Stride frequency in relation to allometric growth in ghost crabs: Running ghost crabs

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Stride frequency in relation to allometric growth in ghost crabs

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Abstract

Body size has significant impacts on fundamental processes of locomotion, including the operational frequency of skeletal muscle contraction, which declines systematically with increasing size. Although this shift in operation frequency and contractile kinetics is well documented in the literature, the mechanisms responsible for these changes are still incompletely understood. One important factor is that the mechanical properties of the musculoskeletal system possess resonant properties that favor higher frequencies in small animals. Another significant element is the physiological properties of the skeletal muscles, which may be tuned for faster contractions in smaller animals. These two components are interrelated, but precisely how muscle physiology and musculoskeletal mechanics interact to shape patterns of locomotion is complex. Ghost crabs *Ocypode quadrata* present an interesting model to study these processes because they are proficient runners that exhibit systematic changes in stride frequency as they grow. In the current study, we focused on anatomical changes that might occur with allometric growth in ghost crabs to test the hypothesis that changes in mechanical parameters contribute to the slowing of stride frequency. We paired basic anatomical measurements with kinematic analyses of crabs running at top speeds on a treadmill and experimentally weighted crabs to determine if the relative mass of larger crabs affects running frequency. We found that biologically relevant mechanics of the leg joints do not change with growth, as mechanical advantage and muscle fiber length relative to joint moment arm were unaffected by body size. Loading crabs had similar effects on stride frequency in both large and small animals alike. In contrast, muscle shortening velocity, estimated directly from angular velocity of the leg joints, decreased significantly with increasing body size. These data suggest that fundamental changes to the contractile properties of skeletal muscles during growth are primarily responsible for the changes in stride frequency observed in ghost crabs.

Introduction

Body size has several significant effects on skeletal muscle function in locomotion. One of the most obvious impacts is that smaller animals run, fly and swim with higher frequencies of movement than larger animals (Full, 1997; Medler & Hulme, 2009). There has been general acceptance that the physiological properties of the skeletal muscles should 'match' the resonant mechanical properties of the system, but the evidence for this coupling has mainly come from correlational analyses (Hill, 1950; McMahon, 1975; Lindstedt *et al*., 1985; Medler, 2002). For example, muscle shortening velocities from a wide range of animal groups exhibit scaling patterns consistent with patterns known for operational frequency, being approximately proportional to mass$^{-0.15}$ (Medler, 2002). Additionally, the contractile properties of orthologous isoforms of the myosin heavy chain (MHC) motors that drive contraction scale in a similar pattern among mammals ranging in size over several orders of magnitude (Pellegrino *et al*., 2003; Marx, Olsson & Larsson, 2006). Nevertheless, the precise principles governing the 'fit' between mechanical resonance and contractile physiology have been largely undetermined. For example, limb dimensions clearly establish the resonant operating frequency of locomotor systems (McMahon, 1975; Ahlborn, Blake & Megill, 2006), but when an animal changes size does this mean that the physiological properties of the muscles driving locomotion *must* adapt to match the new dynamics?

Ghost crabs provide a compelling model for investigating the linkages between body size and skeletal muscle organization. These active animals are arguably the fastest terrestrial invertebrates, capable of running at more than a meter per second (Hafemann & Hubbard, 1969; Burrows & Hoyle, 1973; Blickhan & Full, 1987; Perry *et al*., 2009). Moreover, the crabs grow in size by orders of magnitude over their lifetime, affecting multiple aspects of locomotion including running velocity

(Burrows & Hoyle, 1973; Perry *et al*., 2009), stride frequency (Burrows & Hoyle, 1973; Perry *et al*., 2009) and the metabolic efficiency of movement (Full, 1987; Tullis & Andrus, 2011). The relatively simple geometry of the muscles that drive extension and flexion of the leg joints also facilitates kinematic analyses of muscle dynamics (Perry *et al*., 2009). The largest crabs grow to sizes approaching 75 g and exhibit stride frequencies of about 5 Hz to achieve top speeds of more than 1 m s^{-1}. Smaller crabs (<30 g) cannot achieve the same maximal speeds, but run with frequencies of up to *c*. 10 Hz at their top speeds (Perry *et al*., 2009). This shift in operational frequency suggests changes to skeletal muscle phenotype accompanying growth. In a previous study, we documented potential associations between isoforms of myofibrillar proteins MHC and troponin T and body size (Perry *et al*., 2009). Although plasticity in muscle phenotype may be responsible for the shift in frequency, an alternate hypothesis is that changes to the muscle and joint mechanics occurring as a result of allometric growth may lead to fundamental differences in kinetics.

