

# Mouse Hallucal Metatarsal Cross-Sectional Geometry in a Simulated Fine Branch Niche

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**ABSTRACT** Mice raised in experimental habitats containing an artificial network of narrow “arboreal” supports frequently use hallucal grasps during locomotion. Therefore, mice in these experiments can be used to model a rudimentary form of arboreal locomotion in an animal without other morphological specializations for using a fine branch niche. This model would prove useful to better understand the origins of arboreal behaviors in mammals like primates. In this study, we examined if locomotion on these substrates influences the mid-diaphyseal cross-sectional geometry of mouse metatarsals. Thirty CD-1/ICR mice were raised in either arboreal (composed of elevated narrow branches of varying orientation) or terrestrial (flat ramps and walkways that are stratified) habitats from weaning (21 days) to adulthood ( $\geq 4$  months). After experiments, the hallucal metatarsal (Mt1) and third metatarsal (Mt3) for each individual were isolated and micro-computed tomography (micro-CT) scans were obtained to calculate mid-shaft cross-sectional area and polar section modulus. Arboreal mice had Mt1s that were significantly more robust. Mt3 cross sections were not significantly different between groups. The arboreal group also exhibited a significantly greater Mt1/Mt3 ratio for both robusticity measures. We conclude that the hallucal metatarsal exhibits significant phenotypic plasticity in response to arboreal treatment due to habitual locomotion that uses a rudimentary hallucal grasp. Our results support the hypothesis that early adaptive stages of fine branch arboreality should be accompanied by a slightly more robust hallux associated with the biomechanical demands of this niche. *J. Morphol.* 276:759–765, 2015. © 2015 Wiley Periodicals, Inc.

**KEY WORDS:** arboreal; foot; hallux; pedal grasping; micro-CT; cross-sectional geometry

## INTRODUCTION

It is commonly recognized that Primates (within Eutheria) represent one of a few adaptive radiations (many belonging to the Euarchonta) with specialized appendicular anatomy that yields functional advantages in the terminal branches of

arboreal substrates (Le Gros Clark, 1959; Cartmill, 1972; Szalay and Drawhorn, 1980; Sussman, 1991; Schmitt and Lemelin, 2002; Bloch et al., 2007; Sargis et al., 2007). There are also examples of rodent (Orkin and Pontzer, 2011), carnivoran (Fabre et al., 2013), marsupial (Lemelin and Schmitt, 2007; Shapiro et al., 2014), and nonmammalian vertebrates including frogs, lizards, and birds (Herrel et al., 2013; Sustaita et al., 2013) that are effective in this niche without primate-like specializations. For most small mammals, a key functional demand of this niche is to counteract lateral instability while balancing above very narrow horizontal supports. Manual and pedal prehension is important in this context because it makes counterbalancing torques possible to maintain above branch postures (Napier, 1967; Cartmill, 1974, 1985; Preuschoft et al., 1995; Lemelin and Schmitt, 2007).

Evidence from extant (Scandentia) and fossil (Plesiadapiformes) groups suggest that grasping with an opposable hallux is a common feature of fine branch arboreal taxa even when other (nonpedal grasping) specializations seen in primates are absent (e.g., stereoscopic vision; Bloch et al., 2007). Therefore, one interpretation is that adaptations for hallucal grasping represent an early transitional evolutionary stage toward better exploiting a fine branch niche. Such multistaged evolutionary sequences have been proposed in

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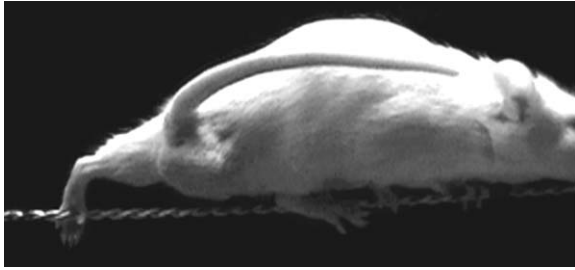


