The Journal of Experimental Biology 205, 1907–1916 (2002)
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JEB4195

Fast muscle in squid (*Loligo pealei*): contractile properties of a specialized muscle fibre type

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Accepted 19 April 2002

Summary

The contractile properties of the transverse muscle of the tentacles and the transverse muscle of the arms of the squid *Loligo pealei* were investigated using small muscle fibre bundle preparations. In addition, transmission electron microscopy was used to measure the length of the thick myofilaments of the two muscle fibre types. The thick filament length of the cross-striated tentacle fibres was 0.81±0.08 μm (mean ± S.D., *N*=51) while that of the obliquely striated arm muscle fibres was 7.41±0.44 μm (*N*=58). The difference in thick filament length of the two muscle types was predicted to result in a much higher shortening velocity of the tentacle muscle compared with the arm muscle. This was tested by investigating the force/velocity relationship for isotonic shortening of the two muscle types. Fitting Hill's equation to the results gave a maximum shortening velocity (V_{max}, the intercept on the velocity axis) of 15.4±1.0 L_0 s^{-1} (mean ± S.D., *N*=9) for the tentacle fibres and of 1.5±0.2 L_0 s^{-1} (*N*=8) for the arm fibres, where L_0 is the length at which peak isometric force was recorded. The difference in thick filament length was also predicted to result in lower peak tension in the tentacle *versus* the arm muscle. For the tentacle, the mean peak tetanic tension during a brief isometric tetanus (0.2 s) of 131±56 mN mm^{-2} cross-sectional area (mean ± S.D., *N*=12) was observed at a stimulus frequency of 80 Hz, whereas the mean peak tetanic tension of the arm fibres during a brief isometric tetanus (0.2 s) was 468±91 mN mm^{-2} (*N*=5) and was observed at a stimulus frequency of 160 Hz. The length/force relationships (expressed relative to L_0) of the two muscle types were similar. The ratio of twitch force to peak tetanic force was 0.66 in the tentacle fibres, but only 0.03 in the arm fibres.

Key words: cephalopod, muscle, cross-striated muscle, force/velocity relationship, *Loligo pealei*, muscle contraction, muscle specialization, obliquely striated muscle, prey capture, squid, thick filament length, tentacle.

Introduction

Muscle fibre specialization in the vertebrates is achieved primarily through variation in the isoforms of the proteins of the myofilament lattice (Moore and Schachat, 1985; Schiaffino and Reggiani, 1996; Sweeney et al., 1988; Wigmore and Dunglison, 1998). The arrangement and dimensions of the thick myofilaments and sarcomeres of different vertebrate muscle fibre types are relatively invariant (Eisenberg, 1983; Offer, 1987). We have studied the specialized cross-striated muscle fibres that cause the remarkably rapid elongation (50–80 % increase in length, 20–40 ms duration, 250 m s^{-2} acceleration) of the prey-capture tentacles of the squid *Loligo pealei* (Kier and van Leeuwen, 1997). Unlike vertebrate muscle fibres, squid tentacle fibres do not show significant differences in the proteins of the myofilament lattice compared with the obliquely striated fibres found in the arms, which are the developmental and probable evolutionary precursors of the tentacle fibres (Kier and Schachat, 1992; Kier, 1996). Instead, the tentacle fibres show dramatic differences in the dimensions and arrangement of the myofilaments (Kier, 1985, 1991). The mechanism of specialization is thus in stark contrast to that observed in the vertebrates. In the present study, we show that, in spite of the similarity in biochemistry, the difference in structure results in a dramatic difference in the performance of the tentacle fibres.

The muscles compared in this study include the transverse muscle mass of the eight arms and the transverse muscle mass of the two tentacles (Fig. 1). In the arms, this muscle provides support for the relatively slow and forceful bending and torsional movements used in swimming, prey handling and behavioural displays. In the tentacles, the transverse muscle generates the force that causes the extremely rapid elongation used by squid during the prey-capture strike (Kier, 1982; Kier and Smith, 1985). Previous work has shown that specialization of the tentacle fibres is not reflected in differences in biochemistry of the contractile proteins. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of the proteins of

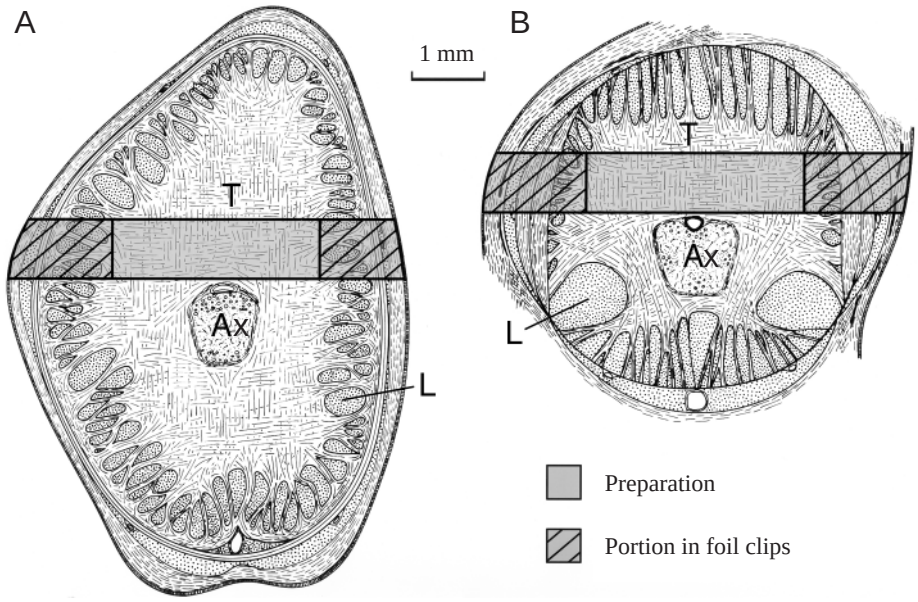

Fig. 1. Schematic diagram of cross sections of the tentacular stalk (A) and arm (B) of the squid *Loligo pealei*. The core of the tentacle and the arm consists of a densely packed mass of transverse muscle fibres (T) oriented perpendicular to the long axis of the appendages. The axial nerve cord (Ax) and longitudinal muscles (L) are also visible in the diagram. The muscle fibre bundle preparations (shaded) were obtained by cutting a small section from a transverse slice of the arm or tentacle. The foil clips used to hold the preparation in the testing apparatus were attached to the ends of the preparation (shaded and cross-hatched) in such a way that only transverse muscle fibres were present between the clips.

the myofilament lattice and peptide mapping of the myosin heavy chains revealed no significant differences between tentacle and arm muscle (Kier and Schachat, 1992). Instead, it is at the level of the ultrastructure that specialization is observed (Fig. 2). The tentacle fibres exhibit cross-striations with unusually short thick filaments and short sarcomeres while the arm fibres show the typical cephalopod obliquely striated pattern with much longer thick filaments (Kier, 1985). The remarkably short myofilaments and sarcomeres of the tentacle fibres result in more elements in series per unit length of fibre. Since shortening velocities of elements in series are additive (Huxley and Simmons, 1972; Josephson, 1975; van Leeuwen, 1991), this ultrastructural specialization is hypothesized to increase greatly the shortening velocity of the tentacles fibres relative to the arm fibres (van Leeuwen and Kier, 1997). The increase in shortening velocity comes at a price; since shorter myofilaments have fewer cross-bridges operating in parallel per half-sarcomere, the tentacle fibres are hypothesized to generate lower tensions. The goal of the present study was to compare the contractile properties of the two fibre types in order to test these hypotheses.

