The Journal of Experimental Biology 212, 673-683
Published by The Company of Biologists 2009
doi:10.1242/jeb.023481

Skeletal muscle fiber types in the ghost crab, *Ocypode quadrata*: implications for running performance

Michael J. Perry[1,*], Jennifer Tait[1,*], John Hu[1,*], Scott C. White[2] and Scott Medler[1,†]

[1]Department of Biological Sciences and [2]Department of Exercise and Nutrition Sciences, University at Buffalo, Buffalo, NY 14260, USA

*These authors contributed equally to this work
†Author for correspondence (e-mail: smedler@buffalo.edu)

Accepted 4 December 2008

SUMMARY

Ghost crabs possess rapid running capabilities, which make them good candidates for comparing invertebrate exercise physiology with that of more extensively studied vertebrates. While a number of studies have examined various aspects of running physiology and biomechanics in terrestrial crabs, none to date have defined the basic skeletal muscle fiber types that power locomotion. In the current study, we investigated skeletal muscle fiber types comprising the extensor and flexor carpopodite muscles in relation to running performance in the ghost crab. We used kinematic analyses to determine stride frequency and muscle shortening velocity and found that both parameters are similar to those of comparably sized mammals but slower than those observed in running lizards. Using several complementary methods, we found that the muscles are divided into two primary fiber types: those of the proximal and distal regions possess long sarcomeres (6.2±2.3 μm) observed in crustacean slow fibers and have characteristics of aerobic fibers whereas those of the muscle mid-region have short sarcomeres (3.5±0.4 μm) characteristic of fast fibers and appear to be glycolytic. Each fiber type is characterized by several different myofibrillar protein isoforms including multiple isoforms of myosin heavy chain (MHC), troponin I (TnI), troponin T (TnT) and a crustacean fast muscle protein, P75. Three different isoforms of MHC are differentially expressed in the muscles, with fibers of the mid-region always co-expressing two isoforms at a 1:1 ratio within single fibers. Based on our analyses, we propose that these muscles are functionally divided into a two-geared system, with the aerobic fibers used for slow sustained activities and the glycolytic mid-region fibers being reserved for explosive sprints. Finally, we identified subtle differences in myofibrillar isoform expression correlated with crab body size, which changes by several orders of magnitude during an animal's lifetime.

Key words: ghost crab, muscle fiber type, skeletal muscle.

INTRODUCTION

Ghost crabs are among the most remarkable of invertebrate athletes, possessing running capabilities that rival those of comparably sized mammals. These mouse-sized crustaceans can run at burst speeds of 2–4 m s^{-1} (Burrows and Hoyle, 1973; Hafemann and Hubbard, 1969) and at slower speeds can maintain activity for more than an hour without rest (Full, 1987). Sustained slow locomotion relies on aerobic metabolism whereas short sprints depend on glycolytic muscle activity and result in lactic acid build-up (Full, 1987). The factorial scope of exercising ghost crabs (approximately 8–12 times the resting metabolic rate) is more typical to that of active ectothermic vertebrates and mammals than to an invertebrate (Full, 1987; Full and Herreid, 1983). Biomechanical studies reveal that in spite of some obvious differences between crabs and terrestrial vertebrates, ghost crabs exhibit many of the same features of locomotion as other running animals. These include similar stride frequencies during running and a trot-to-gallop transition at critical speeds (Blickhan and Full, 1987; Full and Weinstein, 1992). Crustacean muscles exhibit an array of diverse physiological properties and it is therefore relevant to ask how skeletal muscle organization might relate to running performance. Furthermore, it is currently unknown how the kinetic properties of skeletal muscles that power ghost crab locomotion might compare with the more extensively studied contractile parameters of terrestrial vertebrate muscles.

In spite of the fact that running capability is derived directly from the specific contractile and metabolic capacities of skeletal muscles in the ghost crab's legs and thorax, very little is known about the cellular and molecular organization of their muscles. Decapod crustaceans exhibit a wide variety of muscle fiber types specialized for a variety of specific functions (Atwood, 1976; Silverman et al., 1987). Several different fiber types have been identified based on differences in fiber ultrastructure, histochemistry, contractile properties and myofibrillar isoform composition. Studies classifying discrete fiber types based on ATPase histochemistry have identified four fiber types in decapod crustaceans (Gruhn and Rathmayer, 2002; Gunzel et al., 1993; Rathmayer and Maier, 1987). Using myofibrillar isoform assemblages to classify specific fiber types, at least three different fiber types can be recognized: slow phasic (S_1), slow tonic (S_2) and fast (Medler et al., 2004; Medler and Mykles, 2003; Mykles, 1985; Mykles, 1988). Each of these types possesses a different myosin heavy chain, as well as different isoforms of tropomyosin, troponin T (TnT), troponin I (TnI) and others. More recently, two types of fast fibers have been identified in a species of crayfish that exhibit differences in sarcomere width, TnI isoform composition and Ca^{2+} activation properties (Koenders et al., 2004). There is currently no common basis for integrating the different crustacean muscle classification schemes into discrete fiber types but it is clear that a number of distinct fiber types exist and that these types are functionally diverse. In fact, classifying crustacean muscles into discrete categories may not reflect the continuum of fiber types actually present in crustacean muscles (Medler et al., 2004).

In the current study, we investigated the organization of the extensor and flexor carpopodite muscles that comprise the muscle mass within the meropodite (Fig. 1). These antagonistic muscles alternately extend and flex the distal segments of the crab legs during running and represent some of the primary muscles involved in locomotion. Our goal was to define physiologically relevant parameters that are known to affect muscle function in other crustacean muscles. These included identification of alternate isoforms of myofibrillar proteins, as well as fiber type – specific aerobic potential identified through histochemical methods. In conjunction with these studies, we made measurements of running speed and stride frequency in crabs that ranged in size from 200 mg to 75 g. We also used kinematic analyses of digital video recordings from running crabs to estimate muscle shortening velocities *in vivo*. Based on these analyses, we report that the active shortening properties of the muscles are about that expected for a similar sized mammal but substantially slower than a comparably sized lizard. In addition, we identify specializations in muscle fiber type that may help explain how the muscles are used for different running speeds. Finally, we report a significant level of complexity in the myofibrillar isoform assemblages present in different muscle fiber types. These include three alternate isoforms of myosin heavy chain (MHC), TnT and TnI expressed in different combinations in single fibers. We discuss the possible significance of these different fiber types within the broader context of integrated locomotory function.

MATERIALS AND METHODS

Animals and maintenance

Ghost crabs (*Ocypode quadrata* Fabricius) were collected from Padre Island National Seashore (TX, USA) (Permit # PAIS-2005-SCI-0001) and brought back to the lab at the University at Buffalo (NY, USA). Animals were housed in individual plastic containers containing either sand or gravel and artificial seawater. They were kept in a constant temperature chamber maintained at 32°C, with a 12 h:12 h light:dark cycle. A vaporizer was operated within the chamber to keep the humidity elevated. Crabs were fed a diet of commercially available crab pellets, reptile pellets and dried krill. They were maintained and sacrificed in accordance with an approved IACUC protocol.

Determination of muscle shortening velocity *in vivo*

We recorded crabs with a digital video camera (60 Hz) while they were running on a treadmill. A small spot of red paint was applied to the distal end of the meropodite and to the distal end of the propopodite of the second walking leg. Treadmill speed was increased gradually until animals reached a trot-to-gallop transition. Stride frequency and angular velocities of the second walking leg were determined afterward either by visual observation of individual frames (stride frequency) or by using motion analysis software for angular velocities [Motus^{TM} v. 8.4 motion analysis software (Vicon-Peak, London, UK)].

We determined the amounts of muscle strain corresponding to angular changes in the meropodite–carpopodite joint by manually rotating the leg through its range of motion by pulling on the central tendon (apodeme) with a pair of forceps. The amount of apodeme movement (mm) was measured under a stereomicroscope as the leg joint was flexed or extended. The simple geometry of the flexor and extensor muscles allowed us to directly convert apodeme movement into absolute muscle shortening. Muscle fiber lengths were measured with digital calipers and absolute muscle shortening was converted to relative muscle shortening (% of fiber length). We assumed that the fiber lengths measured in dissected legs approximated those of resting fiber lengths *in vivo*.

We estimated muscle shortening velocity in two ways. First, we measured angular velocities from digital video recordings of running crabs and then used these to estimate corresponding muscle shortening velocities as described above. Second, for muscles shortening with a sinusoidal strain trajectory, the frequency of contraction is approximated as one half of the ratio of muscle shortening velocity to the total strain amplitude (Medler and Hulme, 2009). Using the strain amplitudes determined from our kinematic analyses, we were then able to estimate muscle shortening velocities as a function of contractile frequency. To estimate maximal shortening velocity (V_{max}), we assumed that the muscles operating *in vivo* were shortening at approximately 30% of V_{max} (Josephson, 1993; Rome and Lindstedt, 1997).

Muscle tissue preparation

Crab legs were quickly frozen by rapid immersion in liquid N_2 and stored at –80°C for later analysis. For protein analyses, we removed the exoskeleton and cuticle from the anterior and posterior regions of the meropodite and frozen legs were freeze-dried under vacuum. The dried legs with muscles intact were stored at –20°C in sealed plastic tubes containing desiccant. This procedure preserves the anatomical orientation of the fibers and allows for the removal and analysis of individual muscle fibers. For the isolation of RNA, whole pieces of frozen muscle were cut with a razor blade and the tissues were processed immediately.

