

Femoral morphology of sciuriform rodents in light of scaling and locomotor ecology

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Abstract

Sciuriform rodents are a monophyletic group comprising about 300 species with a body mass range spanning three orders of magnitude and various locomotor behaviors that we categorized into arboreal, fossorial and aerial. The purpose of this study was to investigate how the interplay of locomotor ecology and body mass affects the morphology of the sciuriform locomotor apparatus. The most proximal skeletal element of the hind limb, i.e. the femur, was selected, because it was shown to reflect a functional signal in various mammalian taxa. We analyzed univariate traits (effective femoral length, various robustness variables and the in-levers of the muscles attaching to the greater, third and lesser trochanters) as well as femoral shape, representing a multivariate trait. An ordinary least-squares regression including 177 species was used to test for a significant interaction effect between body mass and locomotor ecology on the variables. Specifically, it tested whether the scaling patterns of the fossorial and aerial groups differ when compared with the arboreal, because the latter was identified as the ancestral sciuriform condition via stochastic character mapping. We expected aerial species to display the highest trait values for a given body mass as well as the steepest slopes, followed by the arboreal and fossorial species along this order. An Ornstein–Uhlenbeck regression fitted to a phylogenetically pruned dataset of 140 species revealed the phylogenetic inertia to be very low in the univariate traits, hence justifying the utilization of standard regressions. These variables generally scaled close to isometry, suggesting that scaling adjustments might not have played a major role for most of the femoral features. Nevertheless, the low phylogenetic inertia indicates that the observed scaling patterns needed to be maintained during sciuriform evolution. Significant interaction effects were discovered in the femoral length, the centroid size of the condyles, and the in-levers of the greater and third trochanters. Additionally, adjustments in various femoral traits reflect the acquisitions of fossorial and aerial behaviors from arboreal ancestors. Using sciuriforms as a focal clade, our findings exemplify the importance of statistically accounting for potential interaction effects of different environmental factors in studies relating morphology to ecology.

Key words: adaptation; allometry; morphology; Ornstein–Uhlenbeck model; phylogenetic inertia; shape.

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Introduction

It has been demonstrated that functionally relevant aspects of limb bone morphology differ among taxa in a way that they reflect the biomechanical requirements of the taxon's

locomotor ecology (Dublin, 1903; Osburn, 1903; Shimer, 1903; Smith & Savage, 1956; Taylor, 1974, 1976; Van Valkenburgh, 1987; Hildebrand, 1988; Hildebrand & Goslow, 1995; Polly, 2007; Samuels & Van Valkenburgh, 2008; Samuels et al. 2013; Amson et al. 2017; Fabre et al. 2018). The stresses acting on the bones as a result of the animal's weight or forces applied by muscles also are important aspects to consider for the understanding of morphological variability (Galilei, 1637; Thompson, 1917; Schmidt-Nielsen, 1984). These properties are largely dependent on the size of an animal and have been shown to follow underlying scaling patterns (Alexander, 1977; Alexander et al. 1979; Biewener, 1983; Bou & Casinos, 1983; Christiansen, 1999a). Because the scaling of morphological variables may also depend on locomotor ecology, it is useful to analyze the effects of organismal size and habitat on morphology simultaneously (Bou et al. 1987; Casinos, 1989; Castiella & Casinos, 1990; Godfrey et al. 1991). One can ask, for example, how scaling of morphological variables differs among taxa with different locomotor ecologies (comparison of scaling exponents) or whether these taxa differ constantly in these variables over a range of body masses (comparison of intercepts; Alexander et al. 1979, 1981; Steudel, 1982; Bou et al. 1987; Biknevičius, 1993; Runestad & Ruff, 1995; Christiansen, 1999b; Doube et al. 2012; Randau et al. 2017).

In this study, we used morphometrics to investigate the effect of scaling and locomotor ecology on the morphology of the sciurormorph femur while taking into account phylogenetic non-independence in an adaptive hypothesis framework (Hansen, 1997; Hansen & Orzack, 2005; Hansen et al. 2008). Sciurormorph rodents are a monophyletic group (Fabre et al. 2012, termed squirrel-related clade therein), whose approximately 300 extant species occupy a variety of terrestrial habitats, ranging from xeric shrubland to rocky mountainous regions and tropical rainforests (Nowak, 1999; Thorington et al. 2012). In these diverse habitats, they exhibit aerial, arboreal, terrestrial (i.e. ground-dwelling) and fossorial locomotor behaviors to varying extents (Nowak, 1999; Thorington et al. 2012). Additionally, they display a body mass range spanning two orders of magnitude in fossorial/terrestrial (93–8000 g), arboreal (16–1678 g) and aerial species (36–2268 g), respectively (Fig. 1; Table S1). Previous work has already identified a functional signal in the morphology of the femur in various mammalian taxa (e.g. Cercopithecidae: Gebo & Sargis, 1994; Erethizontidae: Candela & Picasso, 2008; Tenrecoidea: Salton & Sargis, 2009; Tupaiidae: Sargis, 2002; Viverridae: Taylor, 1976). We therefore expected the femur to be informative for the question addressed herein. Various studies have been concerned with the comparison of the morphology of sciurormorph

taxa with different locomotor behaviors (Parsons, 1894; Peterka, 1936; Bryant, 1945; Polyakova & Sokolov, 1965; Gambaryan, 1974; Stalheim-Smith, 1984; Swiderski, 1993; Thorington et al. 1997; Scheibe & Essner, 2000; Essner & Scheibe, 2002; Thorington & Santana, 2007; Haysen, 2008; Mielke et al. 2018). However, except for the study of Haysen (2008), which investigated the relation between body mass and body length as well as tail length among different locomotor groups, we know of no publication that has accounted for a possible interplay of ecological and allometric constraints.

We investigated the effective femoral length, various femoral robustness properties and the in-levers of the muscles of the most prominent insertion sites (Table 1). We additionally used geometric morphometrics to explore further functionally relevant differences in femoral shape. Here, we assumed that the robustness properties of the femoral morphology as well as those associated with muscle activity tend to adapt to the peak loads. For example, it was shown experimentally that the landing reaction forces of a small flying squirrel (about 250 g) can rise up to almost nine times the animal's body weight after a flight distance of only 2 m (Paskins et al. 2007). We would expect that the loads of climbing vertically and jumping over longer distances between trees are minor relative to those. Gambaryan (1974) suggested that forces acting on the hind limb are higher in large jumps compared with climbing and with jumps observed during terrestrial running (e.g. the flight phase during galloping). He reported the European tree squirrel *Sciurus vulgaris* to make larger jumps than the similarly sized fossorial/terrestrial runner *Spermophilus citellus*, which was reflected in the force output (1.49 times the body weight in the former and 0.98 in the latter species; Gambaryan, 1974). According to Gambaryan (1974), digging induces even lower loads on the hind limb when compared with running, because loosened soil is kicked backwards by the hind limbs in small batches that do not exceed 25–40 g even in the heaviest fossorial sciurormorphs (*Marmota*; Fig. 1). Another function of the hind limb during digging is to brace the body against the wall, thus stabilizing the body during forelimb digging (Biknevičius, 1993; Stein, 2000). However, no data regarding the peak stresses on the hind limb during this activity are available to our knowledge, and we would expect them to be lower compared with those during jumping. Thus, we hypothesized that peak loads acting on the femur decrease according to the sequence of aerial, arboreal and fossorial/terrestrial locomotion. Consequently, we assigned species that glide, but also climb trees into the

Fig. 1 Phylogenetic tree of Sciuromorpha considered in this study including ancestral locomotor types. The tree was adopted from Zelditch et al. (2015) and completed with information from the TimeTree database (Hedges et al. 2015). Names were updated to reflect the taxonomic changes proposed by Patterson & Norris (2016). Timescale in million years ago (Ma). Pie charts at the internal nodes present the probability of each locomotor type. Larger pie charts indicate transitions among locomotor types at the internal nodes. See text for more information.

Table 1 Definitions of univariate femoral traits.

