

OBLIQUELY-STRIATED MUSCLE IS NOT JUST FOR SUPER-ELONGATION

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ABSTRACT

Julia Olszewski-Jubelirer: Obliquely-striated muscle is not just for super-elongation
(Under the direction of William M. Kier)

Since the discovery of the sliding filament theory, scientists have made strong connections between the structure and function of cross-striated muscle. These connections are tenuous for obliquely-striated muscle both because of its complex geometry and because of a relative lack of research on this exclusively invertebrate muscle type. This paper will review the diversity of obliquely-striated muscle and the theories that aim to connect the structure and function of this muscle. It will then explore these theories through a geometric model that directly relates the structure of obliquely-striated muscle to its length-tension curve. The review and model suggest that obliquely-striated muscle may not be primarily for super-elongation, as previously suggested, and that the initial striation angle of the muscle could be enough to explain the muscle performance in all but one case.

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CHAPTER 1: A REVIEW OF OBLIQUELY-STRIATED MUSCLE DIVERSITY AND FUNCTION

Introduction

One goal of organismal biology is to relate structures to functions. Biologists who study muscle have been successful in establishing a link between structure and function across a large scale- from the level of whole muscles interacting with tendons (Roberts and Azizi, 2010) down to the proteins that build sarcomeres (Kier, 1991). This understanding is particularly well developed in cross-striated muscle. The sliding-filament theory coupled with the relatively simple geometry of the sarcomeres allows scientists to make and test predictions about how the lengths of thin and thick myofilaments and their relative overlap affect the amount of force or velocity a muscle produces (Gordon et al., 1966).

In obliquely-striated muscle, the relationship between structure and function is less well established. Obliquely-striated muscle has Z-bodies that serve as anchors for the thin myofilaments and are linearly aligned at an angle relative to the long axis of the fiber (Rosenbluth 1965, Fig. 1). This angle, termed the 'initial striation angle' here would be 90 degrees for cross-striated muscle and is typically 4-10 degrees for obliquely striated muscle. In obliquely striated muscle, the striation angle increases as the muscle shortens. It has been proposed that this angle and the more complex three dimensional arrangement of myofilaments allows stretched obliquely-striated muscle to produce higher forces than stretched cross-striated muscle (Miller, 1975). This proposal is derived, however, from the analysis of a small sample of the diversity of obliquely-striated muscle (Gerry and Ellerby, 2011; Miller, 1975), and there is no

evidence that other obliquely-striated muscles are used to produce forces at longer lengths.

Obliquely-striated muscle is present across bilateria (Fig. 1, Table 1) in more phyla than has been recognized in previous reviews (Lanzavecchia et al., 1977) but physiological data are only available from annelids and mollusks (Fig. 2). Previous reviews have focused on the structure and implications of the geometry for the function of obliquely-striated muscle (Paniagua et al., 1996). The following is a review of the functional data available for obliquely-striated muscles.

Early observations and sliding versus shearing

Initially, obliquely-striated muscle was thought to contract via two separate mechanisms: sliding and shearing (Rosenbluth, 1967). In the proposed sliding mechanism, the thick and thin myofilaments interacted in a manner similar to that proposed in the vertebrate sliding-filament theory (Gordon et al., 1966; Rosenbluth, 1967). In addition to sliding, a separate shearing mechanism was proposed which involved a change in the stagger of adjacent thick filaments as the muscle shortened and extended (Rosenbluth, 1967). Later, a geometric model of the muscle suggested that the observed shearing was a passive property of the geometry of the muscle fiber. In this case, the fiber actively contracts with a conventional sliding filament mechanism and the shortening of the long axis of the fiber coupled with the fact that muscle is essentially isovolumetric means that the angle of striation and the stagger of the myofilaments (shearing) must change during contraction. It is this passive shearing coupled with the active sliding mechanism that was thought to make obliquely-striated muscle more extensible, or capable of producing forces at longer lengths than cross-striated muscle (Lanzavecchia, 1977).

Length-tension curves

The most direct test of whether a muscle is capable of greater extensibility is to determine the range of lengths at which the muscle is able to produce force using experiments that

document the length-tension relationship. Muscles produce different amounts of tension (tension is the force divided by the cross-sectional area) due to changes in the overlap between thick and thin myofilaments, which depends on muscle length. To understand how force changes with length, scientists stretch a muscle *in vitro* to a particular length, stimulate the muscle to contract isometrically, and measure the amount of force produced. They then repeat this procedure for a range of lengths. Plots of these forces versus length data are known as length-tension curves. Customarily, length (L) is standardized by the length at which the muscle produces maximum force (L_0) and tension (P) is standardized by the maximum tension (P_0) produced by the muscle. Length-tension curves in vertebrate cross-striated muscle consist of an ascending limb in which the force increases as length increases, a plateau at the maximum force, and then a descending limb in which the force decreases with further increase in length.

Changing partners hypothesis: evidence and theory

Historically, the length-tension curve of obliquely-striated muscle was thought to be “broad” in comparison to that of vertebrate cross-striated muscle, based on an early study of leech body-wall muscle (Miller, 1975). The dorsal body wall muscle of a leech, *Haemopsis sanguisuga* produces active forces close to its maximum active force at a broader range of length than frog muscle. This “broadening” of the length-tension curve was observed on the ascending limb. On the descending limb, the leech muscle produces less force relative to the maximum force than the frog muscle. To explain this phenomenon, Miller (1975) proposed the changing-partner hypothesis: because of the staggered pattern of the myofilaments of obliquely-striated muscle (Fig. 1B), as the muscle is extended and a given thin filament is pulled beyond overlap of a thick filament, there is a nearby thick filament of the correct polarity available for interacting with the thin filament (Fig. 3). Miller (1975) proposed that it was this switching of partners that

allowed the muscle to produce relatively high amounts of force until it was stretched to such a length that there was no overlap with a consequent rapid decrease in force with length.

To evaluate the changing partners hypothesis, Lanzavecchia and Arcidiacono (Lanzavecchia & Arcidiacono, 1981) conducted experiments in which they elongated the obliquely-striated muscle of anesthetized and unanesthetized leeches beyond overlap and then examined transverse sections of the muscle using transmission electron microscopy (TEM). They argued that if changing partners occurs, it should occur in the unanesthetized animals, in which the muscle is active, and therefore there should still be thin filaments surrounding the thick filaments. In the anesthetized animals, they proposed that there would not be changing partners, the thin filaments and thick filaments would be stretched beyond overlap, and therefore thin filaments would not be observed surrounding the thick filaments in cross section. Unfortunately, they failed to report how they determined the lengths to which they stretched the leeches, the lengths themselves, how many leeches they used, how they chose which sections of muscle to analyze, and how they quantified the number of thin filaments surrounding each thick filament. They did not include quantitative data or statistics in their results and instead showed electron micrographs of two example pairs of unanesthetized and anesthetized samples. I was unable to convince myself that their conclusion, that there were more thin filaments surrounding the thick filaments in the unanesthetized samples, was true in one of the two provided examples. In the same paper they make several theoretical arguments about the function of obliquely-striated muscle, including a graph in which they plot the length-tension curve reported by Miller (1975) on the same axes as theoretical curves: a cross-striated muscle curve and obliquely-striated muscle curve, both with the same myofilament dimensions of the leeches from this study, not the leech species from the Miller (1975) study. They provide no explanation of how they calculated

the curves. They also do not plot all three of the curves with L_O coincident, nor do they indicate the relative scaling of the length axis for each muscle type. Their conclusion is that based on the TEM data and the discrepancy between their theoretical and the actual curves, changing partners must be occurring (Lanzavecchia & Arcidiacono, 1981). These studies leave the field in a tenuous position. Because they do not provide enough explanation to evaluate or replicate their results, the validity of their conclusions is uncertain and difficult to test. But scientists who might attempt to repeat their experiments run the risk of devoting large amounts of time to a TEM study that fails to obtain novel insights.

In a follow-up study, Lanzavecchia modified the geometric model he used to differentiate sliding versus shearing mechanisms (Lanzavecchia, 1977) to include the effects of changing partners (Lanzavecchia, 1985). Then, in a manner not made explicit in the study, he used this model to predict morphological characteristics of the sarcomere. He compared these parameters, including distance between filaments, number of filaments, ratio of thick and thin filaments, width of the sarcomere, and filament length, to the observed values in TEMs of the leech, *Hirudo medicinalis*, once again a different species than the one used by Miller (1975), and reported that his model agreed with the micrographs. He concluded that in order to explain the Miller (1975) length-tension curve, the thick and thin filaments must change partners twice over the range from fully contracted to fully elongated (Lanzavecchia, 1985).

Additional length-tension curves of annelid muscle

There are length-tension curves reported for earthworms (Tashiro and Yamamoto, 1971). They do not appear to be super elongating (Figs. 4A and B).

In 2011, a study on the leech *Hirudo verbana* reported an even broader length-tension curve than originally found by Miller (1975), suggesting that obliquely-striated muscle in leeches

is capable of super elongation (Gerry and Ellerby, 2011). This study used a portion of the longitudinal muscle of a single body segment rather than the entire dorsal body wall like Miller (1975), and found that the muscle produced active force from about 40-160% of resting length. When serotonin was present the muscle produced greater force at each length, shifting the length-force curve upwards. Additionally, *in vivo* sonomicrometry of suction feeding, crawling, and swimming suggested that this muscle operates at 75-240% of its resting length (Gerry and Ellerby, 2011). A later study that employed work loop procedures for the same muscle found that without serotonin the muscle acted as a brake, absorbing some of the energy, and that serotonin increased the net work of the muscle by decreasing the negative work due to passive stiffness (Gerry et al., 2012).

Length-tension curves and operating lengths of molluscan muscle

All known length-tension curves (Figs. 4A and B) and all known data on the *in vivo* operating lengths suggest that the obliquely-striated muscle found in molluscs is not capable of super elongation.

The obliquely-striated mantle muscles of *Alloteuthis subulata* (squid) and *Sepia officinalis* (cuttlefish) have length-tension and force-velocity curves that are similar to vertebrate cross-striated muscle and do not appear to be super elongating (Milligan et al., 1997).

The obliquely-striated muscle fibers of the arm of the squid *Doryteuthis pealeii* (previously known as *Loligo pealei*) have longer thick filaments (7.41 +/- 0.44 μm) than the thick filaments of the cross-striated muscle found in the tentacles (0.81 +/- 0.08 μm). As expected from the thick filament lengths, the arm muscle fibers produce higher peak tensions and lower maximum shortening velocities than the tentacle muscle fibers. However, both the obliquely-striated arm muscle and the cross-striated tentacle muscle have length-tension curves in which

the length ranges from about 60% to 110% of the resting length of the muscle (Kier and Curtin, 2002).

