Whole-limb scaling of muscle mass and force-generating capacity in amniotes

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1. Supplemental text

Median size-normalized isometric strength (F_{max}^*) v. body mass (comparison 2b)

Qualitatively, results parallel the patterns reported for mean F_{max}^* in the main text, but on a finer quantitative scale there are several differences (Fig. S2). In the forelimb, reptiles exhibit negative allometry, driven by the proximal limb, whereas mammals do not significantly depart from isometry overall or in the proximal limb, but exhibit positive allometry in the distal limb. In the hindlimb, both mammals and bipeds exhibit positive allometry at the level of the whole limb, although in mammals this is driven by the proximal limb whereas in bipeds it is driven by the distal limb, which again displays very strong positive allometry. Reptiles only exhibit a significant departure from isometry in the distal hindlimb (negative), and excluding the CFL does not significantly alter scaling patterns (Fig. S6). Analyses of covariance without accounting for phylogeny produce similar results to mean F_{max}^* above (Tables S2, S6), indicating that mammals, reptiles and bipeds frequently show different allometric trajectories, especially in terms of slope. Again, differences in intercept were mostly detected for the forelimb, where reptiles exhibit a markedly lower intercept than mammals. Every difference between mammals and reptiles disappears after phylogeny is accounted for using pANCOVA; in contrast, most differences between mammals and bipeds, and reptiles and bipeds, were retained following phylogenetic correction.

Total size-normalized isometric strength v. body mass (comparison 2c)

Qualitatively, results parallel the patterns reported for mean F_{max}^* , but on a finer quantitative scale there are a couple of differences (Fig. S3). Both mammals and reptiles ubiquitously do not show significant departure from isometry, and excluding the CFL does not significantly alter the reptile scaling patterns (Fig. S7). Again, bipeds exhibit strong positive allometry throughout the hindlimb, particularly in the distal limb. Analyses of covariance without accounting for phylogeny produce similar results to mean and median F_{max}^* (Tables S3, S7), indicating that mammals, reptiles and bipeds frequently show different allometric trajectories in terms of both slope and intercept. Reptiles exhibit a markedly lower intercept than mammals throughout the forelimb, and also exhibit a lower intercept in the distal hindlimb. Almost all differences between mammals and reptiles disappear after phylogeny is accounted for using pANCOVA, whereas most differences between mammals and bipeds, and reptiles and bipeds, were retained following phylogenetic correction.

2. Supplemental figures



Figure S1. Time-calibrated phylogeny of all taxa analysed in the study, indicating the major locomotor group to which each taxon was assigned for the purpose of statistical analysis. Also labelled are major taxa sampled in the study.



Figure S2. Median size-normalized isometric strength v. body mass across extant terrestrial amniotes. Conventions as in Fig. 2.



log₁₀ body mass (kg)

Figure S3. Total size-normalized isometric strength v. body mass across extant terrestrial amniotes. Conventions as in Fig. 2.



Figure S4. Total hindlimb muscle mass (Σm_{muscle}) v. body mass (m_{body}) across extant terrestrial amniotes, excluding the reptilian caudofemoralis longus. Conventions as in Fig. 2.



Figure S5. Mean size-normalized isometric strength (F_{max}^*) v. body mass (m_{body}) across extant terrestrial amniotes, excluding the reptilian caudofemoralis longus. Conventions as in Fig. 2.



Figure S6. Median size-normalized isometric strength (F_{max}^*) v. body mass (m_{body}) across extant terrestrial amniotes, excluding the reptilian caudofemoralis longus. Conventions as in Fig. 2.



Figure S7. Total size-normalized isometric strength (F_{max}^*) v. body mass (m_{body}) across extant terrestrial amniotes, excluding the reptilian caudofemoralis longus. Conventions as in Fig. 2.



Figure S8. Characteristic fascicle length (L^*) v. body mass (m_{body}) across extant terrestrial amniotes, excluding the reptilian caudofemoralis longus. Conventions as in Fig. 2.



Figure S9. Total physiological cross-sectional area ($\Sigma PCSA$) v. total muscle mass (Σm_{muscle}) across extant terrestrial amniotes, excluding the reptilian caudofemoralis longus. Conventions as in Fig. 2.

