

The Forearm Musculature of the Gray Mouse Lemur (*Microcebus murinus*): An Ontogenetic Study

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ABSTRACT

Although studies have sought to characterize variation in forearm muscular anatomy across the primate order, none have attempted to quantify ontogenetic changes in forearm myology within a single taxon. Herein, we present muscle architecture data for the forearm musculature (flexors and extensors of the wrist and digits) of *Microcebus murinus*, a small Lemuroid that has been the focus of several developmental studies. A quadratic curvilinear model described ontogenetic changes in muscle mass and fascicle length; however, fascicle lengths reached peak levels at an earlier age and showed a stronger decline during senescence. Conversely, physiological cross-sectional area followed a more linear trend, increasing steadily throughout life. As previous studies into the functional role of the primate forelimb emphasize the importance of long muscle fascicles within arboreal taxa in order to maximize mobility and flexibility, the early attainment of peak fascicle lengths may consequently reflect the importance of agility within this mobile and highly arboreal species. Similarly, observed myological trends in forearm strength are supported by previous *in vivo* data on grip strength within *M. murinus* in which senescent individuals showed no decline in forearm force relative to prime age individuals. This trend is interpreted to reflect compensation for the previously reported decline in hind limb grip strength in the hind limb with age, such that older individuals are able to maintain arboreal stability. Interestingly, the ontogenetic trajectory of each architectural variable mirrored previous observations of the masticatory musculature in *M. murinus*, suggesting that ontogenetic trends are relatively conserved between anatomical regions. Anat Rec, 00:000–000, 2019. © 2019 American Association for Anatomy

Key words: PCSA; grip strength; development; aging; senescence; fascicle length; arboreality

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INTRODUCTION

The morphology and functional repertoire of the forelimb across extant primates is highly diverse, encompassing a broad range of locomotor and behavioral categories. In addition to their role in locomotion (both as propulsive levers and to grasp substrates; Cartmill, 1972; Sussman and Raven, 1978; Lemelin and Grafton, 1998; Schmitt, 1998; Reghem *et al.*, 2012), the forelimb is heavily used in the acquisition and manipulation of food items (Reghem *et al.*, 2011; Toussaint *et al.*, 2013), as well as during inter-individual interactions such as grooming (Mitchell and Tokunaga, 1976; Hopkins *et al.*, 2007). Consequently, the manipulative capacity of the forelimb is considered to have undergone positive selection across several primate lineages (Szalay, 1968; Bloch and Boyer, 2002; Reghem *et al.*, 2011; Thomas *et al.*, 2016).

Several attempts have recently been made to characterize variation in forearm muscular anatomy across the primate order (Gibbs *et al.*, 2002; Aversi-Ferreira *et al.*, 2010; Diogo and Wood, 2012; Lemelin and Diogo, 2016; Leischner *et al.*, 2018; Boettcher *et al.*, 2019). Interestingly, while no consistent phylogenetic trends are reported to describe such variation, locomotor repertoire appears closely correlated with forearm functional capacity. Terrestrial taxa demonstrate a significant increase in forearm flexor strength (as estimated by physiological cross-sectional area; see below); meanwhile, arboreal taxa demonstrate significantly longer fascicles across the forearm as a whole and within their digital flexors, suggesting an increase in muscle excursion potential and contractile velocity that is hypothesized to facilitate greater agility and/or speed during locomotion (Leischner *et al.*, 2018).

The relationship between forearm flexibility and arboreality is potentially intriguing from an evolutionary perspective. Manual prehension among primates is thought to have emerged in an arboreal context (Napier, 1967; Cartmill, 1974, 2017) and the ability to grasp and move between arboreal substrates is critical to the locomotor repertoire of all strepsirrhines—the most basal primate suborder (Roos and Zinner, 2016). As such, patterns of grip strength and overall hand and wrist flexibility within strepsirrhines are of importance to our understanding of the evolution of arboreal locomotor patterns. To this end, three recent studies (Hämäläinen *et al.*, 2015; Thomas *et al.*, 2016; Le Brazidec *et al.*, 2017) have sought to examine grasping and pulling strength within the model Lemuroid taxon *Microcebus murinus*: a small-bodied, highly arboreal taxon that favors narrow substrates (Martin, 1972; Tattersall, 1982). While overall pulling strength appears strongly correlated with body mass and forearm length in this species, older individuals appear relatively weaker (Thomas *et al.*, 2016). In terms of grip strength, Hämäläinen *et al.* (2015) report that senescent individuals show a decline in grip strength relative to prime age adults (in both captive and wild individuals), with prime age females being stronger than equivalent males but declining in strength more rapidly, such that no sex differences were observed within senescent individuals. Conversely, no differences are reported between males and females, nor between younger and older adult individuals by Le Brazidec *et al.* (2017).

While ontogenetic patterns of forearm strength and flexibility are not widely reported either for *M. murinus* or for primates more generally, what little data exist would seem to suggest interesting developmental trends within this

region. Immature mouse lemurs are reported to show relatively high grasping strength when adjusted for body mass (Boulinguez-Ambroise *et al.*, 2019), while data on hand usage during food acquisition suggest that younger adults practice unimanual grasps more frequently than senescent individuals, suggesting some variation in forelimb use associated with aging (Le Brazidec *et al.*, 2017). However, as only experimentally derived *in vivo* measurements of grip strength are reported for this species, the myological changes which drive potential variation in forearm strength and flexibility during development remain unknown.

