**RESEARCH ARTICLE**

# Differences in scaling and morphology between lumbricid earthworm ecotypes

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## ABSTRACT

Many soft-bodied invertebrates use a flexible, fluid-filled hydrostatic skeleton for burrowing. The aim of our study was to compare the scaling and morphology between surface-dwelling and burrowing earthworm ecotypes to explore the specializations of non-rigid musculoskeletal systems for burrowing locomotion. We compared the scaling of adult lumbricid earthworms across species and ecotypes to determine whether linear dimensions were significantly associated with ecotype. We also compared the ontogenetic scaling of a burrowing species, *Lumbricus terrestris*, and a surface-dwelling species, *Eisenia fetida*, using glycol methacrylate histology. We show that burrowing species are longer, thinner and have higher length-to-diameter ratios than non-burrowers, and that *L. terrestris* is thinner for any given body mass compared with *E. fetida*. We also found differences in the size of the musculature between the two species that may correlate with surface crawling or burrowing. Our results suggest that adaptations to burrowing for soft-bodied animals include a disproportionately thin body and strong longitudinal muscles.

## INTRODUCTION

Burrowing is a difficult form of locomotion because of the abrasive, heterogeneous and dense nature of many substrates. Despite the challenges, many vertebrates and invertebrates ranging from micrometers to meters in length burrow effectively in a variety of substrates. Their burrowing actions alter the soil environment and aid in nutrient recycling, air and water infiltration, and soil decompaction. Many invertebrate burrowers lack rigid skeletal elements, relying instead on a hydrostatic skeleton consisting of a liquid-filled internal cavity surrounded by a muscular body wall (Chapman, 1958; Kier, 2012). When the muscles in the body wall contract, the internal fluid is pressurized, allowing for skeletal support, muscle antagonism, skeletal leverage, locomotion and other skeletal functions (Chapman, 1950, 1958; Alexander, 1995). The hydrostatic skeleton can also accommodate deformation in the body due to muscle contraction. Earthworms, for example, possess a fully segmented hydrostatic skeleton with two predominant muscle orientations present in each segment – circumferential and longitudinal. Circumferential muscle contraction elongates the worm, allowing it to move forward and excavate a new burrow; the longitudinal muscles expand the worm laterally, allowing for anchorage and burrow consolidation (Trueman, 1975). In addition, the radial straining of the soil by the longitudinal muscles breaks up soil particles ahead of the worm, reducing the pressure required for

axial elongation (Abdalla et al., 1969; Whalley and Dexter, 1994; Keudel and Schrader, 1999; Dorgan et al., 2008).

Soft-bodied burrowing invertebrates range in size from several hundred micrometers in length (e.g. nematodes) to several meters in length (e.g. earthworms) and burrow in a variety of terrestrial and marine environments. The effects of size on burrowing mechanics has not, however, been studied in detail (e.g. Piearce, 1983; Quillin, 2000; Che and Dorgan, 2010). In addition, the impact on subterranean organisms of anthropogenic changes in soil properties from chemicals and heavy machinery has been investigated previously, yet we do not know if there are size-dependent effects on burrowers (e.g. Ehlers, 1975; Roberts and Dorough, 1985; Chan and Barchia, 2007). This research may also provide insights important for the design of burrowing soft robots (e.g. Trimmer, 2008; Trivedi et al., 2008; Daltorio et al., 2013).

The physical characteristics of soil may impose size-dependent constraints on burrowers (Dorgan et al., 2008; Che and Dorgan, 2010; Kurth and Kier, 2014). For example, many soils exhibit strain hardening, in which the modulus of compression (stiffness) of the soil increases with increasing strain (Chen, 1975; Yong et al., 2012; Holtz et al., 2010). As an earthworm grows in cross-section, it must displace more soil radially as it burrows, which may result in an increase in the stiffness of the soil surrounding the burrow. Hatchling worms may avoid the strain hardening effect as a result of the relatively small volume of soil they must displace during burrowing. Adult earthworms, however, are often several times longer and wider than hatchlings and must create wider burrows in order to accommodate their bodies (Gerard, 1967; Quillin, 2000; Kurth and Kier, 2014). The formation of new burrows is common when conditions change or resources become scarce, forcing large worms to encounter and overcome strain hardening (Evans, 1947; Gerard, 1967). Thus, as a burrower grows there may be a selective advantage to becoming relatively thinner to mitigate the strain hardening effect (Piearce, 1983; Kurth and Kier, 2014).

In previous work, we showed that the burrowing earthworm *Lumbricus terrestris* becomes relatively thinner during growth and shows additional allometric changes in the musculature (Kurth and Kier, 2014). We hypothesized that these allometries may help to compensate for changes in strain hardening with growth. In order to examine this issue, we compared the linear dimensions of earthworms across ecotypes, as well as the ontogenetic scaling of a non-burrowing, surface-dwelling earthworm, *Eisenia fetida*. Not all earthworms burrow; there are three main ecotypes of earthworms that are largely differentiated by their burrowing patterns or lack thereof (Bouché, 1977). Surface-dwelling species like *E. fetida* are known as epigeic worms, which do not burrow and are instead found under leaf litter, in manure and under debris. There are also endogeic worms, which create ephemeral horizontal burrows in the upper 10–15 cm of soil and are geophagus (Edwards and Bohlen, 1996). Finally, there are anecic worms such as *L. terrestris* that build deep permanent/semi-permanent vertical burrows and feed on

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**List of symbols and abbreviations**

| | |
|---|---|
| $A$ | muscle cross-sectional area |
| $a$ | scaling constant |
| $b$ | scaling exponent |
| $D$ | diameter |
| $\mathbf{F}$ | force |
| $L$ | body length |
| $L/D$ | length to diameter ratio |
| $M$ | body mass |
| Mech adv | mechanical advantage |

surface litter (Keudel and Schrader, 1999). We refer to these three ecotypes as surface dwellers, horizontal burrowers and vertical burrowers, respectively.

We hypothesized that there would be both interspecific and ontogenetic scaling differences between earthworm ecotypes. We predicted that to mitigate strain hardening, the burrowing species would be thinner for any given body mass during development compared with surface dwellers, resulting in higher length-to-diameter ratios in the burrowing species. We also hypothesized that forces from the longitudinal musculature, which radially expand the worm during contraction, would be relatively larger and would develop more rapidly in the burrowers compared with the surface dwellers. These muscles are believed to be important in burrowing by anchoring the worm (with assistance from projections of hair-like setae), consolidating the burrow, relieving soil compaction ahead of the worm and pulling posterior segments into the burrow (Seymour, 1969; McKenzie and Dexter, 1988; Keudel and Schrader, 1999; Barnett et al., 2009). These muscles also move the bulk of soil during burrow formation and must generate sufficient force to overcome potential strain hardening effects in the soil (Barnett et al., 2009).