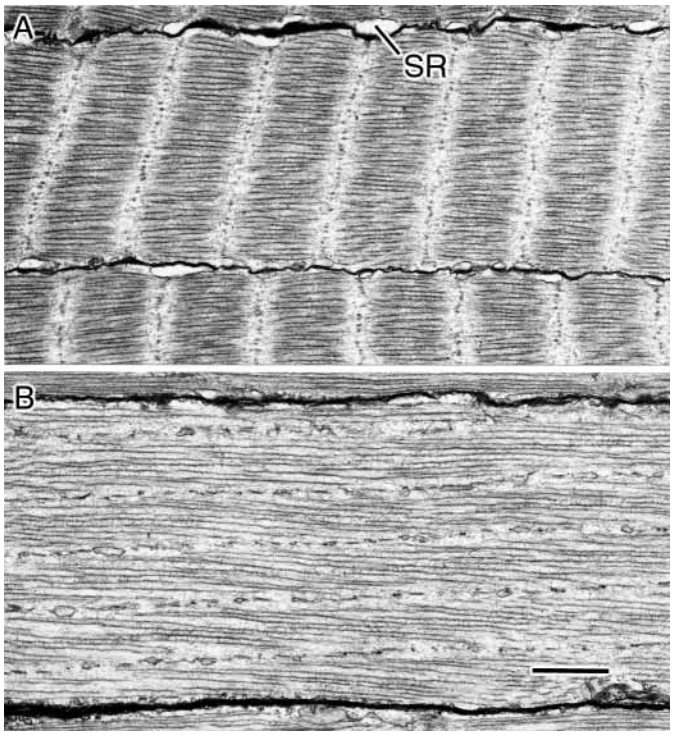

Fig. 2. Transmission electron micrographs of longitudinal sections of the transverse muscle fibres of the tentacle (A) and arm (B) of *Loligo pealei*. The extensive sarcoplasmic reticulum (SR) of the cross-striated tentacle fibres is visible. Note the short sarcomeres and short thick filaments of the tentacle fibres compared with the much longer thick filaments apparent in the obliquely striated arm fibres. Scale bar, 1 μm.

Materials and methods

Experimental animals

Squid (*Loligo pealei* Lesueur; dorsal mantle length 195±20 mm, mean ± S.D., N=35) were captured by trawling off Martha's Vineyard, MA, USA, transported in aerated flow-through holding tanks and maintained in recirculating seawater systems at the Marine Resources Center, Marine Biological Laboratory, Woods Hole, MA, USA. The animals were killed by decapitation, and the tentacles and third arm pair were removed and placed in modified artificial sea water (modified ASW) containing (in mmol l^{-1}): NaCl, 470; KCl, 10; $MgCl_2$, 60; Hepes, 10; pH 7.8, at 4 °C for 5–10 min. Cross-sectional slices 1–2 mm thick were cut from the midpoint along the length of the arm or tentacle with a portion of the edge of a broken double-edged razor blade and placed in standard artificial sea water at 4 °C (standard ASW) containing (in mmol l^{-1}): NaCl, 470; KCl, 10; $CaCl_2{\cdot}6H_2O$, 60; $MgCl_2{\cdot}6H_2O$, 50; glucose, 20;

Hepes, 10; pH 7.8. For an analysis of thick filament lengths in the tentacle muscle fibres, slices were also obtained from the base and tip of the tentacular stalk. Renewing the blades frequently helped to minimize damage and distortion of the tissue during cutting.

Transmission electron microscopy

Cross-sectional slices of the tentacle and arm transverse muscle were fixed in 3.0 % glutaraldehyde, 0.065 mol l^{-1} phosphate buffer, 0.5 % tannic acid and 6 % sucrose for 6–8 h at 4 °C. Following fixation, small blocks of tissue, approximately 1 mm×1 mm×2 mm were cut from the slabs, rinsed overnight in chilled 0.065 mol l^{-1} phosphate buffer and postfixed for 40 min at 4 °C in a 1:1 mixture of 2 % osmium tetroxide and 2 % potassium ferrocyanide. The blocks were rinsed in chilled 0.065 mol l^{-1} cacodylate buffer for 15 min, and then both dehydrated and cleared in a graded series of acetone. Acetone was used in place of ethanol and propylene oxide to minimize dimensional changes in the myofilaments (Page and Huxley, 1963). The tissue blocks were embedded in epoxy resin (Epox 812, Ernest R. Fullam, Latham, NY, USA). During sectioning, special attention was paid to alignment of the blocks to obtain precisely longitudinal sections of the muscle fibres. This was achieved through trial and error by examining 0.5–1.0 μm sections in the light microscope and reorienting the block until individual fibres in the area of interest remained in the section plane. The block was then trimmed for ultramicrotomy of the area of interest and sectioned with a diamond knife. Sections of silver to gold interference colour were stained with saturated aqueous uranyl acetate and Reynolds lead citrate (Reynolds, 1963) and photographed in a Zeiss EM 10CA electron microscope. Thick filament lengths were measured on micrographs using morphometrics software.

Muscle mechanics

Small bundles of fibres (1–2 mm diameter and 5–6 mm long) were dissected from the cross-sectional slices in standard ASW at 4 °C while viewing the slices under a dissecting microscope with transmitted light illumination (Fig. 1). The dominant axis of the transverse muscle fibres in a slice was visible using this approach, and this helped to ensure that the edges of the fibre bundle preparation were cut parallel to the fibres. A T-shaped aluminium foil clip was attached to each end of the preparation by applying a small amount of cyanoacrylate adhesive to the foil clip and then bending the tabs over the ends of the preparation. The foil clips were aligned and attached in such a way that only transverse muscle fibres extended between the clips on each end of the preparation (Fig. 1). A hole in each foil clip provided the means to attach one end of the preparation to a hook on a force transducer (AE801 element; SensorOne Technologies Corp., Sausalito, CA, USA) and the other to a hook on a servomotor arm (model 300B dual-mode lever arm system; Aurora Scientific, Aurora, Canada). The servomotor controlled the length and movement of the preparation during the experiments. The preparation was continuously superfused with aerated standard ASW at 19±0.5 °C. The preparation was stimulated with rectangular current pulses (model DS7A; Digitimer Ltd, UK) *via* large platinum plate electrodes. Stimulation, servomotor lever position and force were controlled and recorded using a ViewDac (Keithley, UK) data-acquisition and control software sequence and an A/D board (DAS-1602 Metrabyte, Keithley, UK).

Isometric contractions

The preparation length was increased until a transient passive force was observed. The stimulus current strength/twitch response relationship was then recorded using 0.2 ms stimuli spaced at intervals of 120 s. Control stimulations of constant current amplitude were used to monitor potential decline of twitch force during the trial. At the end of the trial, the stimulus current was adjusted to a level 10–20 % higher than that required to elicit the maximum twitch force.