In this study, we report anatomical data on the complete forearm musculature of an extensive developmental series of *M. murinus*, ranging from 4 days to 9 years of age. As weaning occurs around 2 months of age (Castanet *et al.*, 2004), sexual maturity is established by an age of 9 months (Lutermann *et al.*, 2006), captive life span can reach 9–11 years of age (Castanet *et al.*, 2004), and the onset of senescence occurs in individuals over the age of 5 years (Bons *et al.*, 2006), this sample spans the entire life history of this taxon from neonates to senescence. Using these data, we describe ontogenetic variation in muscle mass, excursion potential, and contractile force potential across four major functional subgroups: wrist flexors, wrist extensors, digital flexors, and digital extensors. In so doing, we present novel data on the ontogeny of forearm strength and dexterity within a model primate taxon.

Functional Correlates of Forearm Myology

From a functional perspective, both the size and internal configuration of skeletal muscle contribute to their contractile profile. As muscle fibers consist of serially arranged sarcomeres, the lengths of fascicles (bundles of fibers) reflect both contractile velocity and excursion potential (Bodine *et al.*, 1982; Gokhin *et al.*, 2009). Meanwhile, the maximal contractile force that a muscle may generate is proportionally related to its physiological cross-sectional area (PCSA) (Close, 1972; Gans, 1982; Powell *et al.*, 1984; Lieber, 1986; Anapol *et al.*, 2008). As longer muscle fascicles allow greater excursion or speed while shorter, more densely packed fascicles improve force generation potential, there therefore exists a functional trade-off in which a muscle cannot be wholly optimized for both force and excursion potential or velocity for a given volume (Gans and Bock, 1965; Gans, 1982; Lieber, 1986; Lieber and Fridén, 2000).

As noted above, the muscles of the forearm may be neatly subdivided into four functional roles: wrist flexors, wrist extensors, digital flexors, and digital extensors (additionally, dedicated antebrachial muscles allow for supination and pronation of the forearm and one forearm muscle, the brachioradialis, aids in elbow flexion, though these muscles are not the focus of this study.) Combining the architectural profile of muscles that perform similar roles (e.g., extensor carpi ulnaris, extensor carpi radialis longus, and extensor carpi radialis brevis, which collectively constitute the wrist extensors) facilitates functional interpretations of how variation in forearm myology impacts strength and dexterity within the forelimb. Such compartmentalization enables us to better interpret the functional implications of myological variation; for example, while terrestrial primates possess an increase in combined (digital + wrist) flexor PCSA, reflecting a possible increase in grip strength, arboreal species possess longer

fascicles across their combined flexors and extensors, suggestive of a more mobile and flexible hand and wrist (Leischner *et al.*, 2018).

Predictions

While ontogenetic data regarding the forearm musculature have not previously been documented within primates, recent work on ontogenetic scaling within the masticatory muscles of *M. murinus* (Leonard *et al.*, 2019) suggests several myological trends associated with aging in this taxon. Quadratic curvilinear relationships were observed to best describe ontogenetic trends in both muscle mass and PCSA, with these masticatory variables increasing consistently during growth to reach a peak in mid-adulthood. Beyond this point, both mass and PCSA are observed to plateau or exhibit a slight decline in older/senescent adult individuals, a trend interpreted to reflect the physiological effects of age-related sarcopenia. In terms of masticatory fascicle lengths, however, a much more rapid developmental increase is observed within infants and young juveniles, such that peak fascicle lengths are obtained by mid-juvenility. A plateau is maintained throughout adulthood, while some (but not all) muscles subsequently exhibit a decline in fascicle lengths associated with senescence.

On the basis of these documented trends and experimental work on *in vivo* grip strength within adult *M. murinus*, we establish the following *a priori* predictions:

Prediction 1. Across the functional groupings of the forearm musculature (flexors and extensors of the wrist and digits), a quadratic curvilinear relationship will describe ontogenetic changes in both muscle mass and PCSA relative to age—with increases in mass and contractile strength during ontogeny followed by a maintained period of peak mass and strength during adulthood.

Prediction 2. Recent experimental data on *in vivo* grip strength in *M. murinus* (Hämäläinen *et al.*, 2015) report that senescent individuals show a decline in grip strength relative to prime age adults (in both captive and wild individuals). On the basis of these data (and in light of the expected effects of sarcopenia upon muscle strength in senescent individuals), we predict that forearm PCSA will exhibit a decline with the onset of senescence. However, as a more recent *in vivo* study (albeit on a reduced captive sample) demonstrated no significant differences in strength between young and old adult

the relationship between age and fascicle lengths within the forearm musculature. However, following Leonard *et al.* (2019), we predict that adult fascicle lengths will be attained earlier in development than adult muscle masses, such that a plateau is attained earlier and sustained for longer.

MATERIALS AND METHODS

Sample

A full ontogenetic sample comprising 45 specimens of *M. murinus* (of which 34 were male and 11 female) were selected for analysis (Table 1). These specimens ranged in age from 4 days to 9 years. The specimens were born and raised in captivity in the laboratory breeding colony located in Brunoy, France (license number #F91-114-1). None of these animals were euthanized for the purpose of this study. Prior to data collection, all specimens were fixed in 10% formalin and stored in 70% ethanol.

Data Collection

For each specimen, myological data (muscle mass, fascicle length, and PCSA; Tables 1 and S1–S4) were collected for 13 muscles within the forearm, subdivided into the four functional units of the wrist flexors, wrist extensors, digital flexors, and digital extensors (Fig. 1). This sample consisted of both left and right sided muscles, but each individual was represented by only one side. Following skinning, each muscle was individually excised, blotted dry, and weighed to the nearest 0.0001 g. Muscles were then submerged in a 35% aqueous nitric acid solution to dissolve connective tissues, until individual fascicles could be manually separated. Once this process was complete, muscles were stabilized in 50% aqueous glycerol. Manually separated fascicles were then arranged in a petri dish with no overlap and enough distance between each fascicle to ensure clarity and distinguishability. Each dish of fascicles was then photographed using a Nikon D3000 camera and measured using the software package ImageJ (v 1.46) following Cox and Baverstock (2016). A minimum of 40 fascicles were measured for each muscle to ensure a representative sample (or, for smaller muscles, as many fascicles as were visible were measured).

