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RESEARCH ARTICLE

Forelimb muscle architecture and myosin isoform composition in the groundhog (*Marmota monax*)

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ABSTRACT

Scratch-digging mammals are commonly described as having large, powerful forelimb muscles for applying high force to excavate earth, yet studies quantifying the architectural properties of the musculature are largely unavailable. To further test hypotheses about traits that represent specializations for scratch-digging, we quantified muscle architectural properties and myosin expression in the forelimb of the groundhog (*Marmota monax*), a digger that constructs semi-complex burrows. Architectural properties measured were muscle moment arm, muscle mass (MM), belly length (ML), fascicle length (l^F), pennation angle and physiological cross-sectional area (PCSA), and these metrics were used to estimate maximum isometric force, joint torque and power. Myosin heavy chain (MHC) isoform composition was determined in selected forelimb muscles by SDS-PAGE and densitometry analysis. Groundhogs have large limb retractors and elbow extensors that are capable of applying moderately high torque at the shoulder and elbow joints, respectively. Most of these muscles (e.g. latissimus dorsi and pectoralis superficialis) have high l^F/ML ratios, indicating substantial shortening ability and moderate power. The unipennate triceps brachii long head has the largest PCSA and is capable of the highest joint torque at both the shoulder and elbow joints. The carpal and digital flexors show greater pennation and shorter fascicle lengths than the limb retractors and elbow extensors, resulting in higher PCSA/MM ratios and force production capacity. Moreover, the digital flexors have the capacity for both appreciable fascicle shortening and force production, indicating high muscle work potential. Overall, the forelimb musculature of the groundhog is capable of relatively low sustained force and power, and these properties are consistent with the findings of a predominant expression of the MHC-2A isoform. Aside from the apparent modifications to the digital flexors, the collective muscle properties observed are consistent with its behavioral classification as a less-specialized burrower and these may be more representative of traits common to numerous rodents with burrowing habits or mammals with some fossorial ability.

KEY WORDS: Force, Muscle, Myosin, Power, Scratch-digging

INTRODUCTION

Morphological evaluations of scratch-digging mammals often describe large and powerful forelimb muscles and skeletal modifications for increased mechanical advantage for the excavation of earth; however, few studies attempt to quantify the architectural properties of the musculature (e.g. Lehmann, 1963; Gambaryan, 1974; Gambaryan and Gasc, 1993; Lagaria and Youlatos, 2006; Endo et al., 2007). The force and power that a whole muscle can apply at a limb joint are strongly influenced by the arrangement of the muscle fibers relative to the axis of force production within the muscle (Eng et al., 2008; Lieber, 2009). Pennate muscles with short fibers have larger physiological cross-sectional area (PCSA), and thus the ability to produce high isometric force (Alexander, 1984). Alternatively, muscles with long fibers arranged in parallel with the axis of force production have a greater ability to shorten and produce force over a large range of joint motion (Peters and Rick, 1977; Zajac, 1989; Zajac, 1992). A trade-off between these two functional designs indicates that a muscle is capable of performing appreciable mechanical work at high power. To begin identifying traits that represent muscle specializations for scratch-digging, we recently quantified muscle architectural properties in the forelimb of the semi-fossorial American badger and identified the following key modifications: massive humeral retractors, elbow extensors and digital/carpal flexors; two heads of the triceps brachii are biarticular and capable of applying large torque at the shoulder (flexor moment) and elbow (extensor moment) joints; and digital flexors that are pennate and compartmentalized for both high force production and fascicle shortening (Moore et al., 2013).

At the cellular level, the myosin heavy chain (MHC) isoforms expressed within a muscle fiber directly determine fiber isometric tension, unloaded shortening velocity and power (Reiser et al., 1985; Schiaffino and Reggiani, 1996; Schiaffino and Reggiani, 2011). Fast MHC-2X and MHC-2B fibers are more glycolytic in their ATPase metabolism and have much higher power output than fast MHC-2A fibers, which are highly oxidative and generate more power than slow, oxidative MHC-1 fibers. Similar to muscle architectural properties, few studies have evaluated muscle fiber type in the forelimbs of scratch-diggers. Goldstein (Goldstein, 1971) reported that the triceps brachii and teres major of generalized ground squirrels (*Spermophilus*) and chipmunks (*Neotamias*) consist of three 'fiber types,' but were composed of predominately 'slow-contracting' fibers based solely on the presence or absence of stored glycogen in the muscles. Using similar histochemical approaches, Alvarez et al. (Alvarez et al., 2012) found that the same muscles in fossorial tuco-tucos (*Ctenomys*) also contain three fiber types and have a majority of fast oxidative/glycoytic (FOG) fibers as classified by their myosin ATPase reactions. These findings suggest that overall, more oxidative fiber types are required for sustained burrowing activity in rodents, yet comparison of function with homologous muscles from other scratch-diggers is limited because the MHC isoforms of the fiber types are unknown. Moreover, it is not clear whether less heterogeneity in MHC expression represents specialization for variation in muscle force and power between generalized burrowing and fossorial mammals.

The groundhog (or woodchuck) is a terrestrial scratch-digger that belongs to the family Sciuiridae (Steppan et al., 2004) and ranges throughout North America as a result of its flexibility to inhabit

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List of symbols and abbreviations

θ	pennation angle
$\mathbf{F}_{max}$	maximum isometric force
l^F	fascicle length
MHC	myosin heavy chain
ML	muscle belly length
MM	muscle belly mass
PCSA	physiological cross-sectional area
r_m	muscle moment arm
$\mathbf{V}_{max}$	maximum shortening velocity
W	muscle power

numerous ecosystems (Swihart, 1992). It is one of 14 species of marmots (Steppan et al., 1999), all of which are herbivorous, and have the largest body sizes of any of the sciurids. Adult body mass ranges from 2.7 to 5.4 kg (Bezuidenhout and Evans, 2005), with an average dimorphic body mass of 3.8 kg for males and 3.5 kg for females (Snyder et al., 1961). Body mass varies with hibernation behavior, with the peak body mass occurring immediately prior to hibernation (~7 months per year). Groundhogs excavate their own burrows in open pastures and at the edges of forests, and they are primarily used for protection, hibernation and the rearing of kits (Meier, 1992). Burrows may be simple, with no defined structure, or more complex, containing several chambers or dens (Kwiecinski, 1998), and they can be up to 2 m deep and 1–13 m long (Hamilton, 1934). In addition to burrowing, groundhogs have a locomotor repertoire that includes slow walking and running in short intervals, swimming, and climbing to potentially escape predators (Kwiecinski, 1998).

Groundhogs have morphological features more typical of a generalized burrower (Hildebrand, 1985; Kley and Kearney, 2007), including: a reduced nictitating membrane (covers only the medial corners of the cornea); relatively large, shovel-shaped hindfeet that lack webbing between the digits (Bezuidenhout and Evans, 2005); and muscular forelimbs with forefeet that have only four short (~1.5 cm) claws. Although their forelimb osteology [i.e. mechanical advantage (Lagaria and Youlatos, 2006)] and myology (Bezuidenhout and Evans, 2005) have been described in detail, the architectural properties of their forelimb muscles have not been quantified and related to their digging habits. The aims of this study are: (1) to quantify muscle fiber architecture and MHC isoform composition in groundhogs; and (2) to estimate peak isometric force ($\mathbf{F}_{max}$), joint torque and instantaneous power (W) of the forelimb musculature. As a reflection of their relatively generalized lifestyle, we hypothesize that the forelimb muscles of groundhogs will have the capacity to apply only moderate torque and power at the shoulder, elbow and carpal joints, and they will be heterogeneous in their MHC isoform composition. Specifically, we expect the limb retractors and elbow extensors to have long, parallel fibers and be considerably more massive than the carpal/digital flexors, which will have shorter fibers and varying degrees of pennation (and PCSA) that may enhance the application of force at the manus. These functional muscle groups are also expected to have unequal proportions of MHC-1, MHC-2A and MHC-2X, with a predominance of the 1 and 2A isoforms corresponding with both their phylogenetic and functional similarity to ground squirrels and chipmunks. The data obtained serve to clarify the relationship between internal muscle properties and fossorial ability, and further distinguish muscle traits that indicate muscle specializations for scratch-digging among mammals.