Functionally relevant femoral trait	Representative variable
Effective length	Euclidean distance between the centroids of the head and the distal condyles (1)
Robustness of head	Centroid size of all landmarks surrounding the head (2)
Robustness of midshaft in anteroposterior direction	Mean anteroposterior midshaft diameter* (3)
Robustness of midshaft in mediolateral direction	Mean mediolateral midshaft diameter* (4)
Robustness of distal condyles	Centroid size of all landmarks surrounding the distal condyles (5)
Robustness of medial condyle	Euclidean distance between the most medial and the most lateral point of its proximal surface (6)
Robustness of lateral condyle	Euclidean distance between the most medial and the most lateral point of its proximal surface (7)
Robustness of patellar size	Mean width of the patellar groove* (8)
In-lever of muscles attaching to greater trochanter	Euclidean distance between the centroid of all landmarks surrounding the head and the landmark representing the tip of the greater trochanter (9)
In-lever of muscles attaching to third trochanter	Euclidean distance between the centroid of all landmarks surrounding the head and the landmark representing the tip of the third trochanter (10)
In-lever of muscles attaching to lesser trochanter	Euclidean distance between the centroid of all landmarks surrounding the head and the landmark representing the tip of the lesser trochanter (11)

Asterisks indicate variables that were measured directly on the surface scan. Numbers in brackets refer to Fig. 2D–G.

locomotor group ‘aerial’, species that climb trees, but may occasionally dig burrows into the group ‘arboreal’, and those that dig burrows and are otherwise terrestrial into the group ‘fossorial’. We always used the arboreal group as a baseline for our expectations and later discussions, and compared how the two other groups – first the fossorial, then the aerial – deviate from this, because arboreal locomotion is most probably the ancestral sciuriform condition as we will demonstrate via ancestral state reconstruction. This is supported by the oldest known fossil sciurid genera (*Douglasciurus* and *Protosciurus*) exhibiting well-preserved postcrania that very much resemble those of modern tree squirrels (Emry & Thorington, 1982, 1984; Korth & Samuels, 2015).

In congruence with the assumed differences in peak loads, we expected various robustness variables to scale with positive allometry in all groups, but with a smaller slope in the fossorial and a larger slope in the aerial group compared with the arboreal one. We expected a similar pattern for the in-levers of the muscles attaching to the greater, third and lesser trochanters. The greater and third trochanters are the attachment sites for the gluteus muscles, amongst other muscles. These muscles support the abduction and retraction of the limb (i.e. extension of the hip; Goslow et al. 1981). We assumed their maximum force output to increase from digging/terrestrial running to climbing and finally gliding, as the highest loads were expected during running in the fossorial group, during upward vertical climbing against gravity and jumping in the arboreal group, and during take-off jumps as well as absorption of impact forces (i.e. counteracting induced limb flexion) during landing after gliding in aerial species.

Consequently, also the in-levers of the muscles attaching to the greater and third trochanters were expected to increase in relevance along this order. The lesser trochanter is the attachment site for the iliopsoas muscle, which flexes the hip and adducts the limb (Salton & Sargis, 2009). We also assumed this muscle’s in-lever to follow the above-mentioned order among locomotor groups. A specifically high output force might be necessary to counter moments generated by lift that would otherwise rotate the limbs over the back during gliding. Comparably lower forces are expected to be exerted in an arboreal lifestyle to generate adduction forces that counter toppling moments on narrow substrates and flexion forces to control hind limb extension when climbing upside down. The relevance of the iliopsoas muscle for terrestrial locomotion and digging was difficult to assess, but its force output was expected to play a smaller role compared with the other two locomotor types.

We also used the categorization based on the three locomotor groups for the analysis of the effective length of the femur. Femoral length as part of hind limb length is an important determinant of running as well as climbing speed, jumping distance and gliding performance. The largest hind limb length was expected in aerial sciuriforms in order to gain the necessary lift properties during gliding (Runestad & Ruff, 1995). Furthermore, we assumed that it displays positive allometry, as the surface area of the patagium surface needs to scale with positive allometry in order to yield similar lift properties (Runestad & Ruff, 1995). Arboreal species of the same body mass were expected to have a smaller femoral length in order to bring the center of mass close to the substrate as this is important for maintaining balance during climbing (Nakano, 2002; Schmidt & Fischer,

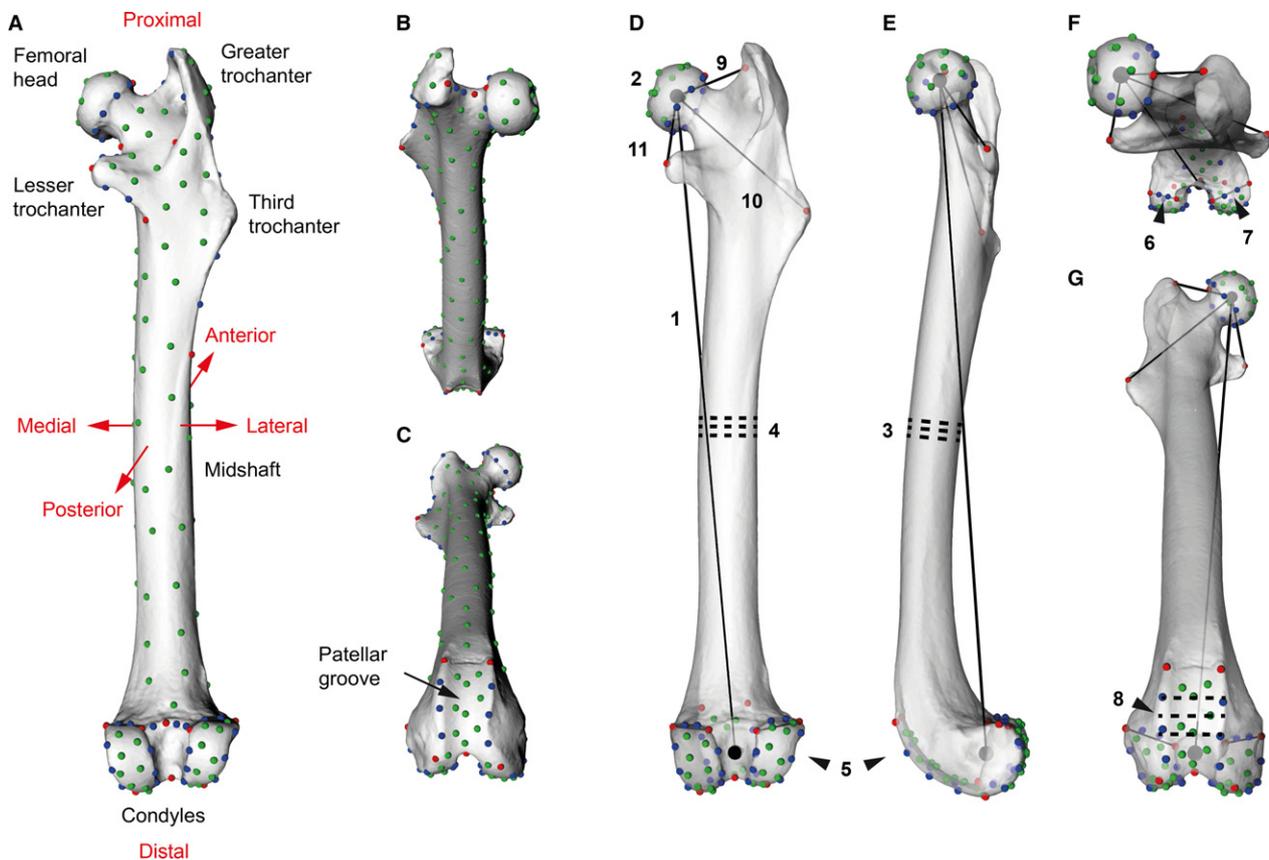


Fig. 2 Landmarks and univariate traits of the sciurormorph femur. Different perspectives of the right femur illustrate the distribution of all landmarks used for shape analysis: (A) caudal; (B) cranioproximal; (C) craniodistal; red: traditional landmarks; blue: curve sliding semi-landmarks; green: surface sliding semi-landmarks. Landmarks used for extraction of univariate traits, Euclidian distances computed from landmarks (solid lines), and Euclidian distances measured directly on the surface scans (dashed lines) are displayed from (D) to (G) (caudal, medial, proximal, craniodistal perspectives, respectively). Black spheres indicate the position of the centroids of the head and distal condyles. Three dashed lines indicate three independent measurements to include uncertainty in the localization of the midshaft diameters and in the width of the patellar groove. Compare with Table 1.