Allometric growth of the musculoskeletal system significantly affects locomotory performance in some animals as they grow and develop (Carrier, 1996). In mammals, younger animals may possess greater mechanical advantage in their joints, which appears to enhance performance in the face of less developed skeletal muscles (Carrier, 1996; Young, 2005). Ontogenetic changes in the musculoskeletal system of grasshoppers have been documented, with juveniles possessing less powerful leg muscles, but with greater capacity for endurance (Kirkton, Niska & Harrison, 2005; Kirkton & Harrison, 2006). Some crustaceans' musculoskeletal systems also exhibit allometric patterns of growth, particularly in the mechanics of their dimorphic claws (Costello & Lang, 1979; Schenk & Wainwright, 2001). In the current study, we tested the hypothesis that changes in the mechanics of growing ghost crabs could account for some of the observed changes in stride frequency. For example, if the joints of larger crabs possessed greater mechanical advantage, this would result in slower angular velocities in favor of higher force production. We ran ghost crabs of different sizes on a motorized treadmill at maximal running speeds while recording with high-speed video to determine their stride frequencies. We also used these recordings to determine angular velocities and used these measurements to estimate muscle shortening velocities. In addition to these performance parameters, we measured limb and muscle dimensions, including muscle fiber length and mechanical advantage, to determine how these parameters change with growth. In a complementary experiment, we experimentally weighted crabs to test the hypothesis that changes in the relative weight of the crabs might be responsible for changes in operational frequency.

Materials and methods

Animals

Twenty-four ghost crabs *Ocypode quadrata* Fabricius ranging in mass from 2.5 to 65 g were obtained from Gulf Specimen Marine Laboratories, Inc. (Panacea, FL, USA). Animals were maintained in aquaria with circulating artificial seawater at 25°C. They were fed daily with dried krill and commercially available pellets formulated to feed crabs. Running experiments were conducted within 1 week of being taken into captivity. In addition, preserved specimens from previous studies were used for some of the morphological measurements.

Morphological measurements

Linear dimensions of crab carapace, legs, muscle fibers and joints were determined to the nearest 0.01 mm using digital calipers. The cross-sectional area of the combined extensor and flexors of the carpopodite were estimated as the product of the merus length and width. This is a different parameter than the cross-sectional area of the *meropodite* (height by width), but is the appropriate measure to include all of the fibers along the muscle length. The mechanical advantage (MA) of the meropodite-carpopodite joint was determined as the ratio of the input lever length to the output lever length. Here, the input lever lengths were the distances from the insertion of the apodemes (extensor or flexor) onto the proximal carpopodite, while the output lever was the length of the carpopodite-propopodite leg segment ($n = 24$). We also measured carapace width and lengths of the meropodite, carpopodite-propopodite and dactylopodite to determine whether crab dimensions change according to geometric growth ($n = 36$).

We also made measurements of the extensors and flexors of the carpopodite, in conjunction with the meropodite-carpopodite joint, to determine MA, muscle cross-sectional area and other functionally relevant parameters. To study these muscles and their joints, the exoskeleton on a lateral side of the merus was removed to expose the extensor (anterior) or flexor (posterior) muscles. To measure fiber lengths and pinnation angles, images of frozen muscles were captured through a stereomicroscope and these images were later used in our measurements. To determine MA and other aspects of the joints, muscles were digested overnight using 1N NaOH solution. This process left the muscle apodemes and the leg joints intact. Images of the joints were captured using a camera attached to a stereomicroscope and these were used in later measurements. For the determination of MA, we estimated the pivot point to lie midway between the extensor and flexor insertion points. This means that the MAs of both the extensor and flexor muscles were estimated to be equal within an individual crab.

Extensor and flexor muscle fiber lengths and pinnation angles have been reported previously (Blickhan, Full & Ting, 1993; Perry *et al*., 2009). In the current study, we confirmed the values of these parameters from images of frozen muscles captured using a stereomicroscope. As in our previous study (Perry *et al*., 2009), the joint was held in a neutral position (~90° as shown in Fig. 1a). We did not detect any differences in pinnation angles as a function of body size.

Running experiments

Crabs were exercised on a motorized treadmill as previously described (Perry *et al*., 2009). The track on the treadmill is

