steady state tensions in activating and rigor solutions for the flight and leg muscles of *D. melanogaster* and *L. indicus* at rest length.

	Active			*Active (+ Pi)*			*Rigor*		
	Mean	*SEM*	*n*	*Mean*	*SEM*	*n*	*Mean*	*SEM*	*n*
D. melanogaster									
DLM	29	5.0	5	23	4.8	5	22	9.0	4
TDT	—	—	—	105	9.9	7	10	3.7	5
L. indicus									
DLM	59	6.9	4	32	3.8	4	60	11.1	4
LEG	—	—	—	156	28.4	5	14	7.0	4

Units are pN A-filament^{-1}. n = number of fibres.

Table 3. Instantaneous stiffnesses of flight and leg muscles of *D. melanogaster* and *L. indicus* in relaxing, active and rigor conditions.

	Relaxed			*Active (+ Pi)*			*Rigor*		
	Mean	*SEM*	*n*	*Mean*	*SEM*	*n*	*Mean*	*SEM*	*n*
D. melanogaster									
DLM	32	8.1	10	48	14.6	10	125	30	12
TDT	4.5	0.9	7	86	11.7	3	53	7.3	4
L. indicus									
DLM	88	9.3	8	186	33	8	573	98	8
LEG	16	4.4	4	201	63	3	166	44	4

Units are pN A-filament^{-1} %$^{-1}$.

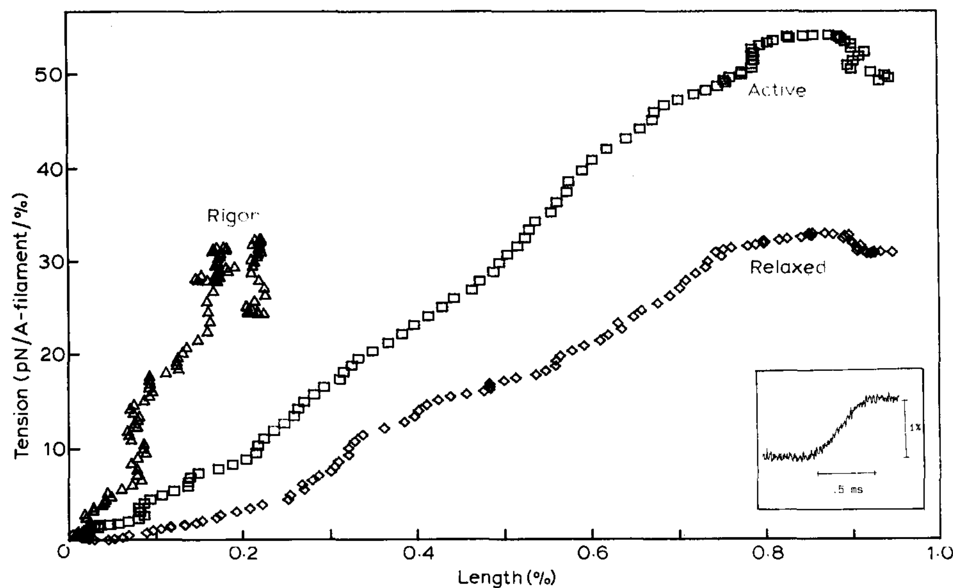

Fig. 5. 'Instantaneous stiffness' in *Drosophila* DLM, measured as described in the Materials and methods. The plots show change in tension during a rapidly applied 0.2% length step in rigor, and 1% length step in relaxing and activating solutions. The instantaneous stiffness is obtained from the slopes. The nonlinearity of the traces is mainly due to noise on the length and tension outputs. Inset: 1% length step vs. time for the step in relaxing solution as sampled. The points in the main figures were drawn from sampling length and tension at this rapid rate.