The length/force relationship of the preparation was investigated using twitches in the tentacle preparations and both twitch and tetanic stimulation (50 Hz, 100 ms) in the arm preparations. The length of the preparation was adjusted to that giving peak twitch or tetanic force (L_0). The stimulus frequency/force relationship was determined using 200 ms tetani at 5–200 Hz with 300 s between tetani.

Force/velocity relationship

For the tentacle preparations, the force/velocity relationship was investigated using isotonic shortening during twitch. The preparation length was adjusted to that giving peak twitch force as described above. Isotonic shortening was produced with the servomotor operating in its force-clamp mode. The velocity of shortening was measured from the recordings of servomotor arm position. Occasional isometric control stimulations were used to monitor force decline, and the experiment was terminated if the force decreased by more than 10 %. Similar procedures were employed for the arm preparations except that the small twitch:tetanus ratio observed for these muscle fibres meant that the twitch force was typically too low for effective force-clamping by the servomotor. The force/velocity relationship of the arm preparations was therefore investigated using isotonic shortening during brief tetani (50 Hz, 100 ms). Perhaps as a result of using tetani rather than twitches, fewer isotonic shortening trials were possible on a given arm preparation compared with the tentacle preparations before a decrease in isometric force occurred.

Curve fitting

Hill's equation (Hill, 1938) was fitted to the force/velocity data using the Solver function of Microsoft Excel to minimize the sum of the squares of the deviations of predicted velocity from observed velocity. We used the following form of Hill's equation:

$$V = V_{max}P^*(P^* - P)/(GP + 1), \quad (1)$$

where P is the force during shortening/peak isometric force, V is the velocity of shortening ($L_0\,s^{-1}$), and the adjustable

constants are V_{max}, P^* and G. V_{max} is the intercept on the velocity axis, P^* is the intercept on the force axis and G is the constant expressing curvature [$=P_0/a$ (Hill, 1938), where P_0 is the peak tetanic force and a is a constant]. All data points were included and given equal weighting. The fit was not constrained to pass through P=1.0.

Physiological cross section

Upon completion of the experiments, the preparation was transferred to a small Sylgard dish and pinned at L_0. When a photomicrograph had been taken (to monitor potential dimensional changes), the preparation was fixed as described above. The foil clips were cut off, and the preparation was dehydrated to 95 % ethanol and infiltrated and embedded in glycol methacrylate plastic (JB4, Polysciences, Fort Washington, PA, USA). Glycol methacrylate embedding was employed because this embedding medium causes minimal distortion and shrinkage during infiltration and polymerization. Transverse sections were cut with a glass knife at four evenly spaced locations along the length of the preparation, and the aggregate area of cross-sectioned muscle fibre bundles was determined from *camera lucida* tracings using morphometrics software (i.e. transverse muscle fibres oriented in the plane of the section or obviously oblique to the plane of the section were excluded since these fibres do not contribute to shortening of the preparation). The specific force was expressed as mN mm^{-2} cross-sectional area of the tissue for the transverse section of minimum area.

Values are presented as means ± S.D.

Results

Thick filament lengths

The mean thick filament length of the cross-striated tentacle fibres was 0.81±0.08 μm (N=51), while that of the obliquely striated arm muscle fibres was 7.41±0.44 μm (N=58). No difference was observed in thick filament lengths measured from tentacle fibres sampled from the base, mid-point and tip of the tentacular stalk (Student's t-test; tip *versus* base, P=0.14; tip *versus* mid, P=0.13; base *versus* mid, P=0.78). Measurement of thick filament lengths in the obliquely striated fibres was challenging because of the difficulty of aligning the section plane precisely with the long axis of the thick filaments. If the section plane is slightly oblique to the long axis of a given thick filament, the filament appears to end at the location where it exits the section plane. In an attempt to avoid this artefact, electron micrographs were taken only of fibres that remained in the plane of the section across the entire opening of a 300 mesh hexagonal grid. In addition, the longest thick filaments from a given micrograph were measured. It is not possible, however, to distinguish between actual variation in thick filament length and artefactual variation in length caused by variability in thick filament orientation relative to the section plane. Nevertheless, the procedures used in the present study to obtain longitudinal sections are improved over those used by Kier (1985) for *Loligo pealei* and thus probably represent a more accurate estimate of their dimensions.

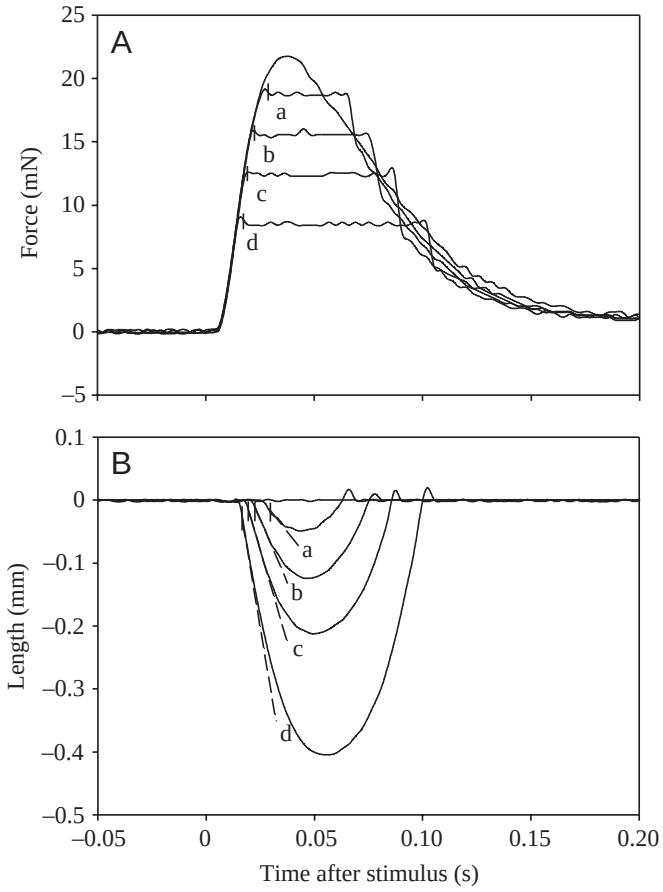

Fig. 3. Twitch force (A) and length (B) of a muscle fibre bundle preparation from the transverse muscle mass of the tentacle of *Loligo pealei*. The recordings of twitch force and length are superimposed after a stimulus at time zero. The highest force recording was obtained during an isometric (constant-length) twitch, and the others (a, b, c, d) were obtained during isotonic twitch with the force clamped to four different levels. The vertical lines mark the point at which force was measured. The slopes of the dashed lines on the length recordings give the velocity of shortening, and the vertical lines are the same times at which force was measured and mark the centre of the section from which the slopes were measured.

Relationship between force and velocity of shortening

The relationship between force and velocity was investigated for nine tentacle and eight arm fibre bundle preparations. Recordings of force and length from a single tentacle preparation shortening in twitch are shown in Fig. 3 for several levels of force-clamp and during an isometric contraction. The force and velocity were measured as a mean value for a period at the beginning of shortening when the force and velocity had stabilized. Identical procedures were used for the arm preparations except that brief (100 ms, 50 Hz) tetani were employed (see Materials and methods).