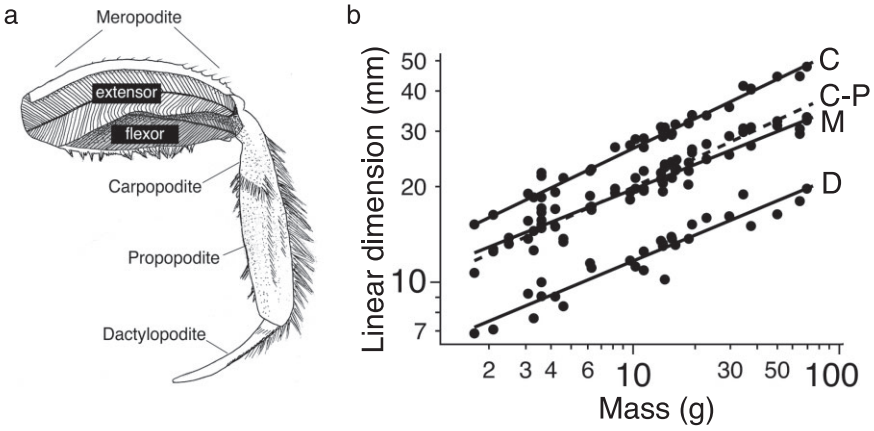

Figure 1 General organization of crab walking legs and scaling of linear dimensions. (a) The meropodite houses the major extensor and flexor carpopodite muscles that extend and flex the meropodite-carpopodite joint. The carpopodite-propopodite leg segment attaches distally to the dactylopodite. (b) The linear dimensions of the carapace (C), meropodite (M), carpopodite-propopodite (C-P) and dactylopodite (D) scale approximately in proportion to mass$^{0.30}$ (n = 36).

48 cm long and crabs were contained within a clear 25-cm long and 8-cm wide plexiglass chamber. After being placed in the chamber, the treadmill speed was gradually increased until the crab was just keeping pace with the speed of the treadmill. After several seconds of maintaining a steady running speed, the speed of the treadmill was turned down to a stop and the crab was removed. These running trials were recorded at capture speeds of 300 frames per second using a Casio Exilim EX-F1 digital video camera (Casio Computer Co., Tokyo Japan).

In a subsequent experiment, weights were attached to the carapaces of crabs to study how added mass affects running dynamics (n = 9). Lead fishing weights were flattened to the approximate dimensions of the crab's carapace and fixed to the exoskeleton using rapidly polymerizing adhesive. Weights were constructed to approximate either 15% or 25% of a crab's native mass. Each crab was recorded running without the weight attached and then with 15% added mass followed by 25% added mass. Each crab was allowed to recover from a sprint for at least 1 h before being tested again.

Kinematic analyses

Individual video recordings were reviewed to determine stride frequencies and angular velocities. To determine frequency, recordings captured at 300 frames per second were played and we counted the number of complete strides for the second walking legs over several stride cycles (n = 24). We then imported recordings of crab running trials into ProAnalyst motion analysis software (XCitex, Inc., Woburn, MA, USA) to determine the changes in joint angles over time (n = 18). Briefly, several hundred frames of video were used to determine angular velocities of the second walking legs over several stride cycles from crabs running at maximal sustained speeds. The second walking leg was chosen because it is used in conjunction with the third leg to generate power during rapid running (Burrows & Hoyle, 1973; Blickhan & Full, 1987) and because it is more visible than the third leg. The angles of the meropodite-carpopodite joints were determined using a three-point angle, constructed by manually identifying points on each video frame. The three points identified were the basipodite-meropodite joint at the proximal end of the meropodite, the distal end of the meropodite just above the joint and the distal end of the propopodite just proximal to the dactylopodite.

Estimation of muscle shortening velocity and muscle strain

Angular velocities determined from our kinematic analyses were used to estimate muscle shortening velocities of the extensor and flexor muscles (n = 18). For a given muscle and joint geometry, muscle shortening velocity and angular velocity (ω, in rad × s^{-1}) are directly connected through the following equation presented by Alexander (2003):

$$\omega = (L/r) \times (\Delta L/\Delta t) \times \cos\theta$$

where L is muscle fiber length, r is the moment arm, $\Delta L/\Delta t$ is the fiber shortening velocity and θ is the angle of fiber insertion. We determined ω directly from our kinematic analyses of leg movements, L and r were determined through morphological measurements and values for θ (27.8° for the extensors and 24.8° for the flexors) were taken from our previous study (Perry *et al.*, 2009). Total muscle strain was estimated from an empirically determined relationship among operational frequency, muscle shortening velocity and total strain where: velocity/strain = 5.3 × frequency (Medler & Hulme, 2009).

Statistical analyses

Linear regression analyses were performed using JMP 10 software (SAS Institute, Cary, NC, USA). For most analyses, linear axes were log-transformed to match the nature of the scaling relationships. Analysis of covariance (ANCOVA) model was used to test the effects of load on stride frequency, where crab body mass was included as a covariate. ANCOVA was also used to compare the scaling relationships of fiber length and joint moment. Where means for parameters are reported, they are reported as mean ± SE.