Table 4. Rate constants (r) and amplitudes (amp) of the delayed increase in tension (phase 3) and its recovery (phase 4) following a 1% stretch in activating solution with or without 10 mM Pi, for the flight and leg muscles of *D. melanogaster* and *L. indicus.*

		Non-Pi				*+ 10mM PI*			
		Phase 3 mean ± SEM (n)		*Phase 4* mean ± SEM (n)		*Phase 3* mean ± SEM (n)		*Phase 4* mean ± SEM (n)	
D. melanogaster									
DLM	r	257 ± 27	(13)	24 ± 3	(13)	478 ± 45	(12)	46 ± 5	(12)
	amp	108 ± 11		124 ± 17		96 ± 15		76 ± 12	
TDT	r	—		—		250 ± 32	(8)	20 ± 5	(7)
	amp					20 ± 4		5 ± 1	
L. indicus									
DLM	r	6.8 ± 0.9	(4)	0.8 ± 0.5	(4)	46.1 ± 8	(4)	3.9 ± 2	(4)
	amp	380 ± 63		330 ± 58		113 ± 21		30 ± 5	
Leg	r	—		—		208 ± 20	(8)	28 ± 7	(7)
	amp					33 ± 8		10 ± 2	

Units for the rate constants are s^{-1}, and for the amplitudes are percentage of the steady-state tension. n = number of fibres.

stiffness in rigor and activating solutions was lower in the flight muscles from *D. melanogaster* than in the flight muscles from *L. indicus.*

The mechanics of the synchronous leg muscles from the two species were similar in relaxing, rigor and activating conditions (Fig. 6; Tables 2 & 3) but different to those for the asynchronous flight muscles. The steady-state tensions in activating conditions were about three to five times higher than those of the asynchronous flight muscle from both species (Fig. 6; Table 2) and were also much greater than the steady-state tensions in rigor. They showed the characteristic sharp dependence on calcium concentration found for vertebrate striated muscle such as rabbit psoas (Fig. 7).

Both leg muscles had a low instantaneous stiffness in relaxing conditions, about 15% of that measured for the flight muscles (Table 3). The instantaneous stiffness in rigor conditions was about the same as in activating conditions. These results are in contrast to the results for the flight muscles described above.

Delayed tension response to a rapid step change in length

Activated fibres from asynchronous flight and synchronous leg muscle fibres from *D. melanogaster* and *L. indicus* all showed a delayed tension increase following a rapid stretch of 1% (Fig. 6). All the fibres were activated from a length in relaxing solution where the muscles was just taut (rest-length). In flight muscles, this delayed increase

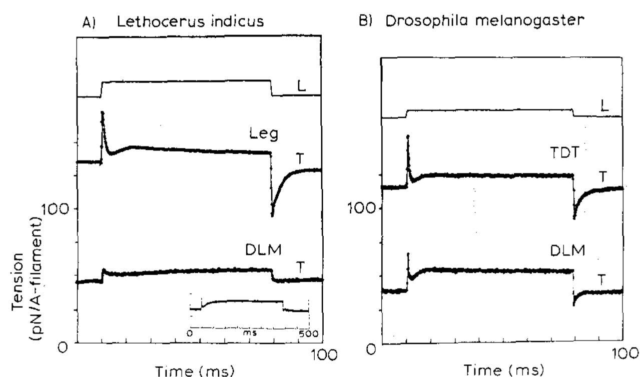

Fig. 6. Comparison of the tension responses to a small rapid stretch of 1% for the asynchronous flight (DLM) and the synchronous leg (TDT) muscles in activating solution containing 10 mM inorganic phosphate. (A) *L. indicus* and (B) *D. melanogaster.* All the responses are shown for the same timebase. Inset: response to a rapid stretch of 1% in the same activating solution for the asynchronous flight muscle from *L. indicus* on a slower time-base.