Fig. 1. Still-frame image of a typical mouse on a horizontal branch (i.e., arboreal group). These animals use a pedal grasp with an opposable hallux (see left foot) in coordination with tail motions that counteract lateral instability.

which a nongrasping, small, clawed euarchontoglian mammal (i.e., Stage 1) transitions to a clawed, pedal grasper like the tree shrew genus *Ptilocercus* (i.e., Stage 2) before evolving a more powerful pedal grasp with a nail-bearing hallux like that found in the opossum genus *Caluromys* (i.e., Stage 3; Gebo, 2004; Sargis et al., 2007; Youlatos, 2008). Accordingly, it should be possible to ascertain functional morphological signals in bones in the foot related to hallucal grasping performance. For example, a hallucal metatarsal (Mt1) with a relatively large peroneal process has been suggested to be an adaptation for powerful hallucal grasping in primates (e.g., Gebo, 2004). This is because strepsirrhine primates and tarsiers, traditionally viewed as capable of strong hallucal grasps, have larger peroneal processes compared to anthropoids that lack this morphology (Gebo, 1986, 1987; Szalay and Dagasto, 1988; Jacobs et al., 2009; Patel et al., 2012; Goodenberger et al., 2015). However, experimental (Boyer et al., 2007; Kingston et al., 2010; Patel et al., 2015) and comparative (Jacobs et al., 2009; Goodenberger et al., 2015) studies have made it clear that the presence of a large peroneal process alone is not consistent with hallucal grasping abilities in primates. Thus, the putative transition between hypothetical evolutionary Stages 1 and 2 may only be accompanied by very modest specializations in Mt1 morphology.

An approach to assess possible morphological changes that may have accompanied an evolutionary transition from Stages 1 to 2 is to use an animal model system. It has been shown that standard laboratory rodents as well as the European Red Squirrel locomote on slender substrates with adaptive behaviors that promote stability (Schmidt and Fischer, 2010, 2011). Recently, Byron et al. (2009, 2011, 2013) also promoted the use of a mouse model system that simulates a fine-branch arboreal habitat. This is because pedal (specifically hallucal) prehension along with tail coordination are observed to help counteract the body's tendency to pitch and roll mediolaterally on narrow

substrates (Fig. 1). The development of this skill for fine branch arboreality is evident at a cerebral and cerebellar somatotopic level (Byron et al., 2013). The habitual use of hallucal grasping commits the mouse to foot postures that place the substrate between the first and second digits. In climbing mice, the transverse processes on caudal vertebrae are relatively larger, the talar head angle is relatively lower, the talar head width and height are more uniform, and the talar neck is relatively shorter (Byron et al., 2011). The talar head is especially pertinent to pedal grasping because its angle relative to the skeletal elements of the more distal regions of the foot (i.e., navicular, entocuneiform, and metatarsals) is decreased and this relates to pedal inversion relative to the climbing substrate.

A similar mechanism for enhanced pedal inversion which helps promote stable above branch quadrupedal postures is reported for some euarchontans (Szalay and Drawhorn, 1980; Sargis, 2001, 2002). Moreover, earlier studies of lab mice and the rodent genus *Peromyscus* have emphasized the role of tail use, increased talar neck angle, and first metatarsal morphology in climbing behaviors (Siegel, 1970; Siegel and Van Meter, 1973; Siegel and Jones, 1975). Recent work with the harvest mouse (*Micromys minutus*; Urbani and Youlatos, 2013) substantiate this approach that early stages of primate evolution can be modeled with living rodent species. By combining tiny-size and secure hallucal grasping, those authors demonstrate that animals not typically viewed as being specialized for the fine-branch niche can nevertheless exploit this habitat to a degree of proficiency that is between the hypothesized Stages 2 and 3 of primate evolution.