2011), but it should still be large enough to facilitate long jumps. We did not make specific assumptions about the scaling of the effective femoral length in the arboreal group, but the slope value should lie between those of the other two groups. Finally, we hypothesized a similarly weighing fossorial sciurormorph to have the shortest femoral length, because jumping is less important in these species and shorter limbs might facilitate locomotion in underground tunnels (Stein, 2000). The fossorial group was expected to exhibit negative allometry or isometry so that tunnel diameters can remain small without forcing the animals to run in a relatively more crouched and energetically less efficient posture (Horner et al. 2016).

Materials and methods

Data acquisition

Femora from 180 extant sciurormorph species were obtained from eleven museum collections (Table S1). Each species was represented

by one specimen, except for *Aplodontia rufa*, for which three specimens were used to yield a shape mean, as it is the only species of its family. All femora were either digitized using a CT- or a surface laser scanner (see Materials S1 for details). We obtained all univariate and multivariate data (femoral shape) from surface models. All left bones were mirrored to obtain a sample of right femora. Species' mass data were obtained from the literature. Hayssen (2008) served as a primary source, and other works were used to complement the data (Table S1). Body mass information was available for 177 species, the remaining three species were removed from the study. If accessible, as in most cases, we used the body mass for the same sex as our sample specimens. Otherwise, we took the information from the other sex and/or specimens with unknown sex as indicated in Table S1.

Landmark placement and extraction of univariate data

We placed landmarks onto the surface models for extracting univariate data as well as for shape analysis via geometric morphometrics. The IDAV Landmark software version 3.0 (Wiley et al. 2005)

was used to place traditional landmarks (Bookstein, 1997b) and sliding semi-landmarks (Bookstein, 1997a; Gunz et al. 2005) onto the models (Fig. 2; for details see Materials S1, Table S2, Fig. S1). The inclusion of semi-landmarks allowed us to capture sub-structures that lack clear homologous marker points such as the femoral head. Additionally, it enabled us to generate 3D models for a functional interpretation of the shape changes as a result of scaling. If not mentioned otherwise, we used the software R version 3.5.1 (R Development Core Team, 2018) for downstream analysis with utility functions of various R packages for data preparation and visualization (GEIGER: Harmon et al. 2008; geomorph: Adams et al. 2018; Morpho: Schlager, 2017; phytools: Revell, 2012; tidyverse: Wickham, 2017).

The univariate data were either extracted from specific raw landmarks (before Procrustes superimposition, see below) or directly measured on the digital scan in Geomagic Studio 2013.0.2 (3D Systems, Rock Hill, South Carolina, USA; Fig. 2; Table 1). The anteroposterior and mediolateral mid-shaft diameters were measured at three slightly displaced positions in the middle of the bone (Fig. 2). These three positions seemed similarly appropriate as representatives of the midshaft diameter and, hence, their values were averaged to account for measurement uncertainty (Fig. 2D,E). Similarly, the patellar groove width was measured at three different positions along the groove (Fig. 2G) to yield a mean value. The centroids of the proximal (head) and the distal (condyles) articulation sites were computed using the landmarks surrounding the respective structures (Fig. 2D–G). They were used as a proxy for the center of rotation of the respective articulation site. The sizes of these two centroids were computed using the *cSize* function of the R package 'Morpho' (Schlager, 2017). The Euclidean distance between these centroids was included as the effective femoral length. The femoral head centroid was furthermore used to compute the in-levers for the muscles attaching to the greater, third and lesser trochanters. We observed the shape of the greater trochanter to differ substantially among the sample specimens so that we decided against homologizing the point of maximum curvature as performed for the other two trochanters as explained below. We decided to use the most proximal fixed (traditional) landmark that we could homologize as a proxy for the endpoint of the in-lever (Fig. 2D,F). Regarding the third and lesser trochanters, we homologized the most lateral and the most medial tips, respectively, to compute the respective muscles' in-lever at each site (Fig. 2D–G).

Shape extraction

We applied a generalized Procrustes superimposition (Rohlf & Slice, 1990) with the criterion of bending energy on the landmark coordinates using the function *gpagen* of the R package 'geomorph' (Adams et al. 2018) to remove differences in size, position and orientation from all specimens (see Materials S1 for a detailed description).

Statistics and visualization

Whereas there seems to be a consensus on the necessity of applying phylogenetic comparative methods when inferring adaptation from interspecific studies, various methods exist that differ in how they infer adaptation and account for phylogenetic constraint (for review, see Hansen & Orzack, 2005). According to Hansen & Orzack

(2005), it is necessary to estimate phylogenetic inertia and adaptation simultaneously, because they are interdependent. Past adaptations would become phylogenetic constraints once a phylogenetic lineage enters a novel environment that imposes a different optimal phenotype on the species (Hansen & Orzack, 2005). The Brownian motion (BM) model of evolution (Felsenstein, 1985) cannot properly account for this interplay in regression analyses (Hansen & Orzack, 2005). Furthermore, if residuals do not fit the assumption of a BM model of evolution, regression parameters are incorrectly estimated when still applying methods that rely on the BM model (Revell, 2010).

We follow Hansen and colleagues, who developed regression methods that integrate the Ornstein–Uhlenbeck (OU) model to describe macroevolution as this interplay of adaptation and phylogenetic inertia acting on the traits of a species (Hansen, 1997; Hansen et al. 2008; Bartoszek et al. 2012). Unfortunately, none of these methods is able to test for an interaction effect of predictor variables. However, an ordinary least-squares (OLS) regression not taking into account phylogeny is able to do so. Furthermore, if trait adaptation happened almost instantaneously, the assumptions of an OLS regression might be less harmful for the estimation of regression parameters than a phylogenetic least-squares regression (Grafen, 1989) adhering to the assumption that residuals reflect a BM-like correlation structure (Hansen, 2014). Thus, we decided to infer estimates from an OLS analysis with an interaction term to analyze the combined effect of locomotor ecology and body mass on the focal variables (explained below), and justified it with an approach inferring adaptation and inertia from fitting OU models to the dataset (outlined thereafter).

Before applying both analyses, we computed the natural logs of the univariate traits as well as body mass to induce a linear scaling relationship. The lowest log value of the body masses was subtracted from all the other mass values so that the comparison of the OLS intercepts represented the difference among locomotor groups for the smallest sampled body mass of a sciuromorph rodent. This was expected to represent the smallest difference, because we predicted relative differences among groups to increase with higher body masses (different slopes without intersection).

Furthermore, for both analyses, it was necessary to reconstruct the ancestral locomotor types. The locomotor group with the ancestral type served as a baseline for the between-group comparisons of the OLS regression. Additionally, a phylogenetic tree needed to be included during the OU modeling. Hence, a fully dichotomous tree based on molecular data was obtained that allowed us to include as many species represented in our dataset as possible. We used the sciurid phylogeny from Zelditch et al. (2015) and added taxa from the TimeTree database (Hedges et al. 2015), using the software Mesquite version 3.04 (Maddison & Maddison, 2017; see Materials S1 and Fig. S2 for details). We pruned the phylogeny and dataset for the OU analysis, so that they only contained those species present in both of them (140 of the 177 species; Fig. 1; Table S1). We used stochastic character mapping (Huelsenbeck et al. 2003; Bollback, 2006) implemented in the *make.simmap* function of the R package 'phytools' (Revell, 2012) to reconstruct ancestral locomotor types, constraining the transition rate matrix to all rates being different. All in all, 1000 character maps were generated, and the most probable ancestral locomotor types at the internal nodes (Fig. 1) were used for the OU regressions.