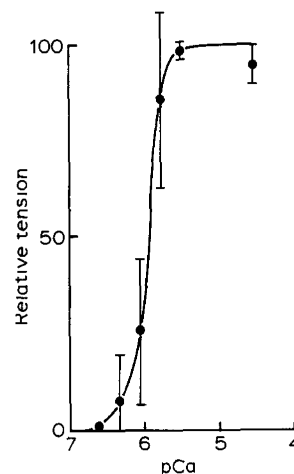

Fig. 7. Force-pCa relationship of TDT muscle from *D. melanogaster.* The results are the averages from three preparations. The error bars represent the standard error of the mean.

in tension was well maintained and large: the rate of recovery of tension to the level before the stretch (r4 in Table 4) was slow, and large in amplitude (A4 in Table 4): the increase in tension following a 1% stretch caused the overall tension to more than double in magnitude on average. In contrast, in the leg muscles this delayed increase in tension was less well maintained and small.

The rate constant of the delayed increase in tension (r3) is about 10 times higher for the flight muscles of *D. melanogaster* than for those of *L. indicus* (Table 4). Addition of phosphate to the activating solution increased this rate constant (r3) in both of the flight muscles, but its effect on the amplitude was more pronounced in *L. indicus* (Fig. 4; Table 3). The delayed-tension response in the leg muscle was only systematically investigated for a 1% length change in the presence of P_i and at full calcium-activation. Under these conditions the value of r3 was approximately the same in both leg muscles.

Sinusoidal Analysis

The ability of asynchronous flight muscles of *D. melanogaster* to perform oscillatory work in activating conditions was clearly shown by sinusoidal analysis experiments. The work output per cycle for five active flight muscles from *D. melanogaster*, in the presence of phosphate, at a temperature of 15°C was greatest at a frequency of 56 ± 11 Hz (mean ± SEM for peak-to-peak length oscillations of 0.5% of the muscle length). This was the frequency at which the in-phase stiffness had a maximum negative value as shown by a Nyquist plot of one of the fibres (Fig. 8). The shape of Nyquist plot for the flight muscles from *D. melanogaster* was similar to that reported for active *L. indicus* flight muscles (Jewell & Rüegg, 1966) except that the frequency for which the work output was greatest was about 10 times greater than in *L. indicus*.

The work output per cycle was determined from the area of the tension versus length loops obtained during sinusoidal oscillation of fibres (Fig. 9). The work per cycle is much less in *D. melanogaster*, in which the work loops

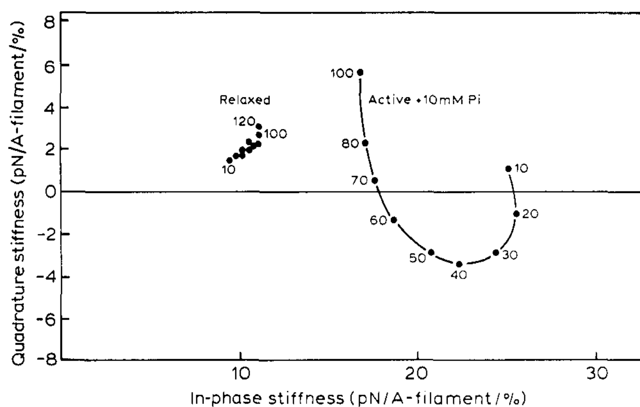

Fig. 8. Sinusoidal analysis using the Transfer Function Analyser (see Methods) of the tension response of *D, melanogaster* flight muscle (DLM) to an imposed length oscillation, of 0.5% peak-peak, plotted as a Nyquist Plot.