Force/velocity curves for one tentacle and one arm preparation are shown in Fig. 4, with force expressed relative

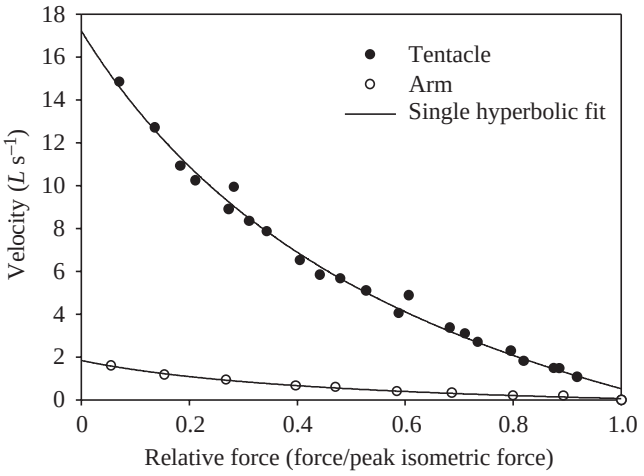

Fig. 4. Force/velocity relationship for a single tentacle transverse muscle bundle preparation (filled circles) and a single arm transverse muscle bundle preparation (open circles). Force is expressed relative to the isometric force of the preparation (mean of repeat twitches for the tentacle and repeat 100 ms, 50 Hz tetani for the arm). Velocity is expressed in $L_0\,s^{-1}$, where L_0 is the length of the preparation at which peak isometric force is produced. The lines were fitted to the data using Hill's single hyperbolic function (see text and Table 1, tentacle preparation 6 and arm preparation 6).

to the isometric force during twitch for the tentacle and relative to the isometric force during tetanus for the arm. A remarkable difference in the force/velocity relationship of the two muscle types is observed. In particular, at 19 °C, the maximum velocity of shortening of the transverse tentacle muscle was estimated to be as high as $17.2\,L_0\,s^{-1}$ (mean ± S.D., 15.4±1.0 $L_0\,s^{-1}$, N=9), while the maximum velocity of shortening of the arm transverse muscle was $1.8\,L_0\,s^{-1}$ (mean ± S.D., 1.5±0.2 $L_0\,s^{-1}$, N=8). Table 1 lists the values of the fitted parameters for Hill's (1938) equation for the tentacle and arm preparations.

Isometric contractile properties

Fig. 5 shows a summary of the results from investigations of the length/active force relationship for twitch stimulation of the tentacle fibre bundle preparations (N=10) and twitch stimulation (N=5) and tetanic stimulation (N=6) of the arm fibre bundle preparations. No difference was evident in the length/force relationship between the two fibre types. In addition, no difference was evident in the length/force relationship during tetanic and twitch stimulation of the arm fibres. Fig. 6 shows the length/passive force relationship and the length/active force relationship for an arm preparation and a tentacle preparation. High levels of resting tension were observed in both the arm and the tentacle preparations when extended beyond optimal length. Indeed, we were unable to explore the length/force relationship in this region because of the damage that occurs to the fibres at these lengths. Near-maximal force is produced over a range of lengths. The ends of this plateau region are smooth curves, suggesting heterogeneity similar to that described by Edman and Reggiani (1987).

Table 1. *Force/velocity parameters of fibre bundle preparations of transverse muscle mass of tentacle and arm of* Loligo pealei

Preparation	P*	V_{max}	1/G	n
Tentacle 1	1.04	14.47	0.61	23
Tentacle 2	1.03	15.50	0.55	11
Tentacle 3	1.04	16.14	0.25	14
Tentacle 4	1.05	15.35	0.49	14
Tentacle 5	1.04	13.90	0.56	14
Tentacle 6	1.08	17.22	0.69	23
Tentacle 7	1.17	15.44	0.47	17
Tentacle 8	0.99	14.68	0.38	19
Tentacle 9	1.06	15.69	0.52	12
Mean	1.06	15.38	0.50	
S.D.	0.05	0.97	0.13	
N	9	9	9	
Arm 1	1.09	1.41	0.67	6
Arm 2	0.98	1.41	0.61	7
Arm 3	0.95	1.22	0.35	9
Arm 4	1.02	1.65	1.09	8
Arm 5	1.10	1.29	0.65	9
Arm 6	1.11	1.84	0.52	10
Arm 7	1.08	1.27	1.06	11
Arm 8	1.16	1.64	0.88	7
Mean	1.06	1.47	0.73	
S.D.	0.07	0.22	0.26	
N	8	8	8	

The values are from fits to Hill's (1938) equation (equation 1; see text).

All forces up to and including isometric values were used in the fitting (see text).

n is the number of data points; N is the number of experiments.

Relationship between stimulus frequency and force

The relationship between stimulus frequency and force was investigated using 200 ms tetani over a range of frequencies up to 200 Hz. The fusion frequency was between approximately 40 and 80 Hz for the tentacle and arm fibres. Multiple stimulation above the fusion frequency produced graded tetani in both the arm and the tentacle preparations (Fig. 7). Fig. 8 shows the relationship between stimulus frequency and force for fibre bundle preparations of arm transverse muscle and tentacle transverse muscle. A striking difference in the response to electrical stimulation in the two fibre types is evident. In particular, the ratio of twitch force to peak tetanic force (the twitch:tetanus ratio) was 0.66±0.06 (N=10) in the tentacle fibres, but only 0.03±0.02 (N=10) in the arm fibres. For the tentacle fibres, the mean peak tetanic tension of 131±56 mN mm^{-2} cross-sectional area (N=12) was observed at a stimulus frequency of 80 Hz. For the arm fibres, the mean peak tetanic tension of 468±91 mN mm^{-2} cross-sectional area

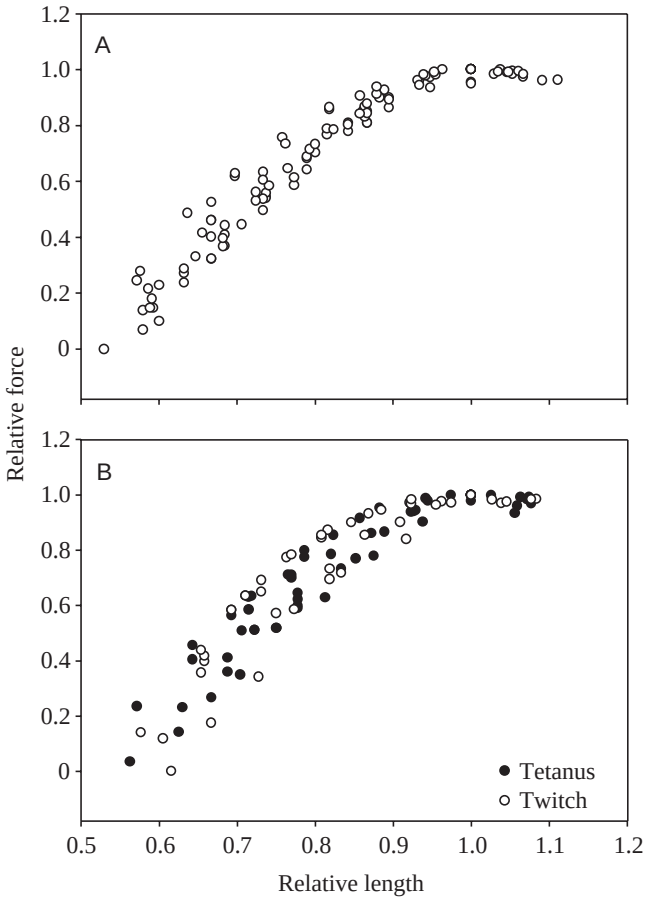

Fig. 5. Summaries of the length/active force relationships for tentacle transverse muscle fibre bundle preparations (A) during twitch stimulation (N=10) and arm transverse muscle fibre bundle preparations (B) during tetanic stimulation for 100 ms at 50 Hz (filled circles, N=6) and twitch stimulation (open circles, N=5). Force is expressed relative to the preparation's peak force for the same pattern of stimulation, and length is expressed relative to that giving this peak force.