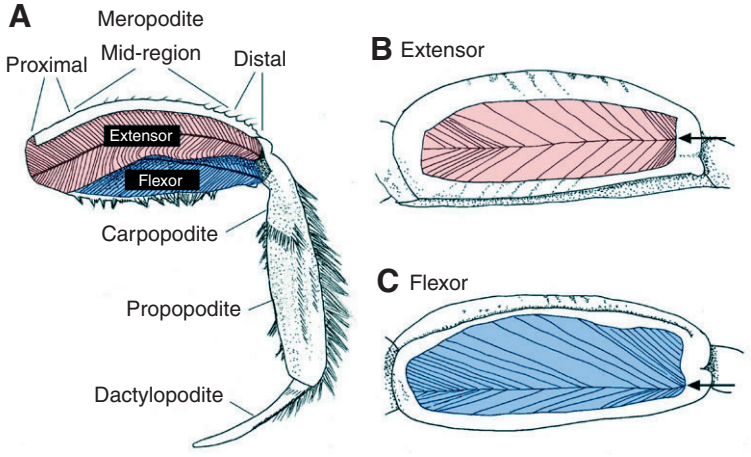

Fig. 1. Anatomical organization of the extensor and flexor carpopodite muscles of the ghost crab. In each figure, the exoskeleton covering the muscles has been removed to allow an unobstructed view of the muscles. (A) General orientation of the muscles within a walking leg viewed from an anterior position. The extensor muscle is partially reflected along its ventral border to allow the flexor to be seen. The relative positions of the proximal, mid and distal regions of the muscles discussed in the text are indicated. (B) More precise organization of the extensor carpopodite, viewed from an anterior position. (C) More precise organization of the flexor carpopodite, viewed from a posterior position. The arrows in B and C indicate the insertion point of the apodeme, with the insertion of the extensor being superior to the flexor.

Measurement of muscle fiber lengths and fiber pinnation angles

To determine fiber lengths, we removed individual dried muscle fibers from intact muscles from different regions along the length of the muscle (proximal → mid → distal). The point of insertion was measured as the distance from the proximal end of the meropodite. Fiber lengths were measured to the nearest millimeter with electronic calipers (VWR Bridgeport, NJ, USA). Fiber lengths were compared statistically using an unpaired *t*-test, pooling values from the proximal and distal regions and comparing their lengths with those from the mid-region.

Muscle fiber pinnation angles were measured from frozen muscles, where the carpopodite–propopodite joint was in a neutral position (~90 deg. as in Fig. 1A). Multiple angles were measured for each extensor and flexor muscle and these were averaged for each individual. Three crabs of different sizes (9.7, 20.6 and 55 g) were used for these measurements and there was no indication that insertion angles varied with animal size.

Determination of sarcomere length

We dissected muscles in a Ringer's solution free of Ca^{2+} and containing EDTA to promote relaxation. Small bundles of fibers were removed from different anatomical regions of the muscles and placed in a small reservoir of the Ringer's solution on a microscope slide and observed at a final magnification of ×200 with either DIC optics or with the condenser diaphragm closed down to increase contrast. Images of the fibers were captured digitally and then printed. Mean sarcomere length was calculated by measuring the distances across several sequential sarcomeres (~10) along a fiber. We calibrated distances with a stage micrometer placed in the same position as the muscle fibers. In addition, sarcomere lengths were measured from histochemically stained muscle sections.

Analysis of myofibrillar proteins by SDS PAGE and western blotting

Individual fibers were removed from different anatomical regions of freeze-dried muscles and placed directly in 30–50 µl of SDS sample buffer [62.5 mmol l^{-1} Tris-HCl (pH 6.8), 12% glycerol, 1.25% SDS and 1.25% β-mercaptoethanol] in 1.5 ml microcentrifuge tubes. We then homogenized fibers in the sample buffer using a plastic hand-held pestle that fitted directly into the tube. Aliquots of the fiber samples (5 µl) were loaded on PAGE gels.

For resolution of MHC isoforms, samples were separated on 16 cm gels for 24 h under constant voltage (200 V) at 8°C following the general protocol of Blough and colleagues (Blough et al., 1996). Resolving gels consisted of 8% polyacrylamide (200:1 acrylamide:N,N′methylenebisacrylamide) and 12% glycerol. Stacking gels were composed of 4% polyacrylamide (20:1 acrylamide:N,N′methylenebisacrylamide) and contained no glycerol. After completion of electrophoresis, gels were fixed overnight in 50% methanol and silver stained according to the methods of Wray and colleagues (Wray et al., 1981). To determine the ratio of multiple MHC isoforms co-expressed in single fibers, we measured the relative amount of each protein for 40 fibers taken from four crabs of different sizes (6.2, 8.75, 18.7 and 50 g) using densitometry of scanned gels (NIH Image 1.63).

Isoforms of TnT, TnI and P75 were identified through SDS-PAGE and western blotting procedures. Samples were separated on gels containing 8% acrylamide (37.5:1 acrylamide: N,N′methylenebisacrylamide) under constant voltage (200 V) for 45 min, using a Bio-Rad Mini-Protean 3 gel apparatus (Hercules, CA, USA). Separated proteins were then transferred to PVDF membranes under constant voltage (100 V) for 1 h at 4°C.

Blots were probed for TnT, TnI or P75 using polyclonal antibodies raised in rabbits against these proteins isolated from lobster muscles (Medler and Mykles, 2003; Medler et al., 2004; Medler et al., 2007). After transfer of proteins, membranes were washed in deionized water and then allowed to air dry and stored for up to several days before probing with antibodies. Dried membranes were wet in 100% methanol for 30 s and then washed in deionized water for 5 min. They were then blocked in a solution of 2% non-fat dry milk in a Tris-buffered saline solution (20 mmol l^{-1} Tris, 500 mmol l^{-1} NaCl, pH 7.5) containing 0.05% Tween detergent (TTBS) for 1 h. Blots were incubated in the same milk solution containing anti-TnT, anti-TnI or anti-P75 antibody (1:10,000 or 1:20,000 dilution) for 1 h. Blots were then washed three times (5 min per wash) in TTBS. They were next incubated in anti-rabbit biotin IgG (Vector Labs, Burlingame, CA, USA; 1:5000 dilution) for 1 h and again washed three times in TTBS. Blots were then incubated in an avidin–biotin complex conjugated to horseradish peroxidase (ABC solution; Vector Labs) for 30 min. Finally, antibody complexes labeling isoforms of TnT, TnI or P75 were visualized by chemiluminescent detection (Covi et al., 1999).

Isolation of cDNAs encoding MHC isoforms

The 3′ end of three MHC isoforms [encoding a portion of the rod region of the MHC molecules and the 3′ untranslated region (3′-UTR)] were cloned using a 3′-RACE procedure. RNA was isolated from the muscles of crab legs with TRIzol reagent (Invitrogen Inc., Carlsbad, CA, USA) and used for first strand cDNA synthesis. The first strand synthesis reaction was carried out using Super Script II RNase-Reverse Transcriptase (Invitrogen Inc.) and contained 100 ng of total RNA, 2.5 µmol l^{-1} dNTP, 1× first strand buffer, 5 mmol l^{-1} dithiothreitol (DTT), 2.5 units of RNase inhibitor and 1 µl of 10 µmol l^{-1} adapter primer (Invitrogen adaptor primer, Cat. No. 10542 – 017): 5′-GGCCACGCGTCGACTAGTAC(T)17-3′. Next, a PCR was performed using the cDNA from the first strand synthesis as a template. The forward primer was designed to anneal to a conserved region of the MHC, as deduced by analysis of three lobster MHC isoforms: 5′-GAAGGCTAAGAAGGCCATGGTTGA-3′. The reverse primer was designed to anneal to a sequence within the adapter primer: 5′-GGCCACGCGTCGACTAGTAC-3′. The PCR reaction consisted of 95°C (5 min) [60°C (30 s), 72°C (30 s), 95°C (30 s)] repeated for 35 cycles and 72°C (10 min). Takara Ex-Taq-HS was used for all PCR reactions (Takara Bio USA, Madison, WI, USA). PCR products were identified on 1% agarose gels stained with ethidium bromide. These bands were gel purified and cloned into the pCR 2.1 TOPO plasmid using the TOPO TA cloning kit (Invitrogen Inc.). Transformed bacteria were grown overnight on pre-warmed LB plates containing ampicillin (100 µg ml^{-1}) coated with X-gal. We identified colonies with PCR inserts by their white color in blue/white screening and the presence of inserts was confirmed by PCR using vector primers (M13 reverse and T7). Several positive clones of each isoform were identified and sequenced by the DNA sequencing lab at Roswell Park Cancer Center (Buffalo, NY, USA).