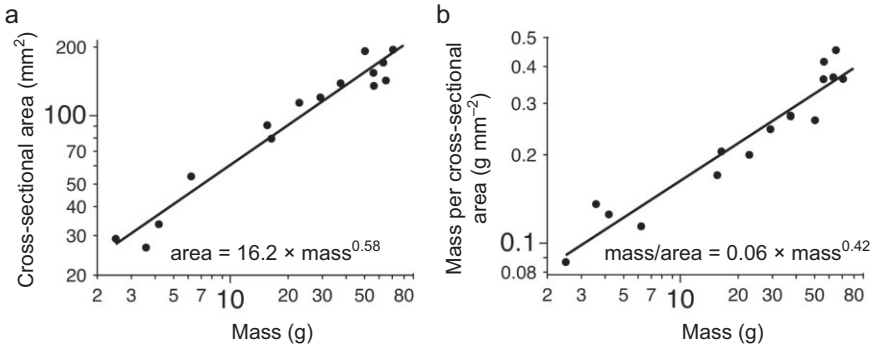

Figure 2 (a) Meropodite cross-sectional area (mm^2) as a function of body mass. Area scaled in proportion to $mass^{0.58}$, meaning that the thickness of the legs does not keep pace with the crab's mass. (b) Crab mass standardized to the cross-sectional area of the meropodite (g mm^{-2}) scaled to $mass^{0.42}$. The largest crabs are more than four times heavier, relative to the cross-sectional area of the legs, compared with the smallest crabs ($n = 16$).

Results

Scaling of limb dimensions

The general anatomical organization of the crab legs are shown in Fig. 1a. According to geometric scaling, the linear dimensions of an animal will scale as a function of $mass^{0.33}$. The linear dimensions of ghost crabs exhibit slightly smaller mass exponents, ranging from 0.31 for the carapace, but only 0.26 for the meropodite (Fig. 1b). The scaling relationships for the various dimensions were as follows: carapace = $12.9 \times mass^{0.31}$, meropodite = $10.7 \times mass^{0.26}$, carpopodite-propopodite = $10.0 \times mass^{0.30}$ and dactyl = $6.3 \times mass^{0.27}$ (masses in g and lengths in mm; $n = 36$). These values are similar to those reported for scaling of the carapace, meropodite and leg length in an earlier study (Blickhan *et al.*, 1993). Overall, the linear dimensions for each of the leg segments scaled with similar patterns as a function of mass (nearly parallel lines).

The cross-sectional area of the meropodite increases as a function of body mass, but with a mass exponent of less than unity: area (mm^2) = $16.2 \times mass^{0.58}$ ($r^2 = 0.96$; $P < 0.0001$; $n = 16$) (Fig. 2a). This relationship means that the cross-sectional area of the meropodite, along with extensor and flexor muscles inside, fail to keep pace with the mass of the crab. In effect, the relative mass of the largest crabs is approximately four times greater than the smallest crabs (Fig. 2b).

Stride frequency

Stride frequency in rapidly running crabs was highest in the smaller crabs, with the highest recorded frequency being just over 12 s^{-1} in a 4-g crab. In the largest crabs, frequencies were between 6 and 7 s^{-1}. Frequency followed a systematic decline as a function of body size, as described by the equation: frequency = $12.6 \times mass^{-0.14}$ ($r^2 = 0.51$; $P < 0.0001$; $n = 24$) (Fig. 3a). This scaling pattern is very similar to those of other terrestrial running animals, including mammals and lizards (Fig. 3b). The mammal line is from Heglund, Taylor & McMahon (1974), while the lizard data are from Huey (1982), Marsh & Bennett (1986) and Swoap *et al.* (1993).

Joint and muscle mechanics

The insertion points of the extensor and flexor apodemes with respect to the point of rotation are shown in Fig. 4. Muscle

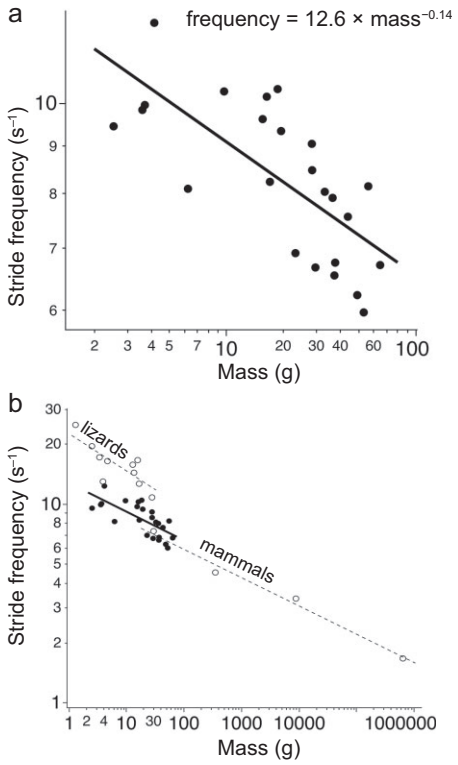

Figure 3 Scaling of maximal stride frequency as a function of body mass. (a) Stride frequency declined systematically with increasing crab size, in proportion to $mass^{-0.14}$. (b) Scaling of crab frequency (filled circles) in relation to mammals (open circles) and lizards (open circles). Each of these terrestrial groups exhibits a similar scaling pattern, with crabs being very close to the extended mammalian line ($n = 24$).