There are two different types of obliquely-striated circular fibers in the mantle muscle of the squid *Doryteuthis pealeii*: centrally located, mitochondria-poor (CMP) and superficially located, mitochondria-rich (SMR). CMP fibers have shorter thick filaments ($1.78 \pm 0.27 \mu\text{m}$) and faster shortening velocities. SMR fibers have longer thick filaments ($3.12 \pm 0.56 \mu\text{m}$) and higher peak forces. For both types of muscle, the length-tension curve ranges 90-105% of the resting length (Thompson et al., 2008). Both CMP and SMR increase in thick filament length and decrease in striation angle as the squid develop from paralarvae to adults (Thompson et al. 2010).

Further work on the CMP muscle fibers found length-tension curves with lengths from about 65% to 105% of resting length. Fibers taken from the inner portion of the mantle produced higher forces after being scaled to P_0 than fibers taken from the outer portion of the mantle. *In vivo* sonomicrometry experiments on CMP muscle fibers from both locations suggest that the muscles work primarily on the ascending limb of the length-tension curve (Thompson et al., 2014).

The funnel refractor muscle of the squid *Doryteuthis pealeii* was initially called helical smooth (Hanson and Lowy, 1957), but subsequent work reclassified it as obliquely-striated (Rosenbluth et al., 2010). *In vivo* sonomicrometry showed that it does not undergo large length changes. Instead, its operating range is around 4% of its resting length. The authors also performed *in vitro* contractile studies, but did not report the length-tension curve (Rosenbluth et al., 2010).

Despite there being no evidence for squid muscle producing force over a large range of operating lengths, authors continue to argue that the squid muscle might be capable of this because of the structural similarities between squid and leech muscle (Thompson et al. 2010). However, kinematic data on squid tentacle muscle shows the muscle is capable of extending 70-100% of its resting length, suggesting it might be capable of super elongation (Kier and Leeuwen, 1997).

Major alternative structures of obliquely-striated muscle

In the nematodes *Caenorhabditis elegans* and *Mermis nigrescens*, unlike in annelids and molluscs, the longitudinal body wall muscles, which are used to bend the body during movement, possess z-bodies that are directly attached to the muscle cell membrane. The z-body/cell membrane attachments are proposed to allow the force of contraction to be transmitted laterally to the sides of the muscle cell rather than longitudinally at the ends of the muscle cell (Burr and Gans, 1998). The oblique striation pattern is thus thought to evenly distribute the attachment sites and force transmission, making it more likely that the worms will bend rather than kink. To my knowledge, there are no length-tension curves reported for nematode muscles.

Crinoids, a group of echinoderms, have obliquely-striated muscle with continuous Z-lines, unlike all other known types of obliquely-striated muscle (Carnevali et al., 1986). These muscles are found in the hinged system of skeletal elements of the crinoid arm (Carnevali & Saita, 1985b), which is not a hydrostatic system as seen in annelids, nematodes, and mollusks. To my knowledge, there are no length-tension curves reported for echinoderm muscle.

Discussion

Obliquely-striated muscle has long been thought to be a specialization for hydrostatic skeletons given its presence in annelids and nematodes (Lanzavecchia, 1981). However,

obliquely-striated muscle can be found across bilateria, including in one non-hydrostatic skeleton: the hinged arms of crinoids (Carnevali & Saita, 1985b). The lack of evidence of a broad length-tension curve for molluscan obliquely-striated muscle coupled with structural differences between different types of obliquely-striated muscle suggests that the functional theories proposed for annelid and nematode muscle should not simply be applied to all obliquely-striated muscle.

Additionally, there are thirteen phyla in which obliquely-striated muscle has been described but for which length-tension information is not available. Currently, the theoretical understanding of obliquely-striated muscle is based on functional data from annelids, and ignores the contradictory data gathered from molluscs. Increasing the number of phyla for which functional data is known could help sort out whether annelids, molluscs, or neither are typical of obliquely-striated muscle as a whole.

Further work involving geometric modeling of the results of sliding, shearing, and changing partners on length-tension curves would provide specific, testable hypotheses that could guide future studies and help elucidate the functional significance of the oblique striation pattern.

Finally, expanding a more rigorous version of Lanzavecchia's TEM studies on changing partners to more phyla would provide crucial evidence for the changing partners theory. These studies must quantify and employ ranges of elongation and contraction observed *in vivo* and quantify the ultrastructural observations for statistical comparison and testing. It would be worthwhile to compare the ultrastructure of stretched leech muscle with and without anesthesia to that of a stretched squid muscle with and without anesthesia. I predict that the squid muscle

would show no evidence of changing partners, given the lack of super-elongation reported in previous studies.

Evidence for super elongation

Only one of six species for which there are physiological data shows evidence of super elongation (Fig. 4). Super elongation should no longer be considered the default function of obliquely-striated muscle. Especially within cephalopods, where all evidence points against super elongation, scientists should assume that the muscles are not capable of super elongation unless proven otherwise.

At the moment changing partners is the only theory offered to explain why leeches are capable of super elongation. This theory was proposed and validated through experimental and theoretical methods before the Gerry (2011) data were available. There is currently no feasible method for testing this theory through imaging, but the Lanzavecchia (1981) anesthetized vs. unanesthetized experiments are worth further exploration.

Table 1. Structural descriptions of obliquely-striated muscle. Asterisks indicate muscles thought to be an intermediary structure between obliquely-striated and smooth muscle.

Phylum	Species name	Citation(s)
Nematoda	<i>Ascaris megalocephala</i>	(Plenk, 1924)
Nematoda	<i>Ascaris lumbricoides</i>	(Rosenbluth, 1965)
Nematoda	<i>Deontostoma californicum</i>	(Hope, 1969)
Nematoda	<i>Caenorhabditis elegans</i>	(Epstein et al., 1985)
Nematoda	<i>Mermis sp.</i>	(Valvassori et al., 1981)
Annelida	<i>Eisenia foetida</i>	(Kawaguti and Ikemoto, 1957a) (Lanzavecchia et al., 1994) (Royuela et al., 1995)
Annelida	<i>Pheretima communissima</i>	(Kawaguti and Ikemoto, 1957a)
Annelida	<i>Lumbricus terrestris</i>	(Hanson and Lowy, 1957) (Heumann and Zebe, 1967) (Mill and Knapp, 1970) (Eguileor et al., 1988) (Lanzavecchia et al., 1994)
Annelida	<i>Rinchemis limosella</i>	(Lanzavecchia & Eguileor, 1987) (Eguileor et al., 1988)
Annelida	<i>Pelodrilus leruthi</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Glycera dibranchiata</i>	(Rosenbluth, 1968)
Annelida	<i>Neanthes sp</i>	(Matsuno & Kawamura, 1991)
Annelida	<i>Syllis amica</i>	(Wissocq, 1967)
Annelida	<i>Hirudo niponia</i>	(Kawaguti and Ikemoto, 1958a)
Annelida	<i>Hirudo medicinalis</i>	(Röhlich, 1962) (Pucci and Afzelius, 1962) (Faller, 1964) (Lanzavecchia, 1985) (Eguileor et al., 1988) (Rowlerson and Blackshaw, 1991)
Annelida	<i>Branchiura sowerbyi*</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Pontobdella muricata</i>	(Royuela et al., 1999) (Royuela et al., 2001)
Annelida	<i>Pisicola geometra</i>	(Pucci and Afzelius, 1962)
Annelida	<i>Haemopsis sanguisuga</i>	(Pucci and Afzelius, 1962)
Annelida	<i>Erpobdella octoculata</i>	(Pucci and Afzelius, 1962)

Phylum	Species name	Citation(s)
Annelida	<i>Glossiphonia complanata</i>	(Pucci and Afzelius, 1962)
Annelida	<i>Enchytraeus albidus</i>	(Eguileor et al., 1988)
Annelida	<i>Prinospio caspersi</i>	(Eguileor et al., 1988)
Annelida	<i>Magelona papillicornis</i>	(Wissocq and Boilly, 1977)
Annelida	<i>Tubifex tubifex</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Limnodrilus udekemianus</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Monopylephorus sp.</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Peloscolex sp.</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Rynchelmis limosella</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Lumbriculus variegatus</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Bythonomus lemani</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Kincaidiana sp.</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Phagodrilus sp.</i>	(Lanzavecchia et al., 1994)
Annelida	<i>Eiseniella tetraedra</i>	(Lanzavecchia et al., 1994)
Mollusca	<i>Sepia esculenta</i>	(Kawaguti and Ikemoto, 1957b)
Mollusca	<i>Doryteuthis pealeii</i>	(Kier 1985) (Rosenbluth et al., 2010)
Mollusca	<i>Illex illecebrosus</i>	(Kier 1985)
Mollusca	<i>Alloteuthis subulata</i>	(Bone et al., 1981)
Mollusca	<i>Sepia officinalis</i>	(Amsellem and Nicaise, 1980) (Kier 1989)
Mollusca	<i>Pecten albicans</i>	(Kawaguti and Ikemoto, 1958b) (Nunzi and Franzini-Armstrong, 1981)
Mollusca	<i>Crassostrea virginica</i>	(Morrison and Odense, 1974)
Mollusca	<i>Artica islandica</i>	(Morrison and Odense, 1974)
Mollusca	<i>Tridacna crocea</i>	(Matsuno & Kuga, 1989)
Mollusca	<i>Mitilus crassitesa</i>	(Kawaguti and Ikemoto, 1957c)
Mollusca	<i>Spondilus cruentus</i>	(Kawaguti and Ikemoto, 1959)
Mollusca	<i>Crassostrea angulata</i>	(Hanson and Lowy, 1961)
Mollusca	<i>Fragum onedo</i>	(Matsuno, 1988)
Mollusca	<i>Sepiella japonica</i>	(Matsuno, 1987)
Brachiopoda	<i>Lingula unguis</i>	(Kuga and Matsuno, 1988)