(N=5) was observed at a stimulus frequency of 160 Hz. As can be seen in Fig. 7B, the force of the arm preparations was still rising at the end of the 200 ms tetani used in our experiments. Although we did not investigate the increase in force from longer tetani in detail, in three arm preparations we used 300, 500 and 700 ms tetani. The force did not increase after 500 ms of stimulation. The peak force with 500 ms of stimulation was 28.5±0.5 % higher than with 200 ms of stimulation. The isometric mechanical properties of the arm and tentacle fibre bundle preparations are summarized in Table 2.

Discussion

Thick filament length and its implication for velocity of shortening

A remarkable difference was observed between the thick filament lengths of the cross-striated tentacle fibres (0.8 μm) and the obliquely striated arm fibres (7.4 μm). The difference in ultrastructure is expected to affect the contractile properties of the two fibre types, in particular with respect to the velocity of shortening and the force of contraction. In an earlier study, sodium dodecyl sulphate polyacrylamide electrophoresis of the

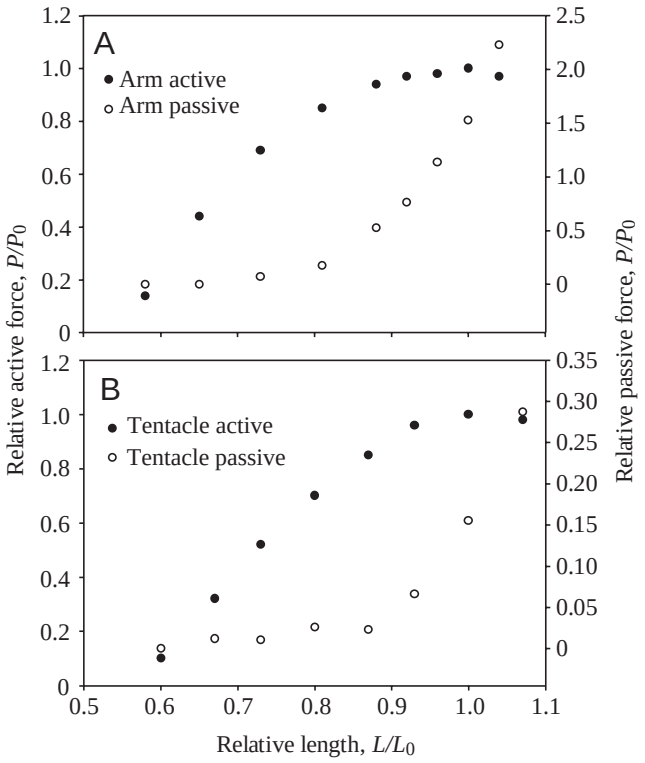

Fig. 6. Length/force relationship of a single arm (A) and a single tentacle (B) transverse muscle fibre bundle preparation. Force is expressed relative to the peak force P_0 during twitch stimulation, and the preparation length is expressed relative to the optimal length for active force production, L_0. Filled circles represent the active force produced in response to twitch stimulation and open circles represent the passive force produced in the absence of stimulation.

Table 2. *Isometric mechanical properties of fibre bundle preparations of transverse muscle mass of tentacle and arm of* Loligo pealei

	Tentacle	Arm
P_0 (mN mm^{-2})	130.9±55.6 (12)	468.3±91.2 (5)
P_{tw}/P_0	0.66±0.06 (10)	0.03±0.02 (10)
TPT (ms)	33.6±3.0 (28)	59.6±10.2 (17)
RT_{50} (ms)	52.7±7.3 (28)	153.4±65.9 (17)

Values are means±S.D. (N).

P_0 is the peak force in a brief tetanus (0.2 s stimulation at 80 Hz for the tentacle and 160 Hz for the arm, the stimulus frequencies that showed the highest force for each type); P_{tw}/P_0 is the twitch:tetanus force ratio; TPT is the time from onset of stimulation to development of maximum force during a twitch; RT_{50} is half-relaxation time from maximum force during a twitch.

Note that P_0 is biased low, especially for the arm preparations (see Discussion).

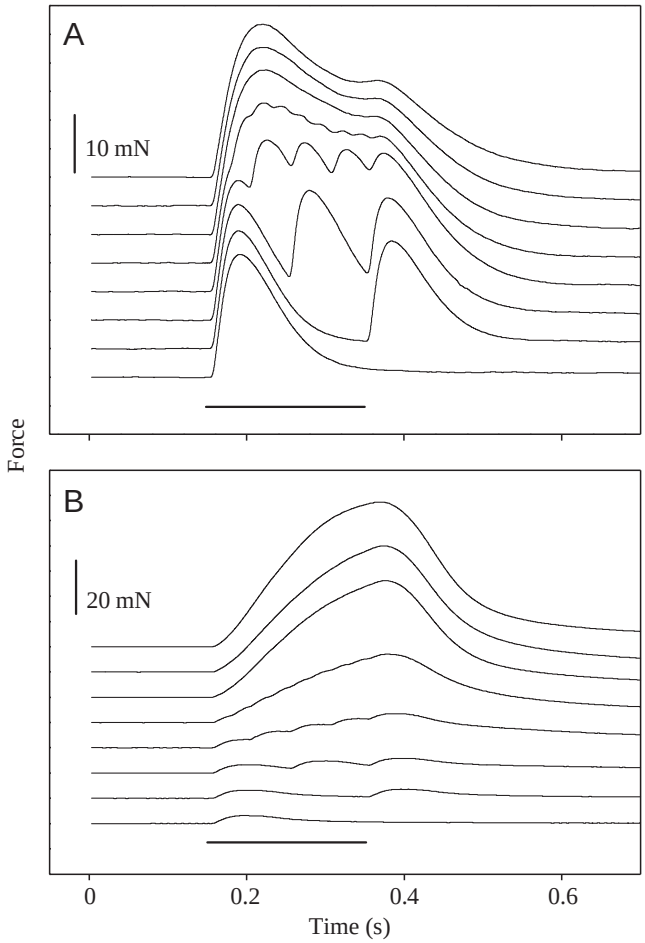

Fig. 7. Superimposed recordings of force produced in response to stimulation at frequencies of 1 (twitch), 5, 10, 20, 40, 80, 120 and 160 Hz for 0.2 s (indicated by the horizontal bar) for a tentacle transverse muscle fibre bundle preparation (A) and an arm transverse muscle fibre bundle preparation (B).

myofilament proteins of the tentacle and arm fibres did not reveal any significant difference in biochemistry (Kier and Schachat, 1992). Peptide mapping of the myosin heavy chains from the arm and tentacle fibres also failed to show any specialization of the tentacle fibres (Kier and Schachat, 1992). This lack of biochemical difference between the arm and tentacle fibres implies that the cross-bridge cycling rate and the interfilamentary sliding velocity are similar. Thus, differences in shortening velocity between the muscle types are predicted to be due primarily to differences in the thick filament length. Because the tentacle fibres have thick filaments that are one-tenth of the length of those of the arm fibres, the tentacle fibres have ten times as many elements (sarcomeres) in series, per unit length. Since the shortening velocities of elements in series are additive (Huxley and Simmons 1972; Josephson, 1975), the maximum velocity of shortening of the tentacle fibres is predicted to be ten times that of the arm fibres. Our results from the isotonic experiments on the two muscle types (V_{max} of 15.4 L_0 s^{-1} for the tentacle fibres and 1.5 L_0 s^{-1} for the arm fibres; see above) are consistent with this prediction.