First, regarding the OLS analysis, univariate regressions were fit to the univariate data and a multivariate regression to femoral shape, setting the alpha level to 0.05. For each variable, we used

the *lm.rpp* function of the package 'RRPP' (Collyer & Adams, 2018) with the option *SS.type = 'III'*. The arboreal group was used as a baseline, as it was the most probable locomotor type of the sciurormorph ancestor (see Results). This means that the model tested whether the intercept and the slope of this group are significantly different from zero, and whether the intercepts and slopes of the fossorial and aerial groups are significantly different from the respective parameter of the arboreal group. We then ran the *ANOVA* function on the fitted model to evaluate the significance of the interaction effect. The more different the slopes were among groups, the more probable the interaction. If the interaction was not significant, we fit a linear model without interaction to obtain model coefficients. Regarding shape, we tested post hoc whether the angles between the shape trajectories of the different locomotor groups differed significantly with the function *pairwise* of the 'RRPP' package. This was necessary, because in the case of a multivariate regression, this property is not directly tested by the *lm.rpp* function (instead, this function tested whether the slopes have significantly different lengths). We created 3D surface models using the fitted shape variables for the lightest and the heaviest specimens of each locomotor group (i.e. those that were predicted for the lightest and heaviest species according to the slope of the regression) with the function *tps3d* of the R package 'Morpho' (Schlager, 2017). Hence, we were able to compare how the average shape changes with body mass among the three locomotor groups.

Second, we used the *slouch.fit* function of the 'slouch' package (Kopperud et al. 2018) to fit an OU model to each univariate dataset *sensu* Hansen et al. (2008): A variable is modelled as evolving toward an optimum imposed by a current primary selective regime (the respective locomotor type in our case) with a certain adaptive rate. All optima themselves depend on a covariate (body mass in our case) in the same way (i.e. no interaction effect between locomotor type and body mass). The species' trait values are normally distributed around the optima, with the scale and phylogenetic

distribution of the residuals reflecting the phylogenetic constraint (because of maladaptation inherited from past selective regimes; pleiotropic effects; secondary, less influential selective regimes; etc.). These constraints are hypothesized to hinder the species from reaching their trait optimum. This interplay between adaptation and constraint results in trait values displaying the evolutionary regression slope (equivalent to an OLS estimate) and not the optimal regression slope. The evolutionary regression slope is estimated as the optimal regression slope times a mean phylogenetic correction factor (MPCF). This factor can range from 0 to 1, and was the most relevant model parameter for us. If it was close to 1, it meant that the evolutionary slope approximated the optimal one, and the slope from an OLS regression could be used as a reliable estimate of an adaptive relationship between the optimal (fitted) trait values and body mass. In order to prevent overfitting involving a MPCF value biased toward 1, we also fit an OU model with one optimum for all species to each variable. This would indicate no differences among locomotor groups. This resulted in a larger residual variance and, hence, in a smaller MPCF. The fit of both models was then compared via the Schwarz information criterion (SIC), with lower values indicating higher model likelihood (Burnham & Anderson, 2002). If the difference between the two models' SIC values was below 2, both MPCFs were reported (Table 2).

As all variables yielded a MPCF of ~0.97 or higher (see Results and Table 2) irrespective of showing an interaction effect during the OLS analysis or not, we considered that the OLS estimates are reliable for inferring adaptive relationships between the response and the predictor variables. We think it is reasonable to assume that if the OU model could have accounted for an interaction effect, the MPCF would have even been closer to 1 as a result of the interaction term explaining an additional percentage of the residual variance.

This approach was not transferable to the multivariate regression of shape. A multivariate equivalent of the *slouch.fit* function does exist (Bartoszek et al. 2012); however, it is not applicable to high-

Table 2 Significance of the effects of the OLS analysis and relevant estimates of the OU analysis.

Variable	OLS analysis			OU analysis		
	<i>P</i> (mass)	<i>P</i> (groups)	<i>P</i> (mass × groups)	SIC (OU3)	SIC (OU1)	MPCF
Femur EL	0.0001	0.1820	0.0001	-203	-125	0.994
Head CS	0.0001	0.4691	0.0813	-252	-246	0.995
Midshaft apD	0.0001	0.4497	0.4821	-174	-167	0.987
Midshaft mLD	0.0001	0.6436	0.8382	-159	-158	0.982/0.969
Condyles CS	0.0001	0.3359	0.0110	-217	-211	0.994
Medial condyle W	0.0001	0.4656	0.5454	-185	-165	0.995
Lateral condyle W	0.0001	0.2583	0.0866	-202	-187	0.979
Patellar groove W	0.0001	0.3147	0.3728	-164	-149	0.989
Greater trochanter I-L	0.0001	0.7283	0.0015	-184	-162	0.993
Third trochanter I-L	0.0001	0.0004	0.0036	-154	-110	0.987
Lesser trochanter I-L	0.0001	0.3223	0.5527	-170	-167	0.994
Shape	0.0032	0.0002	0.0001	-	-	-

apD, anteroposterior diameter; CS, centroid size; EL, effective length; I-L, in-lever; mLD, mediolateral diameter; W, width. *P*-values (*P*) for the mean squares values of the regression effects (mass, groups) and their interaction from an ordinary least-squares (OLS) fit. Significant *P*-values (≤ 0.05) are displayed in bold. See Table S3 for the *ANOVA* tables including all statistical properties for each variable. Values of the Schwarz information criterion (SIC) for an Ornstein–Uhlenbeck (OU) model assigning species to the three locomotor groups (OU3) and for a model pooling them into a single group (OU1). The values of the more likely model are displayed in bold and the estimate for the mean phylogenetic correction factor (MPCF) is presented.