Phylum	Species name	Citation(s)
Brachiopoda	<i>Terebratalia transversa</i>	(Eshleman et al., 1982)
Urochordata	<i>Doliolum gegenbauri</i>	(Bone and Ryan, 1974)
Tardigrada	<i>Macrobiotus hufelandi</i> *	(Walz, 1974)
Tardigrada	<i>Milnesium tardigradum</i> *	(Walz, 1974)
Platyhelminthes	<i>Notoplana acticola</i>	(MacRae, 1965)
Platyhelminthes	<i>Grillotia erinaceus</i>	(Ward et al., 1986)
Echinodermata	<i>Antedon mediterranea</i>	(Carnevali and Saita, 1985a) (Carnevali et al., 1986)
Echinodermata	<i>Ophioderma longicaudum</i> *	(Saita et al., 1982)
Priapulida	<i>Priapululus caudatus</i>	(Mattisson et al., 1974)
Rotifera	<i>Philodina roseola</i>	(Clément and Amsellem, 1989)
Rotifera	<i>Brachionus urceolaris sericus</i> , <i>B. calyciflorus</i> , <i>B. plicatilis</i>	(Clément and Amsellem, 1989)
Rotifera	<i>Rhinoglena frontalis</i>	(Clément and Amsellem, 1989)
Rotifera	<i>Asplanchna brightwelli</i>	(Clément and Amsellem, 1989)
Rotifera	<i>Notommata copeus</i>	(Clément and Amsellem, 1989)
Nemertea	<i>Phalloneimertes cf. murrayi</i>	(Norenburg and Roe, 1998)
Nemertea	<i>Nectonemertes cf. mirabilis</i>	(Norenburg and Roe, 1998)
Nemertea	2 species of protopelagonemertid	(Norenburg and Roe, 1998)
Sipunculida	<i>Sipunculus nudus</i>	(deEguileor & Valvassori, 1977)
Nematomorpha	<i>Gordius aquaticus</i>	(Lanzavecchia et al., 1979)
Nematomorpha	<i>Gordius panighettensis</i>	(Lanzavecchia et al., 1979)
Gastrotricha	<i>Turbanella cornuta</i>	(Teuchert, 1974)
Gastrotricha	<i>Chordodasys antennatus</i>	(Rieger et al., 1974)
Gnathostomulida	<i>Haplognathia rosacea</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Labidognathia longicollis</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Semaeognathia sterreri</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Gnatbostomula jenneri</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Gnatbostomula microstyla</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Gnatbostomula mediterranea</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Gnatbostomula axi</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Austrognatharia kirsteueri</i>	(Rieger and Mainitx, 1977)

Phylum	Species name	Citation(s)
Gnathostomulida	<i>Austrognathia sp. I</i>	(Rieger and Mainitx, 1977)
Gnathostomulida	<i>Austrognathia riedli</i>	(Rieger and Mainitx, 1977)
Chaetognatha	<i>Sagitta setosa</i>	(Duvert, 1969; Duvert and Salat, 1979; Duvert and Salat, 1980)

Figure 1. Diagram of cross-striated (A) and obliquely-striated (B) muscle.

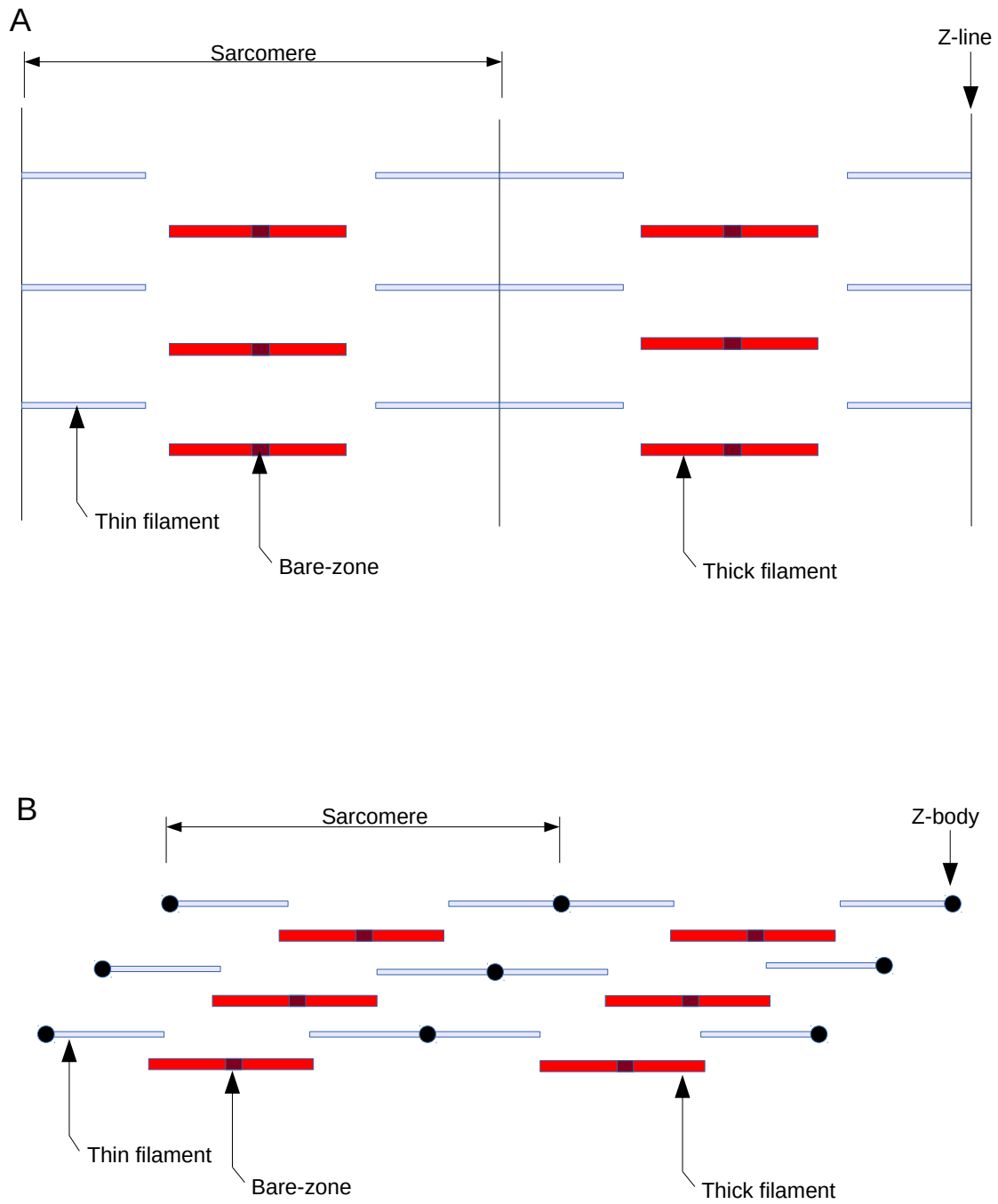


Figure 2. Bilaterians with obliquely-striated muscle (in bold and red). Asterisks indicate muscle thought to be in between obliquely-striated and smooth in structure. Phylogeny is based on (Lartillot and Philippe, 2008).

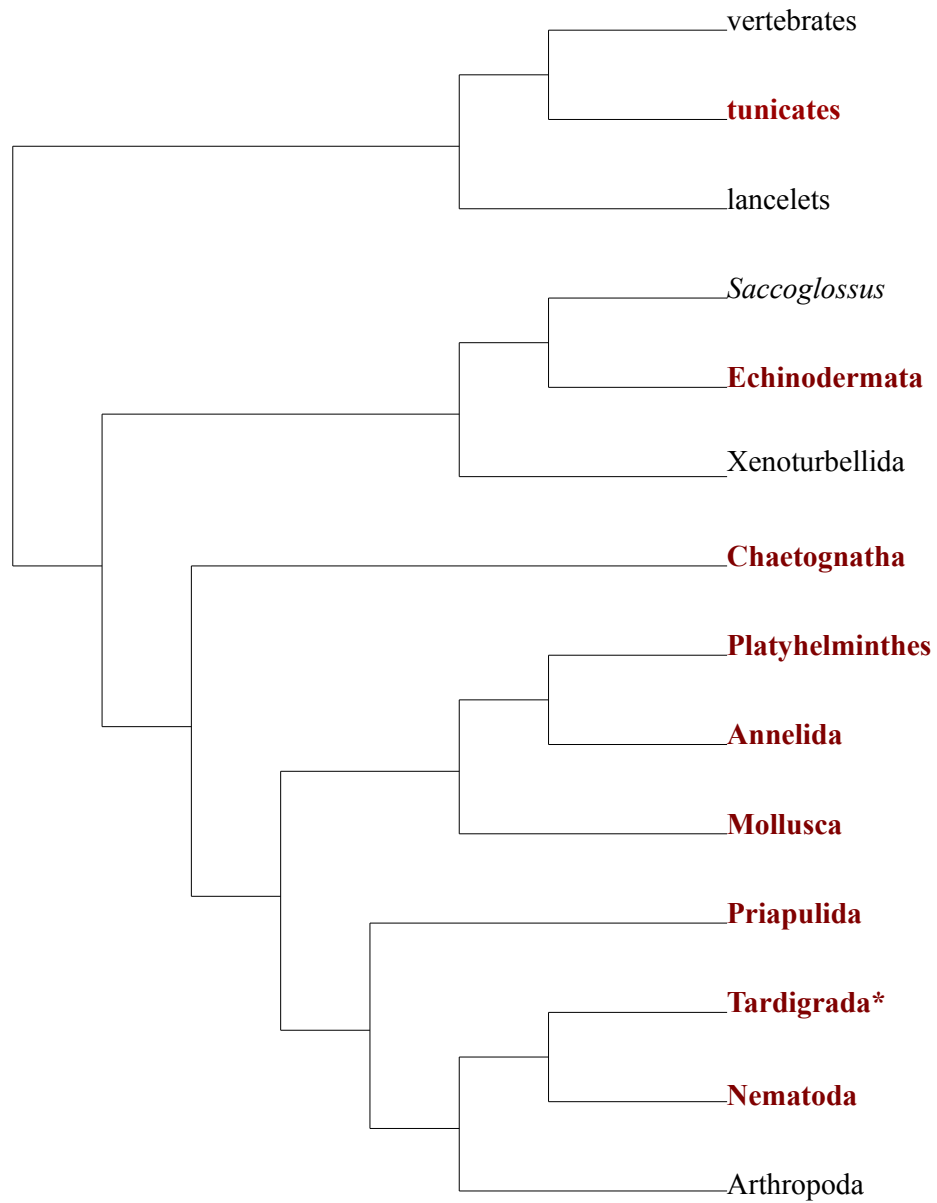
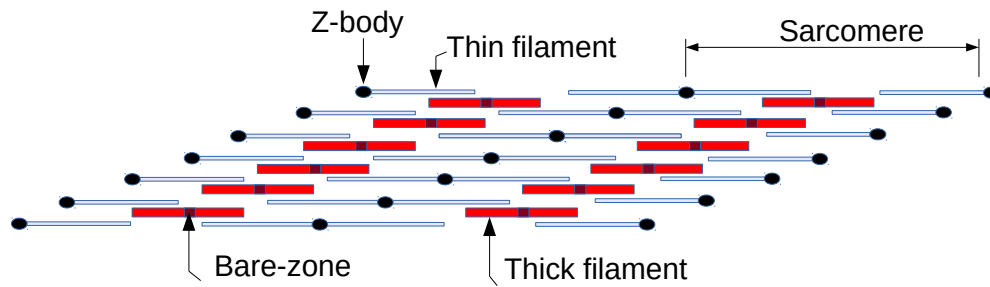
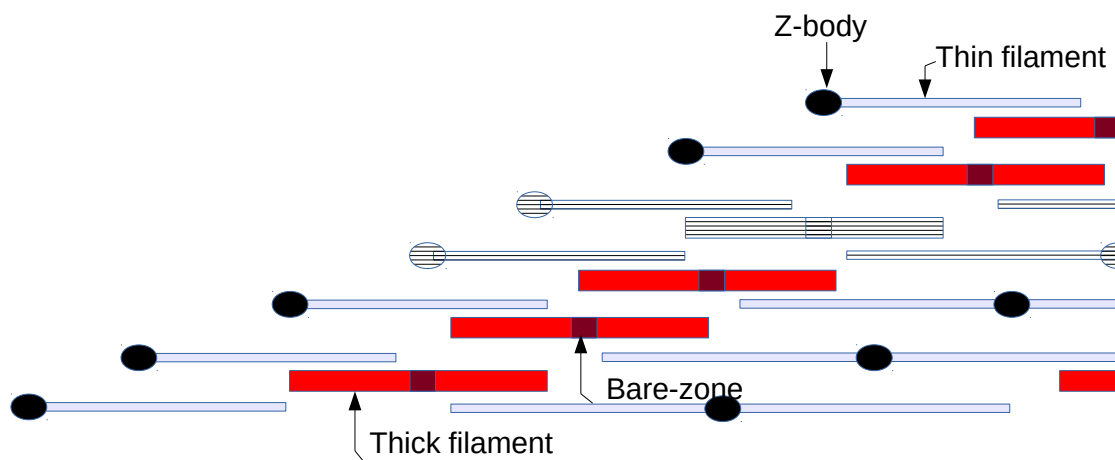


Figure 3. Illustration of changing partners.