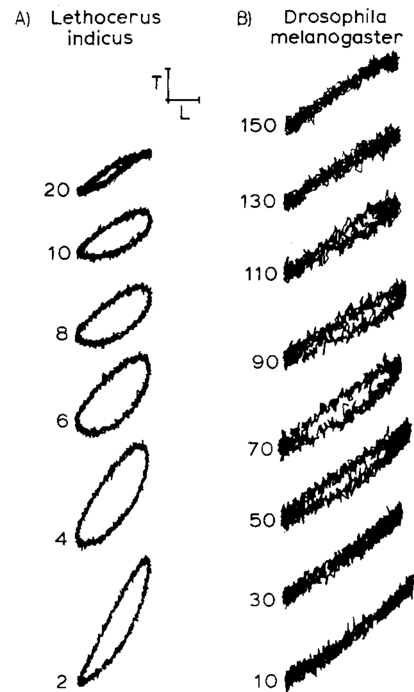

Fig. 9. Work loops (plots of tension versus length) at the stated different frequencies of oscillation (Hz) in activating solution + 10 mM inorganic phosphate for (A) *L. indicus* and (B) *D. melanogaster*. Calibration bars: (A) Tension (T), 30 pN per A-filament; Length (L) 0.2%, (B) Tension (T), 10pN per A-filament; Length (L) 0.2%.

are virtually closed, than in *L. indicus*, in which very open work loops can be obtained (Steiger & Rüegg, 1969; White & Thorson, 1972). However, in *D. melanogaster* the frequency of oscillation at which maximum power output was obtained was about ten times greater than that for *L. indicus*, resulting in similar power outputs per unit mass of the two muscles types. At the frequency of oscillation for which the work loops were most open, for length oscillations of 0.5% peak-to-peak, the power output for 3 fibres from *D. melanogaster* was 2.1 ± 0.4 fW/A-filament (Fig. 9), and for 3 fibres from *L. indicus* was 1.4 ± 0.5 fW/A-filament.

PROTEIN COMPOSITION OF THE FIBRES

SDS-PAGE gels (Fig. 10) of skinned fibres from flight muscles and leg muscles from *D. melanogaster* and *L. indicus* showed differences in proteins expected between fibrillar and non-fibrillar muscle. Identification of proteins in *L. indicus* flight muscles is taken directly from Bullard (1983). In *D. melanogaster* arthrin and troponin-H were present in the flight muscles but not in the leg muscles. The identity of these proteins has been confirmed by Western blotting similar gels (not shown) with monoclonal antibodies to actin and troponin-H respectively (Cripps & Sparrow, unpublished observations). All the muscles contained different amounts of a protein, which on molecular weight is believed to be paramyosin. It has been identified in this position on 1-D SDS-PAGE gels