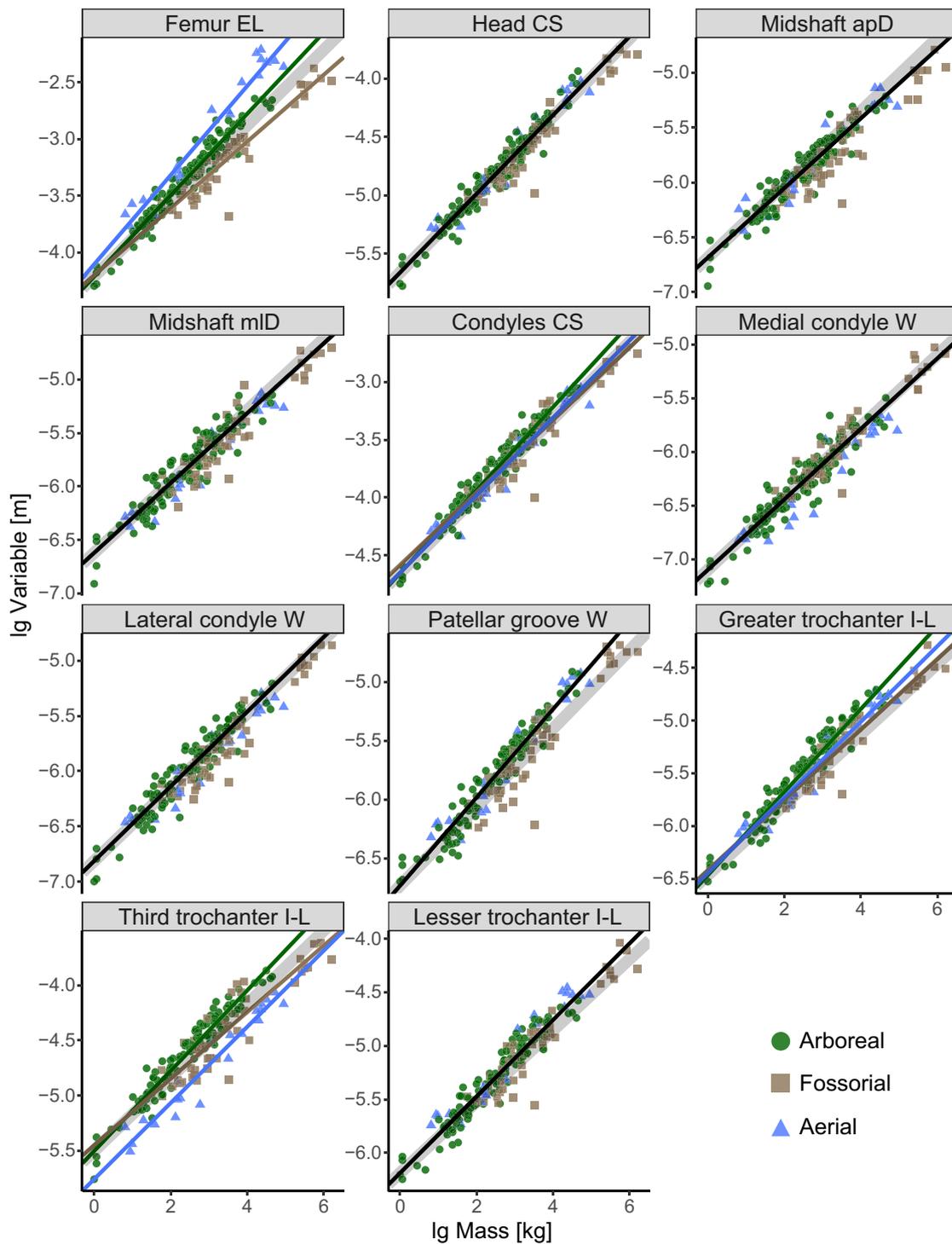


Fig. 3 Ordinary least-squares (OLS) regression plots for all natural log-transformed univariate femoral traits on natural log-transformed body mass. Three slopes for the different locomotor groups are shown in the respective color in case of a significant interaction effect (see Table 2), one slope in black for all species in case of no interaction effect according to OLS regressions. Thick light gray bars indicate the slope expected under isometric scaling. See Table 2 for explanations of abbreviated variable names.

dimensional data such as shape (Adams & Collyer, 2017). Because the MPCF value was close to 1 in all univariate analyses covering various aspects of the femur, we assumed that its shape (by

integrating all these aspects) would also be only slightly affected by phylogenetic inertia. Hence, we used an OLS regression for femoral shape as well.

Table 3 Coefficients of the OLS analysis.

Variable	Arboreal group				Fossorial group				Aerial group								
	IC	UCL	P	SL	UCL	P	SL	UCL	P	IC	UCL	P	SL	UCL	P		
Femur EL	-4.215	4.224	0.0948	0.359	0.056	0.0001	0.100	0.8199	0.295	0.032	0.0005	-4.107	0.116	0.0698	0.393	0.039	0.0895
Head CS	-5.660	5.681	0.3282	0.336	0.051	0.0001	0.036	0.0005	-	-	-	-5.636	0.047	0.3175	-	-	-
Midshaft apD	-6.685	6.711	0.3191	0.317	0.049	0.0001	0.046	0.0001	-	-	-	-6.678	0.058	0.8191	-	-	-
Midshaft mID	-6.620	6.657	0.4906	0.326	0.051	0.0001	0.046	0.0060	-	-	-	-6.694	0.060	0.0148	-	-	-
Condyles CS	-4.654	4.649	0.0312	0.359	0.056	0.0001	0.096	0.1488	0.313	0.030	0.0033	-4.651	0.112	0.9513	0.335	0.036	0.1880
Medial condyle W	-7.098	7.147	0.6883	0.328	0.051	0.0001	0.047	0.2588	-	-	-	-7.221	0.061	0.0001	-	-	-
Lateral condyle W	-6.813	6.845	0.4209	0.338	0.052	0.0001	0.045	0.0001	-	-	-	-6.890	0.058	0.0088	-	-	-
Patellar groove W	-6.732	6.757	0.2852	0.375	0.058	0.0001	0.049	0.0001	-	-	-	-6.728	0.065	0.9146	-	-	-
Greater trochanter I-L	-6.465	6.449	0.0157	0.393	0.061	0.0001	0.107	0.4307	0.333	0.034	0.0005	-6.444	0.124	0.7596	0.358	0.041	0.0913
Third trochanter I-L	-5.504	5.496	0.0304	0.364	0.058	0.0001	0.122	0.4046	0.302	0.036	0.0008	-5.760	0.141	0.0003	0.346	0.044	0.4070
Lesser trochanter I-L	-6.189	6.214	0.3102	0.358	0.055	0.0001	0.046	0.0031	-	-	-	-6.130	0.060	0.0533	-	-	-
Shape	-0.017	1.001	0.6328	-0.001	0.005	0.0032	0.030	0.0060	-0.001	0.009	0.0067	-0.027	0.034	0.0005	-0.004	0.011	0.0038

Intercepts (IC) and slopes (SL) of the OLS fits with their upper confidence limits (UCL) and corresponding P-values (P). Variables without a significant interaction effect (see Table 2) are represented by a single common slope reported under the arboreal group. P-values for the slopes of the fossorial and aerial groups refer to the significance of being different from the slope of the arboreal group. Actual slope values and not the differences of the fossorial and aerial groups relative to the arboreal group are presented in order to facilitate comparison with isometry. For consistency, the same applies to the intercepts. Significant P-values (≤ 0.05) are displayed in bold. See Table 2 for abbreviated variable names.

Results

Reconstruction of ancestral locomotor types

The sciurormorph ancestor was most probably arboreal (Fig. 1). According to the most probable reconstruction at the internal nodes, aerial locomotion was acquired once and fossorial locomotor behavior three times independently.

Consistency of OLS and OU regressions

According to the OLS analysis, only five of 12 variables showed a significant interaction effect: the effective femoral length, the centroid size of the condyles, the in-levers of the muscles attaching to the greater and third trochanters, and femoral shape (Table 2). All variables were significantly affected by body mass (Fig. 3; Table 2). The coefficients and their upper confidence limits as well as their P-values for all linear models are presented in Table 3.

For the seven variables of the univariate analyses that did not recover a significant interaction effect, an MPCF value of ~ 0.97 or higher was observed. The MPCF value of a univariate trait with significant interaction effect was never below ~ 0.99 . These results indicate that phylogenetic inertia must have played a minor role in the morphological adjustment of the femur to new loading regimes and scaling.

Effective femoral length

The arboreal and aerial groups scaled with positive allometry (~ 0.36 and ~ 0.39 , respectively), whereas the fossorial group scaled with negative allometry (~ 0.30). The intercepts and slopes of the fossorial and aerial groups did not deviate significantly from these coefficients of the arboreal group, except for the slope of the fossorial group.

Robustness variables

All variables except for the centroid size of the distal condyles in the arboreal group (~ 0.36) and the patellar groove width (~ 0.38) scaled close to isometry (~ 0.31 – 0.34). The size of the condyles in the fossorial groups displayed a significantly lower slope (~ 0.31) compared with the arboreal group (~ 0.36), whereas the aerial group showed no significant difference (~ 0.34).

The fossorial group exhibited significantly lower values on average (smaller intercept) for all robustness variables, except for the size of the condyles and the width of the medial condyle (no significant differences). The only differences shown by the aerial group were significantly lower mean trait values (smaller intercepts) for the medial-lateral midshaft diameter and for the width of each distal condyle.

In-levers

The slopes for the in-levers of the muscles attaching to the greater and third trochanters in the arboreal group scaled with positive allometry (-0.39 and 0.36 , respectively). In the fossorial group, the slopes of these two in-levers did significantly differ from the arboreal group, with isometric scaling regarding the greater trochanter (-0.33) and negative scaling regarding the third trochanter (-0.30). The intercepts did not significantly differ from the arboreal group. Hence, the higher the body mass, the relatively smaller on average were these in-levers for the fossorial group compared with the arboreal one. The slopes for these two in-levers did not significantly differ in the aerial group (-0.36 and 0.35 , respectively). Only the in-lever of the muscles attaching to the third trochanter was on average significantly different to that of the arboreal group.

The slope of the in-lever of the iliopsoas muscle was the same for all groups (no interaction effect) and displayed slightly positive allometry (-0.36). The intercept of the fossorial group was significantly lower, whereas that of the aerial group did not significantly differ from that of the arboreal group.

Femoral shape

The shape trajectories differed significantly between the arboreal and fossorial groups, as well as between the fossorial and aerial groups (Table 4). This was not the case concerning the arboreal and aerial groups, suggesting that their average shape changes in a similar fashion with increasing body mass.