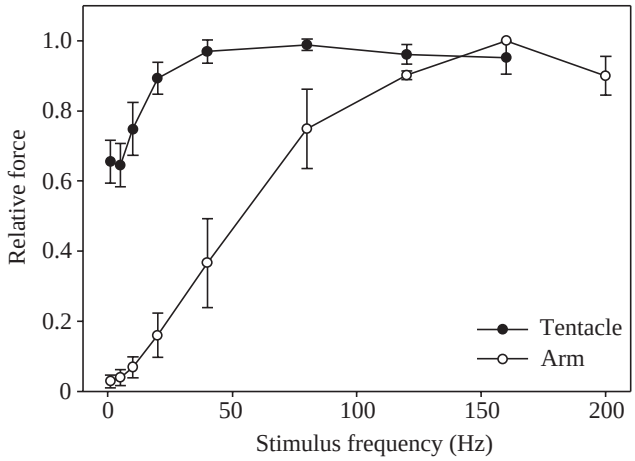

Fig. 8. Pooled stimulus frequency/force relationship data for tentacle muscle fibre bundle preparations (filled circles, *N*=10) and arm muscle fibre bundle preparations (open circles, *N*=10). Force is expressed relative to the preparation's maximum observed force. Values are means ± S.D.

Thick filament length and its implication for peak tension

While the effect of reducing thick filament length is an increase in the maximum velocity of shortening of the tentacle transverse muscle fibres, this change in dimension is likely to reduce the tension (stress) produced by these fibres. This is because shorter thick filaments have fewer myosin cross-bridges operating in parallel per half-sarcomere. A precise prediction of the magnitude of the difference in force is not possible at this time because we need additional information on other aspects of myofilament structure, including for instance, cross-bridge arrangement, thick:thin filament ratio, etc. Nevertheless, the results of the isometric experiments were consistent with the prediction of reduced tension in the tentacle fibres (approximately 130 mN mm^{-2}) relative to the arm fibres (approximately 470 mN mm^{-2}). In addition, as described above, the value of peak tetanic tension reported here for the arm fibres during a 200 ms tetanus is lower by approximately 28.5 % than the peak tetanic tension recorded with 500 ms tetani. By taking into account this effect of longer stimulation, we estimate the maximum tension in the arm muscle fibres to be approximately 600 mN mm^{-2}.

Note that the values reported here are likely to be underestimates of the peak tension for both the arm and tentacle muscle because fibres on the cut surfaces of the preparations are likely to have been damaged and thus will not contribute to the force, even though they are included in the measurement of physiological cross-sectional area. Unfortunately, the results of experiments designed to label the damaged fibres of the preparations with horseradish peroxidase or Lucifer Yellow were inconclusive and, thus, we are uncertain of the proportion of damaged fibres on the surface of the preparations. In addition, the estimation of functional cross-sectional area for the bundle preparations is complicated by the more-or-less orthogonal arrangement of the transverse muscle fibres. It was straightforward to recognize and exclude fibres

oriented parallel to the section plane (and thus perpendicular to the long axis of the preparation) or fibres aligned at a relatively large angle to the long axis of the preparation. It was more difficult, however, to recognize and exclude fibres oriented at a relatively small angle to the long axis of the preparation. We therefore view the values of peak tension reported here as estimates only and hope that single fibre techniques will be developed in order to provide a definitive measure of tension in these cells.

The estimates of peak tension for the cross-striated tentacle fibres and the obliquely striated arm fibres are also of interest in the context of the values of maximum tension reported previously for vertebrate cross-striated fibres and cephalopod obliquely striated mantle muscle fibres. Curtin and Woledge (1988) reported a peak tetanic tension of 255 mN mm^{-2} for white muscle fibre bundles from dogfish *Scyliorhinus canicula*, and Curtin and Edman (1994) measured a peak tension of 287 mN mm^{-2} for single fast-twitch fibres of the frog *Rana temporaria*. The thick filament length of 1.58 μm of vertebrate cross-striated fibres is relatively invariant (Offer, 1987). As described above, the thick filament length of the cross-striated squid tentacle fibres was measured to be half as long (0.81 μm) and, thus, the relative tension of the vertebrate cross-striated fibres and the cross-striated squid tentacle fibres is also consistent with the difference in thick filament dimensions.

Milligan et al. (1997) estimated that the obliquely striated fibres from the mantle of the squid *Alloteuthis subulata* (the fibres sampled were from the central, mitochondria-poor fibres) produce a peak isometric tension of 400 mN mm^{-2}. We estimate above that the obliquely striated squid arm fibres from *L. pealei* may generate 600 mN mm^{-2}. Unfortunately, measurements of thick filament length from squid mantle muscle have not yet been reported (Milligan et al., 1997). A comparison of peak tension from the arm and the mantle muscle suggests, however, that the thick filaments of the mantle muscle are likely to be shorter than those of the arm muscle. It is of interest in this regard to compare the maximum velocity of shortening of the two obliquely striated fibre types. Milligan et al. (1997) estimate the maximum velocity of shortening of the squid mantle fibres to be 2.4 L_0 s^{-1} at 11 °C. As described above, we estimate the maximum velocity of shortening of the arm fibres to be 1.5 L_0 s^{-1} at 19 °C. Thus, the relative shortening velocity of the mantle fibres and the arm fibres is consistent with the prediction of shorter thick filaments in the mantle muscle. Note that, although the Q_{10} of cephalopod muscle has not yet been estimated, it is likely that the maximum velocity of shortening of the mantle fibres would be even higher if measured at the same temperature as for the arm fibres.