fiber length (L; mm) increased with body mass as: $4.61 + mass^{0.30}$ ($r^2 = 0.99$; $P < 0.0001$; $n = 24$) (Fig. 4b). The joint moment arm (r; mm) increased with a similar slope: $0.50 + mass^{0.32}$ ($r^2 = 0.95$; $P < 0.0001$; $n = 24$) (Fig. 4b). The slopes of the two lines were not significantly different from one another ($F = 0.59$; $P > 0.45$; d.f. = 1) and the ratio of fiber length to joint moment arm (L/r) was determined to be 8.6 ± 0.33 and was independent of body mass ($r^2 = 0.08$;

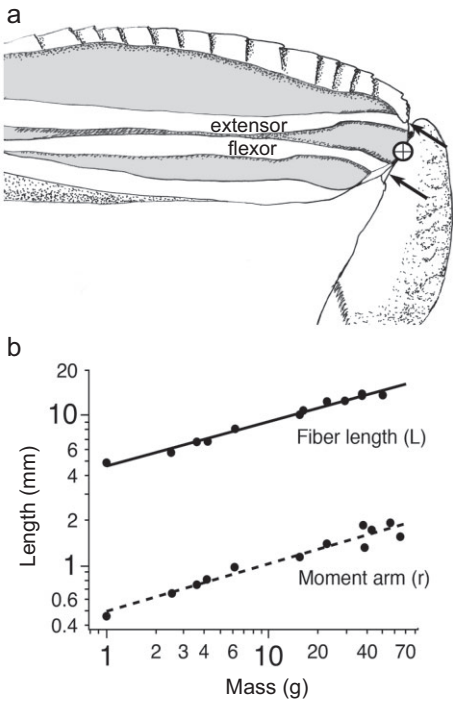

Figure 4 Meropodite-carpopodite joint characteristics and scaling patterns. (a) Orientation of apodeme insertions (arrows) onto the proximal carpopodite as seen with muscles removed. The point of rotation of the carpopodite about the meropodite is indicated by the circle. The distance between the insertion point and the point of rotation represents the input lever distance (r). This distance was estimated to be equal for the extensor and flexor muscles. (b) Scaling of muscle fiber length (L) and input lever distance (r). Both lengths scale approximately as mass$^{0.30}$ and the parallel nature of these lines means that the ratio L/r does not change with body size (n = 24).

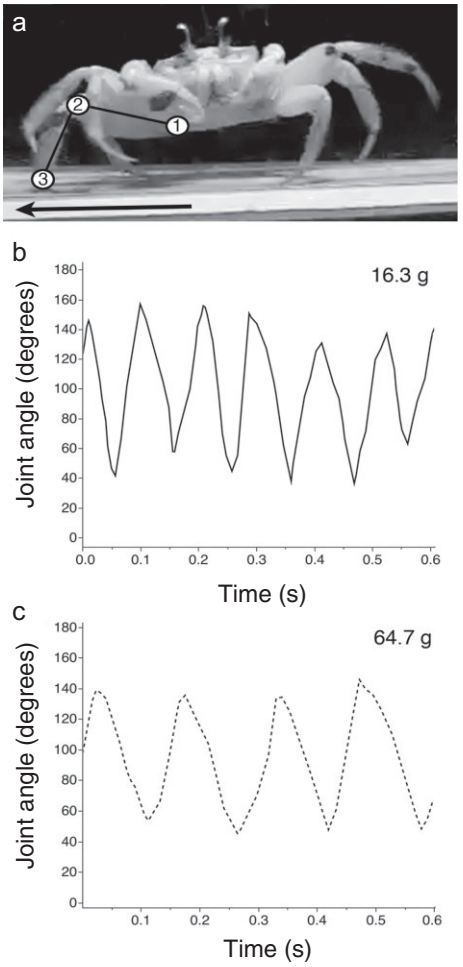

Figure 5 Patterns of joint angle change in running crabs. (a) Ghost crab during rapid running, with points used for angle determination identified on the second walking leg on the leading side. Points marked for identification (dark spots) are also visible on the second walking leg of the trailing side. The arrow at the bottom of the figure indicates the direction of movement. (b) Patterns of angle changes in a 16.3-g crab compared with (c) those of a 64.7-g crab. The fundamental pattern of angular changes is similar between large and small crabs, but the rate of change is greater in the smaller animals (n = 18).

$P > 0.40$; $n = 11$). MA was determined to be 0.052 ± 0.002 and was also independent of body mass ($r^2 = 0.00$; $P > 0.99$; $n = 11$). This MA is only a fraction of that observed in the claws of ghost crabs, which is 0.312 for the crusher and 0.116 for the cutter (Schenk & Wainwright, 2001). The lower MA in the leg joint likely reflects that the legs have evolved for speed, rather than force generation.