RESULTS

Functional distribution of forelimb muscle mass

The digging apparatus of the forelimb has 44 muscles (excluding muscles intrinsic to the manus) for which muscle architecture is quantified (Tables 1, 2). Mean total forelimb muscle mass is 164.9±30.1 g, accounting for 3.3% of body mass (i.e. per single limb). Overall, the limb retractors and elbow extensors are the two

Table 1. Functional muscle groups of the digging apparatus of *Marmota monax*

	Muscle group	Muscles studied
Extrinsic muscles	Scapula elevator/stabilizers	Trapezius (parts: cervical, thoracic) Rhomboideus (heads: capital, cervical, thoracic)
	Scapula/limb retractors	Trapezius thoracica, rhomboideus thoracis, latissimus dorsi, [a]pectoralis superficialis, [b]pectoralis profundus
	Scapula/limb protractors	Trapezius cervicalis, rhomboideus capitis, rhomboideus cervicis
	Limb adductors	Pectoralis superficialis, pectoralis profundus
Intrinsic muscles	Limb retractors (shoulder flexor/stabilizers)	Deltoideus (parts: scapular, acromial, clavicular), teres major, teres minor, infraspinatus, triceps brachii-long head
	Limb protractors (shoulder extensor/stabilizers)	Coracobrachialis, supraspinatus, [c]subscapularis, cleidobrachialis
	Elbow flexors	Biceps brachii, brachialis, cleidobrachialis
	Elbow extensors	Triceps brachii (heads: long, lateral, [d]medial/accessory), anconeus, [e]tensor fasciae antebrachii
	Carpal flexors	Flexor carpi radialis, flexor carpi ulnaris
	Carpal extensors	Extensor carpi radialis (heads: longus, brevis), extensor carpi ulnaris
	Digital flexors	Flexor digitorum superficialis (heads: epicondylar, condylar), *flexor digitorum profundus (heads: humeral medial, humeral profundus, radial, ulnar)
	Digital extensors	Extensor digitorum communis, extensor digitorum lateralis, [f]extensor digiti II, [g]extensor digiti III [h]Abductor digiti I longus
	Pronators	Pronator teres, pronator quadratus
	Supinators	Supinator, [i]brachioradialis

Muscle nomenclature follows Bezuidenhout and Evans (Bezuidenhout and Evans, 2005).
[a]Consists of descending and transverse parts (measured as a single muscle); [b]consists of cranial and caudal parts (measured as a single muscle); [c]subscapularis may also adduct the humerus; [d]measured as a single muscle; [e]common name: m. epitrochlearis; [f]common name: m. extensor indicis; [g]only identified in one animal (data not included in analysis); [h]common name: m. abductor pollicis longus; [i]not indicated to be an elbow flexor.
*Humeral profundus head not previously identified in *M. monax*.

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Table 2. Architectural properties for groundhog forelimb muscles

Muscle	Abbrev.	*N*	Muscle mass (g)	Belly length (cm)	Fascicle length (cm)	Pennation angle (deg)	Volume (cm^3)	PCSA (cm^2)	F_{max} (N)	Power (W)	Fiber architecture
Trapezius pars cervicalis	TC	7	10.7±4.5	7.4±1.2	6.3±1.4	0	10.1	1.6	48.2	2.4	Parallel
Trapezius pars thoracica	TT	8	4.9±1.2	8.5±1.7	6.3±1.5	0	4.6	0.7	22.2	1.1	Parallel
Rhomboideus capitis	RCP	6	5.4±2.4	6.8±1.1	6.8±1.1	0	5.1	0.7	22.4	1.2	Parallel
Rhomboideus cervicis	RCR	6	3.5±1.1	5.1±1.4	4.6±1.5	0	3.3	0.7	21.5	0.8	Parallel
Rhomboideus thoracis	RT	7	2.0±0.6	3.4±0.6	3.1±0.7	0	1.9	0.6	18.5	0.5	Parallel
Latissimus dorsi	LAT	8	18.2±3.3	15.1±2.4	13.7±2.0	0	17.1	1.4	40.5	4.0	Parallel
Pectoralis superficialis	PS	8	16.7±2.9	7.0±1.0	5.9±1.3	0	15.8	2.7	80.3	3.7	Parallel
Pectoralis profundus	PP	8	5.9±2.1	11.6±2.5	10.3±2.7	0	5.6	0.5	16.2	1.3	Parallel
Deltoideus scapularis	DS	8	2.3±0.4	4.5±0.7	2.1±0.5	27±5	2.2	1.0	28.6	0.5	Unipennate
Deltoideus acromialis	DA	8	1.6±0.3	3.4±0.2	2.3±0.5	0	1.5	0.7	20.4	0.4	Parallel
Deltoideus clavicularis	DC	8	2.1±0.8	4.0±0.9	3.4±0.7	0	2.0	0.6	17.6	0.5	Parallel
Teres major	TMJ	8	3.8±0.8	6.2±0.7	2.9±0.7	26±4	3.5	1.1	32.7	0.8	Unipennate
Teres minor	TMN	7	1.5±1.0	5.6±0.7	1.9±1.2	26±4	1.4	0.7	20.1	0.3	Unipennate
Infraspinatus	ISP	8	3.9±1.0	5.5±0.7	1.4±0.4	30±6	3.7	2.3	68.9	0.8	Unipennate
Supraspinatus	SSP	8	7.7±1.6	5.5±0.7	2.3±0.4	29±5	7.2	2.8	83.3	1.5	Bipennate
Subscapularis	SUB	8	7.5±1.9	4.9±0.5	1.5±0.5	30±7	7.1	4.1	123.6	1.5	multipennate
Coracobrachialis	CCB	8	1.3±0.5	5.0±0.4	1.5±0.6	28±4	1.2	0.7	21.3	0.3	Unipennate
Cleidobrachialis	CB	8	3.4±1.1	6.6±0.8	5.4±0.7	0	3.2	0.6	17.9	0.8	Parallel
Biceps brachii	BB	8	2.9±0.4	5.0±0.5	2.2±0.6	25±3	2.7	1.1	33.8	0.6	Unipennate
Brachialis	BCH	8	1.6±0.7	4.9±0.8	1.9±0.8	25±4	1.5	0.7	21.3	0.3	Unipennate
Triceps brachii, long	TBLO	8	13.6±1.7	6.7±0.4	2.3±0.4	30±6	12.9	4.7	141.8	2.6	Unipennate
Triceps brachii, lateral	TBLA	8	8.3±1.6	5.7±0.5	4.4±0.6	0	7.8	1.8	53.9	1.8	Parallel
Triceps brachii, med./acc.	TBMA	8	4.1±1.0	5.6±0.4	4.0±0.7	0	3.8	0.9	28.4	0.9	Parallel
Anconeus	ANC	8	1.0±0.4	3.2±1.3	2.1±1.0	0	0.9	0.4	13.2	0.2	Parallel
Tensor fasciae antebrachii	TFA	8	3.0±0.6	7.5±1.0	6.3±0.9	0	2.8	0.4	13.5	0.7	Parallel
Brachioradialis	BCR	8	2.4±0.5	7.0±0.5	6.0±0.6	0	2.2	0.4	11.2	0.5	Parallel
Pronator teres	PT	8	1.5±0.2	4.8±0.3	1.4±0.4	29±6	1.4	0.9	26.8	0.3	Unipennate
Flexor carpi radialis	FCR	8	1.0±0.2	5.0±0.5	1.5±0.3	24±4	0.9	0.6	16.7	0.2	Bipennate
Flexor carpi ulnaris	FCU	8	1.0±0.2	5.2±0.3	1.8±0.6	25±5	0.9	0.5	14.1	0.2	Unipennate
Flexor dig. sup., epicondylar	FDSE	8	2.8±0.3	5.7±0.5	1.6±0.4	32±7	2.6	1.4	41.0	0.5	Bipennate
Flexor dig. sup., condylar	FDSC	8	3.0±0.5	6.0±0.4	1.2±0.3	29±7	2.8	2.0	60.7	0.6	Bipennate
Flexor dig. prof., medial	FDPHM	8	1.7±0.4	5.1±0.7	1.7±0.3	24±4	1.6	0.9	26.1	0.3	Bipennate
Flexor dig. prof., profundus	FDPHP	8	0.4±0.1	4.2±0.6	2.9±0.6	0	0.4	0.1	4.0	0.1	Parallel
Flexor dig. prof., radial	FDPR	8	1.2±0.3	4.4±0.5	1.7±0.6	25±5	1.1	0.6	18.2	0.2	Unipennate
Flexor dig. prof., ulnar	FDPU	8	1.6±0.3	5.4±0.5	1.7±0.5	24±5	1.5	0.8	23.2	0.3	Unipennate
Extensor carpi rad., longus	ECRL	8	1.4±0.4	5.6±0.6	3.3±1.1	23±3	1.3	0.4	10.9	0.3	Unipennate
Extensor carpi rad., brevis	ECRB	8	1.4±0.2	5.4±0.5	4.2±0.6	0	1.3	0.3	9.3	0.3	Parallel
Extensor carpi ulnaris	ECU	8	1.3±0.3	5.4±0.6	1.3±0.3	25±5	1.2	0.9	26.1	0.3	Bipennate
Extensor dig. communis	EDC	8	1.2±0.2	6.1±0.4	1.4±0.3	29±5	1.1	0.7	20.4	0.2	Unipennate
Extensor dig. lateralis	EDL	8	0.7±0.1	5.6±0.4	1.3±0.3	24±5	0.6	0.4	13.1	0.1	Unipennate
Extensor digiti II	ED2	5	0.3±0.2	3.9±0.8	1.4±1.1	21±5	0.3	0.2	6.1	0.1	Unipennate
Abductor digiti I longus	ADL	7	0.9±0.3	4.6±0.8	1.2±0.4	22±4	0.9	0.7	20.2	0.2	Unipennate
Pronator quadratus	PQ	8	0.1±0.02	1.2±0.2	0.8±0.1	0	0.1	0.1	2.8	0.02	Parallel
Supinator	SUP	8	0.5±0.3	3.9±0.7	0.8±0.3	28±6	0.5	0.5	16.4	0.1	Unipennate