Histochemical identification of metabolic fiber types

The NADH–diaphorase reaction was used to identify aerobic fibers in the crab leg muscles. A solution containing NBT (0.8 mg ml^{-1}) and NADH (0.64 mg ml^{-1}) in 50 mmol l^{-1} sodium phosphate (pH 7.3) was made fresh. Frozen muscles were embedded in OCT compound

(Sakura Finetek, Torrance, CA, USA) and sections (10–20 μm) were cut on a Cryostat (Leica Microsystems, Wetzlar, Germany). Sections were mounted on microscope slides and stored at –20°C until ready for staining. Prior to staining, sections were thawed and allowed to air dry for 15–50 min. Sections were then rinsed quickly by immersing in deionized water to remove any residual OCT compound. Tissues were then covered with the NBT/NADH solution and incubated in the dark at room temperature for 30 min. After staining, sections were rinsed in several washes of deionized water and then dehydrated through a graded ethanol series. Sections were then covered with mounting medium and covered with a coverslip. We identified fibers with positive staining regions by their distinct purple color.

Statistical analyses

Linear regression analyses were performed on running velocity and frequency as a function of body mass, with all values being log-transformed prior to analysis. An unpaired *t*-test was used to compare the lengths of muscle fibers from different muscle regions. In all cases, significance was accepted at $\propto<0.05$. For descriptive statistics, values are means±standard deviation unless otherwise noted. All statistical analyses were performed with Statview 5.0.1 (SAS Institute, Cary, NC, USA).

RESULTS

General muscle organization

The extensor and flexor carpopodite muscles completely fill the meropodite, with the extensor being anterior to the flexor (Fig. 1). The apodeme of the extensor is superior to that of the flexor and is approximately coincident with the mid-line of the meropodite. The flexor apodeme is located closer to the inferior side of the leg, making the muscle asymmetrical. Muscle fibers of both the extensor and flexor originate from ridges of the exoskeleton that lie directly superior and inferior to the apodeme. This orientation means that the anterior and posterior surfaces of the meropodite are not involved in muscle attachment, as they are in the claw closer muscles. The muscles are oriented in parallel sheets of fibers that insert into the apodeme at angles of 27.8±1.9 deg. and 24.8±1.9 deg. for the extensor and flexor muscles, respectively.

Two distinct fiber types comprise both the extensor and flexor muscles. The proximal and distal fibers of the extensor and flexor are visibly light brown to pink in coloration whereas those occupying the mid-regions of the muscles are distinctly white. These differences are consistent with the oxidative capacity of the muscles, as indicated by NADH–diaphorase histochemistry. The pigmented fibers stain intensely from this reaction, indicating high numbers of mitochondria within the fibers (Fig. 2). These fibers also appear to possess internal subdivisions similar to those reported for other aerobic crustacean muscles (membrane in-folding) (Fig. 2B). The fibers from the mid-regions of the muscles were much larger in diameter and showed weak staining with the NADH–diaphorase reaction, which is limited to the fiber periphery (Fig. 2C). In addition, the proximal and distal fibers are significantly shorter than those of the mid-region ($P<0.0001$) (Fig. 3). Sarcomere lengths within fibers were variable and depended on the anatomical location of the fibers. The large diameter fibers of the muscle mid-region possessed mean sarcomere lengths of 3.5±0.4 μm (range: 2.65–4.0 μm) whereas the proximal and distal fibers were more variable. Within the proximal and distal muscle regions, some of the fibers possessed short sarcomeres like the mid-region fibers but most had longer sarcomeres, with a mean length of 6.2±2.3 μm (range: 4–10 μm).

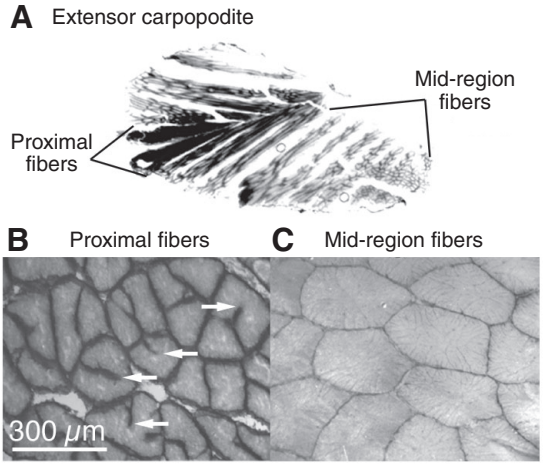

Fig. 2. Histochemical staining of oxidative capacity with NADH–diaphorase reaction. (A) Longitudinal section of extensor carpopodite muscle demonstates that the greatest staining is limited to the fibers of the proximal muscles fibers (distal fibers are not included in this section). (B) Cross section of fibers from the proximal region of the muscle, demonstrating intense staining around the fiber periphery. These fibers are small in diameter (~100 μm) as compared with the mid-region fibers. What initially appears to be the outer muscle cell membrane delineating a single fiber is actually an in-folding of the membranes resulting in a functional subdivision of the fibers (arrows). (C) Cross section of fibers from the mid-region of the muscle (scale as in B). These fibers are significantly larger in diameter (~200 μm) compared with the proximal fibers and possess significantly less staining.

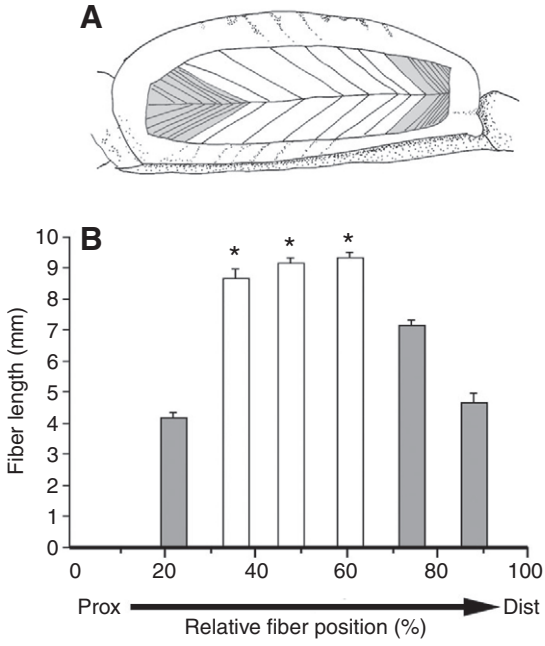

Fig. 3. Muscle fiber length as a function of anatomical position in the extensor carpopodite of a 27 g crab. (A) Extensor muscle illustrating the position of the proximal (prox) and distal (dist) fibers (shaded) in relation to the larger mid-region fibers (unshaded). (B) Relative fiber lengths of fibers from different regions (shaded bars correspond to shaded regions in A). On average, the mid-region fibers are significantly longer than the proximal/distal fibers (*$P<0.0001$; *t*-test comparing lengths from the mid-region fibers with the proximal and distal fibers; $N=18$; ±s.e.m.). The relative lengths of fibers in the flexor carpopodite muscles are similar to the pattern shown here.

Running performance

Running speed was significantly correlated with body size in ghost crabs ranging in size from 200 mg to 63 g, with the fastest animals attaining speeds of just over 100 cm s^{-1} (Fig. 4A). Running speed was a function of mass$^{0.31}$, which means that change in speed as a function of size became less among the larger crabs (Fig. 4A, inset). In the current study, speed became essentially size independent in crabs of 30 g and larger (86.7±11.9 cm s^{-1}; N=14). By contrast, stride frequency declined as a function of body size in crabs, with the smallest crabs (~10 g) measured reaching frequencies of just over 8 Hz whereas larger crabs (~60–70 g) ran at approximately 5 Hz (Fig. 4B). Overall, frequency was found to be a function of body mass$^{-0.13}$. Comparison with stride frequencies of a sister species of ghost grab, *Ocypode ceratopthalma*, revealed a similar pattern of declining frequencies in larger animals, although the slope of the relationship was considerably steeper (mass exponent=–0.49) (Fig. 4C) [data for *O. ceratopthalma* derived from Burrows and Hoyle (Burrows and Hoyle, 1973)].