The setae that project outward during longitudinal muscle contraction are necessary in both crawling and burrowing to prevent backslip of the animal, and are similarly arranged in a 'lumbricine' pattern in all lumbricid earthworms (Sims and Gerard, 1985). Therefore, we focused our research on differences in longitudinal musculature instead of seta morphology or arrangement, although exploration of this topic may be of interest in future work.

In contrast to the longitudinal muscles, we predicted that forces from the circumferential muscles, which cause thinning and elongation of the worm, would be larger in the surface dwellers. The circumferential muscles are particularly important in surface crawling, extending the worm forward during each peristaltic wave of contraction and aiding penetration into litter and under debris; in fact, the largest pressures exerted in surface-crawling earthworms occur during circumferential muscle contraction (Gray and Lissman, 1938; Arthur, 1965; Seymour, 1969).

We also found significant interspecific and ontogenetic differences in scaling, consistent with our hypotheses (Kurth and Kier, 2014). Our results demonstrate that many aspects of the hydrostatic skeleton of earthworms develop in different ways between species, reflecting the ecological context of the organism.

### Scaling of functionally relevant morphological features

A variety of organisms, including *L. terrestris*, exhibit allometric growth, in which the relative proportions change with body size rather than remaining constant, as in isometric growth (Huxley and Tessier, 1936; Schmidt-Nielsen, 1997). Since the density of an animal typically does not change with size, the mass ($M$) is proportional to the volume ($V$). If an organism scales isometrically,

linear dimensions such as length ($L$) or diameter ($D$) are predicted to scale to the animal's $V^{1/3}$ and thus $M^{1/3}$ and any area, such as surface area or muscle cross-sectional area, will scale as $V^{2/3}$ and thus $M^{2/3}$.

In earthworms, the size and scaling of morphological features (e.g. diameter, muscle cross-sectional area) can vary from segment to segment down the length of the body (Quillin, 2000; Kurth and Kier, 2014). If an earthworm exhibits isometric growth, these morphological features must scale isometrically across all segments. To account for potential scaling differences across segments, multiple segments were measured in this study.

### Scaling of linear dimensions

In an isometrically scaling vermiform animal, the $L/D$ ratio will not change with size. This is because both $L$ and $D$ are linear dimensions and should scale as $M^{1/3}$ across segments. However, Kurth and Kier (2014) found allometry in the overall dimensions of *L. terrestris*, which changes the relative force and displacement of the musculature during growth (Kier and Smith, 1985; Vogel, 2013). An increase in the $L/D$ ratio during growth, as is found in *L. terrestris*, increases the distance advantage (the ratio of distance output to distance input) for the circumferential muscles and increases the mechanical advantage (the ratio of force output to force input) for the longitudinal muscles (Kier and Smith, 1985; Vogel, 2013; Kurth and Kier, 2014). Since mechanical advantage and distance advantage are reciprocal, an increase in the $L/D$ ratio decreases the mechanical advantage of the circumferential musculature and decreases the distance advantage of the longitudinal musculature.

We predict that burrowing species will have higher $L/D$ ratios than surface dwellers because surface dwellers are not under selective pressure to minimize their diameters for burrowing. We also predict that this trend will be reflected ontogenetically, with *L. terrestris* having a higher $L/D$ ratio and smaller diameter for a given body mass than *E. fetida*. A lower $L/D$ means that surface dwellers will have lower mechanical advantage during longitudinal muscle contraction and higher mechanical advantage during circumferential muscle contraction for a given size than burrowers.

### Scaling of muscle cross-sectional areas and force output
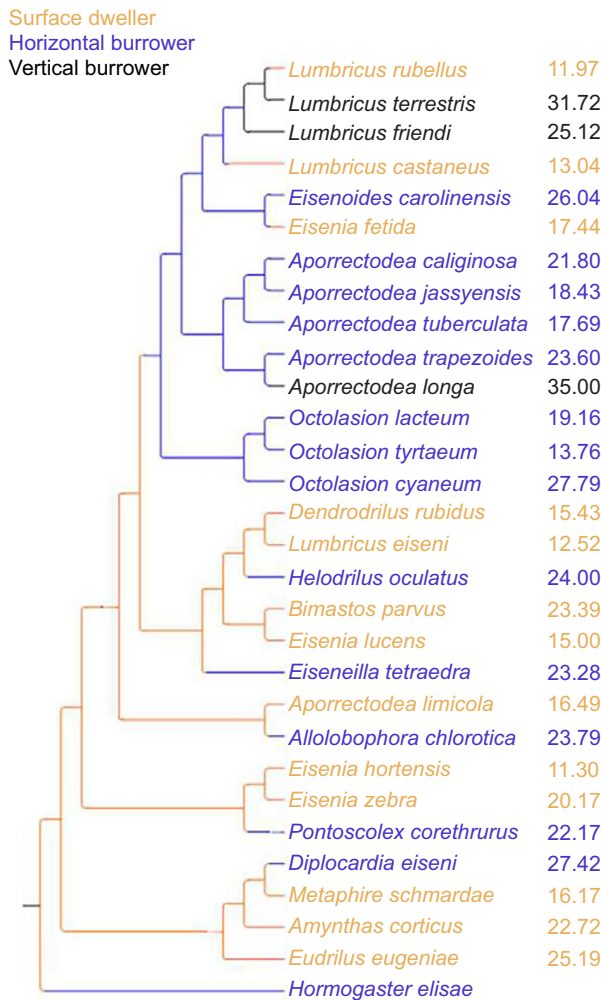
The scaling of muscle physiological cross-sectional area ($A$) determines how relative force production by the musculature changes with size, because force due to muscle contraction is proportional to cross-sectional area. If the circumferential and the longitudinal musculature scales isometrically, the cross-sectional area in each segment will be proportional to $M^{2/3}$. The final force output the animal exerts on the environment, however, is a product both of the force generated by the muscles and the mechanical advantage produced by the skeleton itself:

$$\mathbf{F} \alpha A \cdot \text{mech adv}, \tag{1}$$

where $\mathbf{F}$ is the force output to the environment, mech adv is the mechanical advantage and $A$ is the muscle cross-sectional area (Kurth and Kier, 2014). We predict that the scaling of force output for *E. fetida* will be lower during longitudinal muscle contraction but higher during circumferential muscle contraction than *L. terrestris*.
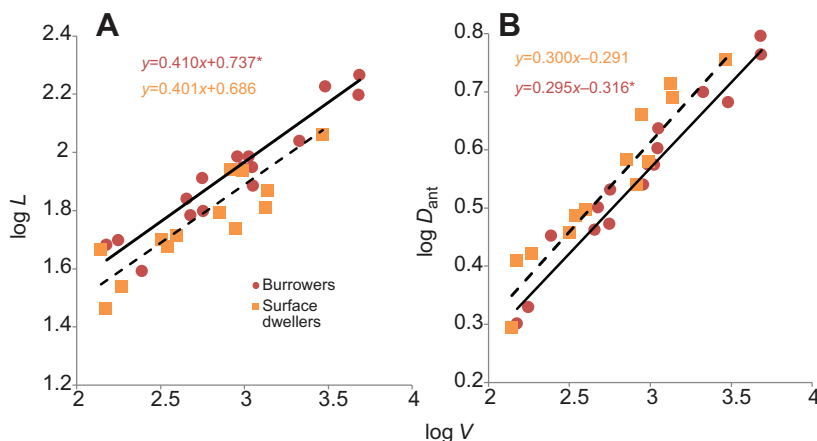
### RESULTS
#### Interspecific scaling of linear dimensions