Data are means ± s.d. acc., accessory; dig., digitorum; med., medial; prof., profundus; rad., radialis; sup., superficialis.

most massive functional muscle groups of the digging apparatus. Of these muscle groups, the latissimus dorsi (LAT) and pectoralis superficialis (PS) are the two largest muscles of the forelimb, and together, they account for 10.6% of total forelimb muscle mass. The triceps brachii long head (TBLO) is also large, and combined with the lateral and medial/accessory heads, the triceps brachii accounts for 5.2% of total forelimb muscle mass.

The distribution of muscle group mass relative to total forelimb muscle mass is shown in Fig. 1. Muscles with synergistic functions are combined into one functional group, and muscles with multiple actions (e.g. pectoralis) and biarticular muscles (e.g. TBLO) are included in more than one functional group. Notably, the largest functional group is the limb retractors, which account for 47.2±1.6% (mean ± s.d.) of total forelimb muscle mass (Fig. 1). The second and third largest functional groups are the limb protractors and elbow extensors, respectively, which account for 25.7±1.7% and 18.0±0.5% of the forelimb muscle mass. Along the antebrachium, the digital flexors are a relatively large functional group and account for 6.5±0.5% of the forelimb muscle mass, whereas the carpal flexors and pronators are much smaller and have masses that each account for ~1% of total forelimb muscle mass (Fig. 1).

Muscle architectural properties

Extrinsic muscles acting on either the scapula or humerus all have long fascicles arranged in a parallel fiber architecture, whereas the intrinsic muscles generally become progressively more pennate along the length of the groundhog forelimb. The muscles with the longest fascicles are two of the main limb retractors, LAT (13.7±2.0 cm) and pectoralis profundus (PP: 10.3±2.7 cm) (Table 2). Other muscles spanning the shoulder joint, including PS and cleidobrachialis, and several elbow extensor muscles (e.g. lateral and medial heads of the triceps brachii) also have relatively long fascicles, each with a mean fascicle length greater than 4 cm. With

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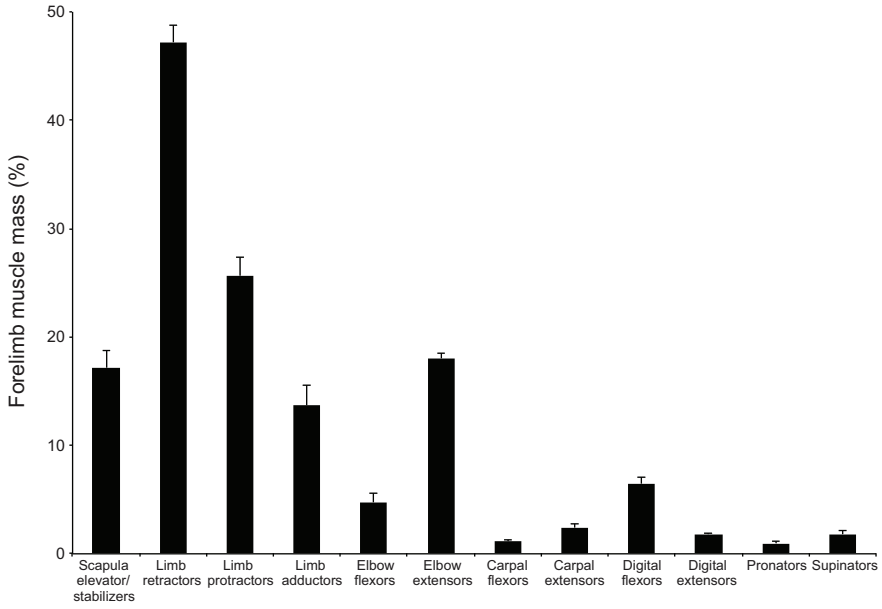

Fig. 1. Architectural index of the distribution of functional group muscle mass to total forelimb muscle mass in the groundhog forelimb. Total forelimb muscle mass was calculated as the summed mass of all individual muscles studied. Proximal-to-distal muscle group mass is expressed as a percentage, with bars representing means for each functional group. Error bars represent the s.d. Muscles with synergistic functions are combined in one functional group. Biarticular muscles are also included in more than one functional group.

the exception of brachioradialis, flexor digitorum profundus-humeral profundus (FDPHP), and both heads of the extensor carpi radialis, the remainder of the muscles of the brachium and antebrachium have relatively short fascicles 2.3 cm or less in length. The flexor digitorum superficialis condylar head (FDSC: 1.2±0.3 cm) is among the muscles with the shortest mean fascicle lengths (Table 2).

Ratios of fascicle length (l^F) to muscle length (ML) are shown in Fig. 2, where higher values indicate greater range of contraction and fascicle shortening capability. Nearly half of the muscles of the forelimb have an l^F/ML ratio of 0.6 or greater. There is a consistent pattern among some functional muscle groups, for example, the scapular elevator/stabilizers, which all have very high l^F/ML ratios.

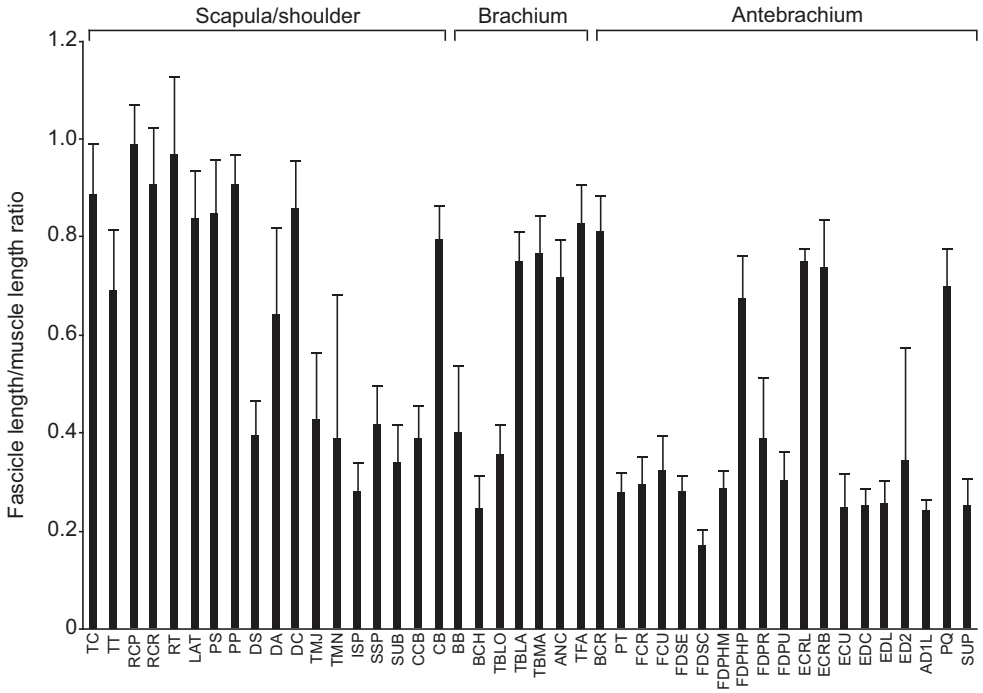

Fig. 2. Fascicle length (l^F) to muscle length (ML) ratios of groundhog forelimb muscles. High mean values indicate greater range of contraction and greater shortening capability. Muscle abbreviations (as listed in Table 2): TC, trapezius pars cervicalis; TT, trapezius pars thoracica; RCP, rhomboideus captis; RCR, rhomboideus cervicis; RT, rhomboideus thoracis; LAT, latissimus dorsi; PS, pectoralis superficialis; PP, pectoralis profundus; DS, deltoideus scapularis; DA, deltoideus acromialis; DC, deltoideus clavicularis; TMJ, teres major; TMN, teres minor; ISP, infraspinatus; SSP, supraspinatus; SUB, subscapularis; CCB, coracobrachialis; CB, cleidobrachialis; BB, biceps brachii; BCH, brachialis; TBLO, triceps brachii-long; TBLA, triceps brachii-lateral; TBMA, triceps brachii-medial/accessory; ANC, anconeus; TFA, tensor fasciae antebrachii; BCR, brachioradialis; PT, pronator teres; FCR, flexor carpi radialis; FCU, flexor carpi ulnaris; FDSE, flexor digitorum superficialis-epicondylar; FDSC, flexor digitorum superficialis-condylar; FDPHM, flexor digitorum profundus-humeral medial; FDPHP, flexor digitorum profundus-humeral profundus; FDPR, flexor digitorum profundus radial; FDPR, flexor digitorum profundus ulnar; ECRL, extensor carpi radialis-longus; ECRL, extensor carpi radialis-brevis; ECU, extensor carpi ulnaris; EDC, extensor digitorum communis; EDL, extensor digitorum lateralis; ED2, extensor digiti II; AD1L, abductor digiti I longus; PQ, pronator quadratus; SUP, supinator. Values are means ± s.d.