Data Analysis

Muscle mass and fascicle length data were combined to calculate the PCSA of each muscle, using the following formula modified from Schumacher (1961):

$$\text{PCSA} = \frac{\text{muscle mass (g)}}{\text{average fascicle length (cm)} \times \text{specific density of muscle (g/cm}^3\text{)'}}$$

individuals (Le Brazidec *et al.*, 2017), such a relationship might instead characterize our sample. In light of the conflicting nature of this literature, we evaluate both possibilities when testing this prediction.

Prediction 3. As with muscle mass and PCSA, we predict that a quadratic curvilinear relationship will describe

in which a constant of 1.0564 g/cm³ was used to represent muscle density (following Murphy and Beardsley, 1974). As recent publications on the forearm musculature of primates have demonstrated the negligible impact of pennation angle on muscle force (van Leeuwen *et al.*, 2018; Boettcher *et al.*, 2019), a conversion into RPCSA (as per Anapol and Barry, 1996) was not used.

TABLE 1. Specimens included in the sample and data for each variable (muscle mass, average fascicle length, and PCSA)

Specimen ID	Sex	Age (years)	Body mass (g)	Wrist extensors				Digital extensors				Wrist flexors				Digital flexors			
				Muscle mass (g)	Average fascicle length (cm)	PCSA (cm ²)	Muscle mass (g)	Average fascicle length (cm)	PCSA (cm ²)	Muscle mass (g)	Average fascicle length (cm)	PCSA (cm ²)	Muscle mass (g)	Average fascicle length (cm)	PCSA (cm ²)	Muscle mass (g)	Average fascicle length (cm)	PCSA (cm ²)	
4 days old	M	0.011	5	0.004	0.351	0.012	0.003	0.275	0.010	0.004	0.208	0.017	0.011	0.017	0.291	0.036			
5 days old	M	0.014	6	0.006	0.237	0.024	0.005	0.292	0.017	0.002	0.260	0.007	0.008	0.007	0.353	0.023			
6 days old	M	0.016	8	0.007	0.249	0.027	0.002	0.230	0.009	0.007	0.239	0.027	0.007	0.302	0.023	0.023			
Infant of 162H	M	0.030	10	0.006	0.323	0.018	0.001	0.268	0.003	0.005	0.276	0.017	0.009	0.322	0.028	0.028			
Infant from 172IB	M	0.110	36	0.036	0.713	0.050	0.010	0.522	0.017	0.022	0.586	0.036	0.051	0.719	0.069	0.069			
Infant from 198F	M	0.120	44	0.035	0.627	0.053	0.005	0.553	0.008	0.029	0.612	0.046	0.074	0.573	0.125	0.125			
Infant from 276B	M	0.160	41	0.047	0.631	0.075	0.020	0.473	0.040	0.036	0.485	0.072	0.111	0.619	0.182	0.182			
Infant from 943GF	F	0.220	38	0.042	0.771	0.053	0.020	0.560	0.034	0.025	0.544	0.044	0.080	0.726	0.104	0.104			
206CA	M	0.240	43	0.028	0.901	0.032	0.016	0.616	0.026	0.050	0.571	0.084	0.047	0.604	0.077	0.077			
102CA	F	0.360	50	0.021	0.519	0.040	0.005	0.489	0.010	0.011	0.475	0.022	0.039	0.595	0.063	0.063			
147EE	M	0.370	98	0.033	0.627	0.051	0.010	0.623	0.015	0.026	0.433	0.058	0.060	0.507	0.113	0.113			
276AC	F	0.500	52	0.014	0.594	0.022	0.006	0.560	0.011	0.008	0.554	0.014	0.034	0.718	0.046	0.046			
139BB	M	0.970	87	0.027	0.680	0.040	0.012	0.626	0.019	0.024	0.514	0.045	0.079	0.683	0.110	0.110			
921BG	F	1.540	70	0.042	0.858	0.047	0.014	0.796	0.016	0.030	0.514	0.054	0.115	0.684	0.159	0.159			
278ACC	F	2.000	78	0.165	0.787	0.203	0.025	0.594	0.040	0.180	0.463	0.370	0.092	0.653	0.137	0.137			
911FBJ	F	2.220	128	0.037	0.670	0.064	0.015	0.697	0.020	0.014	0.504	0.026	0.074	0.684	0.103	0.103			
223GA	M	2.360	85	0.046	0.724	0.067	0.021	0.599	0.033	0.045	0.649	0.066	0.112	0.750	0.144	0.144			
219E	M	2.380	102	0.058	0.830	0.064	0.009	0.627	0.014	0.033	0.607	0.051	0.115	0.920	0.120	0.120			
241AA	M	2.790	62	0.038	0.396	0.091	0.012	0.405	0.028	0.034	0.567	0.058	0.084	0.808	0.103	0.103			
189GAA	M	3.330	81	0.048	0.798	0.066	0.022	0.610	0.035	0.045	0.616	0.072	0.135	0.682	0.193	0.193			
119CBB	M	3.660	91	0.028	0.638	0.043	0.018	0.544	0.032	0.009	0.474	0.018	0.095	0.652	0.138	0.138			
893AAJ	M	3.660	116	0.080	0.638	0.120	0.034	0.540	0.064	0.059	0.628	0.092	0.165	0.848	0.192	0.192			
153F	F	3.920	86	0.087	0.601	0.138	0.037	0.551	0.067	0.077	0.637	0.114	0.200	0.780	0.247	0.247			
206DBA	M	3.930	80	0.057	0.833	0.068	0.019	0.630	0.029	0.043	0.577	0.070	0.113	0.748	0.147	0.147			
189H	M	4.320	87	0.046	0.627	0.072	0.020	0.579	0.032	0.041	0.590	0.066	0.125	0.684	0.176	0.176			
101A	M	4.400	72	0.047	0.588	0.081	0.029	0.559	0.050	0.033	0.436	0.074	0.116	0.689	0.162	0.162			
921BAC	F	4.420	136	0.010	0.886	0.011	0.005	0.620	0.008	0.009	0.572	0.015	0.023	0.704	0.032	0.032			
989EB	F	4.700	118	0.044	0.541	0.078	0.017	0.542	0.030	0.029	0.454	0.062	0.109	0.698	0.152	0.152			
184BBB	M	5.150	66	0.049	0.609	0.078	0.019	0.663	0.027	0.036	0.366	0.035	0.115	0.820	0.135	0.135			
225A	M	5.420	86	0.048	0.723	0.066	0.047	0.600	0.075	0.044	0.521	0.082	0.201	0.639	0.307	0.307			
143CAD	M	5.880	74	0.039	0.688	0.058	0.019	0.540	0.033	0.036	0.497	0.068	0.094	0.693	0.135	0.135			
143CAC	M	5.920	73	0.041	0.413	0.103	0.028	0.349	0.082	0.026	0.444	0.058	0.138	0.699	0.196	0.196			
163DE	M	5.950	90	0.094	0.751	0.126	0.047	0.603	0.074	0.067	0.530	0.120	0.220	0.572	0.368	0.368			
100DBA	M	6.000	70	0.061	0.790	0.075	0.028	0.666	0.042	0.064	0.496	0.123	0.156	0.535	0.283	0.283			
223A	M	6.200	66	0.060	0.731	0.082	0.024	0.813	0.031	0.046	0.496	0.062	0.131	0.843	0.148	0.148			
113B	M	6.280	53	0.043	0.879	0.046	0.037	0.705	0.052	0.040	0.584	0.066	0.187	0.595	0.299	0.299			
129A	M	6.840	80	0.068	0.518	0.129	0.025	0.515	0.049	0.038	0.468	0.081	0.132	0.629	0.210	0.210			
245BB	F	7.000	56	0.040	0.639	0.062	0.011	0.596	0.017	0.019	0.550	0.034	0.123	0.740	0.159	0.159			
143CAA	M	7.010	96	0.035	0.619	0.056	0.019	0.481	0.037	0.035	0.517	0.064	0.107	0.657	0.154	0.154			
883DEM	M	8.000	98	0.052	0.705	0.076	0.031	0.543	0.056	0.050	0.498	0.095	0.144	0.649	0.213	0.213			
965FDC	M	8.000	98	0.056	0.688	0.081	0.023	0.594	0.039	0.047	0.493	0.092	0.136	0.771	0.171	0.171			
1721BA	M	8.000	58	0.122	1.085	0.107	0.009	0.792	0.011	0.027	0.682	0.038	0.076	0.814	0.089	0.089			
141B	M	8.280	86	0.047	0.658	0.071	0.018	0.553	0.031	0.034	0.479	0.068	0.108	0.753	0.139	0.139			
163DCB	M	9.000	27	0.033	0.672	0.049	0.018	0.562	0.030	0.033	0.695	0.046	0.071	0.656	0.103	0.103			