Functional significance of oblique striation

It is apparent from Fig. 5 that the length/tension relationships of the cross-striated tentacle fibres and the obliquely striated arm fibres are quite similar within the range of lengths that could be investigated and are likely to be functionally relevant. Previous analyses of the structure and function of obliquely striated muscle fibres suggested that the oblique striation pattern may be important in allowing a greater range of elongation and shortening than that observed in cross-striated muscle fibres (Hidaka et al., 1969; Miller, 1975). In particular, a superelongation mechanism of ‘changing partners’ between thick and thin filaments at extreme elongation has been proposed (Lanzavecchia, 1977, 1981; Lanzavecchia and Arcidiacono, 1981). We found, however, high levels of resting tension as both the tentacle and arm fibre bundles were extended beyond optimal length (see Fig. 6). We were not able to investigate the length/tension relationship at lengths beyond optimal length because of the damage that typically occurred to the preparation when elongated to this extent. Since the preparations used in our study were bundles of fibres, it is likely that the passive mechanical properties of the cells are derived in large part from connective tissues surrounding the fibres. The mechanical properties we measured therefore probably reflect the properties of the muscle tissue in a whole squid arm and imply that superelongation is not relevant for the obliquely striated muscle fibres of the arms. Milligan et al. (1997) also found high levels of resting tension in squid mantle muscle fibres. Thus, the functional significance of oblique striation for muscle function in squid is unclear.

Constancy of thick filament length as a function of position in the tentacle

Van Leeuwen and Kier (1997) proposed a forward dynamics model for the elongation of the tentacular stalk during prey capture. The model accurately predicts the changing geometry of the tentacle, the pressure and stress distribution in the tentacle and the velocity and kinetic energy distribution on the basis of a comparison with kinematic measurements from high-speed films of prey capture (Kier and van Leeuwen, 1997). In addition, the model demonstrates that the short thick filaments and sarcomeres of the transverse muscle of the tentacles are necessary for the observed performance. If thick filament lengths typical of vertebrate cross-striated muscle sarcomeres were present, a significant reduction in performance during the strike would result. Further, an analysis employing the model was used to predict optimal thick filament lengths for the cross-striated tentacle muscle in two cases: the first case allowed thick filament length to vary as a function of position from base to tip in the tentacle; the second case required that thick filament length be constant from base to tip. In the first case, the model predicts that, to maximize the peak velocity of the tip of the tentacle during the strike, the thick filaments of the transverse tentacle muscle should be longer at the base of the tentacle and shorter at the tip (0.97 μm at the base and 0.50 μm at the tip for *Loligo pealei* of similar size to those analyzed here). Longer thick filaments are predicted at the base of the tentacle because of higher dynamic loading in this region. (Fibres at the base of the tentacle must accelerate a larger mass than those at the tip; see Van Leeuwen and Kier, 1997.) In the second optimization with constant-length thick filaments, the model predicts that, to optimize the peak velocity of the tentacular stalk, the thick filaments should be 0.74 μm long.

The peak velocity of the tentacular strike in the case of constant thick filament length was predicted to be 99 % of the peak velocity in the case of variable thick filament lengths (Van Leeuwen and Kier, 1997).

The measurements of the thick filament length reported above showed no evidence of differences in thick filament length as a function position in the tentacle, so the situation modelled in the first case of the optimization does not occur in *Loligo pealei*. Instead, the thick filaments are constant in length as a function of position in the tentacular stalk. It is notable that the thick filament length measured in the present study (0.81 μm) is within 10 % of the value of (0.74 μm) predicted by the theoretical forward dynamics model.

Excitation/contraction coupling

The isometric contraction experiments revealed a striking difference in the response to electrical stimulation of the two fibre types (see Figs 7, 8). Of particular interest was the observation that the twitch/tetanus ratio was 0.66 in the tentacle fibres but only 0.03 in the arm fibres. This difference in response is important in the context of how these two muscle fibre types function in the animal. The high speed and acceleration of the tentacles during the prey-capture strike require a simultaneous and essentially all-or-none contraction of the transverse muscle. The force produced by the transverse muscle of the arms, however, must be precisely modulated to provide the support required for the bending and manipulative movements used for prey handling, behavioural displays and steering movements while swimming (Kier and Smith, 1985). The structural and biochemical differences responsible for this dramatic difference in response to electrical stimulation have not yet been explored in detail for these two fibre types. Although the fast-contracting tentacle fibres include a more extensive sarcoplasmic reticulum than the arm fibres, additional work on the physiology of the cell membrane and excitation/contraction coupling mechanisms is needed (see, for example, Rogers et al., 1997).

Concluding remarks

Our measurements of the contractile properties of the specialized cross-striated tentacle fibres of the squid and of the obliquely striated fibres from the transverse muscle of the arms are consistent with predictions based on previous ultrastructural and biochemical analyses of the two fibre types. In the absence of differences in the biochemistry of the proteins of the myofilament lattice of the two muscle fibre types (Kier and Schachat, 1992), specialization of the tentacle transverse muscle fibres for fast contraction was predicted to be due primarily to shorter thick filaments (Kier, 1985, 1991). Since the thick filament length of the transverse muscle fibres of the tentacles was found to be one-tenth of that of the transverse muscle fibres of the arm, the velocity of the tentacle fibres was predicted to be ten times that of the arm fibres, and our measurements are consistent with this prediction. In addition, because of the shorter thick filaments of the tentacle fibres, the peak tension of these cells was predicted to be lower than that of the arm fibres. Our measurements are also consistent with this prediction.

It appears that modulation of the performance of muscle fibres in the arms and tentacles of squid has occurred through variation in myofilament and sarcomere length in the absence of variation in biochemistry. This mechanism of specialization is thus in stark contrast to that observed in vertebrate muscle, in which myofilament dimensions are constant but a great diversity of myofilament protein isoforms exists. It is unclear, however, whether the similarity in biochemistry observed in the arms and tentacles is an example of a general phenomenon in cephalopod muscle or whether it reflects the shared developmental and evolutionary history of the arms and tentacles. Additional studies of the biochemistry, ultrastructure and mechanics of a wider diversity of cephalopod muscle are required to determine whether the mechanism of specialization observed in the arms and tentacles is general for cephalopod molluscs. Nevertheless, the results presented here, in conjunction with previous studies of arthropod muscle (Cochrane et al., 1972; Costello and Govind, 1983; Günzel et al., 1993; Gronenberg et al., 1997; Jahromi and Atwood, 1969; Marden, 2000; Marden et al., 1998, 1999, 2001; Stephens, et al., 1984; Stokes et al., 1975), demonstrate a greater diversity of mechanisms of specialization of muscle than is generally recognized.

We thank Q. Bone, B. Milligan and the staff of the Laboratory of the Marine Biological Association of the UK for assistance during the initial phase of this project. We thank R. Hanlon and the staff of the Marine Biological Laboratory for providing space and supplying animals while in Woods Hole. S. Guarda provided excellent technical assistance, and L. Rome provided valuable discussion during troubleshooting of the techniques. We thank J. Thompson and T. Uyeno for comments on the manuscript. This work was supported by grants from the NSF (IBN 9219495), NATO (CRG 971179) and NASA (NAG5-8759).

References

Cochrane, D. G., Elder, H. Y. and Usherwood, P. N. R. (1972). Physiology and ultrastructure of phasic and tonic skeletal muscle fibres in the locust, *Schistocerca gregaria*. *J. Cell Sci.* **10**, 419–441.

Costello, W. J. and Govind, C. K. (1983). Contractile responses of single fibers in lobster claw closer muscles: correlation with structure, histochemistry and innervation. *J. Exp. Zool.* **227**, 381–393.

Curtin, N. A. and Edman, K. A. P. (1994). Force–velocity relation for frog muscle fibres: effects of moderate fatigue and of intracellular acidification. *J. Physiol., Lond.* **475**, 483–494.