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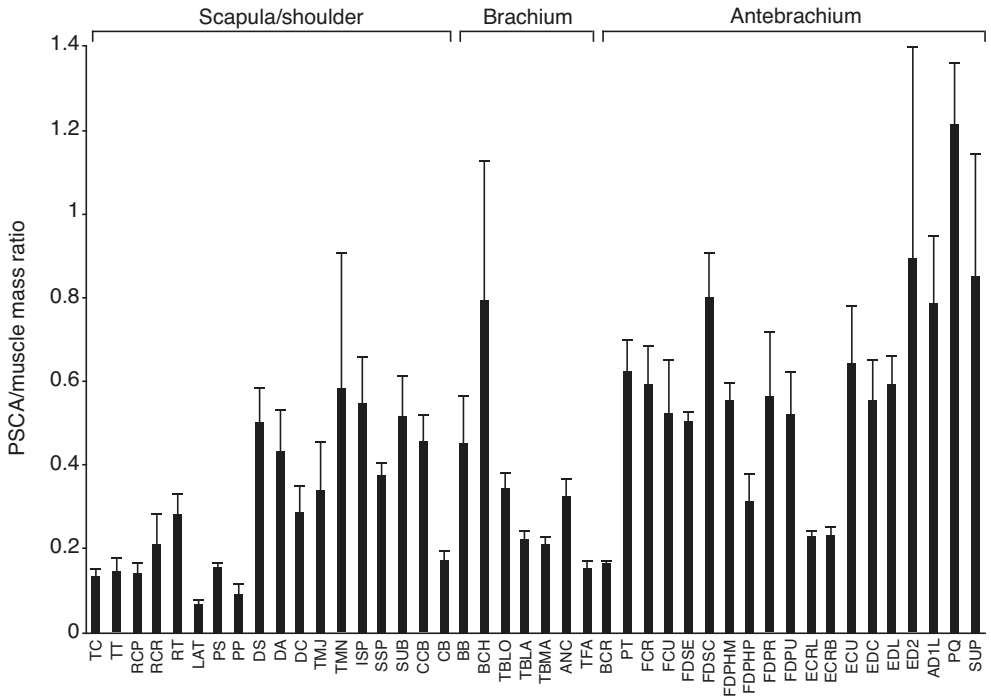

Fig. 3. Physiological cross-sectional area (PCSA) to muscle mass (MM) ratios of groundhog forelimb muscles. High mean values indicate either higher degrees of pennation and force production capability. The combination of both higher PCSA/MM and l^F/ML ratios (see Fig. 2) indicates that a muscle is capable of performing appreciable muscle work. Abbreviations are the same as in Fig. 2. Values are means ± s.d.

The rhomboideus captis has the single highest ratio of all muscles with a mean of 0.99±0.1 (Fig. 2). Of the limb retractors, LAT, deltoideus clavicularis and both heads of the pectoralis each have a high l^F/ML ratio exceeding 0.8. Except for the unipennate TBLO, which has a relatively low l^F/ML ratio, the elbow extensors as a functional group have also have high ratios ranging between 0.72 and 0.83. In general, muscles of the antebrachium are pennate and are calculated to have ratios less than 0.35, with the bipennate FDSC having the lowest ratio of all muscles with a mean of 0.17±0.03 (Fig. 2).

On average, resting pennation angles (θ) range from 0−32°, with many muscles displaying unipennate fiber architecture. Muscles with the highest mean pennation angles are the bipennate flexor digitorum superficialis epicondylar head (FDSE: 32±7 deg) and the multipennate subscapularis (SUB: 31±7 deg) (Table 2). A number of unipennate muscles, including the deltoideus scapular is, teres major, infraspinatus and TBLO, all have mean pennation angles greater than 25°. Corresponding with their relatively high values of θ and short fascicles, the two muscles with the highest PCSA are the SUB and TBLO (Table 2). Additional muscles functionally grouped as limb retractors have modest PCSA (~2.5 cm^2), whereas all other muscles have relatively low PCSA with values ranging from 0.2 to 2.0 cm^2.

Ratios of PCSA to muscle mass (MM) (or size-adjusted PCSA) are shown in Fig. 3, where higher values indicate greater force production capability. The digital extensors, pronator quadratus and supinator have low mass, and correspondingly, have the highest PCSA/MM ratios. The FDSC and brachialis also have high ratios of ~0.8 (Fig. 3). In contrast, the major muscles that act to retract the forelimb and those that extend the elbow joint have the lowest PCSA/MM ratios (range: 0.07−0.35). Despite its relatively low mean PCSA/MM ratio of 0.35±0.04, the massive TBLO has the highest estimated isometric $\mathbf{F}_{max}$ of 141.8 N (Table 2). The intrinsic shoulder muscles and carpal/digital flexors show intermediate PCSA/MM ratios, generally ranging between 0.3 and 0.6 (Fig. 3). Among these muscle groups, only the SUB (123.6 N) and supraspinatus (83.3 N) have relatively high estimates of $\mathbf{F}_{max}$, whereas no other single muscle in the entire forelimb is estimated to produce greater than 80 N of isometric force (Table 2). Fig. 4 shows the estimated summed total isometric force each functional muscle group is capable of producing. The shoulder joint flexors have an average summed isometric $\mathbf{F}_{max}$ of nearly 500 N, which is twice as large as the total force of both the shoulder extensors and elbow extensors. The elbow extensors have a mean summed isometric $\mathbf{F}_{max}$ that is nearly three times greater than the elbow flexors, and this similar to the comparison between the digital flexors and digital extensors. The carpal flexors and extensors have the lowest summed isometric $\mathbf{F}_{max}$ values of all functional groups (Fig. 4).

Muscles with both relatively high force and shortening capabilities indicate higher work and power capacity. As shown in Fig. 5, no muscles of the groundhog forelimb are capable of high power output. The muscles with the highest individual estimates of instantaneous power are the LAT (4.0 W), PS (3.7 W), TBLO (2.6 W) and trapezius cervicis (2.4 W), and these are the same muscles that have the highest masses and volumes (Table 2). As a functional group, the elbow extensors have appreciable power capacity (6.2 W), while the elbow flexors are capable of generating low power (1.7 W). The carpal and digital flexor muscles have a modest combined power of 2.4 W (Table 2).

Last, few muscles of the groundhog forelimb have appreciable muscle moment arms (r_m) and estimated joint torques (Table 3). Despite having a longer mean r_m (2.8±0.6 cm) at the shoulder joint, the PS has a lower joint torque (223 N cm) than the TBLO, which has the highest estimated joint torque of 263 N cm. All other limb retractor muscles have relatively little ability to apply a flexor torque at the shoulder joint. Except for the LAT, which has a modest joint torque value, muscles with the lowest estimated joint torque generally have the highest l^F/r_m ratios (Table 3). At the elbow joint, again the massive, unipennate TBLO is estimated to be able to apply a high joint torque of 236 N cm, whereas the remaining elbow extensors have considerably lower values of estimated joint torque and much greater ability to move the elbow joint through a large range of motion. Surprisingly, the FDS (both heads) and FDP (all heads combined with a common tendon of insertion) each have