The shape of the condyles and their orientation did not appear to change with body mass in any of the three groups (Fig. 4). In heavier species of the arboreal group, the femoral head appeared to be tilted more medially, whereas it was the opposite pattern in the fossorial group and no difference could be observed in aerial species of different mass. The lesser trochanter appeared to change its orientation from caudomedial to more medial in the arboreal and

fossorial groups. However, the lesser trochanter of the fossorial species always projected more caudally and did not stretch as far medially as in the arboreal group. On the contrary, the aerial group's lesser trochanter was observed to project more medially in lighter aerial species and more caudally in heavier ones. Furthermore, it protruded further medially in lighter aerial species, whereas the opposite seemed to be the case in the arboreal group.

Discussion

The aim of this study was to understand how femoral morphology is affected by the interplay of locomotor ecology and body mass in sciuromorph rodents. We hypothesized that peak loads determine femoral robustness variables and lengths of in-levers, and that these loads decrease in the sequence of aerial, arboreal and fossorial locomotion and increase with body mass within each locomotor group. We hypothesized that the effective femoral length would follow a similar trend based on assumptions of the locomotor performance among these groups. We used an OLS regression model with an interaction effect to compare the slopes and intercepts of the fossorial and aerial groups with those coefficients of the arboreal group. The OLS model was justified on the basis of the evaluation of the phylogenetic inertia by the means of fitting OU models to the data. In contradiction to our expectations, most of the variables scaled close to isometry in all three locomotor groups (Table 3). Our expected scaling differences were only supported for the effective femoral length.

Functional inferences

The effective length of the femur scaled with positive allometry in the aerial group, with slightly positive allometry in the arboreal group and with negative allometry in the fossorial group. An OLS slope value of -0.35 has been estimated for various rodents and insectivores representing all kinds of locomotor ecologies (Bou et al. 1987). The slope that we found for the arboreal group matches this overall slope reported by Bou et al. (1987). Furthermore, fig. 6 in Bou et al. (1987) suggests that gliding rodents display a slightly higher slope and the fossorial European mole (*Talpa europaea*) a slightly lower slope compared with the overall slope. This is consistent with our results and implies a common pattern among small mammals. The fact that the fossorial group exhibits a slightly shorter femur on average for all given body masses contributes to an overall shorter hind limb. This could have facilitated locomotion in underground tunnels by reducing the animal's height (Stein, 2000) and, consequently, the necessary tunnel size. It also could have allowed for a more erect limb posture, which in turn increased the effective mechanical advantage (EMA) of the hind limbs (Biewener, 1991). An increase in the EMA, while keeping the overall height of the animal low, might

Table 4 Scaling differences of femoral shape among locomotor groups.

Groups compared	Angle (°)	UCL	P
Arboreal vs. fossorial	74.171	63.645	0.0176
Arboreal vs. aerial	59.198	73.319	0.1468
Fossorial vs. aerial	109.425	77.546	0.0017

The angle between the slopes is compared between each pair of locomotor groups.

UCL, upper confidence limit.

P-values ($P \leq 0.05$) for angles being significantly different from zero are shown in bold.

especially be more advantageous the heavier the fossorial animal becomes. This keeps the stresses in the limb bones and muscles low, as well as energy expenditure during locomotion, while minimizing the increase in tunnel size (Horner & Biknevičius, 2010; Horner et al. 2016). These assumptions are supported by the fact that not only the length of the femur, but also the length of the tibia, humerus and ulna have scaled with negative allometry in

fossorial caviomorph rodents (i.e. 0.29, 0.26, 0.30 and 0.24, respectively: Casinos et al. 1993).

The intercepts and the slope of the effective length in the aerial group were not significantly different from these coefficients in arboreal sciurormorphs. This is surprising as Runestad & Ruff (1995) have reported positive allometry (-0.42) across all gliding mammals, and a significant difference in intercept when compared with the arboreal sister taxa. From a functional perspective, relatively longer limbs

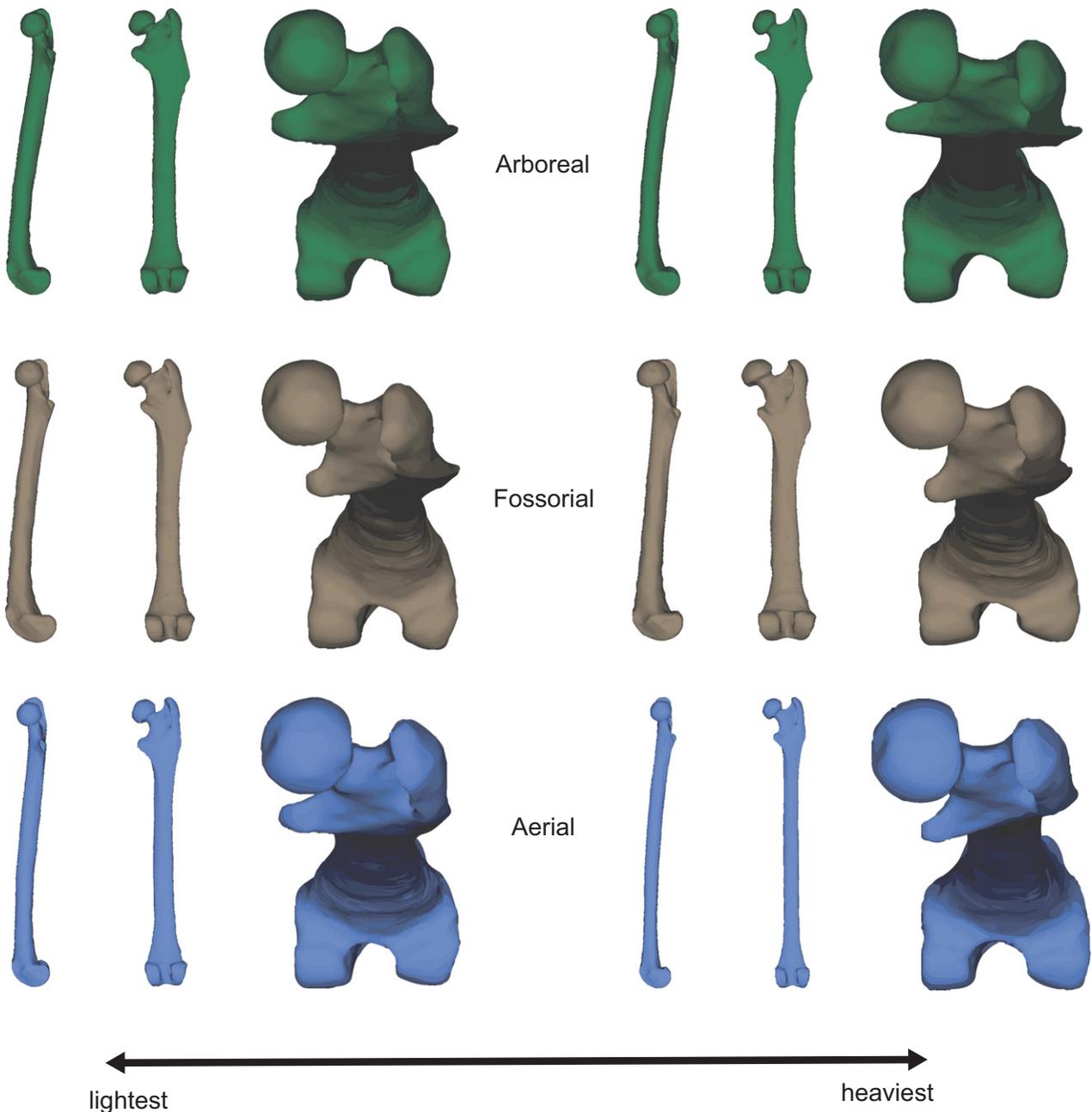


Fig. 4 Femoral shapes for the lightest and heaviest species of each locomotor group as predicted by body mass according to the ordinary least-squares regression. Orthogonal projections of 3D surface models of the right femur are displayed using the fitted shape variables of the lightest and heaviest specimens. From left to right: medial, caudal and proximocaudal views.