3. Supplemental tables

Table S1. The raw architectural data used in this study, presented in an Excel worksheet. This file contains four tabs: raw hindlimb data, raw forelimb data, hindlimb data collated into the parameters investigated in the study, and forelimb data collated into the parameters investigated in the study. The raw data for the opossum (*Didelphis virginiana*) and tegu lizard (*Salvator merianae*) dissected here are reported as mean \pm one standard deviation for fascicle length and pennation angle.

Table S2. Results of comparisons between each major group via pANCOVA and ANCOVA, for the analysis of median F_{max}^* v. m_{body} . Each pairwise comparison was tested for differences in slope (S), intercept (I) and slope and intercept (S + I). Results for analyses without controlling for phylogeny are also presented (ANCOVA, †); significant results are in boldface; df = degrees of freedom.

anatami	anatomical region	test		mamm	nals v. rep	otiles			mamn	nals v. bij	peds			rept	iles v. bip	eds	
	cal region	test	df	F	Р	F†	P†	df	F	Р	F†	P †	df	F	Р	F†	P †
		S	2,25	4.94	0.036	13.428	0.001										
	whole	Ι	2,25	0.967	0.335	6.115	0.021										
		S + I	2,24	2.492	0.104	6.65	0.005										
		S	2,26	3.448	0.075	10.782	0.003										
forelimb	proximal	Ι	2,26	1.997	0.17	8.244	0.008										
		S + I	2,25	1.714	0.201	5.321	0.012										
		S	2,27	3.709	0.065	9.487	0.005										
	distal	Ι	2,27	0.747	0.395	4.319	0.047										
		S + I	2,26	1.919	0.167	4.855	0.016										
		S	2,27	2.492	0.126	16.152	<0.001	2,21	7.619	0.012	14.396	0.001	2,15	27.093	<0.001	50.166	<0.001
	whole	Ι	2,27	0.133	0.718	4.335	0.047	2,21	2.738	0.113	10.571	0.004	2,15	4.879	0.043	14.79	0.002
		S + I	2,26	1.2	0.317	7.815	0.002	2,20	3.651	0.045	7.763	0.003	2,14	13.277	0.001	25.83	<0.001
		S	2,27	1.704	0.203	12.452	0.002	2,21	2.262	0.148	5.501	0.029	2,15	13.515	0.002	32.281	<0.001
hindlimb	proximal	Ι	2,27	0.252	0.62	4.133	0.052	2,21	1.229	0.28	6.399	0.02	2,15	6.123	0.026	17.189	0.001
		S + I	2,26	0.847	0.44	5.995	0.007	2,20	1.083	0.358	3.478	0.051	2,14	7.668	0.006	18.881	<0.001
		S	2,27	2.3	0.141	14.534	0.001	2,21	13.104	0.002	19.487	<0.001	2,15	37.946	<0.001	73.6	<0.001
	distal	Ι	2,27	2×10 ⁻⁴	0.989	3.822	0.061	2,21	4.24	0.052	10.257	0.004	2,15	2.915	0.108	13.415	0.002
		S + I	2,26	1.157	0.33	7.054	0.004	2,20	6.367	0.007	9.725	0.001	2,14	17.71	<0.001	35.683	<0.001