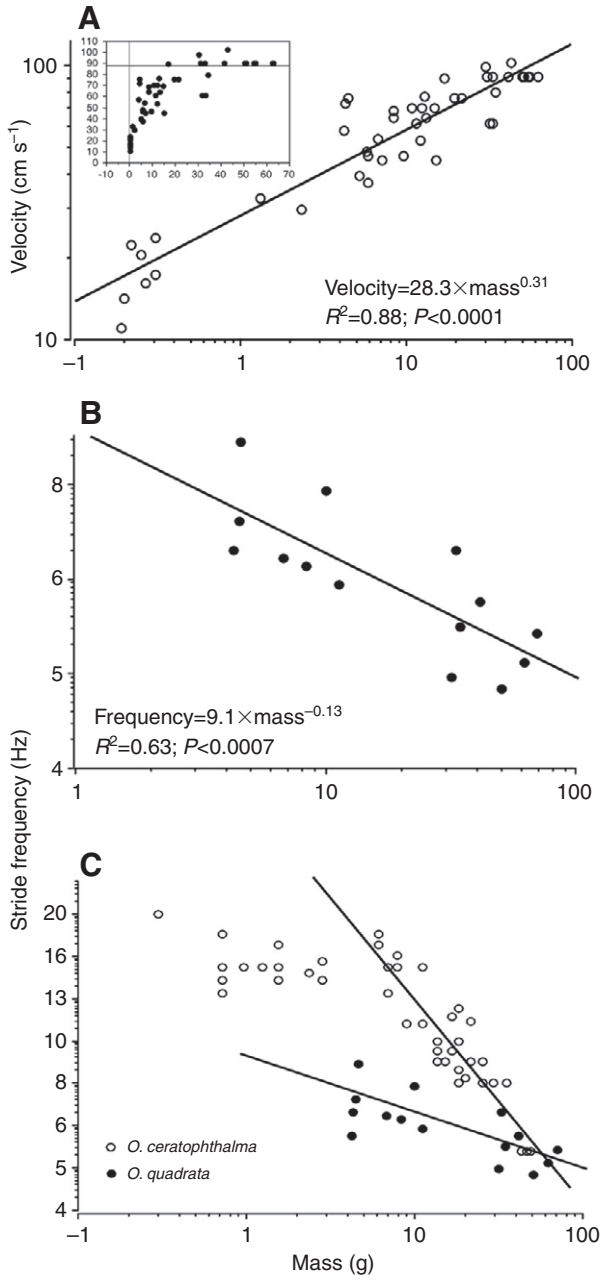

Fig. 4. Running velocity and stride frequency as a function of body mass. (A) Running velocity increases as a function of mass$^{0.31}$ [velocity (cm s^{-1}) = 28.3 × mass (g)$^{0.31}$; R^2=0.88; P<0.0001). Inset shows the same relationship on linear axes to demonstrate the asymptote, with crabs of approximately 30 g and larger reaching speeds of approximately 1 m s^{-1}. (B) Stride frequency in running crabs decreases as a function of mass$^{-0.13}$ [frequency (Hz) = 9.1 × mass (g)$^{-0.13}$; R^2=0.63; P<0.0007) with crabs of ~10 g operating at ~8 Hz, and crabs with a mass of about 50 g and larger running at about 5 Hz. (C) Comparison between stride frequencies of *O. quadrata* (current study) with those of *O. ceratophthalma* (Burrows and Hoyle, 1973).

Muscle shortening velocity and strain *in vivo*

Shortening velocities of the extensor and flexor carpopodite muscles were determined from angular velocity during running and from muscle geometry (Fig. 5). Alternatively, we used stride frequency to estimate shortening velocity, assuming that the shortening cycle was sinusoidal. Using the former method, we found shortening velocities ranged from 0.8–3.1 muscle lengths per second (l s^{-1}) in crabs ranging in size from 10–55 g. For estimates directly from stride frequencies, shortening velocities ranged from 1.1–3.2 l s^{-1} in crabs ranging from 4.3–62.7 g. Shortening velocity declined significantly as a function of body size using either method of estimation (data not shown). To provide a single point of comparison, we estimated the shortening velocity of both muscles for a mouse-sized crab

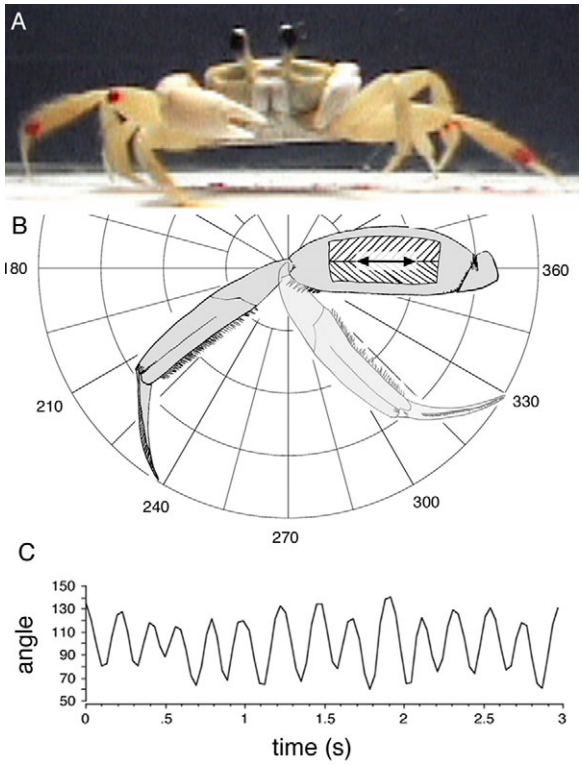

Fig. 5. Determination of muscle shortening velocity *in vivo*. (A) Single frame of a crab running on the treadmill. Second walking legs were marked with red paint at the distal portion of the meropodite and at the distal end of the propopodite. These points were subsequently used to follow angular changes in the meropodite–carpopodite joint during rapid running. (B) Dissected legs were used to measure changes in muscle length (arrows along apodeme) associated with measured changes in joint angle. (C) Joint angle as a function of time is used to determine angular velocity during running.

(~25 g; Table 1). We estimated maximum shortening velocity (V_{max}) by assuming that the muscles were operating near a shortening velocity that would provide maximum power output (~30% of V_{max}). V_{max} for the extensor carpopodite was estimated to be approximately 4.6–4.7 $l\,s^{-1}$, depending on which method was used to estimate shortening velocity in running crabs. For the flexor carpopodite, our estimate of V_{max} was 5.6–7 $l\,s^{-1}$. The mean muscle strain during rapid running was 11.6±5.8% of muscle length for the extensors and 17.6±8.8% for the flexors.

Myofibrillar isoforms

We identified several isoforms of myofibrillar proteins that differed among fiber types in ghost crab muscles. These included multiple isoforms of MHC, TnT and TnI (Fig. 6). There were at least three isoforms of MHC, three of TnT and two of TnI.

We identified three different isoforms of MHC, which were expressed differentially in different regions of the extensor and flexor muscles. We refer to these simply as MHC_1, MHC_2 and MHC_3 based on increasing mobility on SDS-PAGE gels (Fig. 6A). We observed complex patterns of MHC isoform expression, related not only to the anatomical region of the fibers but also to the size of the crabs (Fig. 7). Isoforms 1 and 3 were almost always co-expressed in single fibers whereas isoform 2 was generally expressed as the single isoform within a fiber. When co-expressed in single fibers, isoforms 1 and 3 were consistently expressed in nearly equal proportions (MHC_1 was 95±12% of MHC_3 as determined from densitometry). In some cases, minor levels of co-expression of different isoforms were present in these MHC_2 fibers. The larger glycolytic fibers from the mid-region of the muscles generally expressed isoforms 1 and 3 and some of the smaller oxidative fibers also expressed these isoforms. In particular, it seemed that smaller crabs (<50 g) tended to express these two isoforms in both fiber types, although isoform 2 was sometimes expressed in the oxidative fibers. Isoform 2 was generally expressed in the smaller oxidative fibers, particularly in largest crabs (>50 g) (Fig. 7).

Three unique cDNAs encoding the C-terminal sequence encoding the rod region of three different isoforms of MHC were isolated from the extensor and flexor carpopodite muscles of the ghost crab (Fig. 8) (GenBank accession numbers: DQ534440, EU676338 and DQ534441). The cDNAs were 791, 707 and 706 bp, respectively, and each sequence contained and open reading frame (ORF), 3′ untranslated region (3′-UTR) and poly A tail (Fig. 8). The sequence identity within the ORF was approximately 78–81% among the three isoforms (Table 2) and was 78–88% among the sequences reported here and the MHC isoforms of the lobster (Table 2). This level of similarity is about the same as when the lobster isoforms are compared with one another (78–79%) (Medler and Mykles, 2003; Medler et al., 2004). We believe that these three sequences encode the MHC proteins identified in the muscles but further work is needed to establish which nucleotide sequence encodes each of the three proteins.

We identified three isoforms of TnT, which mirror the expression of MHC isoforms (Fig. 6B; Fig. 9). TnT_3 was expressed in the mid-region fibers whereas TnT_2 was expressed in the more proximal and distal fibers of both large and small crabs. In largest crabs (>50 g), the TnT_1 isoform was co-expressed with TnT_2. Based on our analyses, we believe that the fibers expressing TnT_1 are likely to be the same fibers that express the MHC_2 isoform.

We identified two isoforms of TnI, which were less distinct in their regional distribution than the isoforms of MHC and TnT (Fig. 6C; Fig. 10). In most cases, both of these isoforms were expressed together in single fibers in roughly equal proportions. An exception to this pattern was observed in some crabs where the TnI_1 isoform is predominantly expressed, with little, if any, TnI_2 being present (Fig. 10A, mid-region fibers). This expression pattern in the mid-region fibers, like other patterns in myofibrillar isoform expression, seemed to be size-dependent. Larger crabs generally expressed equal propotions of the isoforms whereas fibers in smaller crabs were more likely to express greater amounts of TnI_1 (Fig. 10A).