Patterns of leg movements

During rapid running, crabs extended and flexed the meropodite-carpopodite joint through a full range of movement (Fig. 5a and b). The mean angular excursion was 81.5 ± 2.6° for the leading legs and 91.7 ± 2.1.9° for the trailing legs. This 10% greater excursion for the trailing leg was statistically significant ($t = 3.67$; $P < 0.0009$; $n = 18$) as determined by a paired t test. The range of movement was independent of body size for both the leading and trailing legs (data not shown). The patterns of angular rotation were in a 'saw tooth' pattern, with very abrupt changes between joint extension and flexion (Fig. 5a and b). These patterns were consistently observed for both leading and trailing legs. Paired comparisons from individual crabs showed that angular velocity during extension was 15% faster than during flexion ($t = 4.31$; $P < 0.0005$; $n = 18$). However, no differences were observed between leading and trailing legs during extension ($t = 0.57$; $P > 0.57$; $n = 18$) or flexion ($t = 0.11$; $P > 0.91$; $n = 18$). Overall, the patterns of leg movements were consistent in crabs of different sizes, but with the movements being compressed into a shorter time scale in the smaller crabs (Fig. 5).

Scaling of muscle shortening velocity

We determined that both the extensor and flexor carpopodite muscles shorten with greater speeds in smaller crabs.

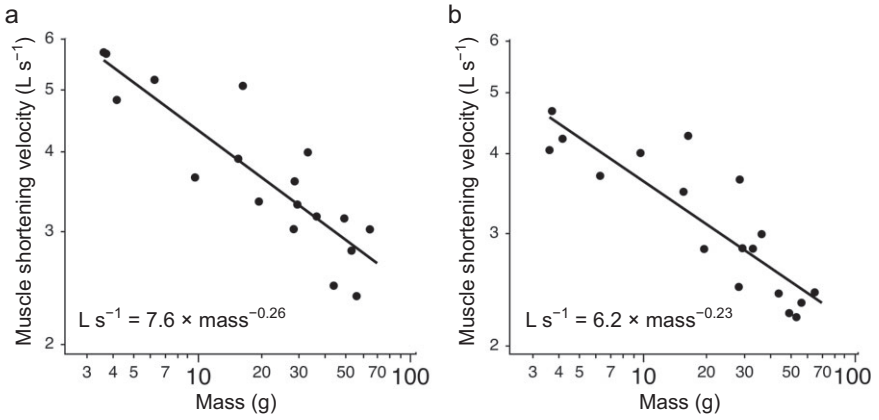

Figure 6 Estimated muscle shortening velocity during rapid running. (a) Extensor muscle shortening velocity was dependent on animal size, with velocity being proportional to mass$^{-0.26}$. (b) Flexors exhibited a similar, although slightly slower velocity, being proportional to mass$^{-0.23}$. Overall, the smallest crab muscles shortened with velocities approximately twice as fast as those of the largest crabs (n = 18).

Shortening velocity in the extensor muscles scaled with a mass exponent of –0.26 ($7.6 \times \text{mass}^{-0.26}$; $r^2 = 0.77$; $P < 0.0001$; $n = 18$), while the velocity in the flexors scaled with a mass exponent of –0.23 ($6.2 \times \text{mass}^{-0.23}$; $r^2 = 0.77$; $P < 0.0001$; $n = 18$) (Fig. 6). The extensors from the smallest crabs (~2–5 g) shortened with speeds of *c*. 5 L s^{-1}, while those in the largest crabs (~50–65 g) contracted at rates of *c*. 2.5 L s^{-1}. The flexors of the carpopodite were slightly slower, with the muscles in the smallest crabs shortening at about 4 L s^{-1} and those in the largest crabs at *c*. 2 L s^{-1}. These patterns are generally consistent with the size-dependent patterns reported previously (Perry *et al.*, 2009), although they are slightly faster. The estimated total strain for the extensor muscles was 0.08 ± 0.003, while that for the flexors was 0.075 ± 0.002, and these were independent of body size.

Effects of load on stride frequency

The slopes of the relationships between stride frequency and body mass were not different in crabs carrying different loads ($F = 0.29$; $P > 0.75$; d.f. = 2). However, ANCOVA indicated that the effect of carrying a load had a significant effect on the magnitude of stride frequency ($F = 14.2$; $P < 0.0001$; d.f. = 2). A Tukey's *post hoc* comparison of the least squares means indicated that frequency was significantly reduced in the crabs carrying 25% load (by *c*. 13%), but was not different from unloaded controls for crabs carrying 15% load (Fig. 7).

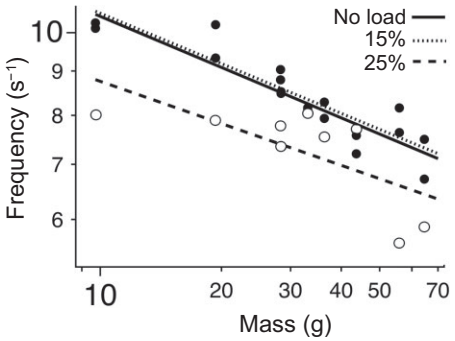

Figure 7 Effects of load carrying on crab stride frequency. Adding an additional 15% of a crab's own weight had no effect on stride frequency. Adding 25% of the crab's weight significantly slowed stride frequency by *c*. 13%. The reduction in stride frequency was not dependent on crab size, as the slopes for all three conditions were not significantly different from one another ($P > 0.75$) (n = 9).