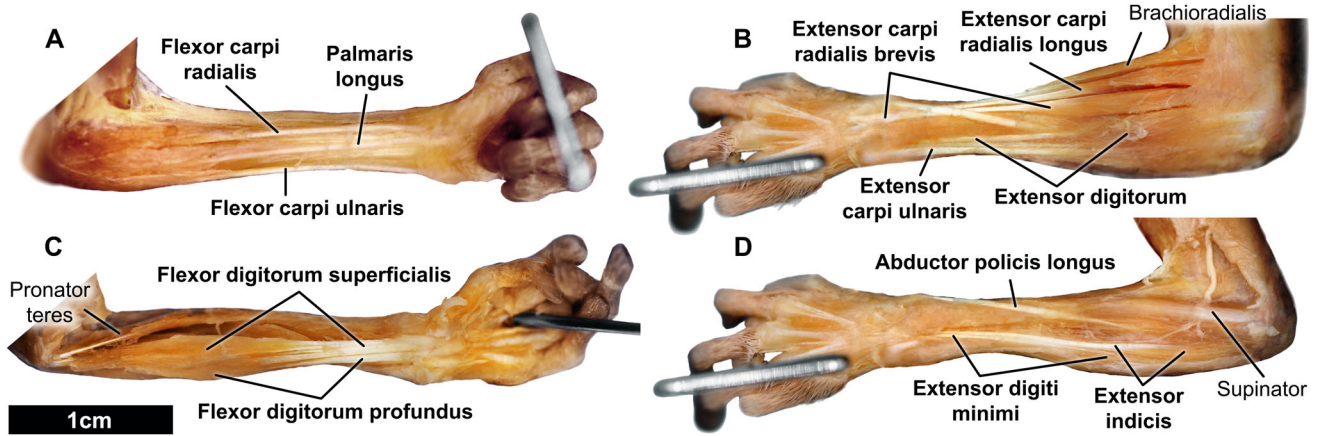


Fig. 1. Photographs of typical *Microcebus murinus* forearm anatomy. (A) Wrist flexors (superficial, anterior); (B) wrist extensors (superficial, posterior); (C) digital flexors (deep, anterior); (D) digital extensors (deep, posterior). Focal muscles are bold. Extensor pollicis longus and pronator quadratus are deeper and not show.

Myological data for individual muscles were then combined into their functional subgroups. Muscle mass and PCSA for each constituent muscle were summed to describe each subgroup. For fascicle length, an average weighted fascicle length was calculated using the following formula adapted from Hartstone-Rose *et al.* (2012):

$$\text{Weighted fascicle length} = \frac{FL_{x_1}M_{x_1} + FL_{x_2}M_{x_2} + FL_{x_3}M_{x_3} + \dots}{M_{x_1} + M_{x_2} + M_{x_3} + \dots},$$

in which FL_x and M_x represent the average fascicle length and mass of each muscle, respectively.