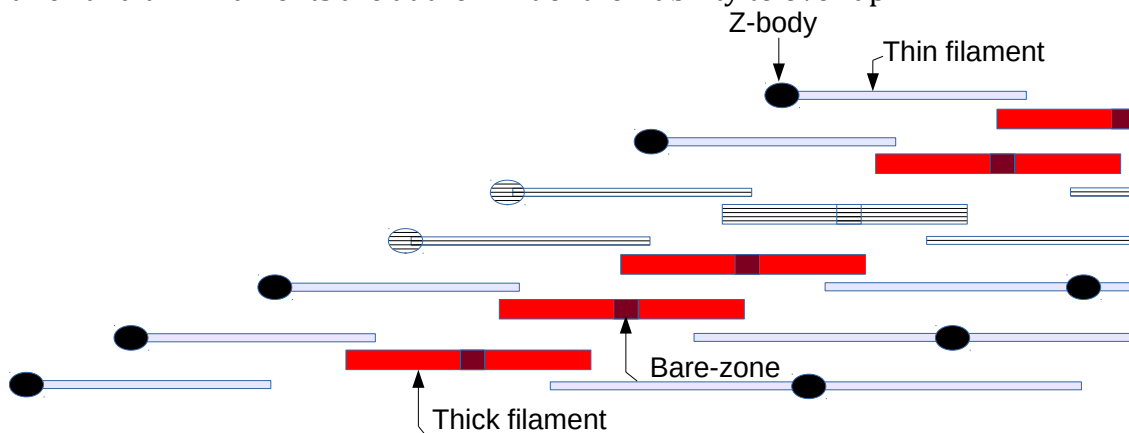
A. Schematic of two obliquely striated sarcomeres.



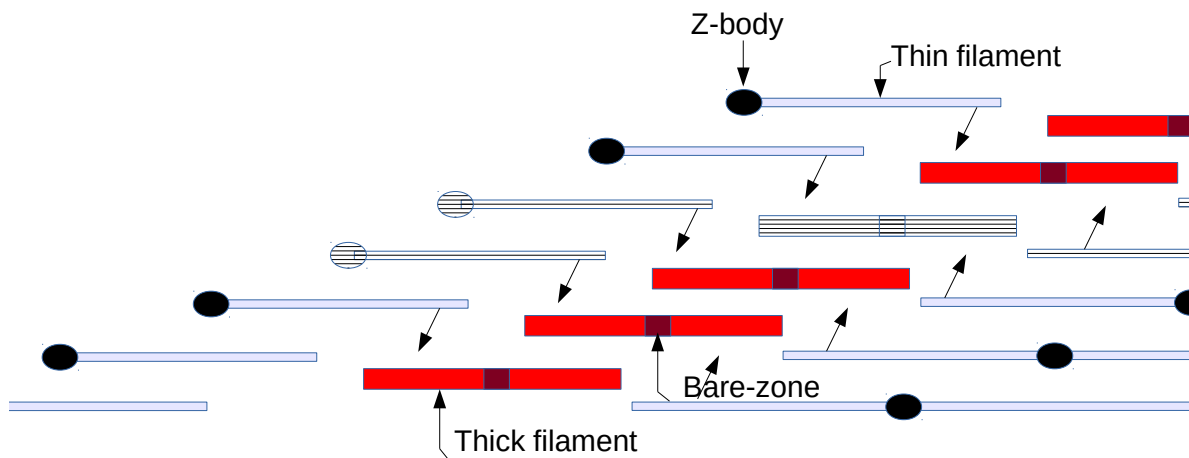
B. Close-up of obliquely striated sarcomere. One pair of thick and thin filaments are white so that they are easy to track throughout the changing partners process.



C. Close-up of obliquely striated sarcomere at a longer sarcomere length. At this length, the thick and thin filaments are at the limit of their ability to overlap.



D. Close-up of obliquely striated sarcomere pulled beyond overlap. Arrows indicate the movement of filaments if changing partners occurs.



E. Close up of sarcomere after changing partners. The white thin filaments are no longer paired with the white thick filament.

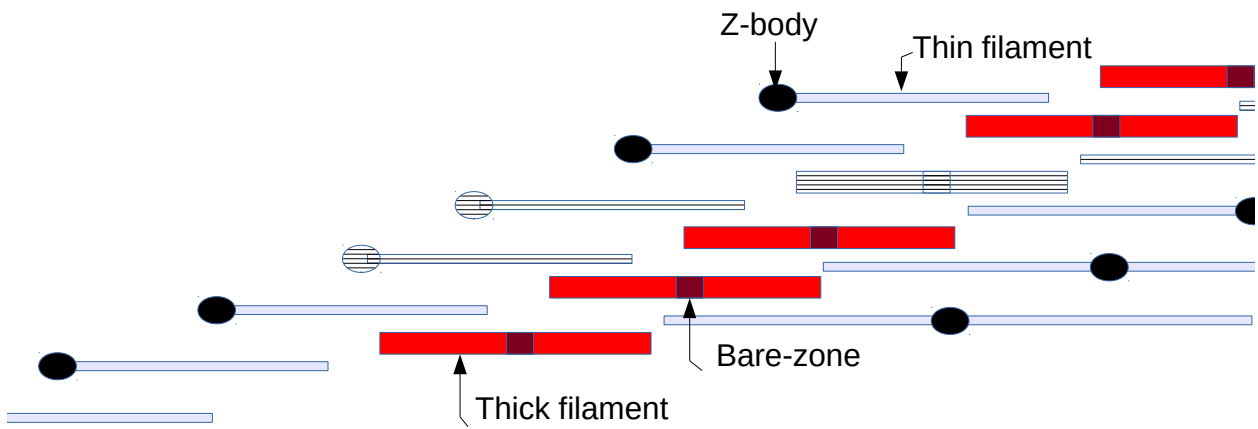
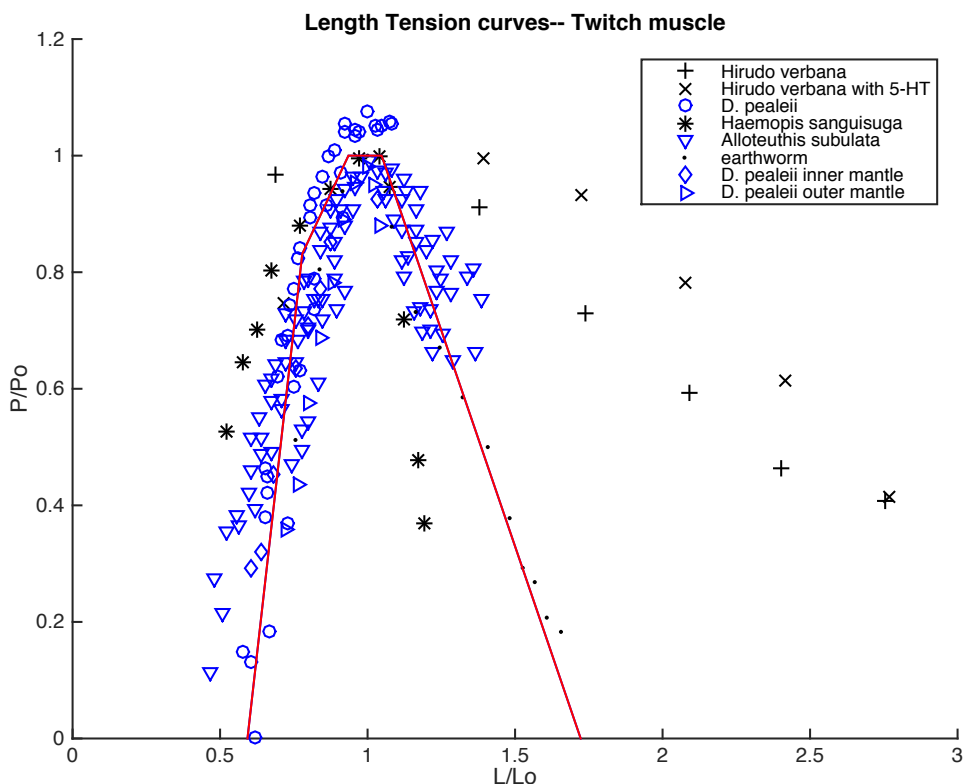
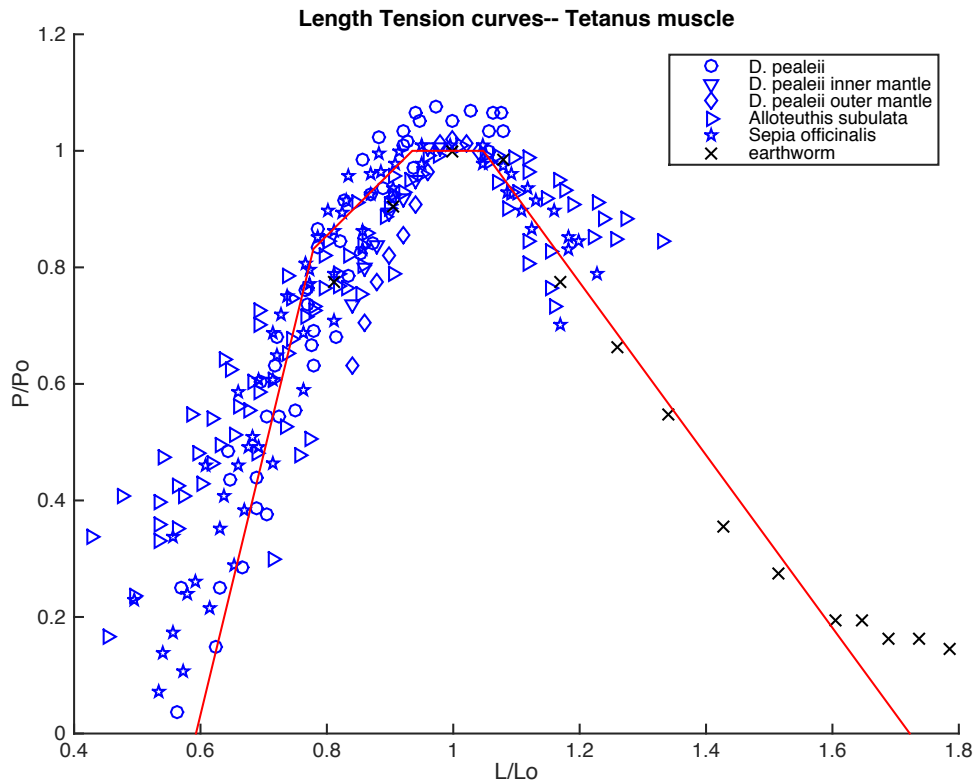


Figure 4. All known length-tension curves of obliquely-striated muscle.