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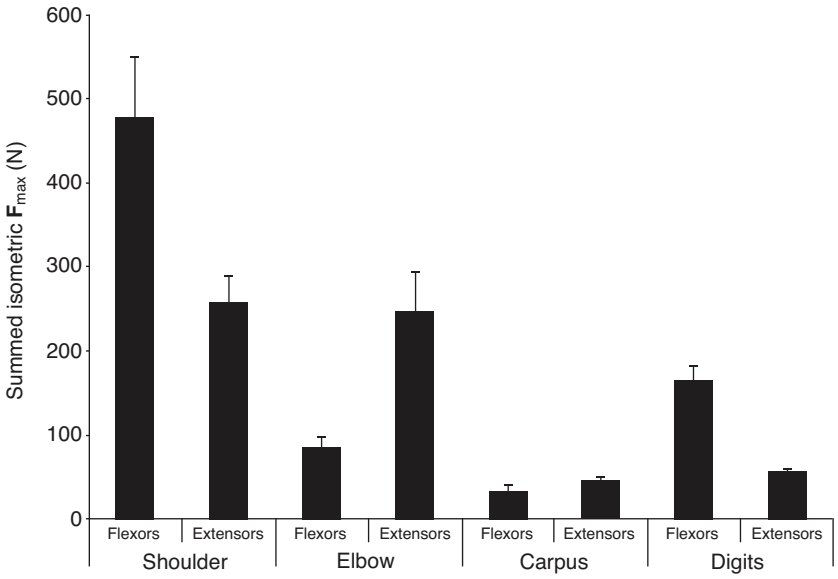

Fig. 4. Mean summed isometric force (F_{max}) across the functional muscle groups in the groundhog forelimb. The functional muscle groups are subdivided by their actions at each limb joint or segment and include the shoulder flexors (*N*=10 muscles), shoulder extensors (*N*=4 muscles), elbow flexors (*N*=3 muscles), elbow extensors (*N*=5 muscles), carpal flexors (*N*=2 muscles), carpal extensors (*N*=3 muscles), digital flexors (*N*=6 muscles) and digital extensors (*N*=5 muscles). Values are means ± s.d.

relatively low estimated values of joint torque at the carpus that collectively do not exceed a total of 100 N cm (Table 3).

MHC isoform composition

Forelimb muscles showed expression of three MHC isoform bands: MHC-1, MHC-2A and MHC-2X. Slow MHC-1 and fast MHC-2A bands were clearly resolved in all muscles from each individual; however, the fast MHC-2X isoform was not expressed in all muscles sampled from the groundhog (Table 4). Across all muscles studied, MHC-2A was the predominant isoform expressed and the relative mean percentage of this isoform was fairly consistent (range: 63–80%) along the forelimb (Table 4). The limb retractors are composed of nearly equal percentages of the MHC-1 and MHC-2X isoforms (Fig. 6). The elbow extensors have an overall faster MHC isoform composition than that of the limb retractors with a mean of 20.7±2.6% for fast MHC-2X isoform, which is twice the mean for slow MHC-1 in these muscles. Finally, MHC isoform composition for the carpal/digital flexors shows a trend of increasing slower-contracting fibers in the distal forelimb by the lack of expression of the fast MHC-2X isoform (Table 4; Fig. 6).

DISCUSSION

The relationship between muscle architectural properties and the observed scratch-digging habits of mammals is not well established. Building on our previous study of the American badger (Moore et al., 2013), we evaluated internal muscle properties in the forelimb of a generalized burrower to distinguish muscle traits (e.g. muscle mass, fascicle length and MHC expression) that indicate fossorial specialization from basic traits common to mammals that have some digging ability. A large investment of mass in shoulder muscles suggests the importance of limb retraction for scratch-digging in groundhogs. In particular, the massive extrinsic muscles (e.g. LAT, PS and PP) have a high capacity to shorten and a low capacity for force production because of their long, parallel fascicles, and this reflects an ability to retract the forelimb through a large range of motion during the power stroke. With the exception of the deltoideus clavicularis, the intrinsic shoulder muscles have moderate shortening and force capacity (AI ratios: 0.3–0.6) indicating the ability to appreciably supplement work and power at the shoulder joint for burrowing. However, the architectural properties of the intrinsic limb retractors (e.g. infraspinatus, ISP) and protractors (e.g.

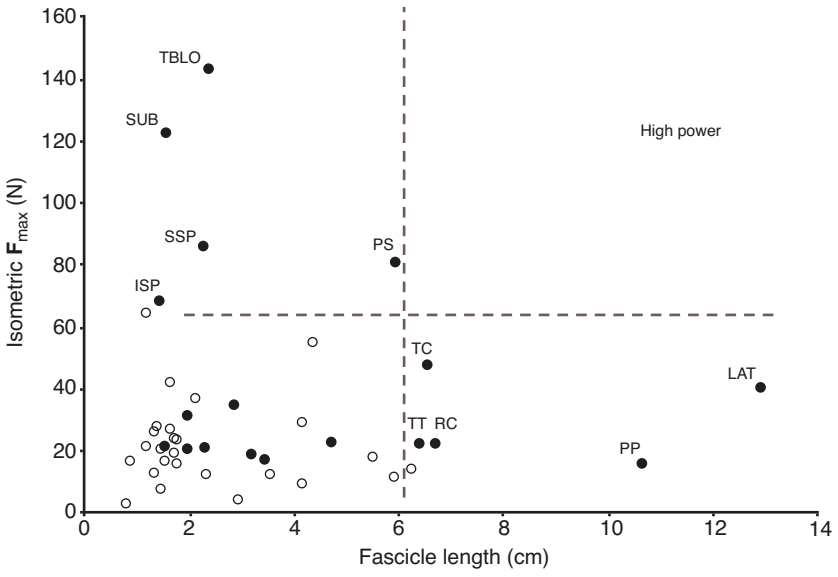

Fig. 5. Estimated muscle F_{max} as a function of resting fascicle length. Filled data points represent proximal limb muscles and open data points represent distal muscles. Dashed horizontal line separates muscles with high (above) versus low (below) force capability, while the dashed vertical line separates muscles with high (right) versus low (left) shortening capability. Only muscles with relatively high force and or fascicle length are labeled. Muscles that both produce high force and shorten substantially (at high velocity) are capable of high power output (upper right quadrant). Abbreviations are the same as in Fig. 2.

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Table 3. Muscle moment arms (r_m), joint torques and architectural indices for groundhog forelimb muscles

Joint	Muscle	Mean r_m (cm)	Joint torque (N cm)	l^F/r_m	l^F/ML
Shoulder	Latissimus dorsi	1.8±0.5	74.5	6.90	0.84
	Pectoralis superficialis	2.8±0.6	223	2.12	0.84
	Pectoralis profundus	1.2±0.3	18.7	8.92	0.89
	Deltoideus scapularis	1.3±0.4	37.6	1.56	0.45
	Deltoideus acromialis	0.7±0.2	14.6	3.17	0.66
	Deltoideus clavicularis	1.5±0.4	25.8	2.32	0.86
	Teres major	1.6±0.3	53.6	1.79	0.47
	Teres minor	1.0±0.4	19.9	1.91	0.34
	Infraspinatus	1.0±0.3	68.1	1.41	0.25
	Supraspinatus	0.9±0.3	79.1	2.40	0.42
	Subscapularis	0.8±0.2	93.6	1.98	0.30
	Triceps brachii, long	1.9±0.5	263	1.26	0.35
Elbow	Cleidobrachialis	1.5±0.4	26.6	3.67	0.82
	Biceps brachii	1.2±0.3	42.2	1.76	0.44
	Brachialis	1.1±0.2	22.5	1.78	0.38
	Triceps brachii, long	1.7±0.4	236	1.41	0.35
	Triceps brachii, lateral	1.2±0.5	67.3	3.49	0.76
	Triceps brachii, medial/accessory	1.1±0.2	32.1	3.57	0.72
	Anconeus	0.8±0.3	10.6	2.63	0.65
	Tensor fasciae antebrachii	1.3±0.3	17.1	4.94	0.84
Carpal	Flexor carpi radialis	0.9±0.2	14.5	1.76	0.31
	Flexor carpi ulnaris	0.8±0.1	11.7	2.12	0.34
	Flexor digitorum superficialis, epicondylar	1.1±0.3	45.9	1.46	0.28
	Flexor digitorum superficialis, condylar	0.7±0.2	42.8	1.71	0.20
	Flexor digitorum profundus, humeral medial	0.5±0.2	14.1	3.05	0.33
	Flexor digitorum profundus, humeral profundus	0.7±0.3	2.6	4.35	0.69
	Flexor digitorum profundus, radial	0.5±0.1	9.7	3.16	0.38
	Flexor digitorum profundus, ulnar	0.6±0.1	14.6	2.75	0.32

Data are means ± s.d. l^F, mean fascicle length; r_m, mean moment arm; ML, muscle belly length. l^F/r_m ratios>2.0 indicate a high ability of the muscle to move a joint through a large range of motion. l^F/ML ratios>0.5 indicate a high ability of the muscle to shorten and contract at appreciable velocity.

subscapularis, SUB) also indicate roles in shoulder joint stabilization. On average, no muscles acting at the shoulder joint (or on the scapula) have a high isometric $\mathbf{F}_{max}$, and numerous muscles have the capability to shorten at moderate velocity based on both their long fascicle length and high percentages of the fast MHC-2A isoform. Correspondingly, all muscles of the groundhog forelimb are capable of generating only moderate-to-low power, as we hypothesized. By a comparison of mass-normalized values, power capacity of badgers (Moore et al., 2013) exceeds that of the same muscles in groundhogs, and yet no badger forelimb muscle is capable of markedly high power output as estimated for some hindlimb muscles of cursorial mammals (Williams et al., 2007a; Williams et al., 2008). In addition to digging shallow burrows for shelter, American badgers actively hunt ground-dwelling rodents by rapid excavation of their burrows (Michener, 2004), whereas as groundhogs may burrow at a slower rate to dig deeper, more-complex burrow systems. Therefore, differences in digging strategy may reflect selection for differences in muscle power capacity and fossorial ability between these two scratch-digging species.