Multidimensional variables were linearized to a consistent power (i.e., the cubic- and square-roots of volumetric and area variables were taken respectively), and all data were subsequently \log_{10} transformed. For each

variable, we evaluated the fit of quadratic curvilinear regressions against age. To evaluate the point at which age-associated changes in myological variables were observed to plateau, we further calculated for each quadratic expression the point at which slope $a = 0$ (Table 2). All statistical analyses were conducted using JMP Pro 14 (SAS).

RESULTS

Muscle Mass

Quadratic curvilinear models were observed to describe the ontogenetic scaling of muscle mass within the wrist extensors, wrist flexors, and digital flexors of *M. murinus*, in addition to the summed mass of all forearm groupings (Fig. 2). In each instance, muscle mass

TABLE 2. Quadratic equations associated with the graphs of each variable for each muscle group and age at which slope changes from positive to negative (vertex)

Muscle group	Variable	Quadratic Equation ($ax^2 + bx + c$)	Age (years) when slope changes from positive to negative (observed or projected)
Total forearm muscles	MM	$-0.0508x^2 + 0.0548x - 0.2143$	5.2
	FL	$-0.0823x^2 + 0.0191x - 0.1779$	2.0
	PCSA	$-0.0344x^2 + 0.0741x - 0.2215$	18.0 ^a
Wrist extensors	MM	$-0.0515x^2 + 0.0423x - 0.4513$	3.9
	FL	$-0.0971x^2 + 0.0042x - 0.1358$	1.6
	PCSA	$-0.0271x^2 + 0.0673x - 0.6128$	26.4 ^a
Digital extensors	MM	$-0.0241x^2 + 0.0820x - 0.6136$	76.9 ^a
	FL	$-0.0861x^2 + 0.0041x - 0.2097$	1.6
	PCSA	$0.0091x^2 + 0.1240x - 0.8240$	0.0
Wrist flexors	MM	$-0.0575x^2 + 0.0355x - 0.4911$	3.1
	FL	$-0.09x^2 + 0.0060x - 0.2407$	1.4
	PCSA	$-0.0426x^2 + 0.0514x - 0.6278$	6.1
Digital flexors	MM	$-0.0511x^2 + 0.0662x - 0.3445$	6.8
	FL	$-0.0788x^2 + 0.0168x - 0.1419$	1.9
	PCSA	$-0.0361x^2 + 0.0907x - 0.4535$	27.5 ^a

^a These data points are outside the life span of the species and were not observed in our data set, but rather are projected ages.

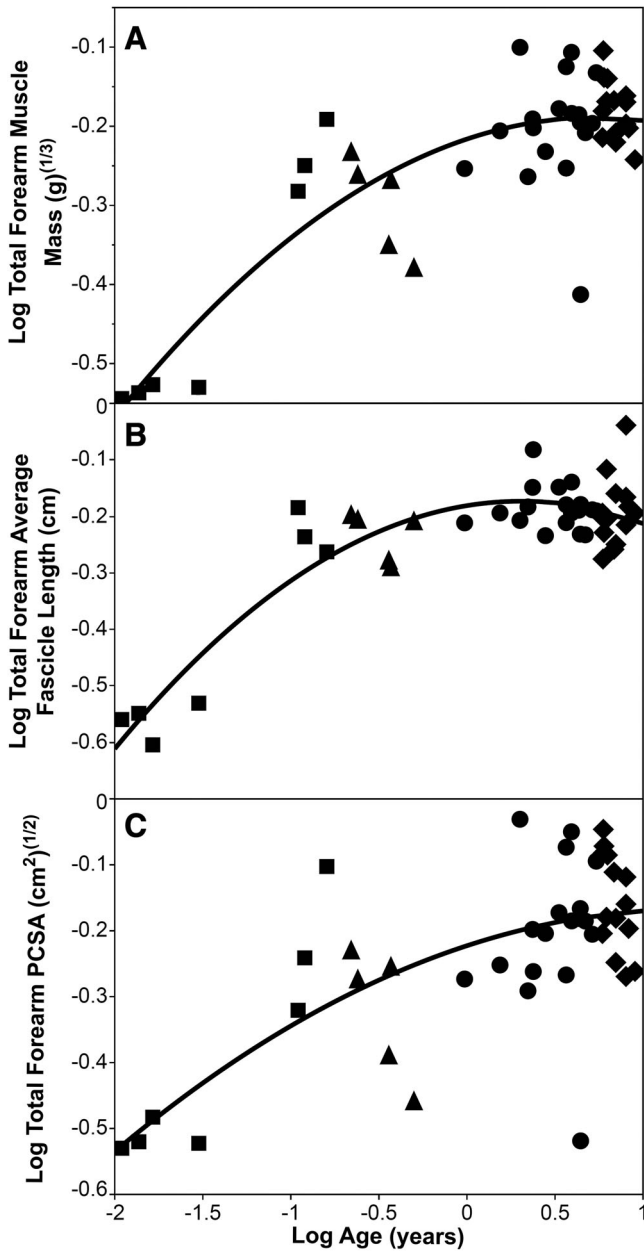


Fig. 2. Quadratic curvilinear regression of age and the total forearm architectural variables (**A**: muscle mass; **B**: average fascicle length; **C**: PCSA). All variables were logged and linearized. Squares: infants; triangles: juveniles; circles: adults; diamonds: senescent individuals.

was observed to increase rapidly between infancy and adulthood, before reaching a plateau at the ages of 3.1 years (wrist flexors), 3.9 years (wrist extensors), 5.2 years (total forearm), and 6.8 years (digital flexors). No obvious decline in mass was associated with senescence within any functional group. One muscle group (digital extensors) demonstrated an alternative trend, being best described by a linear growth model ($P < 0.0007$) in which no plateau (indicating arrested growth in muscle mass) was projected to occur within the life span of the animal (Fig. 3).