A. Twitch contractions. The red line is the theoretical length-tension curve of cross-striated muscle (Gordon et al., 1966). Blue, open symbols are the length-tension curves of cephalopods. Black, closed symbols are the length-tension curves of annelids. *Hirudo verbana* and *Hirudo verbana* with 5-HT are from (Gerry & Ellerby 2011). *D. pealeii* is from (Kier and Curtin, 2002). *Haemopsis sanguisuga* is from (Miller, 1975). *Alloteuthis subulata* is from (Milligan et al., 1997). Earthworm is from (Tashiro and Yamamoto, 1971). *D. pealeii* inner and outer mantle are from (Thompson et al., 2014).



B. Tetanus contractions. The red line is the theoretical length-tension curve of cross-striated muscle (Gordon et al., 1966). Blue, open symbols are the length-tension curves of cephalopods. Black, closed symbols are the length-tension curves of annelids. *D. pealeii* is from (Kier and Curtin, 2002). *D. pealeii* inner and outer mantle are from (Thompson et al., 2014). *Alloteuthis subulata* and *Sepia officinalis* is from (Milligan et al., 1997). Earthworm is from (Tashiro and Yamamoto, 1971).



CHAPTER 2: GEOMETRIC MODEL

Introduction

For cross-striated muscle it is possible to explain the shape of the length-tension curve based on the dimensions of the thick filaments, thin filaments, bare zones and the Z disk thickness (Gordon et al., 1966). When the sarcomere is most extended, there is no overlap between the myofilaments and thus the myosin heads cannot interact with the thin filaments; the muscle cannot produce force. At shorter lengths, the thin filaments overlap with the myosin heads on the thick filaments to produce force. The amount of force the muscle produces increases as the amount of overlap increases until thin filaments overlap with every myosin head. This change in force as a function of overlap creates the descending limb of the length-tension curve. When the muscle has shortened to the extent that the thin filaments project into the bare zone of the thick filaments, there are no additional myosin heads and thus no additional force, resulting in a plateau for this region of the length-tension curve. When the muscle contracts to even shorter lengths, the thin filaments on one side of the sarcomere begin to interfere with the thin filaments on the other side of the sarcomere, decreasing the amount of force the muscle can produce. Additionally the thin filaments on the right side of the sarcomere are only able to produce force with the myosin heads on the right side of the bare zone and the filaments on the left side of the sarcomere are only able to produce force with myosin heads on the left side of the bare zone. These interactions in combination with interaction of the thick myofilaments with the Z disc, is thought to be responsible for the relationship between force and length of the ascending limb of the length-tension curve.

In addition to the effects of this change in overlap on the tension, in obliquely-striated muscle there is an additional effect because the angle of striation and thus amount of stagger between adjacent myofilaments changes (shearing) as the muscle elongates and shortens. Although early studies interpreted shearing in obliquely-striated muscle as a separate and supplemental mechanism responsible for shortening (Rosenbluth, 1967), it was recognized subsequently that changes in the angle of striation are due simply to the geometry of an essentially constant volume fiber (Lanzavecchia, 1977). The effects of this shearing on the length-tension behavior of obliquely-striated muscle, however, remain unclear. The goal of this study was to use mathematical modeling to explore the implications of shearing for the mechanics of obliquely-striated muscle. In addition, the model provides a means of predicting the effects of changes in myofilament dimensions and organization on the mechanics of this important striation type.

Methods

The model was created in MATLAB r2014b (The Mathworks, Natick, MA) (App. 1). Broadly, the model calculates the amount of force a single sarcomere could produce based on the overlap of the filaments. It starts by calculating this force for a sarcomere that is the length of one thick filament and increases the length by a unit equivalent to the distance between myosin heads until the sarcomere no longer produces force.

Specifically, the model starts with the following prescribed lengths: thick filament, two thin filaments plus the width of a Z-body, bare-zone, myosin head spacing, number of filament pairs (n), and height of the sarcomere. The initial angle of striation is also prescribed. The area of the sarcomere is calculated by multiplying the height of the sarcomere by the initial length of the sarcomere (which is always the length of the thick filament). The vertical distance between

filaments is calculated by dividing the height of the sarcomere by the number of filaments. The length of the imaginary line that would be produced if there was a line connecting all of the Z-bodies on one side of the sarcomere was calculated by dividing the height of the sarcomere by the sine of the striation angle. This length was called p . The coordinates of the thin filaments and the myosin heads were calculated as indicated in Figure 5.

Once the coordinates are determined, the right thick filament is compared to the right thin filaments on either side of it. If the right thick filament overlaps with a right thin filament, but not with the corresponding left thin filament, a value of 1 is added to the total force for that length (Fig. 6). This is repeated on the left hand side and for the thin filaments on one n lower than the thick filament. When both right and left thin filaments are present, no force is added to reflect the hypothesis that the thin filaments interfere with each other, preventing cross-bridges from forming (Gordon et al., 1966).

For each new sarcomere length, the height of the sarcomere was recalculated by dividing the area of the sarcomere by the new length. Additionally, the angle of striation was recalculated by taking the inverse sine of the height of the sarcomere divided by p .

The total force at each length was divided by the maximum force the muscle produced (P_0), as is customary with empirical length-tension curves. The length was standardized by L_0 , defined as the average length at maximum force, to most closely match experimental procedures. Length/ L_0 vs. Force/ P_0 were plotted for various muscle conditions. The initial striation angle, whether or not the striation angle changed during contraction, and the spacing between filaments were all varied to explore the implications of these variables on the force produced during muscle contraction.

Results

The smaller the initial striation angle (the greater the stagger between thick filaments), the greater the maximum length normalized by L_0 at which the muscle is able to produce force (Fig. 6).

Because of the rounding and tangent functions in the model (App. 1), there are jumps in the model that change depending on the values of the parameters (Fig. 7). Additionally, below an initial striation angle of about 3 or 4 degrees, there are spacing issues in the model that cause the muscle to no longer behave in a biologically relevant way (Fig. 8).

Discussion

Though changes in myofilament and bare zone lengths can affect the shape of the length-tension curve, changes in the initial angle of striation increases the maximum length at which the muscle can produce force in the most predictable way. This suggests that obliquely-striated muscle could have evolved in order to increase the operating length of the muscle. This might be especially important to animals without hinged skeletons. If an animal has a hinged skeleton it can use the skeleton as a lever to amplify the force or displacement created by the muscle. Absent such a system, animals must have muscle that is capable of operating over the full length of the animal. Thus in animals with hydrostatic skeletons, it would be advantageous for the longitudinal muscle to be obliquely-striated and capable of long extensions.

The primary limitation to my model is that most of the parameters considered in the model (filament length, filament spacing, bare zone length, the spacing of the myosin heads, and the initial angle of striation) are not known for obliquely-striated muscle from most species, though some of these parameters like filament length and spacing are known for *Hirudo medicinalis* (Lanzavecchia, 1985). It is therefore not generally possible to input the values for a

species and then predict the length-tension curve. However, it is possible to determine which variables are likely to have the largest effects on the length-tension curve. Because these parameters are difficult to collect, as a next step it would be a better to focus not on determining their values for additional species but on collecting additional length-tension curves and kinematic data in order to gain a broader understanding of obliquely-striated muscle.

Additionally my model is two-dimensional and sarcomeres are actually three-dimensional. I maintain a constant area of the muscle by adjusting the height of the sarcomere as the length of the sarcomere changes. In reality, the depth of the sarcomere could also be changing. Because at every length I recalculate the angle of striation based on the length and height of the sarcomere, if much of the change in dimension occurs in the depth dimension rather than the height dimension, it is possible that the angle is not changing in a realistic way.

My model is expressed in terms of parameters that scientists can measure experimentally. Some of these parameters could be expressed in terms of each other. For example, all lengths including the length of the sarcomere, thin filaments, and bare-zone, could be expressed in terms of the length of the thick filament. A future version of this model might want to express all lengths as a ratio in order to better explore the effects of varying these lengths.

Comparing Literature and Model Results: Leeches

Lanzavecchia (1985) compared his model with the Miller (1975) leech data and concluded that the results can only be explained through super elongation. My model predicts that extensibility greater than that reported in Miller (1975) can be achieved with a small initial angle of striation (Fig. 9). The extensibility reported by Gerry (2011), however, exceeds that which can be explained by my model (Fig. 9). An alternative mechanism, perhaps changing partners, is necessary to explain the super elongation found by Gerry (2011). It is important to

note that many of the parameters considered in my model are known only for *Hirudo medicinalis* (Lanzavecchia, 1985). Nevertheless, it is possible to explain the length-tension behavior reported by Miller (1975) with reasonable filament dimensions and spacing, myosin head spacing, and the angle of striation.

Figure 5. Obliquely-striated sarcomeres with dimensions considered in the model. L = the length of the sarcomere, b = the length of two thin filaments and one Z body, x = the horizontal distance between two vertically adjacent thin filaments, d = distance between two thin filaments, n = number of filament pairs, BZ = the length of the bare zone, A = the length of a thick filament, θ = angle of striation.