Because $L/D$ is dimensionless, we first compared this value across species without respect to body size. We found a significant

Surface dweller
Horizontal burrower
Vertical burrower

| Species | L/D |
|---|---|
| *Lumbricus rubellus* | 11.97 |
| Lumbricus terrestris | 31.72 |
| Lumbricus friendi | 25.12 |
| *Lumbricus castaneus* | 13.04 |
| *Eisenoides carolinensis* | 26.04 |
| *Eisenia fetida* | 17.44 |
| *Aporrectodea caliginosa* | 21.80 |
| *Aporrectodea jassyensis* | 18.43 |
| *Aporrectodea tuberculata* | 17.69 |
| *Aporrectodea trapezoides* | 23.60 |
| Aporrectodea longa | 35.00 |
| *Octolasion lacteum* | 19.16 |
| *Octolasion tyrtaeum* | 13.76 |
| *Octolasion cyaneum* | 27.79 |
| *Dendrodrilus rubidus* | 15.43 |
| *Lumbricus eiseni* | 12.52 |
| *Helodrilus oculatus* | 24.00 |
| *Bimastos parvus* | 23.39 |
| *Eisenia lucens* | 15.00 |
| *Eiseneilla tetraedra* | 23.28 |
| *Aporrectodea limicola* | 16.49 |
| *Allolobophora chlorotica* | 23.79 |
| *Eisenia hortensis* | 11.30 |
| *Eisenia zebra* | 20.17 |
| *Pontoscolex corethrurus* | 22.17 |
| *Diplocardia eiseni* | 27.42 |
| *Metaphire schmardae* | 16.17 |
| *Amynthas corticus* | 22.72 |
| *Eudrilus eugeniae* | 25.19 |
| *Hormogaster elisae* | |

**Fig. 1. Simplified phylogenetic tree comparing *L/D* ratio and ecotype.** The phylogeny is based on a tree built by Pérez-Losada et al. (2012). Text colors indicate ecotype. The numbers adjacent to the phylogeny indicate the *L/D* value for each species. Each *L/D* value is an average from three adult specimens per species. No *Hormogaster elisae* specimens were available for analysis, so it was only used to root the phylogenetic tree.

relationship between *L/D* and ecotype across species and clades (independent contrasts, *P*<0.05; Fig. 1). Surface-dwelling worms generally had the lowest *L/D* of the three ecotypes, whereas vertical burrowers had the highest *L/D* ratios of the three ecotypes.

Horizontal burrowers had moderate *L/D*, which were significantly higher than surface dwellers and significantly lower than vertical burrowers (*P*<0.05).
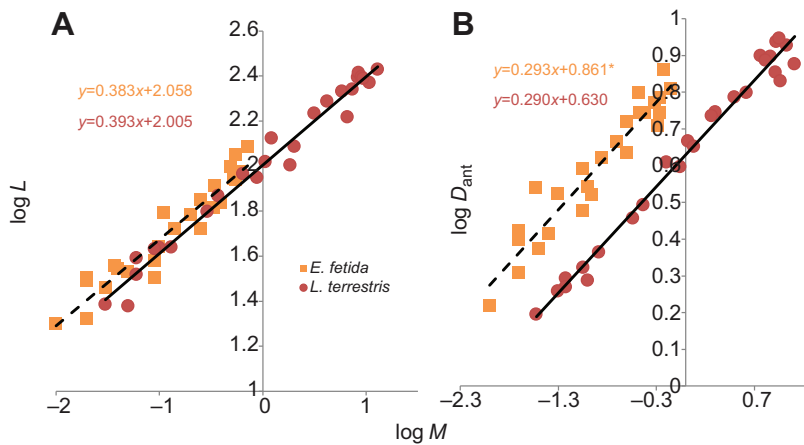
These differences in *L/D* ratios result from dissimilarities in the scaling of length and diameter between ecotypes (Fig. 2). We found that while both burrowing and surface-dwelling species increased in length with similar scaling exponents (*b*=0.410 and 0.401 for burrowers and surface dwellers, respectively), burrowing species were significantly longer for a given body volume than surface dwellers (*a*=0.737 and 0.686 for burrowers and surface dwellers, respectively). Burrowers and surface dwellers also increased in diameter at similar rates (*b*=0.295 and 0.300 for burrowers and surface dwellers, respectively), but burrowers were thinner for a given body volume than surface dwellers (*a*=−0.316 and −0.291 for burrowers and surface dwellers, respectively).

## Ontogenetic scaling of linear dimensions

We found a significant difference between the scaling of *L/D* between the two species (Fig. 3; Table 1). While both *L. terrestris* and *E. fetida* grew disproportionately long ($b_{Lt}$=0.393, $b_{Ef}$=0.383) and disproportionately thin ($b_{Lt}$=0.290, 0.275, 0.277; $b_{Ef}$=0.293, 0.300, 0.308; for anterior, middle and posterior segments) at similar rates, *E. fetida* was always significantly wider at a given body mass than *L. terrestris,* as shown by the differences in log *a*, the *y*-intercept of the log-transformed graph (log $a_{Lt}$=0.630, 0.605, 0.550; log $a_{Ef}$=0.861, 0.883, 0.850 for anterior, middle and posterior segments, respectively) (Table 1; supplementary material Fig. S1). Because of these differences in diameter, *E. fetida* had a lower *L/D* for any given body mass compared with *L. terrestris* (log $a_{Lt}$=1.407, log $a_{Ef}$=1.202; averaged across segments) despite a similar increase in *L/D* with size for both species ($b_{Lt}$=0.114, $b_{Ef}$=0.087; averaged across segments) (supplementary material Fig. S2).