	1	44		mamn	nals v. rej	otiles			mamn	nals v. bij	peds			rept	iles v. bip	eds	
anatomi	cal region	test	df	F	Р	F†	P †	df	F	Р	F^{\dagger}	P †	df	F	Р	F†	P †
		S	2,25	1.074	0.31	18.185	<0.001										
	whole	Ι	2,25	4.428	0.046	32.703	<0.001										
		S + I	2,24	2.129	0.141	15.819	<0.001										
		S	2,26	0.596	0.447	12.827	0.001										
forelimb	proximal	Ι	2,26	2.498	0.126	18.567	<0.001										
		S + I	2,25	1.212	0.315	9.106	0.001										
		S	2,27	1.909	0.178	21.094	<0.001										
	distal	Ι	2,27	4.056	0.054	37.742	<0.001										
		S + I	2,26	2.066	0.147	18.26	<0.001										
		S	2,27	2.717	0.111	5.046	0.033	2,21	10.504	0.004	14.712	0.001	2,15	19.921	0.001	23.937	<0.001
	whole	Ι	2,27	1.01	0.324	5.152	0.031	2,21	3.517	0.075	7.013	0.015	2,15	6.288	0.024	12.374	0.003
		S + I	2,26	1.406	0.263	3.1	0.062	2,20	5.004	0.017	7.102	0.005	2,14	9.645	0.002	12.757	0.001
		S	2,27	2.433	0.13	2.433	0.13	2,21	9.047	0.007	9.047	0.007	2,15	11.151	0.005	11.151	0.005
hindlimb	proximal	Ι	2,27	1.331	0.259	1.331	0.259	2,21	4.302	0.051	4.302	0.051	2,15	4.82	0.044	4.82	0.044
		S + I	2,26	1.196	0.319	1.196	0.319	2,20	4.323	0.028	4.323	0.028	2,14	5.265	0.02	5.265	0.02
		S	2,27	0.641	0.43	9.07	0.006	2,21	11.839	0.003	17.801	<0.001	2,15	28.666	<0.001	35.859	<0.001
	distal	Ι	2,27	0.533	0.472	23.51	<0.001	2,21	4.122	0.055	8.827	0.007	2,15	6.412	0.023	38.294	<0.001
		S + I	2,26	0.486	0.621	11.588	<0.001	2,20	5.747	0.011	8.719	0.002	2,14	15.896	<0.001	36.533	<0.001

Table S3. Results of comparisons between each major group via pANCOVA and ANCOVA, for the analysis of ΣF_{max}^* v. m_{body} . Conventions as per Table S2.

Table S4. Results of comparisons between each major group via pANCOVA, for the analysis of Σm_{muscle} v. m_{body} in the hindlimb. Here results are reported for analyses where the caudofemoralis longus was excluded from the reptile dataset (cf. Table 2). Conventions as per Table S2.

	40.04	_	man	nmals v. r	reptiles			mar	nmals v.	bipeds			rep	tiles v. bip	eds	
	test	df	F	Р	$F\dagger$	P †	df	F	Р	F^{\dagger}	P †	df	F	Р	F†	P †
	S	2,42	1.11	0.298	28.181	< 0.001	2,36	4.896	0.033	11.763	0.002	2,15	16.211	0.001	37.295	< 0.001
whole (no CFL)	Ι	2,42	1.053	0.311	46.861	< 0.001	2,36	2.605	0.115	10.143	0.003	2,15	12.464	0.003	53.946	< 0.001
	S + I	2,41	0.928	0.404	28.051	< 0.001	2,35	2.389	0.107	6.554	0.004	2,14	13.885	0.001	53.201	< 0.001
proximal (no CFL)	S	2,32	2.84	0.102	34.385	< 0.001	2,26	3.116	0.089	6.595	0.016	2,15	7.949	0.013	16.657	0.001
	Ι	2,32	2.118	0.155	43.572	< 0.001	2,26	1.908	0.179	5.666	0.025	2,15	6.228	0.025	25.947	< 0.001
	S + I	2,31	2.074	0.143	29.787	< 0.001	2,25	1.507	0.241	3.564	0.044	2,14	5.274	0.02	15.599	< 0.001

Table S5. Results of comparisons between each major group via pANCOVA and ANCOVA, for the analysis of mean F_{max} * v. m_{body} in the hindlimb. Here results are reported for analyses where the caudofemoralis longus was excluded from the reptile dataset (cf. Table 3). Other conventions as per Table S2.

	40.54		mammals v. reptiles					mai	nmals v.	bipeds			rep	tiles v. bip	eds	
	test	df	F	Р	F^{\dagger}	P †	df	F	Р	$F\dagger$	P †	df	F	Р	F†	P †
	S	2,27	4.552	0.042	15.537	0.001	2,21	9.839	0.005	14.358	0.001	2,15	36.736	<0.001	70.841	<0.001
whole (no CFL)	Ι	2,27	0.438	0.514	6.135	0.02	2,21	3.198	0.088	6.785	0.017	2,15	5.568	0.032	13.676	0.002
CIL)	S + I	2,26	2.195	0.132	7.559	0.003	2,20	4.698	0.021	6.919	0.005	2,14	17.368	<0.001	34.604	<0.001
	S	2,27	4.565	0.042	13.826	0.001	2,21	7.103	0.015	10.455	0.004	2,15	25.343	<0.001	46.338	<0.001
proximal (no CEL)	Ι	2,27	0.654	0.426	5.392	0.028	2,21	3.053	0.095	5.882	0.024	2,15	6.279	0.024	12.562	0.003
	S + I	2,26	2.213	0.13	6.703	0.005	2,20	3.395	0.054	5.117	0.016	2,14	12.284	0.001	22.773	<0.001