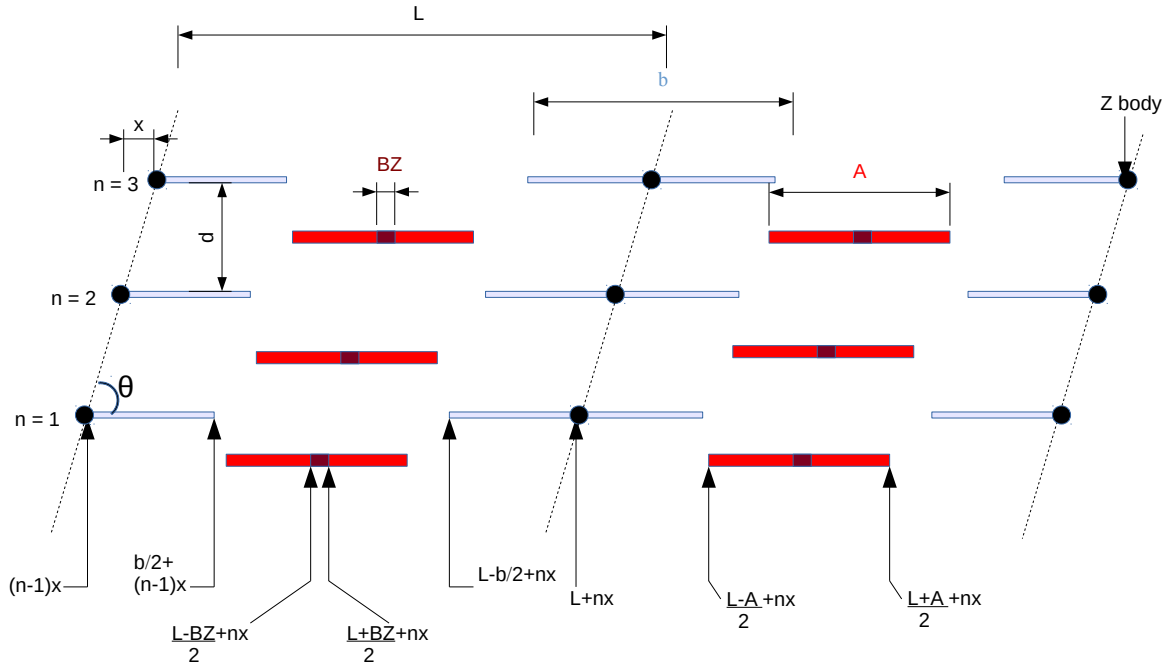


Figure 6. Diagram of one set of thick and thin myofilaments. Dark blue, vertical lines indicate myosin heads that produce force. On the left side of the sarcomere, these are myosin heads that overlap with the left thin filaments and do not overlap with the right thin filaments. On the right side of the sarcomere, these are myosin heads that overlap with the right thin filaments and do not overlap with the left thin filaments. Black, vertical lines indicate myosin heads that do not produce force because they overlap with both left and right thin filaments (B).

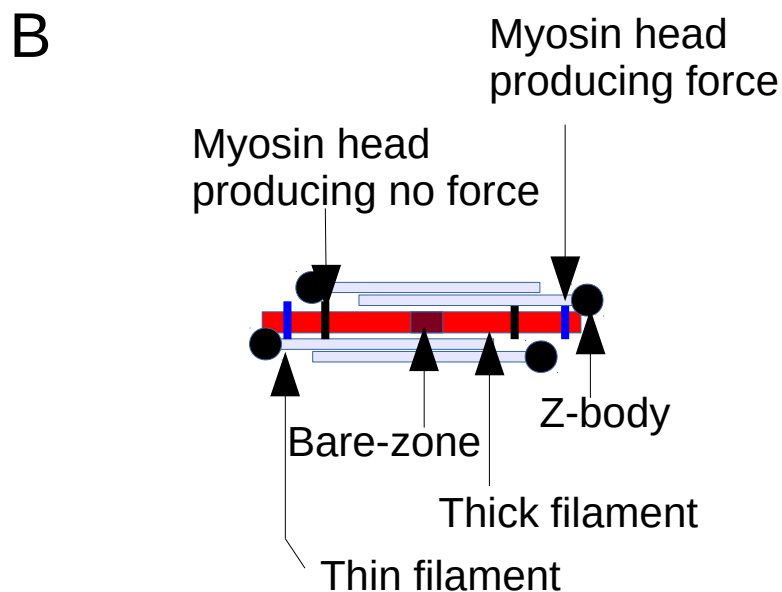
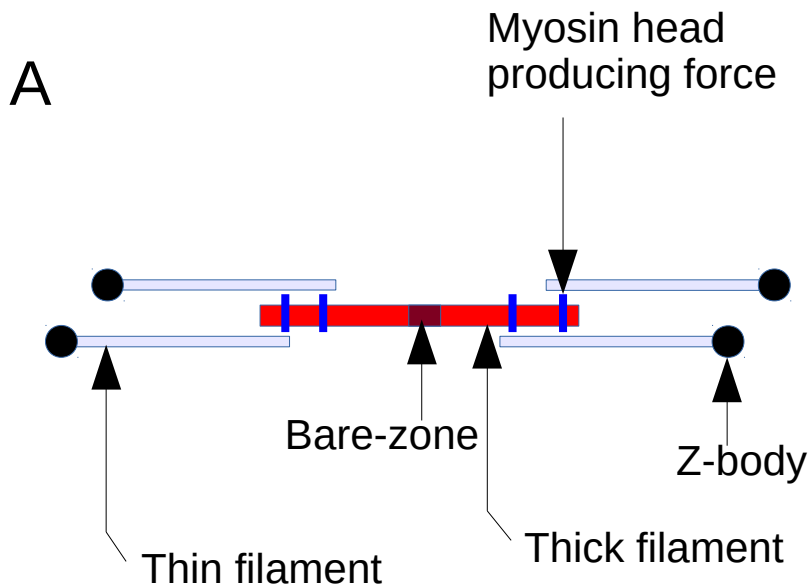


Figure 7. Maximum length/ L_0 changes with initial striation angle. Small initial angles of striation increase the maximum length at which the muscle can produce force.

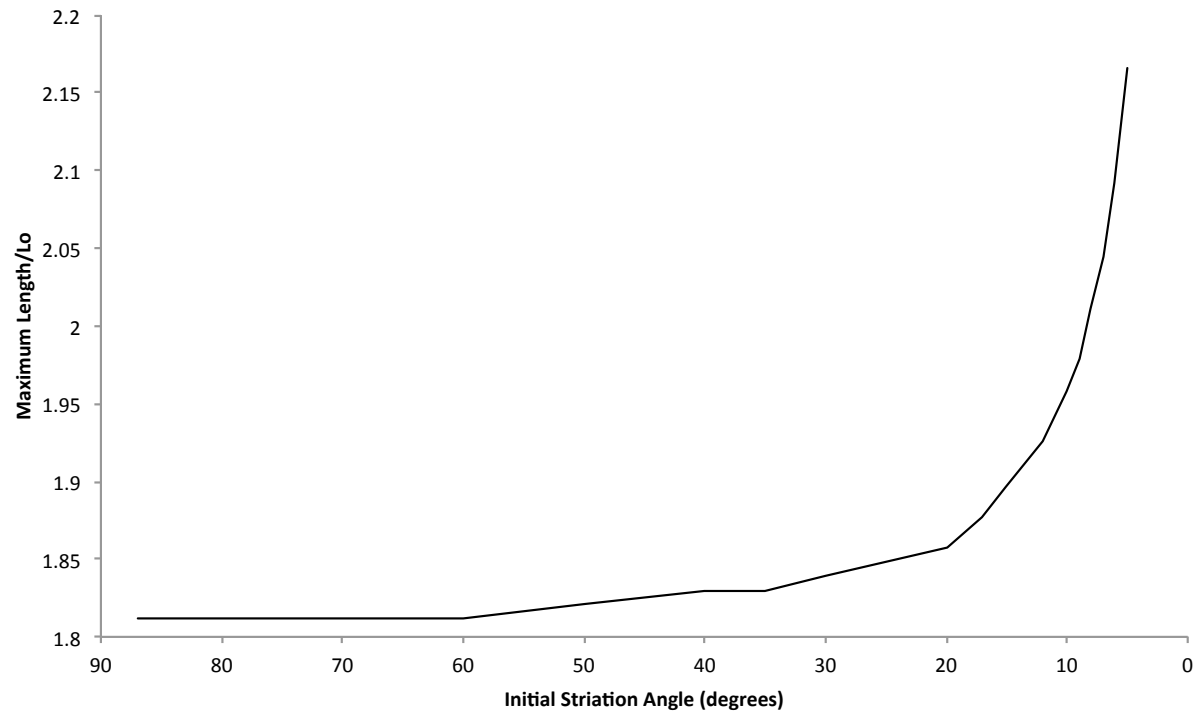
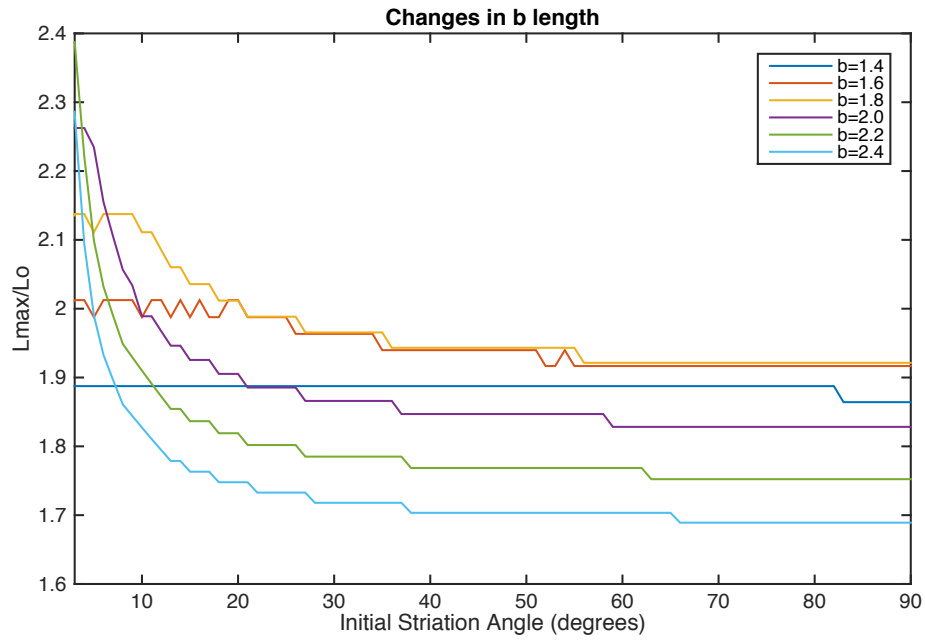
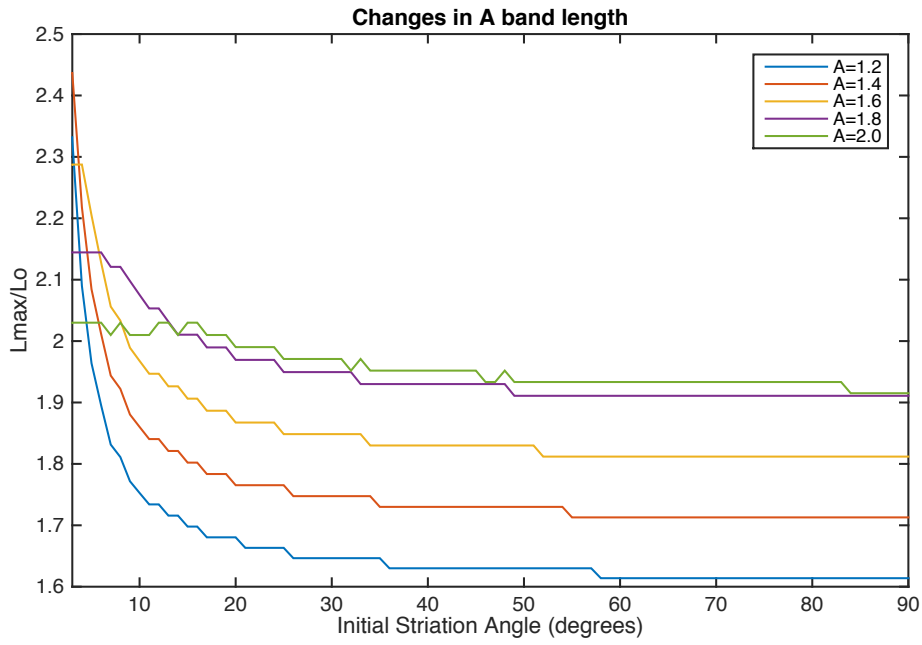
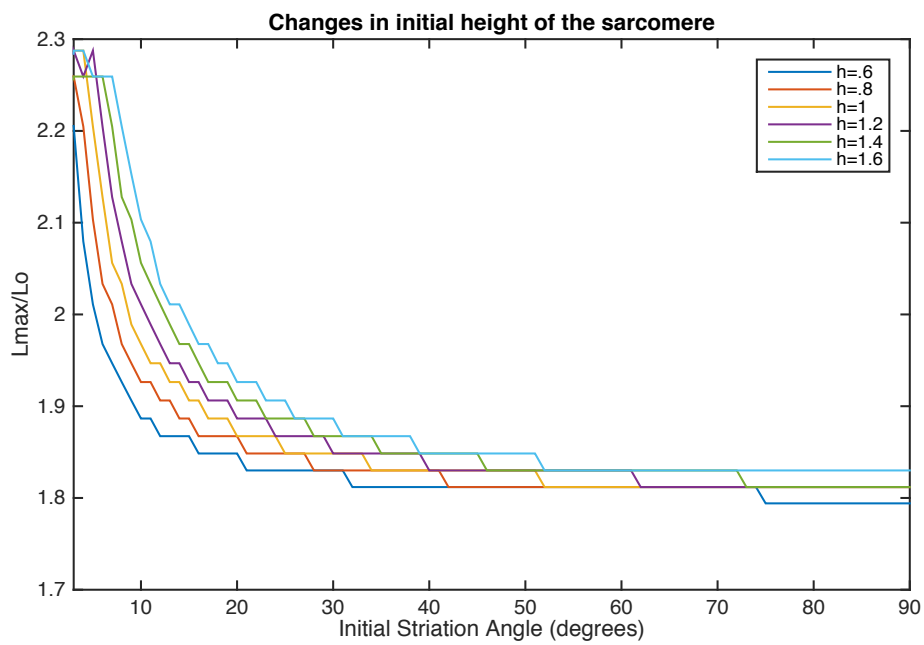
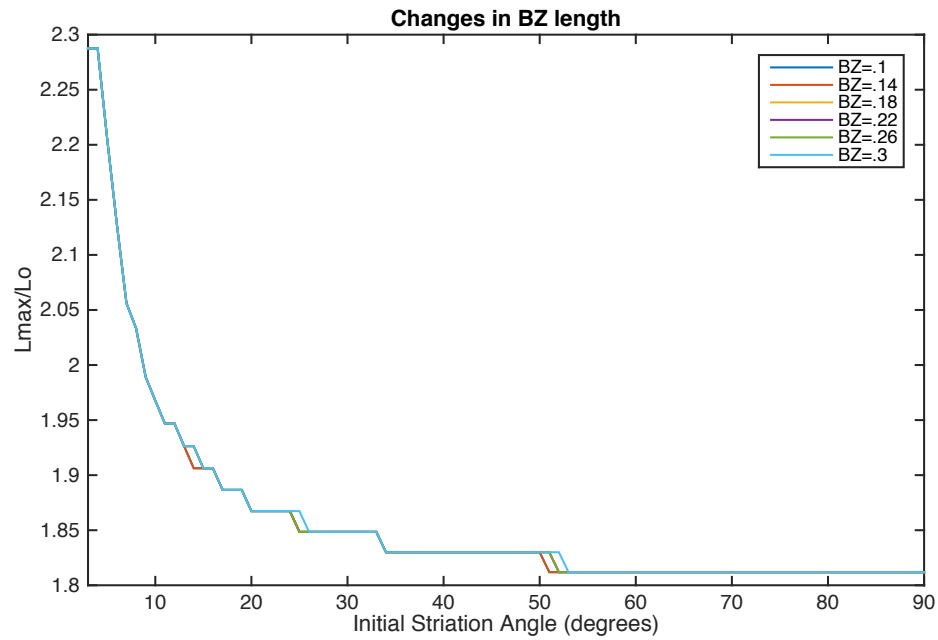