## Ontogenetic scaling of muscle cross-sectional area

We found differences in muscle cross-sectional area between species (Fig. 4; Table 2). In the anterior segments, *L. terrestris* had larger longitudinal muscles for a given body mass than *E. fetida* (log $a_{Lt}$=0.511; log $a_{Ef}$=0.354) and its longitudinal muscles grew at faster rates than those of *E. fetida* ($b_{Lt}$=0.612; $b_{Ef}$=0.539), but these differences were not statistically significant (Fig. 4). Conversely, *E. fetida* had larger anterior circumferential muscles at a given body mass than *L. terrestris* (log $a_{Lt}$=−0.713; log $a_{Ef}$=−0.640), despite a faster growth of these muscles in *L. terrestris* ($b_{Lt}$=0.674; $b_{Ef}$=0.543). Muscles in the middle and posterior segments were more similar in



**Fig. 2. Interspecific differences in the scaling of linear dimensions.** (A) Log-transformed graph comparing body length (*L*) with body volume between burrowing and surface dwelling adult lumbricid species. (B) Log-transformed graph comparing anterior diameter (*D*ant) with body volume between burrowing and surface-dwelling lumbricid species. The regressions shown in A and B were fitted to empirical data using OLS regression (solid line for burrowers, dashed line for surface dwellers) and the regression equations for both ecotypes are shown. Asterisk indicates a significant difference between species with the Bonferroni correction. *N*=13 surface-dwelling species; *N*=15 burrowing species.

In panel A: y=0.410x+0.737*; y=0.401x+0.686. In panel B: y=0.300x−0.291; y=0.295x−0.316*. Legend: Burrowers; Surface dwellers.

**Fig. 3. Ontogenetic scaling of linear dimensions.** (A) Log-transformed graph comparing body length ($L$) with body mass ($M$) for *L. terrestris* and *E. fetida*. (B) Log-transformed graph comparing anterior diameter ($D_{ant}$) with body mass for *L. terrestris* and *E. fetida*. The regressions shown in A and B were fitted to empirical data using OLS regression (solid line for *L. terrestris*, dashed line for *E. fetida*) and the regression equations for both species are shown. Asterisk indicates a significant difference between species with the Bonferroni correction. $N=25$ per species.

the two species (Table 2; supplementary material Figs S3 and S4). The longitudinal muscles from the middle segments scaled similarly ($b_{Lt}$=0.541; $b_{Ef}$=0.552) and were similar in cross-sectional area at a given body mass (log $a_{Lt}$=0.375; log $a_{Ef}$=0.392), whereas the circumferential muscles grew at a faster rate in *L. terrestris* ($b_{Lt}$=0.800; $b_{Ef}$=0.627) but were larger at a given body mass in *E. fetida* (log $a_{Lt}$=−0.974; log $a_{Ef}$=−0.731). The posterior longitudinal segments showed the opposite scaling trend from the anterior segments; the longitudinal muscles of *E. fetida* increased in cross-sectional area at a faster rate ($b_{Lt}$=0.564; $b_{Ef}$=0.640) and were larger at a given body mass (log $a_{Lt}$=0.379; log $a_{Ef}$=0.437) though these differences were not statistically significant. The posterior circumferential muscles showed no significant difference in scaling exponents between the two species ($b_{Lt}$=0.792; $b_{Ef}$=0.743), although the circumferential muscle cross-sectional area of *E. fetida* was larger at a given body mass than that of *L. terrestris* (log $a_{Lt}$=−1.048; log $a_{Ef}$=−0.609).

## Ontogenetic scaling of calculated mechanical advantage and force production

Because the $L/D$ ratio increased in both *E. fetida* and *L. terrestris,* both had similar trends in the scaling of mechanical advantage (Fig. 5). We calculated increases in mechanical advantage during longitudinal muscle contraction for both species ($b_{Lt}$=0.104; $b_{Ef}$=0.078); *L. terrestris* had higher mechanical advantage for a given body mass than *E. fetida* ($a_{Lt}$=1.872; $a_{Ef}$=1.649). The mechanical advantage of the circumferential muscle decreased with growth in both species ($b_{Lt}$=−0.104; $b_{Ef}$=−0.078), but *L. terrestris* exhibited lower mechanical advantage at a given body mass (log $a_{Lt}$=−1.872; log $a_{Ef}$=−1.649).

We also found significant differences in the scaling of calculated force production between the two species (Fig. 6; Table 3). In the anterior segments (Fig. 6), we found calculated force output during longitudinal muscle contraction at any given body mass was greater

for *L. terrestris* than for *E. fetida* (log $a_{Lt}$=2.383; $a_{Ef}$=2.003). Calculated longitudinal muscle force production increased at a greater rate with mass in *L. terrestris* than *E. fetida* (log $b_{Lt}$=0.716; $b_{Ef}$=0.617), although this difference was not statistically significant with the Bonferroni correction. In the case of calculated circumferential muscle force production, however, *E. fetida* had a greater force output at a given body mass than did *L. terrestris* (log $a_{Lt}$=−2.584; log $a_{Ef}$=−2.288), but both species had similar growth rates ($b_{Lt}$=0.568; $b_{Ef}$=0.465).

We found that most of the differences in force production between *E. fetida* and *L. terrestris* were consistent across segments (Table 3; supplementary material Figs S5 and S6). Calculated longitudinal muscle force production in the middle and posterior segments was greater for a given mass in *L. terrestris* than *E. fetida* (log $a_{Lt}$=2.245; log $a_{Ef}$=2.041 in middle segments; log $a_{Lt}$=2.251; log $a_{Ef}$=2.086 in posterior segments), but these segments did not show significant inter-specific differences in the rates of longitudinal muscle force production with mass ($b_{Lt}$=0.649, $b_{Ef}$=0.630 in middle segments; log $b_{Lt}$=0.668, log $b_{Ef}$=0.717 in posterior segments). Circumferential muscle force production in the middle and posterior segments also exhibited similar trends to the anterior segments, with higher intercepts in *E. fetida* (log $a_{Lt}$=−2.838, log $a_{Ef}$=−2.380 in the middle segments; log $a_{Lt}$=−2.920, log $a_{Ef}$=−2.258 in the posterior segments) and similar scaling exponents between the two species ($b_{Lt}$=0.681; $b_{Ef}$=0.550 in the middle segments; $b_{Lt}$=0.688; $b_{Ef}$=0.665 in the posterior segments).