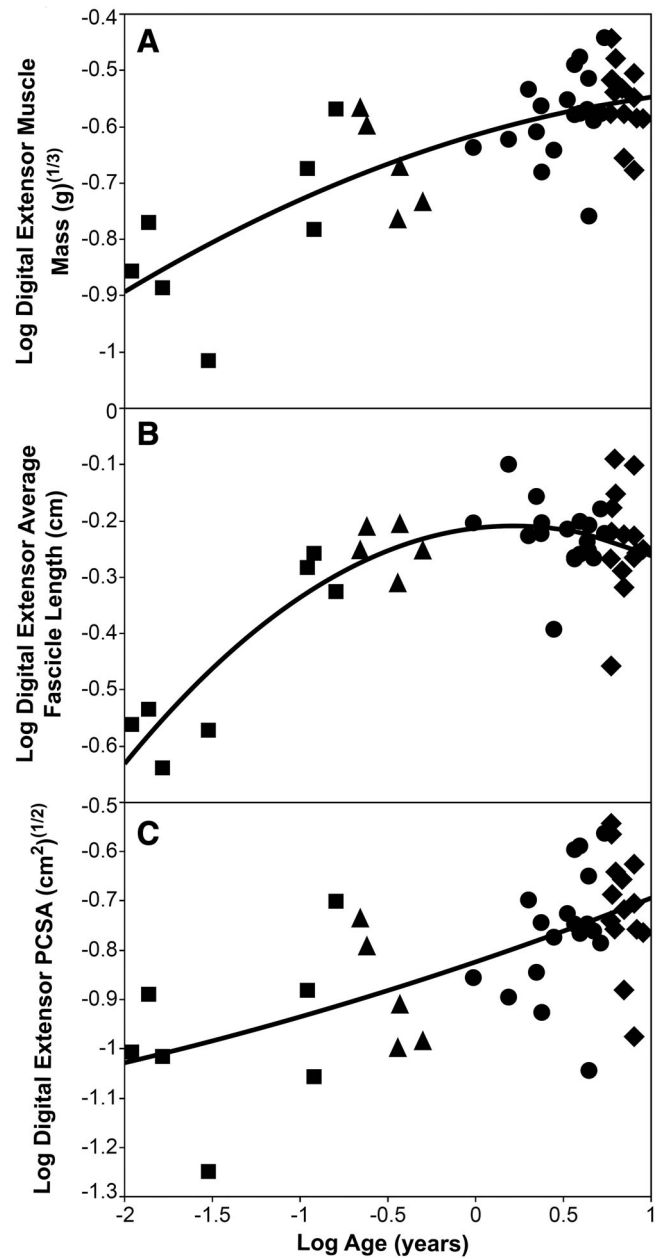


Fig. 3. Quadratic curvilinear regression of age and the digital extensors architectural variables (**A**: muscle mass; **B**: average fascicle length; **C**: PCSA). All variables were logged and linearized. See Figure 2 for key.

Physiological cross-sectional area

A quadratic curvilinear model did not accurately describe the relationship between PCSA and age within any forearm functional group, nor for the summed forearm musculature as a whole. As in the case of muscle mass, PCSA was observed to increase rapidly between infancy and adulthood. However, while three muscle groups (digital flexors, wrist extensors, and wrist flexors) approach a plateau between adulthood and senescence (Figs. 4–6), PCSA continues to gradually increase within these muscles (albeit at

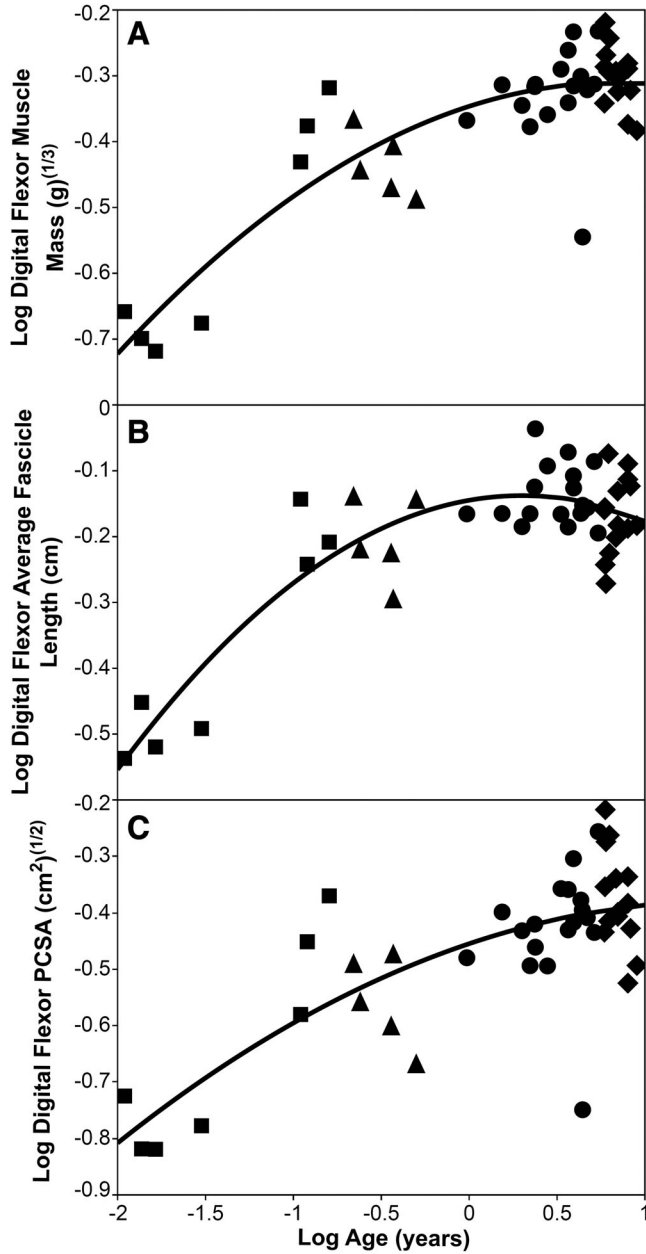


Fig. 4. Quadratic curvilinear regression of age and the digital flexors architectural variables (A: muscle mass; B: average fascicle length; C: PCSA). All variables were logged and linearized. See Figure 2 for key.