Curtin, N. A. and Woledge, R. C. (1988). Power output and force–velocity relationship of live fibres from white myotomal muscle of the dogfish, *Scyliorhinus canicula*. *J. Exp. Biol.* **140**, 187–197.

Edman, K. A. P. and Reggiani, C. (1987). The sarcomere length–tension relation determined in short segments of intact muscle fibres of the frog. *J. Physiol., Lond.* **385**, 709–732.

Eisenberg, B. R. (1983). Quantitative ultrastructure of mammalian skeletal muscle. In *Handbook of Physiology*, section 10, *Skeletal Muscle* (ed. L. D. Peachey), pp. 73–112. Bethesda, MD: American Physiological Society.

Gronenberg, W., Paul, J., Just, S. and Hölldobler, B. (1997). Mandible muscle fibers in ants: fast or powerful? *Cell Tissue Res.* **289**, 347–361.

Günzel, D., Galler, S. and Rathmayer, W. (1993). Fibre heterogeneity in the closer and opener muscles of crayfish walking legs. *J. Exp. Biol.* **175**, 267–281.

Hidaka, T., Kuriyama, H. and Yamamoto, T. (1969). The mechanical properties of the longitudinal muscle in the earthworm. *J. Exp. Biol.* **50**, 431–443.

Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B* **126**, 136–195.

Huxley, A. F. and Simmons, R. M. (1972). Mechanical transients and the origin of muscular force. *Cold Spring Harbor Symp. Quant. Biol.* **37**, 669–680.

Jahromi, S. S. and Atwood, H. L. (1969). Correlation of structure, speed of contraction, and total tension in fast and slow abdominal muscle fibers of the lobster (*Homarus americanus*). *J. Exp. Zool.* **171**, 25–38.

Josephson, R. K. (1975). Extensive and intensive factors determining the performance of striated muscle. *J. Exp. Zool.* **194**, 135–154.

Kier, W. M. (1982). The functional morphology of the musculature of squid (Loliginidae) arms and tentacles. *J. Morphol.* **172**, 179–192.

Kier, W. M. (1985). The musculature of squid arms and tentacles: ultrastructural evidence for functional differences. *J. Morphol.* **185**, 223–239.

Kier, W. M. (1991). Squid cross-striated muscle: the evolution of a specialized muscle fiber type. *Bull. Mar. Sci.* **49**, 389–403.

Kier, W. M. (1996). Muscle development in squid: ultrastructural differentiation of a specialized muscle fiber type. *J. Morphol.* **229**, 271–288.

Kier, W. M. and Schachat, F. H. (1992). Biochemical comparison of fast- and slow-contracting squid muscle. *J. Exp. Biol.* **168**, 41–56.

Kier, W. M. and Smith, K. K. (1985). Tongues, tentacles and trunks: the biomechanics of movement in muscular-hydrostats. *J. Linn. Soc. Lond. Zool.* **83**, 307–324.

Kier, W. M. and van Leeuwen, J. L. (1997). A kinematic analysis of tentacle extension in the squid *Loligo pealei*. *J. Exp. Biol.* **200**, 41–53.

Lanzavecchia, G. (1977). Morphological modulations in helical muscles (Aschelminthes and Annelida). *Int. Rev. Cytol.* **51**, 133–186.

Lanzavecchia, G. (1981). Morphofunctional and phylogenetic relations in helical muscles. *Boll. Zool.* **48**, 29–40.

Lanzavecchia, G. and Arcidiacono, G. (1981). Contraction mechanism of helical muscles: experimental and theoretical analysis. *J. Submicrosc. Cytol.* **13**, 253–266.

Marden, J. H. (2000). Variability in the size, composition and function of insect flight muscles. *Annu. Rev. Physiol.* **62**, 157–178.

Marden, J. H., Fitzhugh, G. H., Girgenrath, M., Wolf, M. R. and Girgenrath, S. (2001). Alternative splicing, muscle contraction and intraspecific variation: associations between troponin T transcripts, Ca^{2+} sensitivity and the force and power output of dragonfly flight muscles during oscillatory contraction. *J. Exp. Biol.* **204**, 3457–3470.

Marden, J. H., Fitzhugh, G. H. and Wolf, M. R. (1998). From molecules to mating success: Integrative biology of muscle maturation in a dragonfly. *Am. Zool.* **38**, 528–544.

Marden, J. H., Fitzhugh, G. H., Wolf, M. R., Arnold, K. D. and Rowan, B. (1999). Alternative splicing, muscle calcium sensitivity, and the modulation of dragonfly flight performance. *Proc. Natl. Acad. Sci. USA* **96**, 15304–15309.

Miller, J. B. (1975). The length–tension relationship of the dorsal longitudinal muscle of the leech. *J. Exp. Biol.* **62**, 43–53.

Milligan, B. J., Curtin, N. A. and Bone, Q. (1997). Contractile properties of obliquely striated muscle from the mantle of squid (*Alloteuthis subulata*) and cuttlefish (*Sepia officinalis*). *J. Exp. Biol.* **200**, 2425–2436.

Moore, G. E. and Schachat, F. H. (1985). Molecular heterogeneity of histochemical fibre types: a comparison of fast fibres. *J. Muscle Res. Cell Motil.* **6**, 513–524.

Offer, G. (1987). Myosin filaments. In *Fibrous Protein Structure* (ed. J. M. Squire and P. J. Vibert), pp. 307–356. London: Academic Press.

Page, S. G. and Huxley, H. E. (1963). Filament lengths in striated muscle. *J. Cell Biol.* **19**, 369–390.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

Rogers, C. M., Nelson, L., Milligan, B. J. and Brown, E. R. (1997). Different excitation–contraction coupling mechanisms exist in squid, cuttlefish and octopod mantle muscle. *J. Exp. Biol.* **200**, 3033–3041.

Schiaffino, S. and Reggiani, C. (1996). Molecular diversity of myofibrillar proteins: gene regulation and functional significance. *Physiol. Rev.* **76**, 371–423.

Stephens, P. J., Lofton, L. M. and Klainer, P. (1984). The dimorphic claws of the hermit crab, *Pagurus pollicaris*: properties of the closer muscle. *Biol. Bull.* **167**, 713–721.

Stokes, D. R., Josephson, R. K. and Price, R. B. (1975). Structural and functional heterogeneity in an insect muscle. *J. Exp. Zool.* **194**, 379–408.

Sweeney, H. L., Kushmerick, M. J., Mabuchi, K., Sréter, F. A. and Gergely, J. (1988). Myosin alkali light chain and heavy chain variations correlate with altered shortening velocity of isolated skeletal muscle fibers. *J. Biol. Chem.* **263**, 9034–9039.

van Leeuwen, J. L. (1991). Optimum power output and structural design of sarcomeres. *J. Theor. Biol.* **149**, 229–256.

van Leeuwen, J. L. and Kier, W. M. (1997). Functional design of tentacles in squid: linking sarcomere ultrastructure to gross morphological dynamics. *Phil. Trans. R. Soc. Lond. B* **352**, 551–571.

Wigmore, P. M. and Dunglison, G. F. (1998). The generation of fiber diversity during myogenesis. *Int. J. Dev. Biol.* **42**, 117–125.