## DISCUSSION

Previous studies found that the hydrostatic skeleton in *L. terrestris* scales allometrically, but the reasons for these growth patterns remain unclear (Quillin, 2000; Kurth and Kier, 2014). We hypothesized that one important factor may be compensation for increases in soil strain hardening as the animal becomes larger. We tested this hypothesis by comparing the hydrostatic skeleton across

**Table 1. Scaling of linear dimensions in the vertical burrower *L. terrestris* and the surface dweller *E. fetida***

| Linear dimensions ($y$) | *L. terrestris* intercept (log $a_{Lt}$) | *E. fetida* intercept (log $a_{Ef}$) | $P$ | *L. terrestris* scaling exponent ($b_{Lt}$) | *E. fetida* scaling exponent ($b_{Ef}$) | $P$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| $L$ | 2.005 | 2.058 | **0.005** | 0.393 | 0.383 | 0.646 | 0.912 |
| $D_{ant}$ | 0.630 | 0.861 | **$2.0 \times 10^{-16}$*** | 0.290 | 0.293 | 0.849 | 0.911 |
| $D_{mid}$ | 0.605 | 0.883 | **$2.0 \times 10^{-16}$*** | 0.275 | 0.300 | 0.215 | 0.909 |
| $D_{post}$ | 0.550 | 0.850 | **$2.0 \times 10^{-16}$*** | 0.277 | 0.308 | 0.134 | 0.958 |

Length refers to body length. Locations sampled: ant, anterior; mid, middle; post, posterior corresponding to segment number 10, 30 and 50, respectively, from the anterior. An ANCOVA was used on empirical data fit by OLS to compare the intercepts (log $a_{Lt}$ and log $a_{Ef}$) and slopes ($b_{Lt}$ and $b_{Ef}$) between the two species. * Indicates a significant difference between species with the Bonferroni correction. $N=25$.
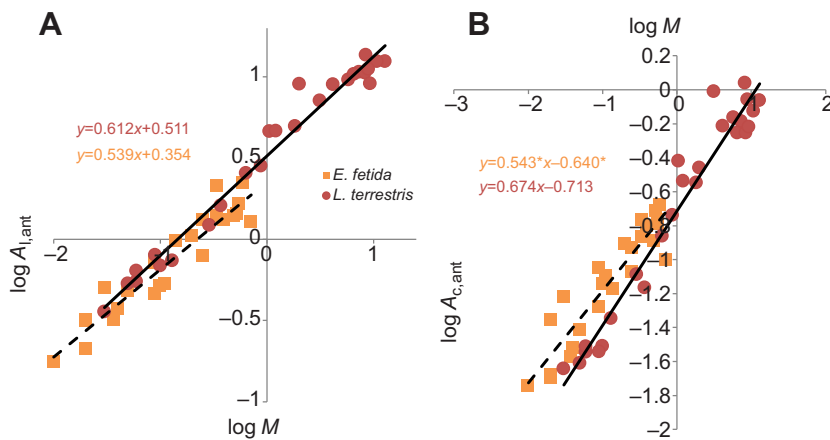
**Fig. 4. Ontogenetic scaling of muscle cross-sectional areas.** (A) Log-transformed graph comparing longitudinal muscle cross-sectional area in the anterior segments ($A_{l,ant}$) to body mass for *L. terrestris* and *E. fetida*. (B) Log-transformed graph comparing circumferential muscle cross-sectional area in the anterior segments ($A_{c,ant}$) with body mass for *L. terrestris* and *E. fetida* in the anterior segments. The regressions shown in Fig. 1A,B were fitted to empirical data using OLS regression (solid line for *L. terrestris*, dashed line for *E. fetida*) and the regression equations for both species are shown. Asterisk indicates a significant difference between species with the Bonferroni correction. $N=25$ per species.

ecotypes in earthworms using interspecific and ontogenetic methods. Our results are consistent with the strain hardening hypothesis and suggest that a disproportionately thin diameter and large forces during longitudinal muscle contractions are key burrowing adaptations in soft-bodied animals.

### Linear dimensions and mechanical advantage

We found that burrowing species across clades had higher $L/D$ ratios than surface dwellers, consistent with previous research by Piearce (1983). These differences in $L/D$ were reflected in both the interspecific and ontogenetic scaling of linear dimensions. The interspecific scaling comparison shows that both ecotypes grew disproportionately long and thin, but burrowing species were significantly longer and thinner than surface-dwelling species. Ontogenetically, both the burrowing *L. terrestris* and surface-dwelling *E. fetida* also grew disproportionately long and thin. At any given body mass, however, *L. terrestris* was significantly thinner than *E. fetida*. Since burrowers would experience greater selective pressures for thin bodies than surface dwellers in order to mitigate strain hardening underground, our interspecific and ontogenetic results are consistent with the strain hardening hypothesis.

Ontogenetic changes in mechanical advantage showed similar trends between species since both increased their $L/D$ ratio during growth. For both species, mechanical advantage increased with body size for longitudinal muscle contraction and decreased with body size for circumferential muscle contraction. The magnitudes of mechanical advantage, however, differed between the two species because of differences in $L/D$ ratios. *Lumbricus terrestris* had greater mechanical advantage during longitudinal muscle contraction, whereas *E. fetida* had greater mechanical advantage

during circumferential muscle contraction. We believe that these differences in mechanical advantage highlight the relative importance of the longitudinal and circumferential muscles in burrowing and crawling, respectively, as discussed below.

We found it surprising that, for *E. fetida*, the mechanical advantage during circumferential muscle contraction decreased with growth, given the importance of circumferential muscles in surface crawling (Gray and Lissman, 1938; Seymour, 1969). As we discuss below, however, an increase in circumferential cross-sectional area appears to compensate for the loss of mechanical advantage so that the circumferential muscles in *E. fetida* are significantly larger than those in *L. terrestris*.

### Differences in calculated force production

The segments measured in *E. fetida* are estimated to produce significantly higher circumferential forces and significantly lower longitudinal muscle forces along the length of the body when compared with similar segments in *L. terrestris*. These differences agree with previous research suggesting that circumferential muscles are of great importance for crawling while the longitudinal muscles are essential for burrowing (Chapman, 1950; Seymour, 1969). Powerful circumferential muscle forces would permit surface-dwelling worms to squeeze in between rocks, litter and debris on the surface, allowing these worms access to new habitats. Conversely, robust longitudinal muscle forces would allow burrowing earthworms to overcome strain hardening in soil by exerting sufficient force to laterally displace soil, expand the burrow walls, break up soil particles ahead of the burrow, anchor the worm (with assistance from the setae) and pull posterior segments into the burrow (Seymour, 1969; McKenzie and Dexter, 1988; Keudel and Schrader, 1999; Barnett et al., 2009).