Because the alterations in morphology documented by Byron et al. (2011, 2013) occur within a single generation of mice, they are not considered to be evolutionary adaptations. Instead, morphological changes in the skeletal, muscular, and nervous systems relate to experience-dependent growth and tissue modeling (nonheritable morphological variance) that is relevant to preprimate-like climbing ability (i.e., phenotypic plasticity; see Stearns, 1989; Scheiner, 1993; Via et al., 1995; Buck et al., 2010). The hallux, ankle, and tail morphology in these mice could mimic the earliest stage of euarchontan evolution into the fine-branched niche. Therefore, relating phenotypic plasticity to morphological adaptations for grasping could lead to a better understanding on the evolution of integrated organ systems related to the early functional demands of the fine-branch niche.

In this study, metatarsals are evaluated because these elements are presumed to play a significant role in pedal prehension (i.e., grasping *sensu lato*) and hallucal opposability. Specifically, we

examined Mt1 and Mt3 midshaft cross-sectional geometry because these variables can directly relate to bone performance during compression, tension, bending, and/or torsion (e.g., Lovejoy et al., 1976; Wainwright et al., 1982; Ruff and Hayes, 1983; Schaffler et al., 1985; Ruff, 1989; Carter et al. 1998; Daegling, 2002; Carlson and Judex, 2007). Generally speaking it is assumed that Mt1s used in habitual hallucal opposition and pedal grasping should experience greater (as well as more complex or variable) mechanical loads than Mt1s that are not used in grasping (Jashashvili et al., 2015). The oppositional (i.e., lateral) metatarsals, as exemplified by Mt3, may or may not also experience altered loading at the mid-shaft. These potentially contrasting loading environments are hypothesized to be caused by differential contraction of digital flexor muscle-tendon complexes acting on the hallux relative to those acting on the rest of the toes in combination with larger bending moments that act on the diaphysis as the hallux opposes the lateral metatarsals around a curved surface. Typically these muscles are identified as adductor hallucis, flexor hallucis longus, and flexor digitorum longus (Popeško et al., 1992) but in primates the digital flexors are referred to as flexor digitorum fibularis and flexor digitorum tibialis, respectively (Gebo, 1993). Thus, if the arboreal mouse Mt1 is subjected to higher mechanical stimuli, we predicted that their Mt1's should have greater geometric section properties relative to the same properties at the Mt3 mid-shaft and compared to the terrestrial mouse's metatarsals. This would be expected due to bone growth and modeling processes that accompany increased multidirectional loading associated with grasping modes of arboreal locomotion. Additionally, we expect that metatarsals in the terrestrial mouse should show greater homogeneity between the hallucal and lateral metatarsals.

## MATERIALS AND METHODS

All experimental manipulations were approved by Mercer University's Institutional Animal Care and Use Committee (protocol #A1001003) and complied with all legal requirements in the United States. Thirty male and female CD-1/ICR mice (Charles River Labs; Wilmington, MA) were euthanized for this study after being raised to adulthood (4–6 months old) in one of two experimental habitats beginning at postnatal day 21 (weaning). These habitats represent terrestrial and arboreal groups. Arboreal animals ( $n = 15$ ), that is those in the experimental or climbing group, were housed in an  $0.057 \text{ m}^3$  terrarium equipped with thin branches articulated into a complex resembling arboreal substrates. Substrates were made from 1 mm stainless steel safety lockwire (type: 302/304; Spencer Aircraft; Puyallup, WA) twisted into doublets and triplets. Branch diameter was between 2.0 and 3.5 mm and mice consistently preferred positional behavior that took place while balancing above branches (Fig. 1). Animals were encouraged to climb by flooding the floor of the terrarium with water and placing food, drinking water, and nest spaces among the branches. Terrestrial, or control animals, ( $n = 15$ ) were housed in an  $0.057 \text{ m}^3$



Fig. 2. A three-dimensional rendering of a representative mouse Mt1 in lateral view showing the location of midshaft (left) and the examined cross-section (right) to calculate cross-sectional geometry variables.