Table 4. Mean percentage MHC isoform composition in selected groundhog forelimb muscles

		MHC isoform (%)		
Muscle	*N*	MHC-1	MHC-2A	MHC-2X
Latissimus dorsi	4	19.1±7.4	65.5±4.6	15.4±5.1
Pectoralis superficialis	4	18.3±4.2	62.6±6.4	19.1±4.3
Deltoideus acromialis	4	20.3±11.8	79.7±11.9	0.0
Teres major	4	13.9±4.9	63.2±5.4	22.9±6.0
Biceps brachii	4	9.7±5.6	69.7±5.1	20.6±3.3
Triceps brachii long	4	9.9±6.6	67.5±6.1	22.6±3.3
Triceps brachii lateral	4	8.5±2.1	72.7±5.2	18.8±3.4
Flexor carpi ulnaris	4	28.2±4.9	71.8±4.9	0.0
Flexor dig. sup., epicondylar	4	35.1±4.6	64.9±4.6	0.0
Flexor dig. prof., medial	4	21.1±5.0	78.9±5.0	0.0

All data are means ± s.d. Means for each muscle were computed from three independent gel experiments per individual. dig., digitalis; prof., profundus; sup., superficialis.

Muscles with long fascicles and high mass also depend on fast MHC isoforms to be powerful. The LAT and PS have the highest values of instantaneous power (~4.0 W) and each muscle is similar in its composition of MHC-1, MHC-2A and MHC-2X. The expression of the 2X isoform in the LAT and PS suggests moderate glycolytic properties for power to retract (or force to support) the limb during digging or terrestrial locomotion. Assuming the presence of MHC-2X and the lack of MHC-2B, our isoform composition for the TMJ is similar to the 'white' and fast glycolytic fiber distributions previously reported for this muscle in ground squirrels (Goldstein, 1971) and tuco-tucos (Alvarez et al., 2012), respectively. Also consistent among scratch-digging rodents is a heterogeneous distribution of fiber types in shoulder and elbow joint muscles, and an overall prevalence either slow or fast oxidative fibers, as predicted. For example, high percentages of FOG fibers in tuco-tucos match well with a primary composition of MHC-2A in all the forelimb muscles of groundhogs that were studied. MHC-2A fibers are highly oxidative and recruited for sustained force and power (Rupert et al., 2014), and although the digging habits and locomotor mechanics of groundhogs are largely unknown, these properties seem appropriate for progressive burrowing. Moreover, fast MHC-2B was not found as expected, and this is consistent with the high metabolic demands of burrowing requiring sustained activity and fatigue resistance. Additional analyses are needed to specifically assess whether a similar composition of fast MHC

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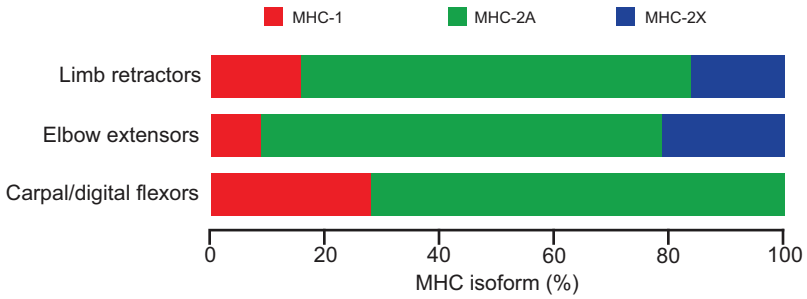

Fig. 6. Myosin heavy chain (MHC) isoform composition in groundhog forelimb muscles. Mean percentage composition of MHC isoforms for the major functional muscle groups associated with scratch digging: limb retractors, elbow extensors and carpal/digital flexors.

isoforms is present in homologous forelimb muscles of other scratch-diggers and whether this is consistently related to a given level of fossorial ability.

Although kinematic data do not exist for groundhogs during burrowing, simultaneous retraction of the limb and extension of the elbow joint occurs at the outset of the power stroke in scratch-diggers (Stalheim-Smith, 1984; Moore et al., 2013). At the shoulder joint, the unipennate TBLO can apply the largest torque (flexor moment) of any muscle studied because of its relatively long moment arm and large PCSA, and thus is hypothesized to function synergistically as a limb retractor. In addition, an equally long moment arm at the elbow joint allows the biarticular TBLO the capacity to apply a similarly large amount of joint torque. These findings are similar to those in the badger where it was estimated that the TBLO could apply the highest shoulder flexor and elbow extensor moments (Moore et al., 2013). However, the somewhat low l^{F}/r_{m} ratios of this muscle at both joints suggest that its role in full rotation of the limb segments may be limited. Therefore, the TBLO might act to stabilize each joint against substrate reaction forces during the power stroke in these two species, but this functional interpretation will need to be verified by *in vivo* measurements of fascicle contractile behavior. In either case, relatively high $\mathbf{F}_{max}$, torque and power properties of the TBLO in particular, may indicate muscle specialization for scratch-digging. Our future investigations of internal architectural properties in the forelimbs of highly fossorial scratch-digging mammals will help to clarify adaptive traits.

Elbow extension throughout the power stroke is also important, and the elbow extensors of the groundhog account for relatively large portion of its total forelimb muscle mass. Specifically, this feature is consistent across scratch-digging rodents for which relative muscle mass has been quantified (Lehmann, 1963; Gambaryan and Gasc, 1993). In addition, the total PCSA of the triceps brachii of groundhogs is in similar high proportion to that measured in the forelimbs of European ground squirrels (Lagaria and Youlatos, 2006), reflecting the importance of force in this functional muscle group to burrowing rodents. Given that the large PS and PP are limb adductors and this action also occurs throughout the power stroke, it is noteworthy to observe that muscles involved in adduction account for less total forelimb muscle mass than the elbow extensors. Adding to the mass of the elbow extensors, the accessory head of the triceps brachii is fused with the medial head, and as a whole muscle, has long, parallel fascicles that provide it with high shortening capability, but low force-production ability. Having similar properties, the lateral and medial/accessory heads of the triceps are best suited to actively extend the elbow joint throughout the power stroke to enable the forelimbs to move soil to the hindlimbs. The modest power of the lateral head (~2.0 W) in addition to a nearly 20% composition of the fast MHC-2X isoform indicates its capacity for appreciable shortening and extending of the elbow joint during the power stroke. However, the relatively low joint torque of lateral and medial/accessory heads, may also suggest a role in elbow joint stabilization during slow terrestrial locomotion. Interestingly, the TBL and TBLO have nearly identical MHC isoform compositions, which may suggest that these muscles perform the synergistic function of elbow extension.

As observed in other scratch-diggers, the digital flexor muscles are relatively massive in groundhogs, and account for the highest percentage of muscle mass in the antebrachium. The difference in mass between the digital and carpal flexors reflects the importance of strong digital flexion for scratch-digging (Hildebrand, 1985). This may be especially true for groundhogs that have short claws. The FDP is a relatively large muscle with four heads and a range of fiber architectures, whereas the FDS has two bipennate heads that combined are more massive than the FDP. With the exception of a small FDP humeral profundus (FDPHP) [also observed in the hare (Williams et al., 2007b), which has high fascicle-shortening capability, the digital flexors as a muscle complex almost uniformly have the functional properties to perform appreciable mechanical work. It is expected that work done to flex the digits would not only maintain the digits in a flexed position throughout the power stroke, but also augment the total force applied to the substrate by exerting moderate joint torque at the carpal, metacarpophalangeal and interphalangeal joints. Overall, the muscle architecture of the digital flexors is as hypothesized, but this muscle group in the groundhog is not as functionally compartmentalized compared with that of the badger (Moore et al., 2013). Both relatively high $\mathbf{F}_{max}$ and long moment arm at the carpal joint indicate that the FDS is mechanically well-suited for flexion of the carpus, which is additionally important for scratch-digging. The flexor carpi radialis (FCR) and flexor carpi ulnaris (FCU), however, have a low combined muscle mass and relatively low force production, power and joint torque capability, suggesting that they are less well-suited for strong carpal joint flexion during the power stroke.