**Table 2. Scaling of muscle cross-sectional area in the vertical burrower *L. terrestris* and the surface dweller *E. fetida***

| Muscle area (y) | *L. terrestris* intercept (log $a_{Lt}$) | *E. fetida* intercept (log $a_{Ef}$) | P | *L. terrestris* scaling exponent ($b_{Lt}$) | *E. fetida* scaling exponent ($b_{Ef}$) | P | $R^2$ |
|---|---|---|---|---|---|---|---|
| $A_{l,ant}$ | 0.512 | 0.354 | **0.034** | 0.612 | 0.539 | 0.080 | 0.903 |
| $A_{l,mid}$ | 0.375 | 0.392 | 0.514 | 0.541 | 0.552 | 0.595 | 0.930 |
| $A_{l,post}$ | 0.379 | 0.437 | 0.511 | 0.564 | 0.640 | 0.472 | 0.962 |
| $A_{c,ant}$ | −0.713 | −0.640 | **0.001*** | 0.674 | 0.543 | **<0.05*** | 0.862 |
| $A_{c,mid}$ | −0.974 | −0.731 | **$6.4×10^{-9}$*** | 0.800 | 0.627 | **<0.05*** | 0.853 |
| $A_{c,post}$ | −1.048 | −0.609 | **$7.2×10^{-12}$*** | 0.792 | 0.743 | 0.090 | 0.838 |

$A_l$ refers to longitudinal muscle cross-sectional area; $A_c$ refers to circumferential muscle cross-sectional area. Locations sampled: ant, anterior; mid, middle; post, posterior corresponding to segment number 10, 30 and 50, respectively, from the anterior. An ANCOVA was used on empirical data fit by OLS to compare the intercepts (log $a_{Lt}$ and log $a_{Ef}$) and slopes ($b_{Lt}$ and $b_{Ef}$) between the two species. * Indicates a significant difference between species with the Bonferroni correction. $N=25$.
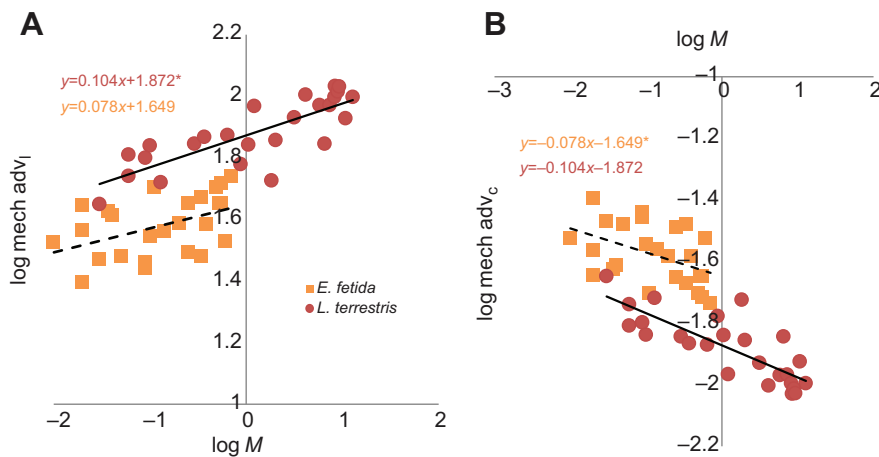
**Fig. 5. Comparison of calculated mechanical advantage with body mass.** Mechanical advantage was calculated by normalizing each worm's average $L/D$ across segments with mass and calculating the reciprocal of distance advantage over 25% radial strain. (A) Mechanical advantage from longitudinal muscle contraction (mech $\text{adv}_l$) and (B) circular muscle contraction (mech $\text{adv}_c$) mechanical advantage as a function of earthworm body mass. Asterisk indicates a significant difference between species with the Bonferroni correction. $N=25$ per species.

## Scaling similarities

Although our results showed significant differences in the magnitude of musculoskeletal dimensions and calculated forces (i.e. different intercepts) between surface dwellers and burrowers, it is unclear why both burrowers and surface dwellers exhibit scaling similarities (i.e. similar scaling exponents). For example, both burrowing and surface-dwelling ecotypes grow disproportionately long and thin and are predicted to exhibit similar increases in circumferential and longitudinal muscle forces with size. These shared scaling trends may be the result of ecological, physiological or functional similarities between the species.

For instance, both ecotypes may growth disproportionately thin because the relative surface area for gas exchange would be enhanced in larger individuals. Since the burrowing earthworms are more likely to encounter hypoxic regions than surface dwellers, there may be increased selection pressure for a high $L/D$ ratio in burrowing species.

Similar increases in the rates of force production with size may result from the shared functions of these muscles across ecotypes. The circumferential muscles in all earthworms must grow sufficiently powerful to push the animal forward and excavate through debris or soil. The longitudinal muscles in all species must provide sufficient forces to anchor the earthworm, prevent backslip,

pull posterior segments forward and push away constrictive soil or debris.

## MATERIALS AND METHODS

### Interspecific measurements and phylogenetic reconstruction

We used sexually mature earthworm specimens preserved in 70–95% ethanol in the collections of the Smithsonian Institution Museum of Natural History (Washington, DC). A phylogeny is available of species in the Lumbricidae family (Pérez-Losada et al., 2012), so we focused our analysis on genera from this family to avoid pseudoreplication (Felsenstein, 1985). We further narrowed the study by only comparing lumbricid species whose ecotypes are well documented, for a total of 29 species studied (Bouché, 1977; Sims and Gerard, 1985; Edwards and Bohlen, 1996). For each species, we chose museum specimens with the most recent fixation dates to minimize tissue distortion from fixation.

Most worms appeared elongate in fixation, and we selected only these elongate worms for measurement to ensure consistent resting lengths across specimens. To measure each species' linear dimensions, we first pulled each worm by the anterior end along the bench surface in order to straighten the body and ensure similar elongation. We used calipers to measure the length and anterior diameter of three adult specimens per species (for a total of 87 worms measured) and calculated an average length and diameter, which was then used to calculate the length-to-diameter ratio. We were also able to compare the interspecific scaling of linear dimensions in burrowing and surface-dwelling ecotypes. Because many specimens we measured had been
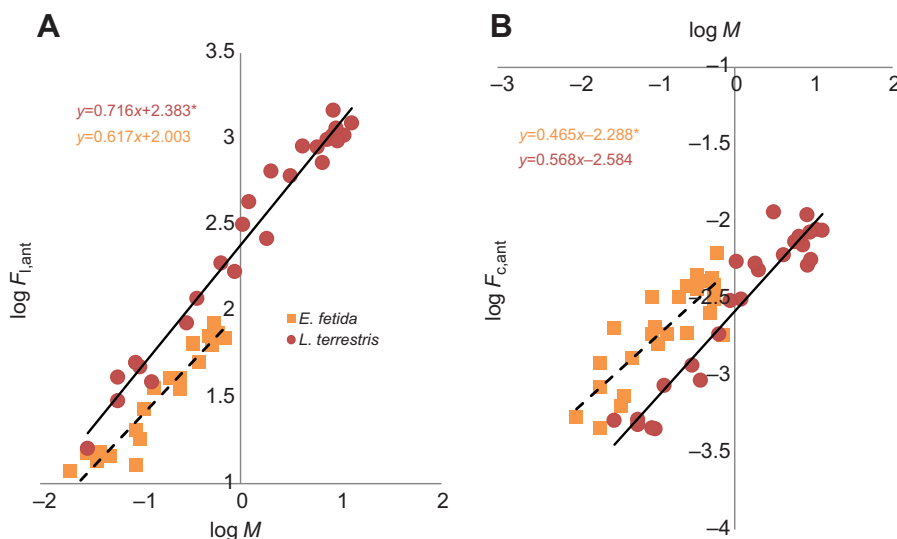