Table S6. Results of comparisons between each major group via pANCOVA and ANCOVA, for the analysis of median F_{max} * v. m_{body} in the hindlimb. Here results are reported for analyses where the caudofemoralis longus was excluded from the reptile dataset (cf. Table S2). Other conventions as per Table S2.

	tost		mammals v. reptiles					mai	nmals v.	bipeds			rep	otiles v. bip	eds	
_	test	df	F	Р	F^{\dagger}	P†	df	F	Р	F^{\dagger}	P †	df	F	Р	$F\dagger$	P †
	S	2,27	2.799	0.106	18.379	<0.001	2,21	7.608	0.012	14.396	0.001	2,15	28.975	<0.001	54.281	<0.001
whole (no CFL)	Ι	2,27	0.217	0.645	5.672	0.025	2,21	2.74	0.113	10.571	0.004	2,15	5.358	0.035	16.666	0.001
CFL)	S + I	2,26	1.356	0.275	8.85	0.001	2,20	3.647	0.045	7.763	0.003	2,14	14.461	<0.001	29.181	<0.001
	S	2,27	1.753	0.197	13.651	0.001	2,21	2.266	0.147	5.501	0.029	2,15	13.012	0.003	31.501	<0.001
proximal (no CFL)	Ι	2,27	0.296	0.591	4.764	0.038	2,21	1.232	0.28	6.399	0.02	2,15	6.176	0.025	17.244	0.001
	S + I	2,26	0.883	0.426	6.579	0.005	2,20	1.084	0.357	3.478	0.051	2,14	7.499	0.006	18.539	<0.001

Table S7. Results of comparisons between each major group via pANCOVA and ANCOVA, for the analysis of ΣF_{max}^* v. m_{body} in the hindlimb. Here results are reported for analyses where the caudofemoralis longus was excluded from the reptile dataset (cf. Table S3). Other conventions as per Table S2.

	toot		mammals v. reptiles					mai	nmals v. l	bipeds			rep	otiles v. bip	eds	
	test	df	F	Р	F^{\dagger}	P †	df	F	Р	F^{\dagger}	P †	df	F	Р	F†	P †
	S	2,27	2.233	0.147	11.462	0.002	2,21	9.148	0.006	14.712	0.001	2,15	24.923	<0.001	39.397	<0.001
whole (no CFL)	Ι	2,27	1.346	0.256	19.545	<0.001	2,21	2.706	0.115	7.013	0.015	2,15	7.568	0.015	28.675	<0.001
CIL)	S + I	2,26	1.432	0.257	10.501	0.001	2,20	4.418	0.026	7.102	0.005	2,14	13.441	0.001	31.071	<0.001
	S	2,27	2.684	0.113	10.109	0.004	2,21	6.167	0.022	9.047	0.007	2,15	17.953	0.001	28.47	<0.001
proximal (no CEL)	Ι	2,27	2.02	0.167	14.135	0.001	2,21	2.071	0.165	4.302	0.051	2,15	7.858	0.013	19.375	0.001
	S + I	2,26	1.784	0.188	7.901	0.002	2,20	2.948	0.076	4.323	0.028	2,14	9.657	0.002	18.261	<0.001

Table S8. Results of comparisons between each major group via pANCOVA and ANCOVA, for the analysis of L^* v. m_{body} in the hindlimb. Here results are reported for analyses where the caudofemoralis longus was excluded from the reptile dataset (cf. Table 4). Other conventions as per Table S2.