Figure 8. Effects of varying parameters on inflection points in the model





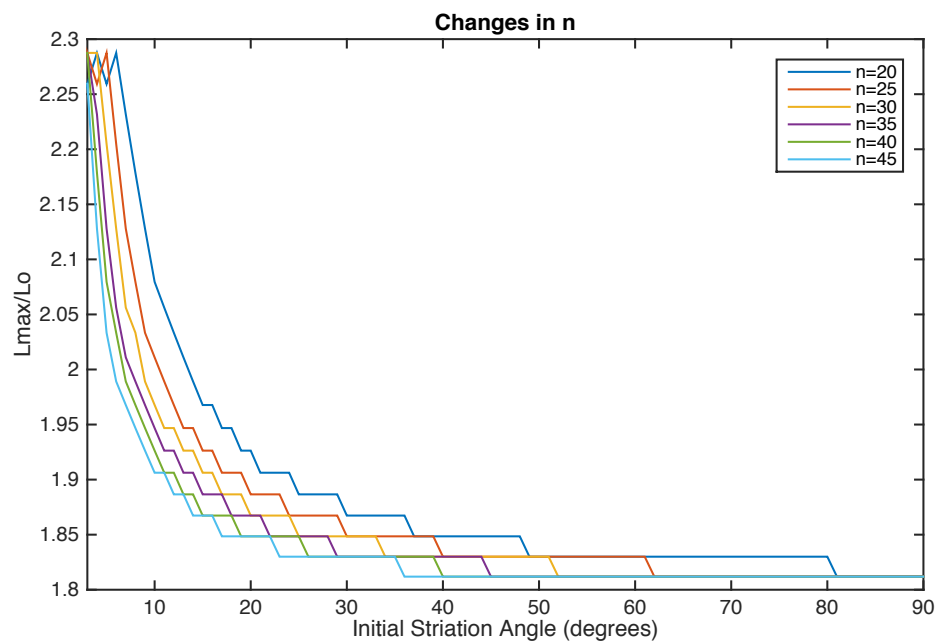
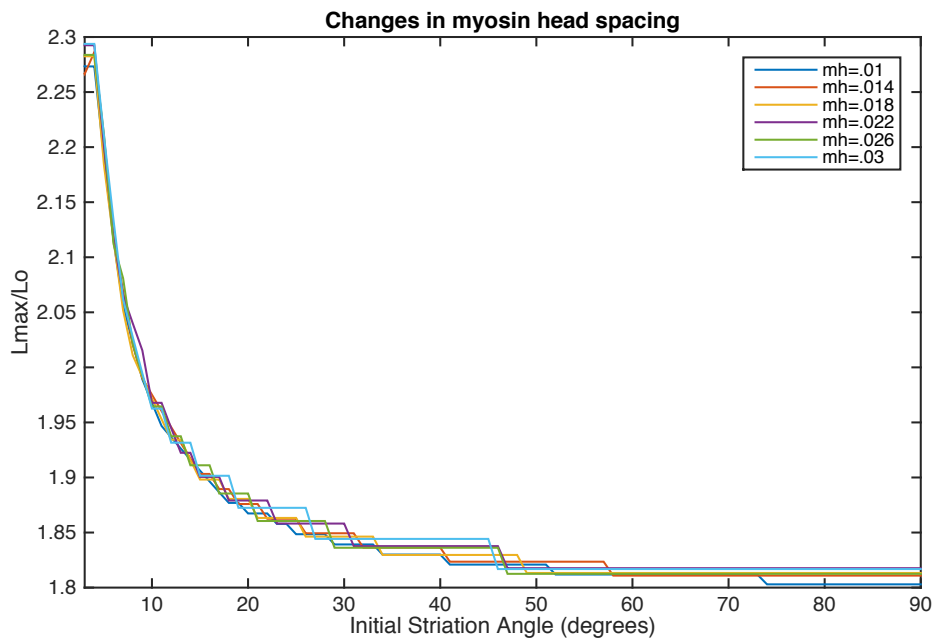
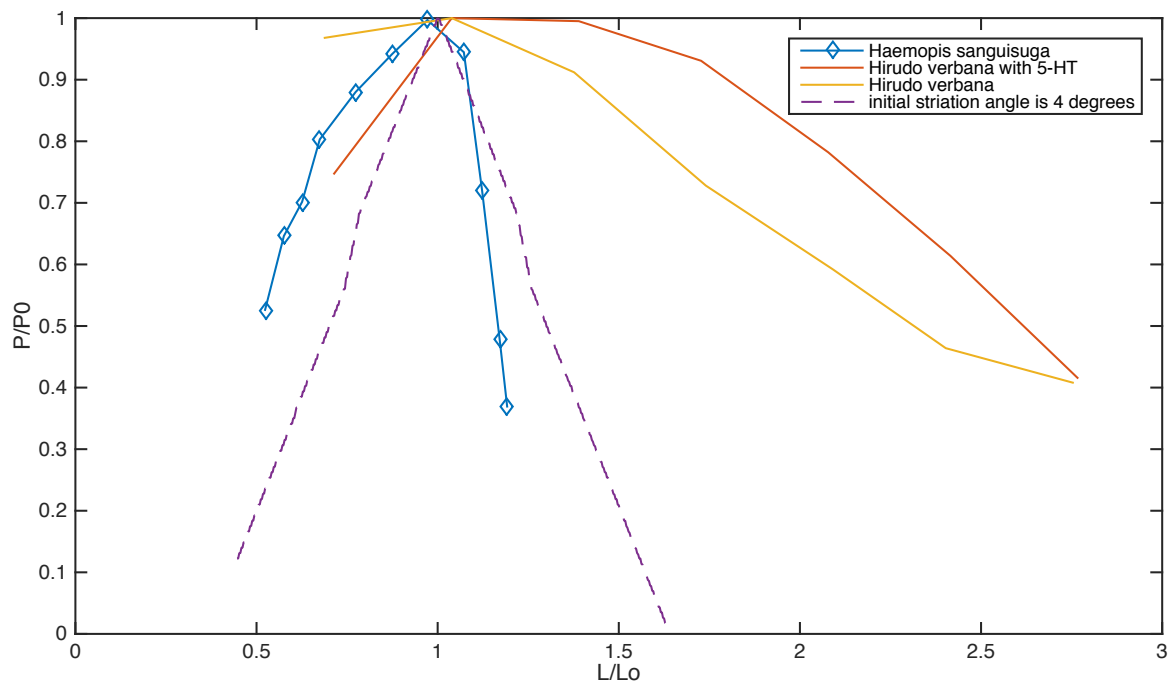


Figure 9. Comparing literature and model results for leeches. All parameters for the dashed purple curve, including the initial striation angle, were assigned experimental values from Lanzavecchia (1985) when possible.