a slowing rate) throughout the life span of the animal. Within the digital extensors, the observed increase in PCSA during senescence mirrors that which occurs during adulthood (Fig. 3), suggesting that muscle strength continues to increase at a relatively consistent rate throughout maturity.

Fascicle Length

Quadratic curvilinear models were observed to describe the ontogenetic scaling of fascicle lengths within all functional groups of the forearm, in addition to the combined forearm musculature as a whole. In all instances, fascicle

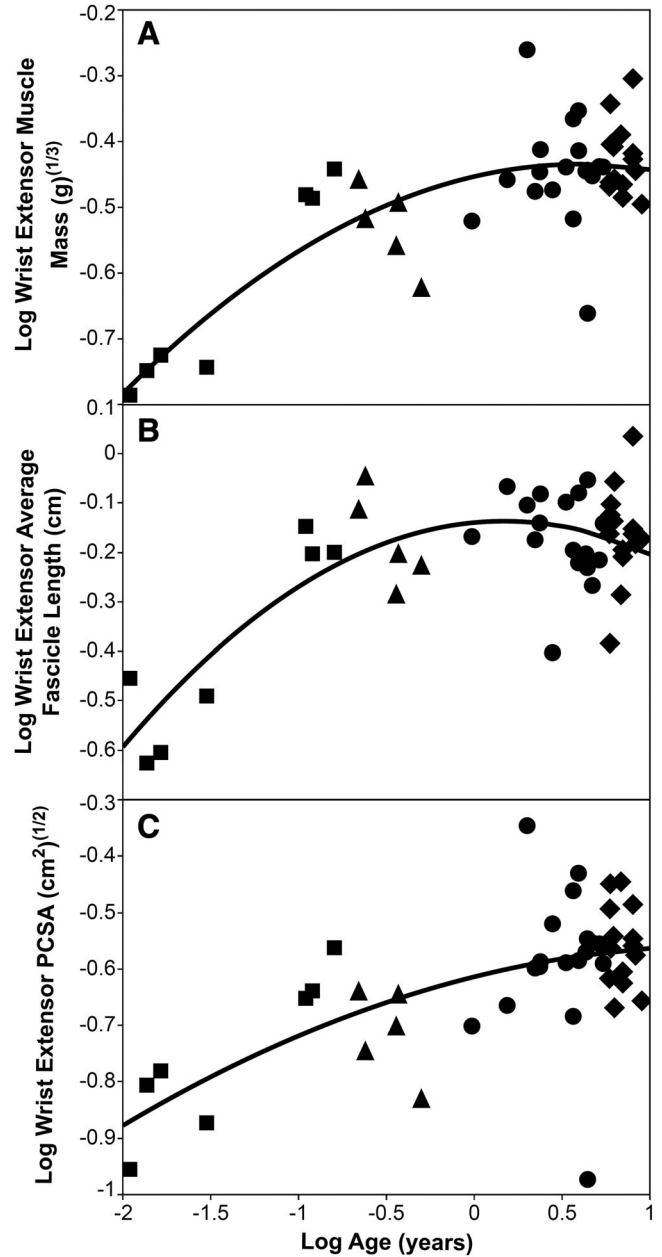


Fig. 5. Quadratic curvilinear regression of age and the wrist extensors architectural variables (A: muscle mass; B: average fascicle length; C: PCSA). All variables were logged and linearized. See Figure 2 for key.

lengths undergo a rapid increase that occurs throughout infancy and juvenility, reaching a peak during early adulthood (Figs. 2–6). Plateaus were observed to occur at the ages of 1.4 years (wrist flexors), 1.6 years (wrist and digital extensors), 1.9 years (digital flexors), and 2.0 years (total forearm musculature). This plateau is maintained until the onset of senescence (which occurs at ~5.5 years; Chazeau *et al.*, 2012), following which point fascicle lengths exhibit a slight decline (Figs. 2–6). However, two-tailed *t*-test comparisons of adult and senescent fascicle lengths demonstrate that this difference is not statistically significant for any functional subgroup ($P = 0.13–0.78$).