are necessary for gliding as they result in a relatively larger patagium to carry the animal's weight. The patagium would otherwise scale disproportionately low with increasing body mass, resulting in a relatively higher wing loading (Runestad & Ruff, 1995). However, it was shown that overall patagium area in gliding sciuriforms scales isometrically (Thorington & Heaney, 1981). Perhaps, gliding squirrels adapted differently to larger body masses than other gliding mammals. Thorington & Heaney (1981) assumed that the larger wing loading in heavier flying squirrels does not affect glide ratio (horizontal glide distance per vertical drop) when the animal glides faster. This assumption is supported by the findings of Scheibe & Essner (2000), who reported differences in the scaling of the pelvic shape between gliding sciuriforms and gliding anomaluriforms. The former group showed a relatively longer ilium in larger species, whereas it was the opposite in the latter group. Scheibe & Essner (2000) hypothesized that the relatively longer ilium in sciuriforms is advantageous for the force output of the originating gluteus muscles, which retract the hind limb during gliding take-offs. This might be mandatory to accelerate the body mass to an appropriate horizontal take-off speed. Indeed, horizontal glide speed appears to increase with increasing body size as it was observed to be $\sim 5 \text{ m s}^{-1}$ for the lighter gliding sciuriform *Glaucomys sabrinus* and $8\text{--}10 \text{ m s}^{-1}$ for two of the heaviest species, i.e. *Petaurista petaurista* and *Petaurista philippensis* (Koli et al. 2011; Krishna et al. 2016). Additionally, Polyakova & Sokolov (1965) as well as Thorington & Heaney (1981) demonstrated that the largest bone length adjustments are to be found in the forelimb of gliding sciuriforms in order to increase the area of the patagium. Thus, our finding fits into this line of argument.

All robustness properties scaled close to isometry in all three groups, only the centroid size of the distal condyles in the arboreal group (-0.36) and the patellar groove width of all three groups (-0.38) exhibited positive allometry. This indicates that increasing loads because of body mass increase might be counterbalanced only to a small degree by the adjustments of outer bone features. Consequently, we have to assume that these are accounted for by an allometric adjustment of the compact bone area and the trabecular architecture of the femur, properties of the attaching muscles, or the posture during climbing, running/digging and gliding as already suggested for terrestrial locomotion (Alexander et al. 1981; Biewener, 1983, 1989, 1991). Mielke et al. (2018) investigated the scaling of the trabecular architecture in the femoral head, but did not specifically look at scaling differences among locomotor groups. Nevertheless, they found the trabecular bone volume to be relatively larger in heavier species, manifested in an increase of trabecular number, with trabeculae being relatively thinner, but more closely packed (Mielke et al. 2018). Interestingly, the size of the condyles was the only robustness variable for which we found a significant

interaction effect, with the fossorial group displaying a significantly lower slope. This might indicate that fossorial sciuriforms experience relatively lower loads compared with arboreal and aerial species with increasing body mass. The crossing of the fossorial slope and the slopes of the other two groups within midrange of body mass (Fig. 3) suggests that the fossorial group only displays a relatively smaller size of the condyles when looking at heavy species, while it would be the opposite for the lightest species. Anyway, the minor difference among slopes proposes that the overall difference and the resulting functional consequences are negligible. This concurs with the fact that no adjustments in the shape and orientation of the condyles appeared to have happened with changes in body mass or locomotor ecology (Fig. 4). The condyles serve as an attachment site for muscles important for generating propulsion, for example, the biceps femoris (Goslow et al. 1981) and the gastrocnemius (Smith et al. 1977) muscles. As these muscles attach via tendinous connections, the size of the attachment site might be less revealing than the architecture of the muscles. For example, it was recently demonstrated that differing functional demands of striding and jumping caviomorph rodent species are evident in the limb muscle architectural properties of the triceps surae (i.e. combined gastrocnemius and soleus muscles; Rosin & Nyakatura, 2017). Although we found no difference in the scaling of the femoral head size, this was the case for its orientation. The more medial orientation in heavy arboreal species compared with lighter ones could indicate a change in hind limb posture, with heavier species having a less abducted limb. Following this assumption, the contrary would be true for the fossorial group, whereas no changes occurred in the aerial group. Allometric studies into the locomotor kinematics are needed to evaluate these hypotheses.

As expected, the fossorial and terrestrial species (fossorial locomotor category) showed generally lower trait values for most of the robustness variables. This is in agreement with Biknevicius (1993), who reported the cross-sectional properties of the femur of the less cursorial (fossorial) caviomorph rodents to reflect less rigidity in bending and torsion compared with those of cursorial caviomorphs known for high running speed. Other studies hypothesized that robust bones are expected to be found in fossorial taxa as they would improve stability and load transfer in the forelimb (Fabre et al. 2015), as well as in the hind limb (Hildebrand & Goslow, 1995). For sciuriforms, we think that it is unlikely that stresses acting on the femur are higher in fossorial than in arboreal species, because the hind limbs are only used to shovel away small batches of soil that had previously been loosened with the forelimbs (Gambaryan, 1974) or to anchor the body during digging with the forelimbs (Stein, 2000). However, the importance of a robust femur might depend on the hind limb's utilization during digging

behavior, which varies among mammalian fossorial taxa (Lehmann, 1963; Lessa, 1990; Reichman & Smith, 1990). Casinos et al. (1993) found negative allometry in long bone length in combination with positive allometry in anteroposterior long bone diameter in fossorial caviomorphs, and interpreted a stouter long bone as an adaptive feature for digging rodents without providing a specific functional explanation. We confirm this observation for the femur of sciurormorph rodents, as femoral length scaled with negative allometry, whereas the two diameters scaled very close to isometry. Whether this can be regarded as an adaptation to a reduction of bending stresses (Christiansen, 1999b), especially as a result of fossorial behavior (Hildebrand & Goslow, 1995), remains to be demonstrated experimentally.

Contrary to our expectations, it was always the aerial group that displayed a lower mean trait value (intercept) compared with the arboreal group regarding the three robustness variables for which a significant difference was revealed (i.e. the mediolateral midshaft diameter and the widths of the two distal condyles). We expect the distal condyles to be highly loaded during landing, and that a broader surface would facilitate a lower pressure induced to the femur. Similarly, the mediolateral midshaft diameter was expected to be thicker compared with the arboreal group, because of bending moments during gliding as a result of lift forces and during landing as a result of impact forces. Perhaps, weight reduction is a more important aspect of gliding optimization (Polyakova & Sokolov, 1965). We hypothesize that adaptations to counter these forces will be manifested in the architecture of the leg muscles or properties of the inner bone. For example, Mielke et al. (2018) demonstrated that certain trabecular variables reflect the loading of the head and thus bear an ecological signal. According to these authors, aerial species display significantly thicker but less dense trabeculae compared with fossorial species (Mielke et al. 2018). This was attributed to high, but less frequent peak loads as experienced when landing after gliding as opposed to lower, but more frequent peak loads during digging (Mielke et al. 2018). The arboreal species tended to fall in between, whereas the semifossorial species (these were assigned to the arboreal group in our study) resembled more often the fossorial than the arboreal species (Mielke et al. 2018). Hence, it seems that the inner structure rather than the outer morphology of the bone was preferentially adjusted to new loading regimes during the evolution of sciurormorph rodents. This is further supported by Polyakova & Sokolov (1965), who found the relation between area of the compact region to the whole cross-sectional area of the long bones' midshaft to be 0.6 in *Pteromys volans* (aerial, body mass ~150 g) and 0.47/0.5 in *S. vulgaris* and *Spermophilus anomalus* (arboreal, body masses ~400 g), respectively.