CHAPTER 3: CONCLUSIONS AND FUTURE DIRECTIONS

Given the scarcity of evidence for super elongation, it would be interesting for researchers to consider alternative explanations for oblique striation. For instance, obliquely-striated muscle may be an adaptation for hydrostatic skeletons. Because animals with hydrostatic skeletons do not have the option of manipulating the displacement output of the muscle by varying the relative lengths of input and output lever arms, it is possible that oblique striation evolved as a way of accommodating the large dimensional changes common in invertebrates. This explanation is the most compelling in animals with highly organized oblique and cross-striated muscle like cephalopods. However, for other animals, that lack cross-striated muscle or lack highly organized obliquely-striated muscle, it is possible that oblique striation is simply an intermediate form between smooth and cross-striated muscle. The sarcomeres of cross-striated muscle allow the muscle to amplify velocity by organizing the sarcomeres in series. Animals without cross striation, are unable to take advantage of this in a systematic manner, and may have evolved obliquely-striated muscle as a solution.

In order to understand the function and evolution of obliquely-striated muscle it would be helpful to consider the variation within obliquely-striated muscle from various phyla.

Lanzavecchia (1977) proposed a classification for the different three-dimensional structures of obliquely-striated muscle, but this scheme has not been widely adapted. When scientists describe obliquely-striated muscle in echinoderms compared with obliquely-striated muscle in annelids, they are talking about two different structures. The crinoid muscle has solid Z-lines and the annelid muscle has only Z-bodies. Though this potentially opens up an avenue to explore the

significance of the angle of striation, up to this point, scientists have been acting on the assumption that these two different structures should have the same function because they have the same name. However, it is challenging to classify the types of obliquely-striated muscle with the information available in the literature. On the two-dimensional level, it is hard to determine the angle of striation, the lengths of the filaments, and the exact proteins present in the sarcomeres. In general, only a few micrographs are published in each study, and even with access to all of the micrographs taken, it is difficult to piece together a 3D structure from 2D images. It would be useful to have complete three-dimensional reconstructions, perhaps through the use of an electron microscope capable of sectioning during the imaging process.

Though obliquely-striated muscle has been documented in over 80 species (Table 1), many of these species are difficult to obtain or manipulate. Some of the species like the nemertean *Phallonemertes cf. murrayi* are only available in the depths of the ocean, and others, like *C. elegans*, are too small to easily measure contractile properties. Other species, like *Glycera*, are only available seasonally or must be collected individually. Thus, in addition to collecting length-tension curves of these species, it would be helpful to have TEM data for more easily available species. Species used for bait can be ordered online in large quantities and easily kept alive. These species would be ideal for exploring contractile properties such as length-tension behaviors. A preliminary test of stretching the muscles of many different species, or even collecting behavioral or kinematic data, might reveal if the muscle is even capable of extending to extreme lengths, helping to select muscles that might yield the most interesting length-tension curves.

APPENDIX 1: MATLAB PROGRAM FOR PREDICTING THE LENGTH TENSION CURVE OF OBLIQUELY-STRIATED MUSCLE

```
function[LTcurve] = sarc_sim(angle,angle_changing,animate,moviename)

% assign filament dimensions in microns
A = 1.6; % length of the thick filament
I = 2.05; % length of two thin filaments and a z-line or body
BZ = 0.20; % length of the bare-zone

mh_spacing = 0.02; % spacing between myosin heads
n = 30; % number of thin filaments
h_initial = 6; % initial height of the sarcomere
area = A*h_initial; % area is the initial length of the sarcomere (A) times
the initial height of the sarcomere (h)
p = h_initial/sin(angle/180*pi); % other side of the parallelogram

% create figure for the animation
if animate
    fig = figure;
    hold on
    h1 = []; % dummy handle for some figure graphic
end

% create movie container if called for
if exist('moviename','var')
    mov = VideoWriter([moviename, '.avi']);
    open(mov);
end

% find the force at each sarcomere length
LF = zeros(round(2*I/mh_spacing)+1,2); % create an array in which to store
Length and Tension
l = 1;
for L = A:mh_spacing:A+4*I
    h = area/L; % calculate the height of the sarcomere

    d = h/n; % calculate the distance between the filaments based on area and
length.

    % if the angle is changing, calculate the angle of striation
    if angle_changing
        theta = asin(h/p);

    else
        theta = angle/180*pi;
    end

    x = d/tan(theta); % calculate the lateral distance between the start of
thin filaments

    % create arrays to hold the locations of the filaments
    TFL = zeros(n-1,round((A-BZ)/2/mh_spacing)+1,2); % create an array to
```

```

hold the locations of the left myosin heads
% fill in array for myosin heads
for i = 1:n-1
    k = 1;
    for j = (L-A)/2 + x*i:mh_spacing:(L-BZ)/2 + x*i
        TFL(i,k,1) = j;
        TFL(i,k,2) = (i-1)*d;
        k = k+1;
    end
end

TFR = zeros(n-1,round((A-BZ)/2/mh_spacing)+1,2); % create an array to
hold the locations of the right myosin heads
% fill in array for myosin heads
for i = 1:n-1
    k = 1;
    for j = (L+BZ)/2 + x*i:mh_spacing:(L+A)/2 + x*i
        TFR(i,k,1) = j;
        TFR(i,k,2) = (i-1)*d;
        k = k+1;
    end
end

tfl = zeros(3,n); % create an array to hold the locations of the left
thin filaments
% fill in array for the thin filaments
for i = 1:n
    tfl(1,i) = (i-1)*x;
    tfl(2,i) = I/2 + (i-1)*x;
    tfl(3,i) = (i-1)*d;
end

tfr = zeros(3,n); % create an array to hold the locations of the right
thin filaments
% fill in array for the thin filaments
for i = 1:n
    tfr(1,i) = L-I/2+i*x;
    tfr(2,i) = L+i*x;
    tfr(3,i) = (i-1)*d;
end

% compare the arrays to calculate the force
Fsumn = zeros(1,n); % create an empty array to count the force produced
by each filament set

for i = 1:n-1 % for each filament
    for k = 1:round((A-BZ)/2/mh_spacing)+1 % along all possible positions
        if (tfl(1,i) <= TFL(i,k,1)) && (TFL(i,k,1) <= tfl(2,i)) % if the
left myosin head overlaps with the left thin filament
            if (tfr(1,i) <= TFL(i,k,1)) && (TFL(i,k,1) <= tfr(2,i)) % if
they left myosin head overlaps with the right thin filament
                Fsumn(i) = Fsumn(i); % keep the force the same because
the thin filaments are interfering with each other
            else
                Fsumn(i) = Fsumn(i) + 1; % if the thin filaments do not
overlap and there is a left myosin head, add one to the force.
            end
        end
    end
end

```

```

end

    if (tfl(1,i+1) <= TFL(i,k,1)) && (TFL(i,k,1) <= tfl(2,i+1)) %
check the thin filament on the other side of the left myosin head
        if (tfr(1,i+1) <= TFL(i,k,1)) && (TFL(i,k,1) <= tfr(2,i+1))
            Fsumn(i) = Fsumn(i);
        else
            Fsumn(i) = Fsumn(i) + 1;
        end
    end
end

    if (tfr(1,i) <= TFR(i,k,1)) && (TFR(i,k,1) <= tfr(2,i)) %do the
same for the right myosin heads
        if (tfl(1,i) <= TFR(i,k,1)) && (TFR(i,k,1) <= tfl(2,i))
            Fsumn(i) = Fsumn(i);
        else
            Fsumn(i) = Fsumn(i) + 1;
        end
    end
end

    if (tfr(1,i+1) <= TFR(i,k,1)) && (TFR(i,k,1) <= tfr(2,i+1))
        if (tfl(1,i+1) <= TFR(i,k,1)) && (TFR(i,k,1) <= tfl(2,i+1))
            Fsumn(i) = Fsumn(i);
        else
            Fsumn(i) = Fsumn(i) + 1;
        end
    end
end

end

end
% store the length and total force in the LF array
LF(1,1) = L;
LF(1,2) = sum(Fsumn);

% create/update the animation if called for
if animate
    if isempty(h1) == false % delete graphics from the previous
        delete(h1);
        delete(h2);
        delete(h3);
        delete(h4);
        delete(h5);
    end

    % plot new components in the bottom panel
    subplot(2,1,1);
    hold on

    h1 = plot(TFL(1:n-1,1:round((A-BZ)/2/mh_spacing)+1,1),TFL(1:n-
1,1:round((A-BZ)/2/mh_spacing)+1,2),'g*');
    h2 = plot(TFR(1:n-1,1:round((A-BZ)/2/mh_spacing)+1,1),TFR(1:n-
1,1:round((A-BZ)/2/mh_spacing)+1,2),'g*');

```

```

q = tfl(1:2,1:n);
w = tfl(3,1:n);
w(2,:) = tfl(3,1:n);
h3 = plot(q,w);

r = tfr(1:2,1:n);
s = tfr(3,1:n);
s(2,:) = tfr(3,1:n);
h4 = plot(r,s);

axis([0 A+2*I 0 h_initial]);

% Adjust the length tension curve for P0 and L0
[m,q] = size(LF);
maxForce = max(LF(1:m,2));
LTcurve(1:m,2) = LF(1:m,2)/maxForce;

[r,c] = find(LTcurve == 1);
[x,y] = size(r);
L0 = sum(r(1:x))/x;

LTcurve(1:m,1) = LF(1:m,1)/LF(round(L0),1);

% plot new components in bottom panel
subplot(2,1,2);
hold on
h5 = plot(LTcurve(1:m,1),LTcurve(1:m,2),'g*');
axis([0 4 0 1])

% add frame to the movie if we're creating one
if exist('movienam', 'var')
    % Write each frame to the file
    currFrame = getframe(gcf);
    writeVideo(mov,currFrame);
end
else
    % Adjust the length tension curve for P0 and L0
    [m,q] = size(LF);
    maxForce = max(LF(1:m,2));
    LTcurve(1:m,2) = LF(1:m,2)/maxForce;

    [r,c] = find(LTcurve == 1);
    [x,y] = size(r);
    L0 = sum(r(1:x))/x;

    LTcurve(1:m,1) = LF(1:m,1)/LF(round(L0),1);
end

pause(.01) % slight pause so the figure animation gets displayed

```

```

        if LF(1,2) == 0 % if the force is 0, stop the program
            break
        end
        l = l+1;

    end
    % close the movie if we created one
    if exist('moviename','var')
        close(mov);
    end

end

```

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