Finally, we identified a 75 kDa protein in all fiber types that is recognized by the anti-P75 antibody raised against lobster P75 (Fig. 11). As a control, deep abdominal flexor muscles from the lobster were run in lanes adjacent to the crab fibers, confirming that the proteins are the same size (not shown). The identity and function of this protein remain unknown but it is expressed exclusively in

Table 1. Comparison of kinetic properties muscles of 25 g ghost crabs with kinetic properties of muscles from similar-sized lizards and mouse

	Maximum speed (cm s^{-1})	Stride frequency (Hz)	Shortening velocity ($l\,s^{-1}$)	V_{max} ($l\,s^{-1}$)	Temperature (°C)
Ghost crabs					
Ocypode quadrata	81	6			22
Extensor carpopodite			1.4[a,b]	4.6[b]–4.7[a]	
Flexor carpopodite			1.7[a]–2.1[b]	5.6[a]–7.0[b]	
Ocypode ceratophthalma	180–230	8			28–32
Lizards					
Dipsosaurus dorsalis	370	13.9		19.6	40
Sceloporus occidentalis	323	15.6		21.9	35
Mammal					
Mus musculus	369	5.9[c]–8.2[d]			37
Soleus				6	26
Extensor digitorum longus				12	26

Crab shortening velocities were estimated either from angular velocity (a) or directly from operational frequency (b). V_{max} estimates for the crab are based on the assumption that the muscles are operating *in vivo* at 30% of V_{max}. Fiber lengths were measured from dissected muscles and were assumed to be the same as muscle resting lengths *in vivo*. Running speed and stride frequency data for *O. ceratophthalma* are from Hafemann and Hubbard (Hafemann and Hubbard, 1969) and Burrows and Hoyle (Burrows and Hoyle, 1973). Data for *D. dorsalis* are from estimates for fast glycolytic iliofibularis (FG-IF) muscle of a 25 g lizard as reported by Marsh (Marsh, 1988). Data for *S. occidentalis* are also for the FG-IF muscle as reported by Marsh and Bennet (Marsh and Bennet, 1986a; Marsh and Bennet, 1986b) for ~13 g lizards. Mouse maximum running speed is from Djawdan and Garland (Djawdan and Garland, 1988), stride frequencies are from James and colleagues (James et al., 1995) and V_{max} for the mouse muscles is from Askew and Marsh (Askew and Marsh, 1997). Stride frequencies for the mouse are determined at the trot to gallop transition (c) or at an all out gallop (d).

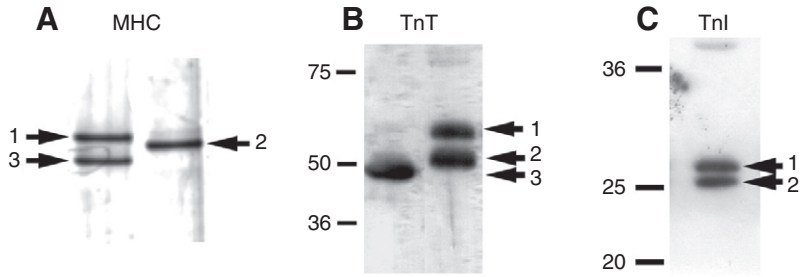

Fig. 6. Summary of alternate myofibrillar protein isoforms identified in the extensor and flexor carpopodite muscles. (A) Three isoforms of myosin heavy chain (MHC) are expressed and numbered in the order from least to greatest migration on SDS-PAGE gels (silver stain). The sample on the left is from a single fiber from the mid-region of the muscle and co-expressed both MHC_1 and MHC_3. The sample on the right is from a single proximal/distal fiber and expressed only MHC_2. (B) Three isoforms of troponin T (TnT) are expressed (western blot). The sample on the left is from a fiber from the mid-region of the muscle and expressed only TnT_3. The sample on the right is from a proximal/distal fiber and expressed both TnT_1 and TnT_2. (C) Two isoforms of troponin I (TnI) are expressed in various ratios (western blot). Shown is a single fiber sample that expressed both (TnI_1 and TnI_2) isoforms.

fast fibers of lobster muscles (Costello and Govind, 1984; Medler and Mykles, 2003; Mykles, 1985). We did not detect any body size related patterns in the expression of this protein.

DISCUSSION

General muscle organization

The extensor and flexor carpopodite muscles represent the bulk of the muscle tissue of the crab meropodite. The extensor muscle lies in the anterior position of the meropodite and its central tendon (apodeme) inserts at a superior position of the carpopodite that results in extension of the joint during muscle contraction (Fig. 1). The flexor muscle is parallel and posterior to the extensor muscle and its apodeme inserts at the inferior border of the carpopodite and facilitates joint flexion (Fig. 1). Both are simple pinnate muscles, with fibers originating along either a dorsal or ventral ridge of the exoskeleton of the meropodite and inserting onto an apodeme that runs the length of the meropodite. During walking or running, the extensor and flexor muscles alternately contract and relax to extend and flex the joint between the meropodite and carpopodite. In both muscles, muscle fiber types are segregated regionally, with shorter aerobic fibers being restricted to the proximal and distal regions and larger diameter putative glycolytic fibers being present in the mid-region of the muscle. The more proximal and distal fibers exhibit histochemical staining and fiber subdivision (membrane in-folding) (Fig. 2B) similar to the aerobic fibers used for swimming by the blue crab, *Callinectes sapidus*, consistent with a role of providing sustained power output (Johnson et al., 2004; Tse et al., 1983). Overall, the fiber type distribution is essentially the same as that described for the flexor carpopodite of the crab, *Carcinus maenas* (Parsons, 1982; Parsons and Mosse, 1982). In *C. maenas*, the more aerobic fibers are slower in their intrinsic contractile properties whereas those of the mid-region are fast (Parsons, 1982). The extensor muscles of crayfish, *Procambarus clarkii* and lobsters, *Homarus americanus*, also exhibit a similar pattern of fiber type distribution, where slow tonic (S_2) fibers are concentrated at the proximal and distal regions of the muscles (Mykles et al., 2002). These S_2 fibers exhibit a high degree of post-synaptic facilitation and are presumably used for sustained postural control (Mykles et al., 2002).

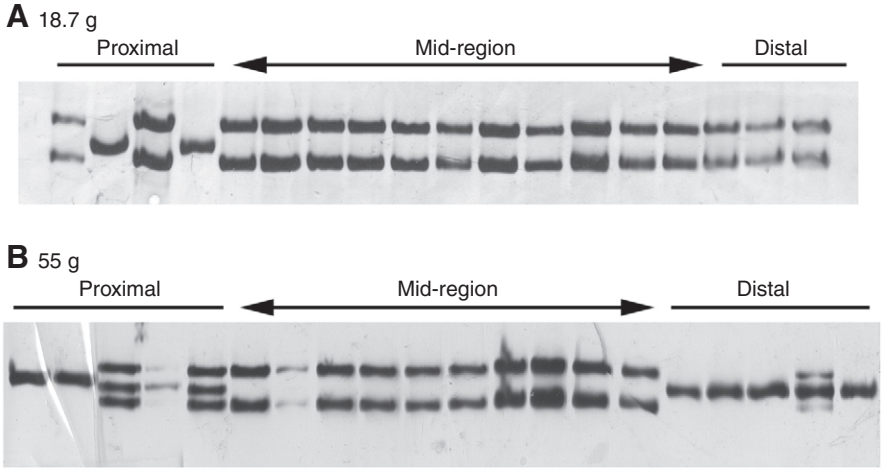

Fig. 7. Myosin heavy chain (MHC) isoform expression as a function of anatomical location in extensor carpopodite muscles. Proximal and distal fibers frequently express MHC_2 but the proportion is variable and is correlated with animal size. In general, MHC_2 is more prevalent in larger sized crabs. Here, fibers from a medium sized crab (18.7 g) exhibits MHC_2 expression in some fibers of the proximal region but not in the distal fibers. By contrast, the muscle from the large (55 g) crab exhibits MHC_2 expression in all of the fibers from the proximal and distal muscle regions. In some of these fibers, MHC_2 is present in combination with MHC_1 and MHC_3. The arrows associated with the mid-region fibers indicate that the samples represent a continuum in anatomical location, with the samples closer to the proximal and distal samples being taken from adjacent to these locations within the muscle.

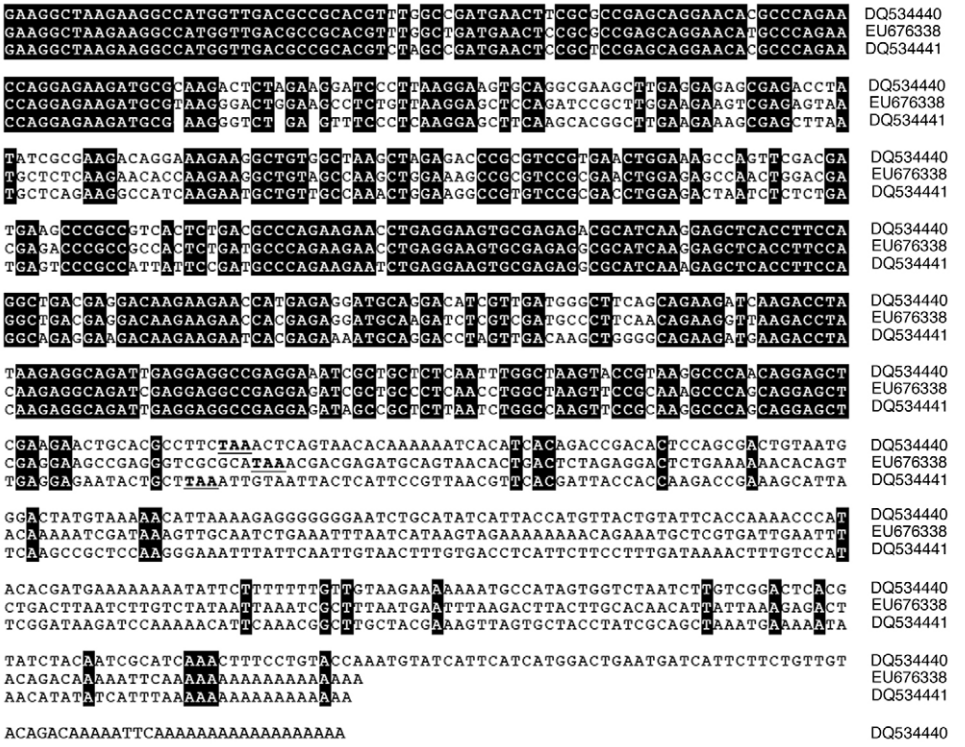

Fig. 8. Sequence comparison of three myosin heavy chain (MHC) cDNAs isolated from muscles of the crab meropodite. Nucleotides that match among all three sequences are shaded in black and the stop codon (TAA) for each sequence is in bold text and underlined. Within the open reading frame, the sequences share approximately 80% sequence identity but show significant divergence in the 3′-UTR.