	test		mammals v. reptiles					mai	mmals v.	bipeds			rep	tiles v. bip	eds	
	test	df	F	Р	F†	P †	df	F	Р	$F\dagger$	P †	df	F	Р	F^{\dagger}	P†
	S	2,27	0.098	0.757	1.157	0.292	2,21	0.371	0.549	0.073	0.789	2,15	0.25	0.624	0.204	0.658
whole (no CFL)	Ι	2,27	0.001	0.974	0.305	0.585	2,21	0.624	0.438	1.437	0.244	2,15	0.681	0.422	1.375	0.259
CIL)	S + I	2,26	0.057	0.945	0.574	0.571	2,20	1.607	0.225	2.24	0.133	2,14	1.134	0.35	0.764	0.485
proximal (no CFL)	S	2,27	0.03	0.865	3.068	0.091	2,21	0.238	0.631	0.175	0.68	2,15	4×10^{-4}	0.985	0.731	0.406
	Ι	2,27	0.196	0.662	2.256	0.145	2,21	0.302	0.588	0.372	0.548	2,15	1.073	0.317	2.227	0.156
	S + I	2,26	0.094	0.91	1.616	0.218	2,20	0.828	0.452	0.966	0.398	2,14	0.805	0.467	1.061	0.372

Table S9. Results of comparisons between each major group via pANCOVA and ANCOVA, for the analysis of $\Sigma PCSA v$. Σm_{muscle} in the hindlimb. Here results are reported for analyses where the caudofemoralis longus was excluded from the reptile dataset (cf. Table 5). Other conventions as per Table S2.

	tost		mammals v. reptiles					mai	nmals v. l	bipeds			rep	tiles v. bip	eds	
	test	df	F	Р	F^{\dagger}	P †	df	F	Р	F†	P †	df	F	Р	$F\dagger$	P †
	S	2,27	0.28	0.601	0.28	0.601	2,21	5.272	0.032	5.272	0.032	2,15	1.264	0.279	1.264	0.279
whole (no CFL)	Ι	2,27	1.5	0.231	1.5	0.231	2,21	0.225	0.64	0.225	0.64	2,15	0.658	0.43	0.658	0.43
CFL)	S + I	2,26	0.725	0.494	0.725	0.494	2,20	2.676	0.093	2.676	0.093	2,14	1.253	0.316	1.253	0.316
proximal (no CFL)	S	2,27	0.073	0.789	0.073	0.789	2,21	2.84	0.107	2.84	0.107	2,15	0.716	0.411	0.716	0.411
	Ι	2,27	0.075	0.787	0.075	0.787	2,21	0.313	0.582	0.313	0.582	2,15	0.095	0.763	0.095	0.763
	S + I	2,26	0.049	0.952	0.049	0.952	2,20	1.743	0.201	1.743	0.201	2,14	0.538	0.596	0.538	0.596

Table S10. Pan-amniote regression (pGLS) coefficients for each comparison, for hindlimb analyses where the caudofemoralis longus was excluded from the reptile dataset. These are reported for data on a log_{10} scale; also reported is the mean percent prediction error (%PE). Note that the coefficients are computed excluding bipeds, given that bipeds frequently differ from quadrupedal species.

	slone under		whole limb			proximal	
comparison	isometry	slope	intercept	mean %PE	slope	intercept	mean %PE
Σm_{muscle} v. m_{body}	1.0	0.9812	-1.3788	63.91	1.0006	-1.4884	55.08
mean F_{max}^* v. m_{body}	-0.333	-0.3116	0.1999	39.35	-0.3181	0.2262	40.22
median F_{max}^* v. m_{body}	-0.333	-0.2926	0.0031	41.14	-0.2763	0.0369	46.03
ΣF_{max}^* v. m_{body}	-0.333	-0.3106	1.6691	50.52	-0.3022	1.5098	45.2
L^* v. m_{body}	0.333	0.2911	-1.5947	21.28	0.2988	-1.535	21.96
$\Sigma PCSA v. \Sigma m_{muscle}$	0.666	0.7118	-1.8378	22.31	0.7122	-1.9303	21.52

4. Supplemental code

Included with the Supplemental Information is R computer code used to perform all analyses in the study, along with necessary input data files (muscle data in .csv format, phylogenetic tree in .nwk format). The code also allows for estimation of a particular variable via the panamniote regression given some user-supplied input (e.g., body mass estimate for an extinct species), along with 95% prediction intervals.