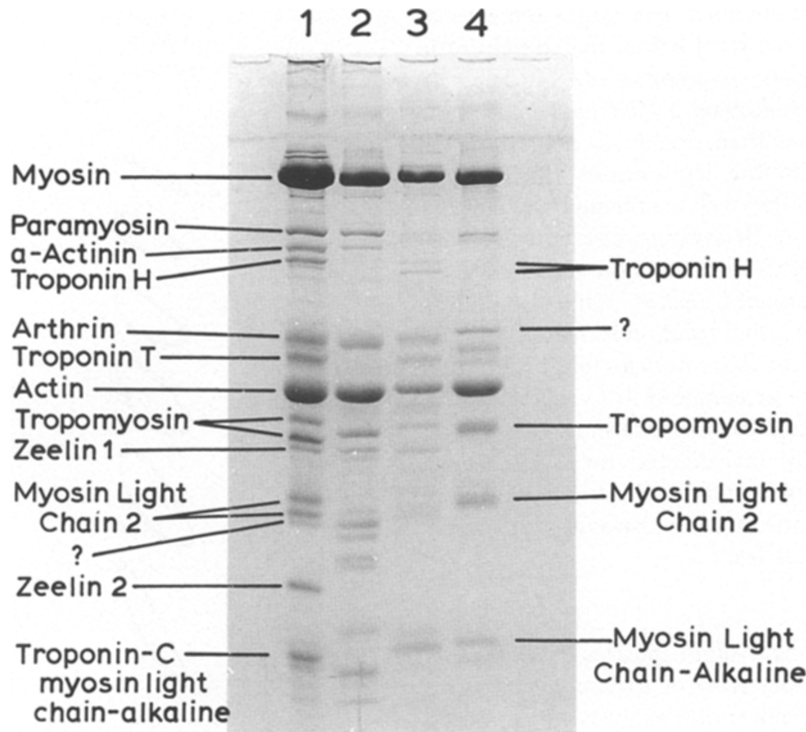

Fig. 10. SDS gels of myofibrils. (Lane 1) *L. indicus* flight muscle (DLM). (Lane 2) *L. indicus* leg muscle. (Lane 3) *D. melanogaster* flight muscle (DLM). (Lane 4) *D. melanogaster* leg (TDT) muscle. Identification of the bands is taken from Bullard (1983), Bullard *et al.* (1988) and Sparrow & Cripps (unpublished observations).

(Bullard, 1983). The light chains were identified by the presence of these bands in myosin isolated from myofibrillar preparations of both *D. melanogaster* and *L. indicus,* and tropomyosin in *D. melanogaster* muscles by its changed mobility in SDS-Urea gels compared to SDS gels (Cripps & Sparrow, unpublished observations).

Discussion

We have found that the mechanics of the asynchronous flight muscles of *D. melanogaster* are similar to those of *L. indicus*; they both have a high relaxed stiffness, are only partially activated by calcium (tension is low), and once activated, they have a delayed-tension response to a rapid stretch which is well maintained and of large amplitude relative to isometric tension. The rate constant of the delayed-tension response is about ten times faster in *D, melanogaster* than in *L. indicus* flight muscle under the same conditions. The mechanics of the synchronous leg muscles of *D. melanogaster* and *L. indicus* are similar but different from those of the asynchronous flight muscles. They have a lower relaxed stiffness than flight muscles, are fully activated by Ca^{2+} (tension is high) and once activated, they have a delayed-tension response to a rapid stretch which is of a small relative amplitude.

The different mechanical properties of the insect flight and leg muscles are related to their different functions. The flight muscles undergo uniform repetitive activity, producing their power from the low-amplitude oscillations of length which drive the wings in flight. Their ability to work asynchronously of nerve input, to produce significant power output at the flight frequency, depends on the intrinsic ability of the contractile proteins to produce large delayed-tension responses with the required rate constant; the faster rate constant of the delayed-tension response for the flight muscle of *D. melanogaster* is related to the faster wingbeat frequency of this insect. The wingbeat frequency of *D. melanogaster* is about 200 Hz (at the flight muscle temperature of 25°C) whereas that of *L. indicus* is 38 Hz (at the flight muscle temperature of 42°C; Molloy (1989).

The leg muscles undergo a much greater variety of magnitude and speed of movement. The TDT muscles are used for jumping in *D. melanogaster*, although it is possible that their use in this movement will be found to strain an elastic element as occurs in fleas (Bennett-Clarke & Lucey, 1967) and locusts (Bennett-Clarke, 1975). Although, in common with many other kinds of synchronous striated muscle (Abbott & Steiger 1977), they do have a delayed-tension increase in response to a stretch, it is not obvious that they utilise this in their normal work production. Its amplitude is relatively small, and its rate constant seemingly inappropriate for the much slower contractions of the leg muscles. These muscles are almost certainly

synchronous, their activity being controlled entirely via changes in the calcium-ion concentration. Their full calcium-activation, correlated with their dense sarcoplasmic reticulum (O'Donnell & Bernstein, 1988), is consistent with this manner of use.