Locomotion kinetics

The exercise physiology of invertebrates has received far less attention than that of vertebrate animals, yet common patterns of function revealed through comparative studies provide a deeper understanding of the foundational principles of physiological function. Ghost crabs exhibit remarkable athletic capabilities that rival those of comparably sized mammals, with maximum running speeds approaching 4 m s^{-1} and an aerobic scope of nearly 12 (Full and Herreid, 1983; Full and Weinstein, 1992; Hafemann and Hubbard, 1969). In the current study, running speed in *O. quadrata* increased as a function of mass$^{0.31}$, meaning that running speed increases as crabs get larger but then levels off at a maximum of just over 1 m s^{-1} for crabs of ~30 g and larger (Fig. 4, inset). These values are similar to those previously reported for *O. quadrata* (Blickhan and Full, 1987; Blickhan et al., 1993), although maximum running speeds of up to 3–4 m s^{-1} have been reported for a sister species of ghost crab, *O. ceratophthalma* (Hafemann and Hubbard, 1969). Running surfaces significantly affect maximum speeds attained by ghost crabs (Hafemann and Hubbard, 1969; Pennisi, 2007) and the smooth surface of our treadmill probably limited maximum speeds attained by our crabs. Comparably sized rodents and lizards are able to reach speeds of approximately 3.7 m s^{-1} (Djawdan and Garland, 1988; Marsh, 1988; Marsh and Bennett, 1986b) (Table 1). Stride frequencies of the crabs are similar to those of mammals, with ~25 g crabs and mice operating at approximately 6–8 Hz whereas running lizards are able to operate at higher frequencies of up to approximately 15 Hz (Marsh, 1988; Marsh and Bennett, 1986b) (Table 1). The stride frequencies of *O. ceratophthalma* are higher than those of *O. quadrata*, with maximum frequencies of 20 Hz, as compared with 8 Hz for *O. quadrata* (Burrows and Hoyle, 1973). Stride frequency as a function of body mass in *O. ceratophthalma* is biphasic, with the smallest crabs (0.1–5 g) exhibiting a size-independent frequency of approximately 15 Hz. For larger crabs (~10–50 g), there is a steeper decline in frequency as a function of body mass than that observed for *O. quadrata* (Fig. 4C). These larger *O. ceratophthalma* showed a decline in frequency as a function of mass$^{-0.49}$ [frequency (Hz) = 38.9 × mass (g)$^{-0.49}$; R^2=0.83; P<0.0001]. Overall, when compared with similar sized vertebrates, ghost crabs exhibit slower running speeds but quite similar stride frequencies (Table 1).

The simple pinnate muscle organization of the extensor and flexor carpopodite muscles facilitated the estimation of muscle shortening parameters from running crabs. Based on these measurements, we estimate that in a mouse-sized crab the extensor muscles have a

Table 2. Comparison among nucleotide sequence identities within the open reading frames of the three myosin heavy chain (MHC) cDNAs in the current study and with those of lobster (*H. americanus*) MHC isoforms

	O. quadrata			*H. americanus*		
	DQ534440	DQ534441	EU676338	S_1	S_2	Fast
DQ534440	–	0.78	0.81	0.82	0.78	0.80
DQ534441	0.78	–	0.81	0.82	0.79	0.79
EU676338	0.81	0.81	–	0.88	0.84	0.81

Crab sequences are listed by GenBank accession numbers. Accession numbers for the lobster are AY232598.1 (S_1), AY521626.1 (S_2) and U03091.1 (fast).
Sequence identities are reported as relative identities, where a value of 1.0 is an exact match.

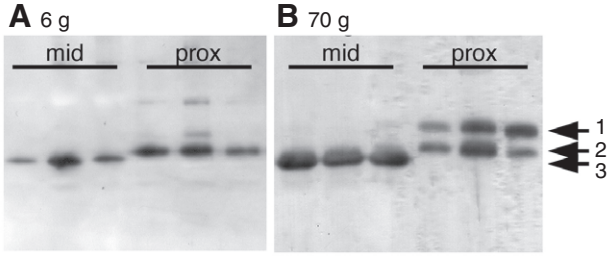

Fig. 9. Troponin T (TnT) isoform expression as a function of anatomical location in flexor carpopodite muscles of a smaller (A) and larger (B) crab. Mid-region (mid) fibers express the TnT_3 isoform whereas the more proximal fibers express either TnT_2 or a combination of TnT_1 and TnT_2. In general, TnT_1 expression is more prevalent in larger crabs. In this example, the proximal (prox) fibers from the 6 g crab express the TnT_2 isoform almost exclusively (A) whereas the fibers of the 70 g crab contain significant levels of TnT_1 (B). Each of the three replicates from different crabs or muscle regions are single fibers from the indicated muscle region.

maximum shortening velocity of approximately 4 $l s^{-1}$ (3.8–4.6 $l s^{-1}$) (Table 1) whereas the flexors possess a V_{max} of approximately 6 (5.6–7 $l s^{-1}$) (Table 1). The mouse soleus, which is composed of a mixture of type I and IIa fibers, has a V_{max} of approximately 6 $l s^{-1}$ whereas the exclusively fast muscles of mice and lizards have a V_{max} of approximately 12 and 20 $l s^{-1}$, respectively (Askew and Marsh, 1997; Marsh, 1988; Marsh and Bennett, 1986a) (Table 1). Overall, the contractile properties of the muscles used for running by the ghost crabs are slightly lower than those of a comparably sized mammal but substantially lower than those of a similar sized reptile. However, the estimates for the lizard muscles were made at temperatures almost 20 deg. higher than for the mouse or the crab and these temperatures probably explain the higher values. Skeletal muscles from diverse species exhibit shortening velocities that differ by more than 100-fold and the velocities of fast and slow fibers from a single species frequently differ by up to 10-fold (Medler, 2002). By comparison, the relatively minor differences in kinetic properties of muscles driving terrestrial locomotion in the evolutionarily divergent species examined here suggests that common design constraints have shaped the organization of these muscles.

Muscle fiber types

Although the architecture of the extensor and flexor carpopodite is that of a simple pinnate muscle, the fiber types present in the different regions of the muscles are complex. It is not currently possible to fit the muscle fibers in the legs of the ghost crab into discrete fiber type categories of other crustaceans. One common classification scheme defines one fast and two slow fiber types [slow phasic (S_1) and slow tonic (S_2)] based on different isoforms of the myofibrillar proteins (MHC, P75, TnT, TnI) and on histochemistry (Medler and Mykles, 2003; Medler et al., 2004; Mykles, 1985; Mykles, 1988). The MHC protein isoforms identified through SDS-PAGE are clearly different between lobster muscles and crab muscles, and the gene sequences from the crab muscles do not offer many clues as to the similarity to other crustacean MHCs. Overall, all three identified sequences share ~80% similarity to each of the identified lobster (*Homarus americanus*) MHC isoforms (fast, S_1, S_2) within the coding sequence but none of the *O. quadrata* sequences matches preferentially with any of the *H. americanus* sequences. A unique pattern observed in the mid-region fibers is the apparently invariant co-expression of two MHC isoforms in single fibers. Single fibers exhibiting polymorphic, or hybrid, expression of multiple MHC isoforms are not uncommon but these are often variable in the levels of different isoforms present. In all of our analyses, the MHC_1 and MHC_3 are present in single fibers in a highly consistent 1:1 ratio. The physiological significance of polymorphic fibers is currently still a matter of debate. Pette and colleagues interpreted these fibers as being transient in nature, being in the process of switching from one fiber type to another (Pette and Staron, 2000; Pette and Staron, 2001). More recently, the common occurrence of these fibers has called this interpretation into question, raising the possibility that these intermediate fibers types provide a continuum of mechanical properties to the muscles (Caiozzo et al., 2003; Medler et al., 2004; Stephenson, 2001). Indeed, single fibers containing two MHC isoforms possess contractile properties intermediate to pure fiber types (Andruchov et al., 2004; Caiozzo, 2002; Caiozzo et al., 2003). In the case of the mid-region fibers in the extensor and flexor carpopodite, it is unclear what advantage might be gained by the roughly equal expression of two isoforms, rather than expressing a single isoform. Nevertheless, many types of crustacean fibers co-express multiple isoforms of other myofibrillar proteins, including TnI and TnT.