**Fig. 6. Comparison of calculated force production with body mass.** Force production was estimated for each worm using its mechanical advantage and muscle cross-sectional area. Mechanical advantage was calculated by normalizing each worm's $L/D$ ratio with mass and calculating the reciprocal of distance advantage over 25% radial strain (A) Force production during anterior longitudinal muscle contraction ($F_{l,ant}$) and (B) force production during anterior circumferential muscle contraction ($F_{c,ant}$) as a function of earthworm body mass. Asterisk indicates a significant difference between species with the Bonferroni correction. $N=25$ per species.

**Table 3. Scaling of calculated force production in the vertical burrower *L. terrestris* and the surface dweller *E. fetida***

| Linear dimension ($y$) | *L. terrestris* intercept (log $a_{Lt}$) | *E. fetida* intercept (log $a_{Ef}$) | $P$ | *L. terrestris* scaling exponent ($b_{Lt}$) | *E. fetida* scaling exponent ($b_{Ef}$) | $P$ | $R^2$ |
|---|---|---|---|---|---|---|---|
| $F_{l,ant}$ | 2.383 | 2.003 | $9.13 \times 10^{-13}$* | 0.716 | 0.617 | <0.05 | 0.946 |
| $F_{l,mid}$ | 2.245 | 2.041 | $2.11 \times 10^{-7}$* | 0.649 | 0.630 | 0.633 | 0.946 |
| $F_{l,post}$ | 2.251 | 2.086 | $1.39 \times 10^{-5}$* | 0.668 | 0.717 | 0.334 | 0.916 |
| $F_{c,ant}$ | −2.584 | −2.288 | $9.59 \times 10^{-8}$* | 0.568 | 0.465 | 0.154 | 0.703 |
| $F_{c,mid}$ | −2.838 | −2.380 | $1.08 \times 10^{-12}$* | 0.681 | 0.550 | 0.066 | 0.759 |
| $F_{c,post}$ | −2.920 | −2.258 | $3.43 \times 10^{-15}$* | 0.688 | 0.665 | 0.759 | 0.783 |

Calculated force production was estimated for each worm using its mechanical advantage and muscle cross-sectional area. Mechanical advantage was calculated by normalizing each worm's $L/D$ ratio with mass and calculating the reciprocal of distance advantage over 25% radial strain. $F_l$ and $F_c$ refer to longitudinal muscle and circumferential muscle force output, respectively. Locations sampled: ant, anterior; mid, middle; post, posterior. * Indicates a significant difference between species with the Bonferroni correction. $N$=25.

dissected and were missing inner organs, we used body volume as a proxy for body mass. No *Hormogaster elisae* specimens were available for analysis, so it was only used to root the phylogenetic tree as discussed below.

We used TreeGraph2™ (Stöver and Müller, 2010) to construct a simplified phylogeny based on a lumbricid earthworm phylogeny by Pérez-Losada et al. (2012). Pérez-Losada et al. (2012) used molecular data from multiple specimens of each species, which resulted in significant variation in branch length and branch placement between specimens within a species. The authors attributed this variation to the sampling of cryptic species. Because we do not know which specimens were misidentified, we simplified the phylogeny by placing each species in the clade where most specimens per species appeared. Because of the high variation and uncertainty in branch length, we also made all branch lengths equal in our simplified tree. Although this reduces our statistical power, the reduction is relatively minor and tends to produce only false negative results (Grafen, 1989; Martins and Garland, 1991; Swenson, 2009).

### *Eisenia fetida* collection and maintenance

*Eisenia fetida* earthworms were supplied by Uncle Jim's Worm Farm (Spring Grove, PA, USA) as well as raised from hatchlings bred in a colony maintained in the laboratory. Adult worms (~0.1–0.7 g) were purchased, raised from purchased juveniles and colony hatchlings. Hatchlings were raised from cocoons deposited by adults bred in the laboratory colony. All worms were housed in plastic bins filled with moist peat moss (Inouye et al., 2006) at 15°C (Presley et al., 1996) and fed dried infant oatmeal (Ownby et al., 2005).

### Histology and morphometrics

The measurements and calculations follow those described in Kurth and Kier (2014) for *L. terrestris* in order to allow consistent comparisons between *E. fetida* and *L. terrestris*. Each *E. fetida* worm was anesthetized in a 10% ethanol solution in distilled water (v/v) until quiescent, patted dry and weighed. The length was obtained after dragging the worm by the anterior end along the lab bench to straighten the body and extend the segments to a consistent resting length. Because *E. fetida* does not add segments with growth, we measured the length of the entire body (supplementary material Fig. S7). The worm was then killed and three blocks of tissue containing 20 segments each were removed (segments 1–20, 21–40 and 41–60, numbering from anterior) to account for morphological differences across segments. We focused on segments in the anterior half of the worm since it is of greatest importance in locomotion (Yapp, 1956).

The tissue blocks were fixed in 10% formalin in distilled water (v/v) for 24–48 h. After fixation, the blocks were further dissected for embedding (segments 9–14, 29–34 and 49–54). We refer to segments 9–14 as 'anterior', segments 29–34 as 'middle' and segments 49–54 as 'posterior'. The anterior, middle and posterior segments were then cut so that both transverse and sagittal sections could be obtained from each location.