We expected that a higher body mass leads to disproportionately higher muscle forces necessary to accelerate the body, for example, in the quadriceps femoris

muscles, which attach to the patella and extend the knee. This would induce relatively higher loads in the knee during extension, which need to be counteracted by a relatively more massive patella. Thus, we expected that a relatively more massive patellar groove would be found in larger sciurormorphs, and that the most massive patellar groove would be found in the gliding sciurormorphs, followed by the arboreal and the fossorial species. Indeed, this variable's slope was the largest compared with the other robustness variables (~0.38). However, all three locomotor groups shared the same slope. Interestingly, only the fossorial species showed a lower mean value compared with the arboreal group, whereas the aerial group did not. The former finding is supported by Gambaryan (1974), who showed that the quadriceps femoris muscles are relatively heavier (in % of hind limb muscle weight) in arboreal sciurormorphs when compared with fossorial species with a similar or greater weight. The latter finding contradicts Polyakova & Sokolov (1965), who found these muscles to be more strongly developed in *P. volans* when compared with *S. vulgaris* and *S. anomalus*. The authors attributed this difference to the functional importance of the quadriceps femoris muscles to adjust the limb position during gliding. Especially the bi-articular rectus femoris muscle that extends the knee and flexes the hip might play an important regulating role during the process (Polyakova & Sokolov, 1965). Taking this evidence into consideration, patellar groove width might only be partly linked to the force output of the quadriceps muscles.

The greater and third trochanters are the attachment sites of the gluteus muscles, which are important for the generation of propulsion (Goslow et al. 1981) and considered to be important for rapid hind limb retraction (Smith & Savage, 1956). Their in-levers scaled with relatively high allometry in the arboreal group (~0.39 and 0.36, respectively), suggesting that a relatively higher force output is needed for limb extension during climbing and jumping as body mass increases. It has been argued that a longer in-lever of the muscles attaching to the third trochanter is typical for fossorial species (Salton & Sargis, 2009). As above, we think that this might depend on the utilization of the hind limb during digging, which has been shown to be quite variable (Lehmann, 1963; Hildebrand & Goslow, 1995). As opposed to the argument of Salton & Sargis (2009), we found a significantly lower slope for the in-levers of the muscles attaching to both trochanters in the fossorial group (~0.33 and 0.30). But it has to be kept in mind that the landmark indicating the insertion of muscles in the greater trochanter was difficult to obtain and might not be representative in our case (Fig. 2D,F). The in-lever of the muscles attaching to the third trochanter being shorter on average (smaller intercept) in the aerial group compared with the arboreal group suggests that heavier gliders have a lower force output for propelling the body against gravity

when climbing trees, but also for stretching the legs during gliding. Polyakova & Sokolov (1965) demonstrated that the gluteus muscles are particularly well developed in *P. volans* as compared with *S. vulgaris* and *S. anomalus*. Hence, adjustments to gliding as opposed to climbing and maybe also to higher body masses in gliding sciuriforms are realized by changes in the muscles themselves and not in the in-levers of those muscles.

The lesser trochanter is the insertion site of the iliopsoas muscle, which facilitates hip flexion and adduction (Sargis, 2002; Salton & Sargis, 2009). We found that the scaling of the in-lever of the iliopsoas muscle is identical in all three groups, but that the change in orientation and protrusion of the lesser trochanter with increasing body mass depends on locomotor ecology. However, these differences are difficult to interpret, because well-grounded data on the exact thigh posture during various locomotor behaviors are, to our knowledge, not available. It was reported that the iliopsoas muscle is relatively more pronounced in arboreal as compared with fossorial sciuriforms of a given weight (Gambaryan, 1974). We could also detect a smaller mean in-lever in the fossorial group. Polyakova & Sokolov (1965) argued that the adductor muscles attaching to the femur possess a functional relevance for absorbing impact forces during landing in gliding sciuriforms, but they did not refer to the iliopsoas muscle. Scheibe & Essner (2000) also assumed adductor forces to relatively increase in larger gliding sciuriforms. A mean larger in-lever in the aerial group was almost significant in our case ($P = 0.053$), which would support the idea of these authors. Peterka (1936) did not report particular differences in the iliopsoas muscle between a gliding and a fossorial sciuriform species, but he did not account for allometric effects, as these species differed in weight. It remains to be demonstrated whether an adjustment of the iliopsoas muscle was important for the shift to gliding behavior and whether its properties had to adapt to increasing body mass in gliding sciuriforms.

Biological assumptions and methodological limitations

Various biological assumptions were made in this study that are worth discussing. We assigned the species into groups based on loading categories for the robustness and muscle variables, and performance categories for the effective femoral length. Previous studies on sciuriforms used different ecological classification schemes. Steppan et al. (2004) introduced the classification of terrestrial, intermediate and arboreal locomotor types, assigning species that were here defined as aerial to the arboreal type and the genera *Paraxerus*, *Funisciurus* and *Tamias* that were here defined as arboreal to their intermediate (terrestrial and arboreal) locomotor type. The terrestrial species were defined as fossorial in our study. Rocha et al. (2016) adopted the categorization from Steppan et al. (2004). We departed from the classification of these authors, because

we did not regard them as appropriate representatives of locomotor regimes that reflect similar peak biomechanical demands. Their classification might well suffice to analyze the evolution of the locomotor behavior, which was the focus of their studies (Steppan et al. 2004; Rocha et al. 2016). However, when conducting eco-morphological studies, we think that it is important to make precise assumptions about the biomechanical demands and derived loading regimes, which might even vary among different morphological traits. We decided to use assumed peak loads as a baseline for our categorization, but other loading patterns, such as loading frequency or loading duration, play an important role as well (Currey, 2002) and might be equally appropriate to define these regimes. Future experimental and behavioral studies concentrating on the loading patterns during various locomotor activities in sciuriforms might shed light on the biomechanical demands of the femur.

We utilized the OU model *sensu* Hansen et al. (2008) in order to assess the relative influence of phylogenetic inertia and adaptation. As outlined above, the OU method cannot account for an interaction effect and, hence, the MPCF is most reliable for those variables that did not display an interaction between the predictor variables (locomotor group and body mass). We assumed that an OLS analysis with interaction effect is appropriate if a variable showed a high MPCF (i.e. low phylogenetic inertia) in the OU analysis (without an interaction effect). This was based on the idea that the theoretical inclusion of an interaction term should reduce the residual variation and thus increase the MPCF even more. The problem is that we do not know whether an OU model with an interaction term would be favored over an OU model without this term, just because this is the case for the OLS analysis. In fact, the phylogenetic correlation of residuals might change with a significant interaction term because of the different estimated slopes. We think it is reasonable to assume that these residual differences would be minor as the difference among slopes itself is rather small for any of the variables with interaction effect. Finally, the variables included in univariate analyses were selected on the basis of their supposed functional relevance for locomotor ecology. This differs from the overall bone shape that might integrate aspects driven by other factors, potentially resulting in a lower MPCF. Thus, the application of an OLS approach for the study of femoral shape based on the low phylogenetic inertia measured in the other variables has to be taken with caution. Keeping these limitations in mind, we conclude that interaction effects appear to play an important role for the study of allometry as demonstrated here and in previous studies (Alexander et al. 1979; Steudel, 1982; Bou et al. 1987; Christiansen, 1999a). Future work on this subject would profit from the development of evolutionary models accounting for this effect independent of the dimensionality of the data.

Conclusion

Overall, we found that most of the examined univariate traits of the sciuriform rodent femur scaled close to isometry in all three locomotor groups. Only the effective femoral length matched our assumptions, indicating that adjustments in this feature played a major role during the evolutionary diversification of sciuriform rodents. The overall similarity in scaling in most features of the femur indicates that this is not the case for allometric effects. Nevertheless, the low phylogenetic inertia found in all of the univariate parameters suggests that it was important during sciuriform evolution to maintain the observed scaling patterns. The comparison of general differences among locomotor groups suggests that more adjustments in the femur were necessary for the fossorial species than the aerial species. Using sciuriforms as a focal clade, our findings exemplify the importance of statistically accounting for potential interaction effects of different environmental factors in studies relating morphology to ecology.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

JW and JAN conceptualized the study. JW, EA, PA and AHvH collected the data. JW conducted, and LBD and A-CF assisted the data analysis. JW drafted the first manuscript. All authors contributed to and reviewed all versions of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Step-by-step landmarking procedure.

Fig. S2 Compound unpruned phylogenetic tree used for phylogenetic regression.

Table S1 List of sampled specimens.

Table S2 Landmark definitions.

Table S3 ANOVA tables.