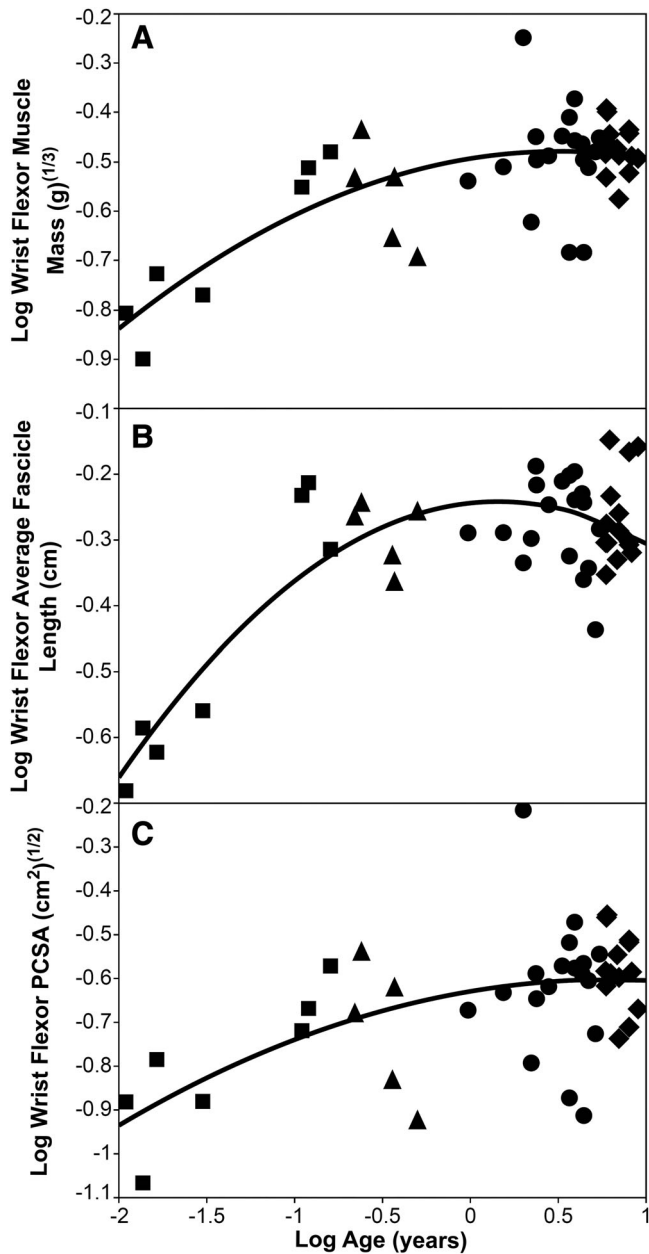


Fig. 6. Quadratic curvilinear regression of age and the wrist flexors architectural variables (**A**: muscle mass; **B**: average fascicle length; **C**: PCSA). All variables were logged and linearized. See Figure 2 for key.

DISCUSSION

Ontogeny of Forearm Musculature in *M. murinus*

Muscle architectural variables were investigated across four functional groups of the forearm (wrist flexors, wrist extensors, digital flexors, and digital extensors), and across the forearm as a whole. In each of these regions (with the exception of the digital extensors; see below), muscle mass followed a quadratic curvilinear model in which mass increased rapidly during early development (infancy and juvenility) before plateauing throughout adulthood and

senescence, supporting Prediction 1. Contrary to the expected impact of sarcopenia, no substantial loss of muscle mass was associated with senescence. However, we did not observe this same trend in PCSA (counter to Prediction 1). Rather, PCSA continues to gradually increase within these muscles (albeit at a slowing rate) throughout the life span of the animal, contradicting Prediction 2.

While counter to the findings of Hämäläinen *et al.* (2015), this ontogenetic trend in forelimb strength is supported by previous *in vivo* data on hand and foot grip strength within *M. murinus*, in which senescent individuals (7–8 years) showed no decline in forearm force relative to prime age (2–3 years) individuals (Le Brazidec *et al.*, 2017). These experimental data were interpreted to reflect a delay in the onset of sarcopenia within the muscles of the forelimb. Interestingly, however, a decline in grip strength was observed within the muscles of the hindlimb, such that foot grip strength decreased significantly between young adult and senescent individuals (Le Brazidec *et al.*, 2017). As this loss of foot strength may result in a diminished ability for older individuals to stabilize on narrow substrates, maintenance of forelimb strength into senescence may therefore be needed to compensate for this loss of gripping ability (Le Brazidec *et al.*, 2017).

In each functional group of the forearm, a rapid increase in average fascicle length was observed from infancy to adulthood, as predicted in Prediction 3. A peak is attained by early adulthood (1.4–2.0 years) and maintained into senescence, at which point a slight (but not statistically significant) decline in fascicle length is observed in all functional divisions of the forearm. Previous studies into the functional role of the primate forelimb emphasize the importance of long muscle fascicles within arboreal taxa in order to maximize mobility and flexibility in the wrist and hand (Kikuchi, 2010; Leischner *et al.*, 2018). The early attainment of peak fascicle lengths may consequently reflect the importance of agility within this small, mobile and highly arboreal taxon.

Finally, in terms of both muscle mass and PCSA, the digital extensors exhibited distinct trends. Both variables were best described by a linear growth model in which no plateau was projected to occur within the life span of the animal. However, the functional interpretation of this scaling relationship is unclear, as neither mass nor PCSA of the digital extensors has been observed to correlate with locomotor mode across primates (Leischner *et al.*, 2018), and other behaviors that might require forceful digital extension are not documented within *M. murinus*.

Broader Scaling Trends within *M. murinus*

To contextualize these data, our findings were compared against a similar study assessing myological scaling within the masticatory musculature during ontogeny within *M. murinus* (Leonard *et al.*, 2019). For each architectural variable, similar trends were observed in both the forearm and masticatory musculature, which suggests that ontogenetic trends are relatively conserved between anatomical regions. Such consistency similarly suggests that the developmental trajectories of architectural variables may be driven less by the functional role of individual muscles or muscle groups and more so by global genetic and hormonal mechanisms.

Future work may investigate ontogenetic trends in other anatomical regions (such as the hindlimb) to better elucidate the extent to which myological variables are

conserved throughout development. Such data would also provide anatomical evidence to evaluate the decline in hindlimb grip strength observed by Le Brazidec *et al.* (2017). Examining these trends in other taxa would further contextualize our findings and clarify the extent to which ontogenetic changes in forearm myology vary between species. Finally, it should be considered that our sample was obtained from a captive breeding colony, which may have impacted food acquisition patterns and locomotor behaviors relating to predator avoidance (i.e., behaviors requiring strong grip strength). The absence of such ecological pressures might also allow older individuals to maintain fitness longer than would be possible in the wild.

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