Discussion

Our goal was to determine whether allometric growth in ghost crabs affects muscle leverages in ways that could account for the observed scaling effects on stride frequency. Overall, we found that the relative proportions of the limb segments, muscle fiber lengths and moment arm, where the muscles insert onto the carpopodite, all maintained similar proportions in small and large crabs (Figs 1 and 4). In particular, the ratio between fiber length and moment arm is a key parameter because this relationship directly determines the gearing of the leg joints (Alexander, 2003). If the fiber length were longer relative to the moment arm in smaller crabs, it could explain their faster angular velocities without requiring any differences in the physiological properties of the muscles themselves. The fact that the length of these structures change in parallel with size (Fig. 4) means that we can rule out anatomical changes as contributing to the slowing of stride frequency with age. Similarly, we found that the mechanical advantage of the leg joint was just over 0.05, but was unaffected by body size. Other animals do experience ontogenetic changes in joint leverages or muscle organization during growth and maturation that influence performance (Carrier, 1996; Kirkton *et al.*, 2005; Young, 2005; Kirkton & Harrison, 2006). Some crustaceans also exhibit developmental changes in mechanics related to specialization of their dimorphic claws. For example, juvenile lobsters' claws begin as isomorphic appendages, but differentiate into a massive crusher and a slimmer cutter claw as the animals grow and mature (Govind, Mellon & Quigley, 1987). The crusher develops into a claw with greater MA than the cutter by the sixth molt and through allometric growth MA continues to increase until it is more than double that of the cutter (Costello & Lang, 1979). By comparison, the cutter

claw does not exhibit any changes in MA over the same time period (Costello & Lang, 1979). In ghost crabs, the muscles and joints appear to follow the growth pattern exhibited by the lobster cutter claw, in that we found no changes in their mechanics in crabs ranging from ~1 to 75 g.

Burrows & Hoyle (1973) reported that a related species of ghost crab *O. ceratophthalma* generated driving forces through extension of the trailing legs and that the leading legs simply functioned as a 'skid' to land on during rapid running. Mechanical studies of running *O. quadrata* confirmed similar patterns of usage, determined from force plate recordings of rapid running crabs (Blickhan & Full, 1987). Additionally, exoskeletal strains experienced during galloping are several times higher in the trailing legs compared with the leading legs (Blickhan *et al.*, 1993). Based on the premise that the trailing legs are disproportionately loaded while running, we anticipated that the extension rate in the trailing legs would be slower than in the leading legs. This hypothesis, however, was not born out by the data. The average rates of both extension and flexion were the same between leading and trailing legs of individual animals. One difference we did observe was that the angular velocity during extension was about 15% faster than flexion in both leading and trailing legs. This difference could be due to differences in the muscle fibers, fiber geometry, differences in mechanical advantage or the fact that the extensor muscles in ghost crabs are greater in mass than the flexors (Hafemann & Hubbard, 1969). The general patterns of leg movement appeared to be independent of crab size. All crabs employed a 'saw tooth' pattern of angular change, with rapid transitions between extension and flexion (Fig. 5). The total angular excursion was also independent of crab size.

Based on the data presented, it seems clear that the basic leverages of the musculoskeletal system in ghost crabs are not affected by allometric growth. Likewise, the overall patterns of limb movement are scale-independent, with the exception that the speed of limb movement is faster in smaller crabs (Figs 5 and 6). This pattern is not unique to ghost crabs, as operational frequency changes systematically as a function of body size in many kinds of animals (Heglund *et al.*, 1974; Full, 1997) (Fig. 3b). One explanation for this phenomenon is that the natural frequency of limb movement is dictated by the dimensions of the limbs (McMahon, 1975; Ahlborn *et al.*, 2006). Although simple limb length likely contributes to differences in stride frequency, there are important exceptions to this pattern. Fiddler crabs (*Uca* spp) share membership in the family Ocypodidae with the ghost crabs, but are far less proficient runners. Fiddler crabs possess much shorter legs than ghost crabs of comparable mass and based on this pattern we might predict that fiddler crabs would run with higher frequencies. However, fiddler crabs (~2–3 g) running at full speed only attain frequencies of ~6 Hz (Whittemore, Morris & Medler, 2013; Gerald & Thiesen, 2014) compared with frequencies approaching 12 Hz in longer-legged ghost crabs of similar body mass (Fig. 3). Another possibility we investigated focused on the relative mass of the crabs, which is greater in the larger crabs (Fig. 2). This pattern is a simple consequence of the basic rules of scaling, whereby volume increases faster than linear dimensions (Calder, 1996). Based on this relationship, we hypothesized that the relative load of the crabs might be responsible for the slower stride frequency in larger crabs. However, our data did not support this hypothesis. Loading crabs with 15% of their mass had no effect on stride frequency (Fig. 7). Loading crabs with 25% of their mass resulted in a reduction in stride frequency by just over 13%, but the magnitude of the reduction in frequency was not significantly related to crab size (Fig. 7). This pattern is consistent with studies of hermit crabs carrying loads that demonstrated that shell mass had little effect on stride frequency (Herreid & Full, 1986). Male fiddler crabs carrying a large claw pay an energetic cost for their load, but stride frequency is not affected when running on a level substrate (Gerald & Thiesen, 2014). Another explanation for the scale-dependent changes in stride frequency is that the contractile physiology of the leg muscles shifts as a function of body size.