The different mechanics of the leg and flight muscles must arise, at least in part, from the presence of different myofibrillar proteins. Obvious differences are the presence of arthrin, a conjugate of actin and ubiquitin (Ball *et al.*, 1987) and troponin-H (Bullard *et al.*, 1988) in the flight muscles but not in the leg muscles (Ball *et al.*, 1987; Cripps & Sparrow, in press). In *D. melanogaster* there is molecular genetic evidence that the two muscle types also contain different isoforms of actin and myosin, and probably other myofibrillar proteins. The actin in the flight muscle is encoded solely by the *Actin88F* gene (Ball *et al.*, 1987, and in preparation), while the actin of the TDT (and other non-fibrillar adult muscles) is encoded for by the *Actin79B* gene (Ball *et al.*, 1987; Courchesne-Smith & Tobin, 1989). Indirect evidence from cytochemistry (Raghavan, 1981) and mutants (O'Donnell & Bernstein, 1988) suggests that the leg and flight muscle contain different isoforms of myosin, which arise from the differential splicing of the single myosin heavy chain gene (Bernstein *et al.*, 1986; Rozek & Davidson, 1986). A knowledge of the mechanics of the flight and leg muscles may therefore help in understanding any relationship between the different isoforms (and their amino acid sequences) and the contractile properties of the muscles in which they occur.

The property of stretch-activation found in asynchronous flight muscles of both *L. indicus* and *D. melanogaster* but not in the synchronous leg muscles could arise from a difference in their type of myosin. The structural basis for stretch-activation has been suggested to arise from the matching of the helix periodicity of the myosin-containing filament to that of the actin-containing filament (Deschereveskii, 1971). In addition, it was demonstrated that a four-start helix for the myosin-containing filament was required (Wray, 1979). Such a recruitment model, in which the number of crossbridges which can interact with actin is altered by stretch, had been suggested earlier (Thorson & White, 1969). However, even if there were a matching of the helix periodicity of the thick and thin filaments in the leg muscles, the alignment of crossbridges and actin monomers required for stretch activation (Wray, 1979) would not be possible in the leg muscles because they have a higher thin to thick filament ratio of 6:1. These ideas require that the rate constants controlling tension generation in the flight muscles be about 10 times faster in *D. melanogaster* than in *L. indicus*. This difference in rate constants must also have a structural basis in the contractile proteins.

It might be expected, at least in active and rigor muscle in which the stiffness is largely due to the crossbridges, that the stiffness of *D. melanogaster* flight muscle fibres would be greater than that of *L. indicus* because the number of crowns per half thick filament (proportional to the number of crossbridges per thick filament) is 40 in *D. melanogaster* (M. Reedy, personal communication) but only 30 in *L. indicus*. The A-filament length is 3.1 μm in *D. melanogaster* (M. Reedy, personal communication) and 2,4 μm in *L. indicus*. However, the stiffness and tensions measured for *D. melanogaster* muscles (Table 2) are significantly less than those from *L. indicus*.

We cannot exclude the possibility that the reduced stiffnesses and tensions arise from the difficulty in mounting these small fibres rather than to any inherent variability in the mechanical properties themselves. The shortness of the fibres makes crimping in the T-clips more difficult than with the longer fibres from other species, because a much shorter length of fibre is held within the clips themselves. Since the ratio of the stiffnesses measured in the different solutions (Table 3) is roughly the same for *D. melanogaster* as for *L. indicus*, the lower individual values measured with *D. melanogaster* can be explained in terms of failure to grip a fraction of the myofibrils measured in the cross-sections. However, the values we obtain with *D. melanogaster* are sufficiently consistent that we think this unlikely to be the complete explanation. If the crossbridge stiffness were lower in *D. melanogaster* than in *L. indicus* then this would increase the values of rate constants between attached states of the crossbridge (Huxley & Simmons, 1971), thereby causing an increased rate constant for the delayed change in tension, as seen in *Drosophila*.

Despite the difficulties of using such small preparations, mechanical experiments on the leg and flight muscles from *D. melanogaster* can be carried out reliably. The mechanical properties of the fibrillar flight muscles are similar to those from the much studied *L. indicus* and those of the leg muscle (TDT) are similar to vertebrate striated muscle. This now allows comparison with muscle mutants from *D. melanogaster* of both these muscle types.

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