In lobster muscles, different isoforms of TnT and TnI provide convenient markers of fiber type and in ghost crab muscles differences also exist in terms of fiber type–specific isoform expression. However, the lobster isoforms do not appear to correspond in a simple way to the ghost crab isoforms. In lobster muscles, three isoforms of TnT are present, with fast muscles expressing T_2, S_1 muscles expressing T_3 and S_2 muscles expressing some combination of T_1 and T_3. In the leg muscles of the ghost crab, the mid-region fibers express T_3 whereas some proximal and distal fibers express T_2 and some express both T_1 and T_2. Overall, the expression of the T_1 isoform is much more restricted than the other two isoforms but when present is always in the proximal and distal regions of the muscles. Therefore, the ghost crab fibers expressing the T_1 isoform might share similarities with S_2 fibers in other crustaceans. Fewer similarities are shared between lobster and ghost crab fiber types for TnI isoforms. In the lobster, five

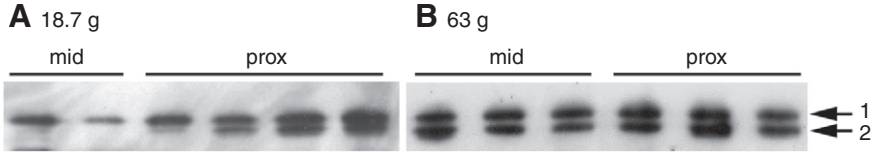

Fig. 10. Troponin I (TnI) isoform expression as a function of anatomical location in flexor carpopodite muscles of a smaller (A) and larger (B) crab. In the 18.7 g crab flexor, the mid-region (mid) fibers express primarily the TnI_1 isoforms, with little or no TnI_2 being present (A). By contrast, the proximal (prox) fibers from the same muscle possess nearly equal amounts of both isoforms (A). In the muscles from the 63 g crab, both TnI isoforms are co-expressed in approximately equal proportions in fibers from both the mid- and proximal regions. The three samples from different crabs or muscle regions are replicates of single fibers from the indicated muscle region.

TnI isoforms exist and are expressed in different proportions in different fiber types. In the crab muscles, we have only identified two isoforms. Both are expressed in the different fiber types, although the mid-region fibers tend to have a greater proportion of the I_1 isoform (Fig. 10A). Finally, in lobster and crayfish muscles the 75 kDa protein (P75) is only found in fast muscle types (Costello and Govind, 1984; Medler and Mykles, 2003; Mykles, 1985; Mykles et al., 2002). Based on western blots, this protein is present in both the proximal/distal and mid-region fibers of the ghost crab muscles (Fig. 11). In the corresponding leg muscles in lobsters and crayfish, these are composed entirely of either S_1 or S_2 fiber types, none of which express P75 (Mykles et al., 2002). The presence of P75 in these fibers raises the possibility that both fiber types represent fast fibers but perhaps with different levels of contractile speed. This would be similar to the pattern observed in mammalian muscles, which possess a single slow fiber type and several fast fiber types (Pette and Staron, 2000; Schiaffino and Reggiani, 1996). Preliminary analyses using ATPase histochemistry are consistent with this possibility, as acid pre-incubation of muscle sections leads to differential staining of the proximal and mid-region fibers that both stain intensely without acid treatment (not shown).

Several of the muscle parameters we observed suggest that these muscles function as a two-geared system, with the more proximal and distal fiber bundles functioning for slow, sustained locomotion and the mid-region fibers being reserved for more explosive power output during sprinting. There are several indicators that the more proximal and distal fibers are slower contracting fibers. First, the fibers are shorter than the mid-region fibers (Fig. 3), which directly impacts muscle shortening velocity, as velocity is directly proportional to the number of sarcomeres operating in series (Josephson, 1975). Second, the muscles possess longer sarcomeres than the mid-region fibers (~6 μm *vs* 3.5 μm), a characteristic typical of slow fibers in crustaceans. Sarcomere length is not simply a convenient descriptor of shortening velocity but has direct effects on the mechanical properties of the muscles. Long sarcomere fibers generate greater forces but at the expense of velocity in comparison with fibers with short sarcomeres (Josephson, 1975; Taylor, 2000). Finally, these fibers possess a distinct MHC isoform not present in the mid-region fibers (Fig. 7). Although we do not currently have any direct data about the ATPase activity of the different MHC isoforms, we can hypothesize that the MHC_2 is a slower isoform. This is consistent with patterns of contractile speed in the different regions of the flexor muscle in the crab, *C. maenas* (Parsons, 1982). In terms of aerobic capacity, the proximal and distal fibers possess greater mitochondrial densities than the mid-region fibers. Furthermore, these fibers are significantly smaller in diameter and possess fiber subdivisions, facilitating the efficient delivery of oxygen during exercise. This type of muscle organization is similar to the two-geared system in fish, where the slower fiber types drive slow sustained swimming and the more powerful fast muscles power escape responses (Rome et al., 1988). Physiological measurements support this idea, as slow locomotion can be sustained by the crabs for over an hour but endurance capacity is greatly reduced at speeds greater than 30 cm s^{-1} and sprints can be sustained for only seconds (Full, 1987; Full and Herreid, 1983; Full and Weinstein, 1992). Exercise leads to rapid accumulation of lactate in ghost crabs but intermittent exercise allows for prolonged activity and greater distance capacity than prolonged slow locomotion (Full, 1987; Full and Weinstein, 1992; Weinstein and Full, 1992).

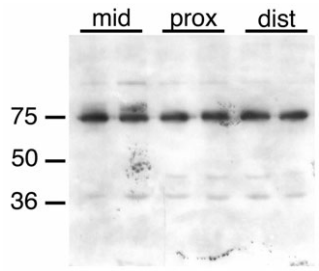

Fig. 11. Expression of 75 kDa protein in fibers from different anatomical regions of flexor carpopodite muscle of a 63 g crab. Expression of this protein is observed in each fiber type in approximately equal amounts. P75 in other decapod crustaceans is expressed exclusively in fast fiber types. Mid, mid-region fibers; prox, proximal fibers; dist, distal fibers.

Scale effects and muscle organization

Running ghost crabs exhibit a systematic decline in stride frequency as a function of increasing body size (Fig. 4B,C). The slope of this decrease is less pronounced in *O. quadrata* than in *O. ceratophthalma* but is similar in magnitude to the general relationship known for quadrupedal mammals (Heglund et al., 1974). Interspecific differences in stride frequencies of running mammals are associated with systematic shifts in the intrinsic contractile properties of the skeletal muscles that produce the power to drive locomotion. In particular, the rate of ATP hydrolysis and shortening velocity of orthologous isoforms of MHCs from different species are fine-tuned to match the appropriate rate of intrinsic muscle shortening (Marx et al., 2006; Medler, 2002; Pellegrino et al., 2003; Reggiani et al., 2000; Seow and Ford, 1991). Similar changes in the contractile properties of muscles from individuals of different size within a single species also exist but the cellular and molecular mechanisms responsible for these shifts in muscle physiology are poorly understood. In this context, we found two notable differences in myofibrillar isoform assemblage in muscle fibers as a function of body size in *O. quadrata*. Although we have yet to define precise patterns of expression correlated with specific sizes of crabs, some general trends are evident. Most prominently, the expression of MHC_2 and TnT_1 is more prevalent in larger crabs than in smaller animals. We also found a potential difference in the expression of TnI, with lower expression of the TnI_2 isoform in the mid-region fibers of smaller crabs. Functionally, differences in MHC isoform expression have clear implications for muscle shortening velocity whereas differences in troponin isoforms may affect the rates of muscle activation and deactivation (Schiaffino and Reggiani, 1996). Myofibrillar isoform composition is clearly correlated with physiology in other decapod crustacean muscles (Koenders et al., 2004; Mykles et al., 2002). We propose that subtle shifts in contractile properties may stem from the continuum of myofibrillar assemblage known to exist in crustacean muscle fibers (Medler et al., 2004; Mykles et al., 2002). Developing lobster claw muscles exhibit a gradual shift in myofibrillar isoform expression, as the muscles differentiate into specialized fast and slow fibers over a period of months (Medler et al., 2007). Further studies are needed to understand precisely how these differences influence the running capability of ghost crabs.

Special thanks to Chase, Ethan and Peyton Medler for their expert assistance in collecting crabs. Thanks to Dr Donald L. Mykles for providing the antibodies used in this study and for discussion about the manuscript. Thanks to Kristyn Anthony, Shannon Palermo, Ogechukwu Etudo and Josh Feuerstein for their dedicated lab assistance. We gratefully acknowledge the insightful comments and suggestions

of two anonymous reviewers. This work was supported by an Interdisciplinary Research Development Fund grant from the University at Buffalo and a United University Professionals Individual Development Award.

REFERENCES

Andruchov, O., Andruchova, O., Wang, Y. and Galler, S. (2004). Kinetic properties of myosin heavy chain isoforms in mouse skeletal muscle: comparison with rat, rabbit, and human correlation with amino acid sequence. *Am. J. Physiol. Cell Physiol.* **287**, C1725-C1732.

Askew, G. N. and Marsh, R. L. (1997). The effects of length trajectory on the mechanical power output of mouse skeletal muscle. *J. Exp. Biol.* **200**, 3119-3131.

Atwood, H. L. (1976). Organization and synaptic physiology of crustacean neuromuscular systems. *Prog. Neurobiol.* **7**, 291-391.

Blickhan, R. and Full, R. J. (1987). Locomotion energetics of the ghost crab II. Mechanics of the centre of mass during walking and running. *J. Exp. Biol.* **130**, 155-174.

Blickhan, R., Full, R. J. and Ting, L. (1993). Exoskeleton strain: evidence for a trot-gallop transition in rapidly running ghost crabs. *J. Exp. Biol.* **179**, 301-321.