Despite the differences in muscle architectural properties between the carpal and digital flexors, these muscles equally do not express the fast MHC-2X isoform. This result somewhat contradicts our hypothesis and instead emphasizes lower force and power, but higher fatigue resistance in the these functional muscle groups. A generalized burrower that uses its forelimbs for additional functional behaviors including terrestrial locomotion and food manipulation was expected to have a heterogeneous composition of MHC-1, MHC-2A and MHC-2X throughout the forelimb musculature. Although published data for the carpal/digital flexors of other digging rodents are not available for comparison, an expression of only MHC-1 and MHC-2A may be related to the potential use of a carpal-only (via carpal flexion) mode of scratch-digging in groundhogs. The combined architecture and MHC isoform properties of the carpal/digital flexors are well suited for this method of digging. Detailed biomechanical evaluations are needed to understand if the internal muscle properties observed in distal forelimb of groundhogs are modifications for enhanced carpal flexion digging.

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Comparative and functional insights

To place the muscle traits observed in the groundhog into a proper evolutionary context, morphological comparisons with the forelimbs of mammals specialized for behaviors other than scratch-digging are also necessary to evaluate traits for fossoriality. Quantitative evaluations of limb muscle architecture and fiber type have mainly focused on cursorial adaptations (e.g. Alexander, 1984; Payne et al., 2005; Toniolo et al., 2007; Williams et al., 2007a); however, functional insights can be gained by interpretation of available muscle data in mammals that climb – a locomotor behavior that shows a number of morphological trade-offs with fossorial habit (Stalheim-Smith, 1984; Stalheim-Smith, 1989; Rose et al., 2014).

Climbing mammals have relatively less intrinsic muscle mass for elbow extension and digital flexion (Gambaryan, 1974; Taylor, 1978; Moore, 2011), and variation in relative extrinsic muscle mass is largely explained by the absence of muscles. For example, the rhomboideus capitis (and profundus) is commonly absent (Fisher et al., 2009), which may indicate less ability to protract the limb and stabilize the scapula cranially. Climbers invest in large pectoralis muscles as do scratch-diggers, but they may show relatively greater division of the pectoralis superficialis and profundus (Harrison, 1882; Julik et al., 2012) for strong adduction and increased grasping control of the forelimb during climbing. A broad caudal origin of the LAT is also generally similar between climbers and scratch-diggers, indicating that long fascicles for shortening and power output are important to both habits. However, there is evidence that some climbing mammals also have a broad and distal insertion of the teres major on the humerus (Taylor, 1978), thus increasing its r_m and ability to apply a large flexor moment at the shoulder joint. We previously found the size-specific mass of the teres major in the opossum to be significantly higher than that of the badger (Moore, 2011), and this may be an alternative strategy to increase the applied flexor moment in more generalized climbers. Moreover, climbing mammals often have both an articularis humeri and a well-developed coracobrachialis (Fisher et al., 2009), indicating the need for shoulder joint stability during arboreal maneuvering, and additionally, emphasizing the importance of limb adduction.

Aside from lower muscle mass, mammals may show modifications to muscle origins and the number of heads of the triceps brachii. A long head originating on the scapula is the typical mammalian condition and is a feature consistent among climbers (Stalheim-Smith, 1984; Thorington et al., 1997; Fisher et al., 2009). However, the presence of additional scapular heads of the triceps as observed in badgers (Moore et al., 2013), skunks (Ercoli et al., 2014) and armadillos (Windle and Parsons, 1899) is not a feature observed in climbers, although the origin of the long head on the scapula may be broad (Harrison, 1882). Carnivores that climb often have a second accessory head associated with the medial head (Fisher et al., 2009; Julik et al., 2012). Multiple accessory heads suggest greater joint position control for precise movements on narrow substrates, whereas two biarticular heads of the triceps can substantially increase of the flexor moment applied at the shoulder joint for retraction of the forelimb to excavate earth (Moore et al., 2013). The lack of each of these modifications in the groundhog is consistent with its classification as a less-specialized burrower.

In contrast to the elbow extensors, climbing mammals have relatively more well-developed elbow flexors than scratch-diggers. Significantly larger flexor mass can help provide the propulsion to move up a vertical substrate (Moore, 2011) and large joint torques applied by the biceps brachii and brachialis have been shown to distinguish elbow flexor function between climbers and scratch-diggers (Stalheim-Smith, 1984). Indeed, a size-specific value of $0.14\ \mathrm{N\ mm\ g^{-1}}$ calculated for the combined joint torque for the elbow flexors of both groundhogs and badgers is low compared with an average value of $0.40\ \mathrm{N\ mm\ g^{-1}}$ reported for scansorial fox squirrels and raccoons (Stalheim-Smith, 1989). The elbow flexors in scratch-diggers may therefore play a role in counterbalancing large elbow extensor torques (Moore et al., 2013), as opposed to initiating limb recovery (via elbow flexion) at the end of the power stroke. Perhaps it is for this function that groundhogs and other sciurids have a separate cleidobrachialis that inserts on the ulna (Thorington et al., 1997), instead of the humeral insertion observed in climbing mammals (Harrison, 1882; Fisher et al., 2009). In addition, the origin of both the brachioradialis and ECR is shifted more proximally on the humerus in some climbers, thus increasing their r_m at the elbow joint and their ability to augment elbow flexor torque. Available data indicate these two muscles are relatively more massive in tamanduas (Taylor, 1978) versus groundhogs and they also may be compartmentalized with a range of fascicle lengths, indicating specialization for elbow joint rotation. For example, the l^F/r_m ratios of the ECR in raccoons is >2.0 (McClearn, 1985) and these data relate to their marked ability to rotate the elbow joint in flexion.

The carpal and digital flexors show marked differences between climbing and scratch-digging mammals. Significantly less mass is dedicated to the carpal/digital flexors compared with the digital extensors (Moore, 2011), and this reflects overall lower force of these functional muscle groups in climbers. Correspondingly, the observed variation in muscle origins, number of muscle bellies (and their mass) and fiber architecture is most likely related to additional dexterity of the digits for grasping in arboreal locomotion. For example, climbers often have a fleshy origin of the FDS from the FDP instead of a strong attachment to the humerus (McClearn, 1985; Fisher et al., 2009). The number of heads of the profundus and the arrangement of the flexor tendons serving the digits also show a wide range of variation. Whereas the groundhog has only four heads of profundus with tendons to digits II–V, climbing mammals have five heads with tendons serving all five digits (McClearn, 1985; Julik et al., 2012). However, carpal/digital flexors with considerable pennation and shorter fascicles is a feature consistent across climbing and digging taxa studied (McClearn, 1985; Julik et al., 2012; Moore et al., 2013), although the ability to move the carpus and digits through a large range of motion may be greater in climbers relating to their enhanced dexterity. In addition, the FCU of the opossum was found to have significantly more mass and PCSA than that of the badger (Moore, 2011). This finding may reflect the importance of carpal abduction when climbing vertical substrates. Despite having potentially shorter r_m lengths at the carpus than diggers, the insertion of the FCU in some climbers extends to the base of metacarpal V (Harrison, 1882), which increases its ability to abduct the carpal joint.

Finally, the lack of studies that have identified MHC expression in the limbs of climbing and digging adapted mammals make comparative interpretations difficult. In general, available data indicate that the forelimb muscles of climbers are faster-contracting and fatigue more easily than those of scratch-digging mammals (Stalheim-Smith, 1984). The predominance of MHC-2A in groundhog forelimbs is interesting with respect to previous findings of large distributions of fast type II fibers in scansorial mammals (Hansen et al., 1987) and studies of MHC isoforms in other species of squirrels (Rourke et al., 2004; Reiser et al., 2009) that did not identify the 2A isoform. Our recent analyses (unpublished data) of MHC isoforms in both tree (*Tamiasciurus hudsonicus*) and ground (*Spermophilus lateralis*) squirrels confirm a primary composition

of MHC-2X and MHC-2B isoforms as the fast Type II fibers in selected forelimb muscles of both species. MHC-2A is expressed in the carpal/digital flexors of ground squirrels, suggesting lower sustained force and power properties in the antebrachial muscles of species that share a similar lifestyle and digital manipulation abilities (Nowak, 1999). The lack of expression of MHC-2A in a tree squirrel may also be reflective of its arboreal lifestyle. Ascending trees is a rapid locomotor behavior in sciurids compared with burrowing, thus fast-contracting MHC-2B fibers match the high power requirements for climbing. A last consideration for the differences in MHC expression among diverse genera of squirrels is body size. The general lack of expression of the fast MHC-2X and MHC-2B isoforms in groundhogs may be due to their larger body mass. *T. hudsonicus* and *S. lateralis* are much smaller (200–400 g), thus a large composition of fast MHC isoforms in their skeletal muscles is important for thermoregulation and is consistent with an inverse relationship between MHC shortening velocity and body size (Toniolo et al., 2007). Nonetheless, the disproportionately high MHC-2A content of groundhog muscles is difficult to reconcile for its size and rodent phylogenetic ancestry. It is possible that variation in MHC expression may have evolved as a way to modify muscle structure and function for the different lifestyles of squirrels.