There are currently no data available for the contractile properties of isolated ghost crab muscles. However, we were able to estimate contractile velocity and muscle strain from our kinematic analyses and we can compare these with previous estimates (Perry *et al.*, 2009). For a given muscle with a given joint moment arm, angular velocity is directly related to muscle shortening velocity (Alexander, 2003). We took advantage of this relationship to translate angular velocities from running crabs into muscle shortening velocity (L s^{-1}) (Fig. 6). Using this approach, we found that muscles shorten with velocities of about 4–6 L s^{-1} in the smallest crabs, but only about 2–3 L s^{-1} in the largest crabs. Because muscles typically operate *in vivo* at *c.* 30% of maximum unloaded shortening velocity (V_{max}) to maximize power output (Josephson, 1993), this suggests that the muscles in the smallest crabs possess a V_{max} of *c.* 15–20 L s^{-1}. These shortening speeds are fast for skeletal muscles, but are within the range of those of other small terrestrial animals, including mammals and reptiles (Medler, 2002; Medler & Hulme, 2009). Our estimates of total muscle strain in the range of 7–8% of fiber length are also similar to muscles from other animals (Medler & Hulme, 2009). Overall, these rates of muscle shortening are greater than those we reported in a previous study (Perry *et al.*, 2009). In our previous study, we directly measured apodeme movement as the leg joint was rotated manually to determine the magnitude of muscle strain associated with angular changes. If we apply these values to current angular velocities, the extensor velocities are reduced to 60% of the values determined in this study and the flexor velocities to 85% of the reported values. Crabs in the current study also achieved higher stride frequencies than crabs in the previous study. This increased frequency may stem from two different factors. First, in the current study, we attached a textured cloth to the treadmill surface, which provided better traction for the crabs. Second, the crabs were obtained from different populations, one from south Texas (Perry *et al.*, 2009) and the other from the panhandle of Florida (current study). It is possible that these population differences might have resulted in the observed differences in frequency.

Skeletal muscle shortening velocity declines with increasing body size in a pattern very similar to that observed for stride frequency in a wide variety of animals (Medler, 2002). This

pattern demonstrates that selective forces have shaped the contractile properties of the muscles to match body dimensions. Our data indicate that comparable shifts in contractile properties of ghost crab muscles occur as they grow (Fig. 6). Similar changes in contractile kinetics occur during ontogeny in lizards, with the physiological properties of isolated muscles being closely matched with operational frequencies *in vivo* (Marsh, 1988; Johnson *et al*., 1993; Swoap *et al*., 1993). A key question is what differences exist between the muscles of smaller versus larger crabs that could provide a mechanism for the observed changes in muscle physiology. Among different mammalian species, selection has resulted in subtle changes in MHC sequences, such that orthologous MHC isoforms are faster in smaller animals (Pellegrino *et al*., 2003; Marx *et al*., 2006). In ghost crabs, at least three alternate MHC isoforms are expressed, along with multiple isoforms of other myofibrillar proteins such as troponin I and troponin T (Perry *et al*., 2009). We previously documented size-related differences in the expression of these isoforms, but these were primarily restricted to fiber populations that appear to be slower, aerobic fibers (Perry *et al*., 2009). Ontogenetic shifts in other crustacean muscle fibers are well documented and may occur over a period of several years (Govind *et al*., 1987; Silverman, Costello & Mykles, 1987; Medler *et al*., 2007). Further work is required to further identify how differences in myofibrillar isoform expression might contribute to the observed changes in contractile properties.

In conclusion, we found that stride frequency and muscle shortening velocity systematically decline with increasing body size in sprinting ghost crabs. However, we did not detect any changes in the basic mechanics of these muscles as a result of allometric growth. The overall patterns of leg movements and muscle shortening were similar between small and large crabs, but the smallest crabs simply performed the movements at approximately twice the speed. These patterns raise questions of how the muscles of the smaller crabs generate these higher contractile speeds. One possibility is that the expression of myofibrillar proteins, such as myosin heavy chain, changes with growth.

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