Blough, E. R., Rennie, E. R., Zhang, F. and Reiser, P. J. (1996). Enhanced electrophoretic separation and resolution of myosin heavy chains in mammalian and avian skeletal muscles. *Anal. Biochem.* **233**, 31-35.

Burrows, M. and Hoyle, G. (1973). The mechanism of rapid running in the ghost crab, *Ocypode ceratophthalma*. *J. Exp. Biol.* **58**, 327-349.

Caiozzo, V. J. (2002). Plasticity of skeletal muscle phenotype: mechanical consequences. *Muscle Nerve* **26**, 740-768.

Caiozzo, V. J., Baker, M. J., Huang, K., Chou, H., Wu, Y. Z. and Baldwin, K. M. (2003). Single-fiber myosin heavy chain polymorphism: how many patterns and what proportions? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **285**, R570-R580.

Costello, W. J. and Govind, C. K. (1984). Contractile proteins of fast and slow fibers during differentiation of lobster claw muscle. *Dev. Biol.* **104**, 434-440.

Covi, J. A., Belote, J. M. and Mykles, D. L. (1999). Subunit compositions and catalytic properties of proteasomes from developmental temperature: sensitive mutants of *Drosophila melanogaster*. *Arch. Biochem. Biophys.* **368**, 85-97.

Djawdan, M. and Garland, T. (1988). Maximal running speeds of bipedal and quadrupedal rodents. *J. Mammal.* **69**, 765-772.

Full, R. J. (1987). Locomotion energetics of the ghost crab.1. Metabolic cost and endurance. *J. Exp. Biol.* **130**, 137-153.

Full, R. J. and Herreid, C. F. (1983). Aerobic response to exercise of the fastest land crab. *Am. J. Physiol.* **244**, R530-R536.

Full, R. J. and Weinstein, R. B. (1992). Integrating the physiology, mechanics and behavior of rapid running ghost crabs: slow and steady doesn't always win the race. *Am. Zool.* **32**, 382-395.

Gruhn, M. and Rathmayer, W. (2002). Phenotype plasticity in postural muscles of the crayfish *Orconectes limosus* Raf.: correlation of myofibrillar ATPase-based fiber typing with electrophysiological fiber properties and the effect of chronic nerve stimulation. *J. Exp. Zool.* **293**, 127-140.

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

Hafemann, D. R. and Hubbard, J. I. (1969). On the rapid running of ghost crabs (*Ocypode ceratophthalma*). *J. Exp. Zool.* **170**, 25-32.

Heglund, N. C., Taylor, C. R. and McMahon, T. A. (1974). Scaling stride frequency and gait to animal size-mice to horses. *Science* **186**, 1112-1113.

James, R. S., Altringham, J. D. and Goldspink, D. F. (1995). The mechanical-properties of fast and slow skeletal-muscles of the mouse in relation to their locomotory function. *J. Exp. Biol.* **198**, 491-502.

Johnson, L. K., Dillaman, R. M., Gay, D. M., Blum, J. E. and Kinsey, S. T. (2004). Metabolic influences of fiber size in aerobic and anaerobic locomotor muscles of the blue crab, *Callinectes sapidus*. *J. Exp. Biol.* **207**, 4045-4056.

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

Josephson, R. K. (1993). Contraction dynamics and power output of skeletal-muscle. *Ann. Rev. Physiol.* **55**, 527-546.

Koenders, A., Lamey, T. M., Medler, S., West, J. M. and Mykles, D. L. (2004). Two fast-type fibers in claw closer and abdominal deep muscles of the Australian freshwater crustacean, *Cherax destructor*, differ in Ca^{2+} sensitivity and troponin-I isoforms. *J. Exp. Zool.* **301A**, 588-598.

Marsh, R. L. (1988). Ontogenesis of contractile properties of skeletal-muscle and sprint performance in the lizard *Dipsosaurus dorsalis*. *J. Exp. Biol.* **137**, 119-139.

Marsh, R. L. and Bennett, A. F. (1986b). Thermal dependence of sprint performance of the lizard *Sceloporus occidentalis*. *J. Exp. Biol.* **126**, 79-87.

Marx, J. O., Olsson, M. C. and Larsson, L. (2006). Scaling of skeletal muscle shortening velocity in mammals representing a 100,000-fold difference in body size. *Pflugers Arch.* **452**, 222-230.

Medler, S. (2002). Comparative trends in shortening velocity and force production in skeletal muscles. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **283**, R368-R378.

Medler, S and Hulme, K. (2009). Power output from striated muscles performing cyclical contractions: patterns and constraints. *Comp. Biochem. Physiol. A* (in press).

Medler, S. and Mykles, D. L. (2003). Analysis of myofibrillar proteins and transcripts in adult skeletal muscles of the American lobster *Homarus americanus*: variable expression of myosins, actin and troponins in fast, slow-twitch and slow-tonic fibres. *J. Exp. Biol.* **206**, 3557-3567.

Medler, S., Lilley, T. and Mykles, D. L. (2004). Fiber polymorphism in skeletal muscles of the American lobster, *Homarus americanus*: continuum between slow-twitch (S-1) and slowtonic (S-2) fibers. *J. Exp. Biol.* **207**, 2755-2767.

Medler, S., Lilley, T. R., Riehl, J. H., Mulder, E. P., Chang, E. S. and Mykles, D. L. (2007). Myofibrillar gene expression in differentiating lobster claw muscles. *J. Exp. Zool.* **307A**, 281-295.

Mykles, D. L. (1985). Heterogeneity of myofibrillar proteins in lobster fast and slow muscles: variants of troponin, paramyosin, and myosin light chains comprise four distinct protein assemblages. *J. Exp. Zool.* **234**, 23-32.

Mykles, D. L. (1988). Histochemical and biochemical characterization of two slow fiber types in decapod crustacean muscles. *J. Exp. Zool.* **245**, 232-243.

Mykles, D. L., Medler, S., Koenders, A. and Cooper, R. (2002). Myofibrillar protein isoform expression is correlated with synaptic efficacy in slow fibres of the claw and leg opener muscles of crayfish and lobster. *J. Exp. Biol.* **205**, 513-522.

Parsons, D. W. (1982). The leg flexor muscle of *Carcinus*. I. Innervation and excitatory neuromuscular physiology. *J. Exp. Zool.* **224**, 157-168.

Parsons, D. W. and Mosse, R. L. (1982). The leg flexor muscle of *Carcinus*. II. Distribution of muscle fiber types. *J. Exp. Zool.* **224**, 169-175.

Pellegrino, M. A., Canepari, M., Rossi, R., D'Antona, G., Reggiani, C. and Bottinelli, R. (2003). Orthologous myosin isoforms and scaling of shortening velocity with body size in mouse, rat, rabbit and human muscles. *J. Physiol.* **546**, 677-689.

Pennisi, E. (2007). Crab's downfall reveals a hole in biomechanics studies. *Science* **315**, 325.

Pette, D. and Staron, R. S. (2000). Myosin isoforms, muscle fiber types, and transitions. *Microsc. Res. Tech.* **50**, 500-509.

Pette, D. and Staron, R. S. (2001). Transitions of muscle fiber phenotype profiles. *Histochem. Cell Biol.* **115**, 359-372.

Rathmayer, W. and Maier, L. (1987). Muscle fiber types in crabs: studies on single identified muscle fibers. *Am. Zool.* **27**, 1067-1077.

Reggiani, C., Bottinelli, R. and Stienen, G. J. M. (2000). Sarcomeric myosin isoforms: fine tuning of a molecular motor. *News Physiol. Sci.* **15**, 26-33.

Rome, L. C. and Lindstedt, S. L. (1997). Mechanical and metabolic design of the muscular system in vertebrates. In *Handbook of Physiology. Comparative Physiology*, Section 13, Vol. 13, pp. 1587-1651. Bethesda, MD: American Physiological Society.

Rome, L. C., Funke, R. P., Alexander, R. M., Lutz, G., Aldridge, H., Scott, F. and Freadman, M. (1988). Why animals have different muscle fibre types. *Nature* **335**, 824-827.

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

Seow, C. Y. and Ford, L. E. (1991). Shortening velocity and power output of skinned muscle fibers from mammals having a 25,000-fold range of body mass. *J. Gen. Physiol.* **97**, 541-560.

Silverman, H., Costello, W. J. and Mykles, D. L. (1987). Morphological fiber type correlates of physiological and biochemical properties in crustacean muscle. *Am. Zool.* **27**, 1011-1019.

Stephenson, G. M. (2001). Hybrid skeletal muscle fibres: a rare or common phenomenon? *Clin. Exp. Pharmacol. Physiol.* **28**, 692-702.

Taylor, G. M. (2000). Maximum force production: why are crabs so strong? *Proc. Biol. Sci. B* **267**, 1475-1480.

Tse, F. W., Govind, C. K. and Atwood, H. L. (1983). Diverse fiber composition of swimming muscles in the blue crab, *Callinectes sapidus*. *Can. J. Zool.* **61**, 52-59.

Weinstein, R. B. and Full, R. J. (1992). Intermittent exercise alters endurance in an eight-legged ectotherm. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **31**, R852-R859.

Wray, W., Boulikas, T., Wray, V. P. and Hancock, R. (1981). Silver staining of proteins in polyacrylamide gels. *Anal. Biochem.* **118**, 197-203.