In conclusion, the groundhog forelimb has the following five features purportedly related to their degree of fossorial ability: (1) humeral retractors, elbow extensors and digital flexors account for a majority of forelimb muscle mass; (2) LAT and PS have long fascicles and are capable of the highest power; (3) pennate triceps brachii long head that has large PCSA and is capable of the highest joint torque at the shoulder and elbow joints; (4) pennate digital flexors capable of appreciable mechanical work to flex the carpus and digits; and (5) primary expression of the MHC-2A isoform in major forelimb muscles associated with scratch-digging function. Overall, the forelimb musculature is capable of relatively low force and power and has limited ability to apply high joint torque at the shoulder and elbow joints, which are consistent with its behavioral classification as a less-specialized burrower. Modification for scratch-digging is most evident in the distal forelimb and is reflected by complex digital flexors containing only MHC-1 and MHC-2A isoform fibers for sustained force development. The findings of this study and our future investigations will further define muscle traits that are specific to fossorial lifestyle and establish whether these traits are adaptive or phylogenetic in nature.

MATERIALS AND METHODS

Study specimens

A total of eight groundhogs (*Marmota monax* Linnaeus 1758) with an average body mass of 4.7±0.8 kg were used for this study (see supplementary material Table S1 for complete morphometric data from the study specimens). Groundhogs were obtained from licensed hunters and trappers in Mahoning and Columbiana Counties in Ohio, USA. Within an hour post mortem, the carcasses were removed from the field (on ice), frozen and stored at −20°C until observation. Specimens were allowed to thaw for 24−36 h at 4°C prior to dissection and measurement. Morphometric data for all specimens are presented as supplementary material Table S1.

Muscle architecture measurements

Muscle names, origin and insertion for *M. monax* followed those of Bezuidenhout and Evans (Bezuidenhout and Evans, 2005) and muscles were grouped based on their main action (Table 1). The forelimbs were skinned and muscles (excluding those of the manus) were identified and systematically dissected. Muscles were periodically moistened with phosphate buffered saline (PBS) to prevent desiccation during dissection and measurement. Muscle architecture was quantified following the procedures used in our previous studies (see Moore et al., 2013; Rose et al., 2013). Briefly, muscle moment arm (r_m) and muscle length *in situ* were measured using digital calipers (CD-8 CSX, Mitutoyo, Japan) with the limb joints placed in a neutral position (i.e. angles in which antagonistic muscles could exert equal joint torque). Following removal of muscles and any free tendons, muscle belly mass (MM) was recorded using an electronic balance (accurate to 0.01 g) (PB4002-S/FACT: Mettler-Toledo, Columbus, OH, USA) and a measurement of resting muscle belly length (ML) was taken. Muscle bellies were then incised along a visible fascial plane to reveal the fiber fascicles. Resting fascicle length (l^F) was measured from 5–10 random fascicles (depending on muscle size) using digital calipers. Resting pennation angle (to the nearest degree) was measured at 5–10 random sites using a goniometer. Last, forelimb bone length and width measurements were recorded and several functional osteological indices (Rose et al., 2014) were calculated (see supplementary material Table S1).

MHC isoform identity and composition

Small blocks of muscle tissue were harvested from the mid-belly region of selected forelimb muscles from a subset of N=4 random specimens after measurement. Muscle tissue was prepared for SDS-PAGE by freezing in liquid nitrogen, grinding to powder, homogenizing 50 mg of muscle powder in 800 ml (ratio 1:16) of Laemmli buffer (Laemmli, 1970; Toniolo et al., 2007) and centrifugation of the homogenates at 13,000 rpm for 10 min (Rupert et al., 2014). Samples for gel loading were diluted (1:500) in gel sample buffer (Mizunoya et al., 2008) to a final protein concentration of ~0.125 µg ml^{-1}. MHC isoforms were identified on SDS-PAGE gels using established methods (Talmadge and Roy, 1993) performed with slight modifications (Mizunoya et al., 2008) as previously described (Hazimihalis et al., 2013; Rupert et al., 2014). Gels were loaded with a total of ~1 µg protein per lane, stained with silver (Bio-Rad, Hercules, CA, USA) and imaged using a Fluor-Chem E Imaging System (Cell Biosciences, Santa Clara, CA, USA). MHC isoform content was quantified by densitometry in ImageJ (v.1.43, NIH) using the brightness area product method (BAP) similar to Toniolo et al. (Toniolo et al., 2008). Band intensity values in each gel lane were summed and used to calculate a percentage for each MHC isoform expressed in a single muscle. Percentages of the MHC isoforms for each muscle were averaged across three independent gel runs per specimen to provide an overall mean percentage composition of slow and fast MHC isoforms.

Muscle functional properties and architectural indexes

Muscle volume was calculated by dividing mean MM by a muscle density of 1.06 g cm^{-3} (Mendez and Keyes, 1960). PCSA was calculated as (muscle volume/mean l^F)×cos θ, where θ is mean pennation angle (in deg). Isometric force ($\mathbf{F}_{max}$) was estimated by multiplying PCSA by a maximum isometric stress of 30 N cm^{-2} (Woledge et al., 1985; Medler, 2002). Joint torque was calculated as $\mathbf{F}_{max}\times r_m$. Muscle power (W) was estimated to be one tenth the product of $\mathbf{F}_{max}$ and $\mathbf{V}_{max}$ (Hill, 1938), where $\mathbf{V}_{max}$ is maximum fiber-shortening velocity (in FL s^{-1}). A size-specific value of 1.97 FL s^{-1} for a 4.7 kg groundhog was predicted using published slack test data for fast MHC-2A fibers (determined to be the primary isoform: see below) at 12°C (Toniolo et al., 2007). Accounting for a Q_{10} (temperature quotient) of 2−6 for $\mathbf{V}_{max}$ (Pate et al., 1994; Ranatunga, 1996), a value of 7.87 FL s^{-1} was calculated as $\mathbf{V}_{max}$ at physiologic temperature for groundhogs [37.8°C (Hayes, 1976)]. Importantly, calculations of $\mathbf{F}_{max}$ and $\mathbf{V}_{max}$ are only estimates, and are used here to indicate muscle functional capacity (Williams et al., 2007a; Smith et al., 2006).

Descriptive statistics for raw measurements are reported as means (±s.d.). Calculated and estimated functional properties are presented as single values consistent with our previous studies (see Moore et al., 2013; Rose et al., 2013). Mass of each muscle group was normalized to total forelimb muscle mass and presented as an architectural index (AI) of proximal-to-distal muscle mass distribution (Smith et al., 2006; Williams et al., 2008). Ratios of PCSA/MM, l^F/ML and l^F/r_m (Moore et al., 2013; Rose et al., 2013) were calculated as additional AIs to assess muscle functional capacity.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
J.E.R. performed experiments and data analysis, interpreted the findings, and prepared the manuscript; J.A.R. performed experiments and data analysis, and prepared the manuscript; J.M.O. interpreted the findings and revised the manuscript; and M.T.B. developed the concepts and approach, supervised data collection and analysis, interpreted the findings, and drafted and revised the manuscript.

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Table S1. Morphometric data for experimental animals

Animal	Sex	Limb	Body mass (kg)	Humerus length (cm)	Ulna length (cm)	Olecranon length (cm)	Metacarpal III length (cm)	Fossorial ability index	Triceps metacarpal out-force index
Mm 1010	F	R	4.6	7.2	7.5	1.2	2.4	0.19	0.14
Mm 1122	M	R	3.9	7.2	7.7	1.7	2.3	0.28	0.21
Mm 0923	F	R	4.4	7.7	8.4	1.6	2.3	0.23	0.17
Mm 0904	F	L	5.5	7.3	7.9	1.6	2.5	0.24	0.18
Mm 0911	F	R	6.2	7.0	7.1	1.7	1.8	0.31	0.23
Mm 0918	F	L	4.9	6.7	7.2	1.3	1.6	0.23	0.18
Mm 1101	M	R	4.4	6.7	7.7	1.5	2.0	0.23	0.18
Mm 1115	M	L	3.9	6.8	7.3	1.4	2.4	0.24	0.17
			4.7±0.8	**7.1±0.3**	**7.6±0.4**	**1.5±0.2**	**2.1±0.3**	**0.24±0.04**	**0.18±0.03**

In bold are mean ± s.d.

Indices are defined as in Lagaria and Youlatos (2006). Fossorial ability index (IFA): ratio of olecranon length to total ulna length minus olecranon length; Triceps metacarpal out-force index (TMOI): ratio of olecranon length to the sum of ulna and metacarpal length minus olecranon length.