The tissue blocks were partially dehydrated in 95% ethanol and embedded in glycol methacrylate plastic (Technovit 7100, Heraeus Kulzer GmbH, Wehrheim, Germany). Sections of 3–7 μm thickness were cut with a glass knife. We used a Picrosirius/Fast Green stain in order to differentiate muscle from connective tissue (López-De León and Rojkind, 1985). We stained the slides at 60°C for 1–2 h then rinsed the slides in distilled water, dried them and mounted coverslips. We used Sigma Scan (Systat Software, San Jose, CA, USA) to make morphological measurements on micrographs. Longitudinal muscle cross-sectional area ($A_l$) and diameter ($D$) were measured from transverse sections, whereas circumferential muscle cross-sectional area ($A_c$) was measured from sagittal sections (Fig. 7). The earthworms prepared in this way were flattened slightly and thus had an elliptical cross-section. To determine an equivalent diameter of a circular cylinder we measured the major and minor axes, calculated the area of the ellipse and then calculated the diameter of a circle of the same area.
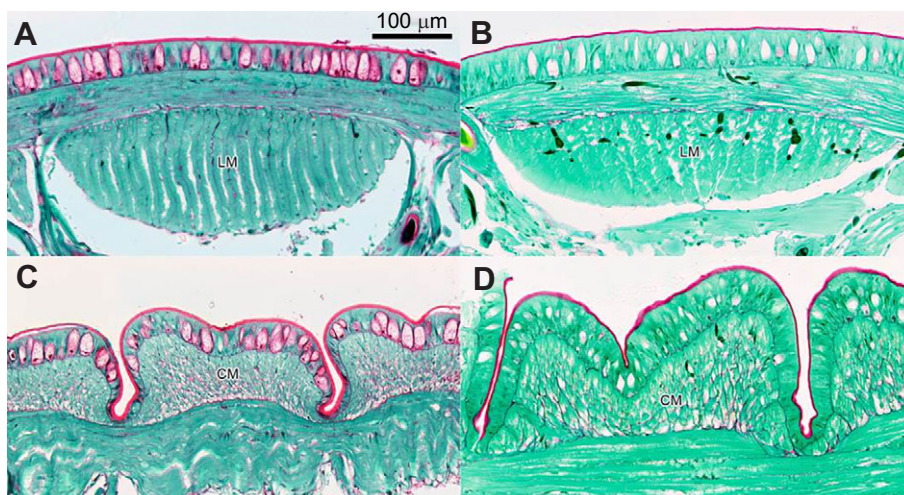


**Fig. 7. Photomicrographs of 0.1 g *Eisenia fetida* and 0.1 g *Lumbricus terrestris* specimens.** All sections were 7 μm in thickness and were stained with Picrosirius/Fast Green. (A) Transverse section of *L. terrestris* showing the cross-sectional area of the longitudinal musculature. (B) Transverse section of *E. fetida* showing the cross-sectional area of the longitudinal musculature. (C) Parasagittal section of *L. terrestris* showing the cross-sectional area of the circumferential musculature. (D) Parasagittal section of *E. fetida* showing the cross-sectional area of the circumferential musculature. LM, longitudinal muscle; CM, circumferential muscle.

## Calculation of mechanical advantage and force output

The scaling of mechanical advantage was calculated using the average $L/D$ ratio across segments for each worm. The average $L/D$ ratio was observed to change as a function of size and thus the mechanical advantage of the musculature changes during growth (supplementary material Fig. S2; Kier and Smith, 1985; Kurth and Kier, 2014). Since the mechanical advantage is the reciprocal of the distance advantage, we calculated the mechanical advantage of the circumferential musculature as the absolute value of the decrease in body diameter ($D$) during circumferential muscle contraction divided by the resulting increase in body length ($L$), as a function of the $L/D$ ratio, for a 25% decrease body in diameter. Kinematic data for *E. fetida* are unavailable but a 25% change in body diameter has been empirically recorded from kinematic data in *L. terrestris* during crawling (Quillin, 1999). Since *L. terrestris* and *E. fetida* are closely related phylogenetically, we believe that a 25% change in diameter is a reasonable assumption for both species. Likewise, the mechanical advantage of the longitudinal muscle was calculated as the absolute value of the decrease in body length of the worm divided by the resulting increase in body diameter, as a function of the $L/D$ ratio:

$$\text{mech adv}_c = \frac{|\Delta D|}{|\Delta L|}; \text{mech adv}_l = \frac{|\Delta L|}{|\Delta D|}. \quad (2)$$

These calculations thus provided estimates of the mechanical advantage of both the longitudinal (l) and circumferential (c) musculature as a function of size.

Force production was calculated in each worm as the product of mechanical advantage and muscle cross-sectional area in both the circumferential and longitudinal muscles. We made the assumption of constant stress in the muscles with ontogeny, though this assumption has not been empirically tested in obliquely striated muscle.

## Statistical analysis

We used R statistical software for both the phylogenetic and ontogenetic analyses (R Development Core Team, 2014). We used the *ape* package in R (Paradis et al., 2004) to perform independent contrasts on the phylogeny for statistical analysis. Independent contrasts allowed us to test for correlations between ecotype and $L/D$ ratio while avoiding pseudoreplication (Felsenstein, 1985). We treated ecotype as a continuous variable to allow for transitional/intermediate ecotypes in ancestral nodes.

We also used linear regression on log transformed interspecific and ontogenetic scaling data. We fitted both sets of scaling data to the power function $y=aM^b$, where $y$ represents the morphological trait of interest, $a$ is the scaling constant, $M$ is body mass and $b$ is the scaling exponent. Log transforming these data allowed us to perform regression analyses, as $b$ becomes the slope of the line and log $a$ becomes the intercept.

We used the *caper* function (Orme et al., 2012) in R (R Development Core Team, 2014) to perform phylogenetically corrected regression on the interspecific scaling data. We pooled horizontal and vertical burrowers together for this analysis because only three vertical burrowing species were measured, and all three were similar in body size. To test for differences in slope and intercept between burrowing and surface-dwelling ecotypes, we performed an ANCOVA analysis on the phylogenetically corrected regression data. Although there may be error in the x-variable (i.e. volume) that is not accounted for in a standard ANCOVA, ANCOVAs using model II regression techniques are not well developed or commonly used (Sokal and Rohlf, 1994). Thus, standard ANCOVAs are still commonly used in scaling studies (e.g. Niven and Scharlemann, 2005; Davies and Moyes, 2007; Snelling et al., 2011; Xu et al., 2014).

We used the *lmodel2* package (Legendre, 2011) in R to perform ordinary least squares (OLS) and reduced major axis regression (RMA) on the ontogenetic scaling data. In our ontogenetic analysis, the symbols $b_{Lt}$ and log $a_{Lt}$ denote the slopes and intercepts of *L. terrestris*, while the symbols $b_{Ef}$ and log $a_{Ef}$ denote the slopes and intercepts of *E. fetida*. To determine differences in slope and intercept between the two species, we used a standard ANCOVA. We also compared RMA scaling exponent $b_{Lt}$ and constant $a_{Lt}$ for *L. terrestris* against the corresponding 95% confidence intervals for *E. fetida* (Heins et al., 2004). Our data generally showed high

coefficients of determination ($R^2>0.85$) and both OLS regression and RMA regression fit similar scaling exponents in our analysis and were consistent in distinguishing significant scaling differences between species. Because of the similarity and agreement between the models, only the ANCOVA and OLS results for both species are reported to remain consistent with the statistical reporting from the interspecific scaling study. To account for multiple comparisons, a Bonferroni correction was used on the $P$-value outputs from the ANCOVAs. Most $P$-values remained significant.

### Supplementary material
Supplementary material available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.119305/-/DC1

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