terrarium consisting of flat substrates arranged as walkways and ramps where plantigrade and/or digitigrade postures were used without any need for pedal grasping postures. All mice had access to food and water ad libitum. Previous use of this model system with arboreal groups, sedentary controls, and stratified walkway controls established that body mass does not significantly differ. Previous comparisons demonstrate that growth rates of arboreal mice are equal to sedentary controls (Byron et al., 2009). Also, each habitat type compared here (simulated fine branch niche vs. stratified walkway controls) promoted active exploration and eliminated stress and aggression commonly associated with housing same-sex groups of multiple individuals. Therefore, it is assumed that the activity levels of the two groups compared here are equivalent and that any differences observed are not caused by increased or decreased exercise, stress, aggressive, or sedentary habits in either group.

Appendicular samples were skeletonized using detergent maceration. One Mt1 from the terrestrial group was lost during this process so that in the final sample of terrestrial Mt1s there are only 14 specimens. Skeletal collections remain in the care of the Byron lab and can be made available on request. From these preparations, the Mt1 and Mt3 were identified and isolated for high-resolution micro-CT scanning. Micro-CT scans were obtained with a voxel size of approximately  $3.9 \mu\text{m}$  for Mt1s using a custom-made scanner (Feinfocus X-ray tube and Varian 2520V Paxscan a-Si flat panel detector) at the “Centre for X-ray Tomography” of Ghent University (Masschaele et al., 2007). The tube was operated at 90 kV and a 1 mm aluminum X-ray beam filter was used. Micro-CT scans for the Mt3 samples were obtained with a different custom-made UGCT scanner (Masschaele et al., 2013). These samples were scanned at a tube voltage of 120 kV and a filtration of 1 mm aluminum, yielding images with  $8 \mu\text{m}$  voxel size. Reconstruction was performed using the UGCT software package Octopus (Vlassenbroeck et al., 2007). To ensure that only a single bone was visible in a stack of images before analysis (because multiple bones were scanned simultaneously) image stacks were first cleaned in ImageJ software (<http://imagej.nih.gov/ij>) by deleting nonessential pixels in the background in each image. Each cleaned image stack was then oriented using the Moment of Inertia protocol in the BoneJ plugin for ImageJ software (Doube et al., 2010). This plugin calculates the three orthogonal principal axes ( $x$ ,  $y$ ,  $z$ ) from the raw pixel data after thresholding for bone pixels in the entire stack and produces a new image stack that is standardized in orientation (i.e., rotation and translation). An automated iterative thresholding algorithm, used by default in BoneJ and as implemented in current versions of ImageJ, was performed on the entire Mt1 and Mt3 image stack histogram. After identifying the new, reoriented slice corresponding to midshaft (Fig. 2), the Slice Geometry protocol in BoneJ was used to calculate the cross-sectional geometry variables (using the same thresholding protocols). For this study, we focused on a measure of cross-sectional cortical bone area at the mid-shaft (CSA) as well as a biomechanical proxy for twice bending strength ( $Z_{\text{pol}}$ : polar section modulus; Ruff, 2003).

Preliminary analyses did not find a significant difference in either body mass or bone length between control and experimental groups even though male and female animals were used in



TABLE 1. Descriptive statistics

Dependent variable	Experimental group	Metatarsal element	N	Mean	Std. deviation	Coefficient of variation (%)
Body mass (g)	Arboreal		15	35.64	5.117	14.3
	Terrestrial		15	38.22	6.139	16.1
Relative CSA: CSA <sup>(1/2)</sup> /bone length	Arboreal	Mt1	15	0.0958	0.0031	3.2
	Terrestrial	Mt1	14	0.0918	0.0060	6.3
Relative Zpol: Zpol <sup>(1/3)</sup> /bone length	Arboreal	Mt1	15	0.0670	0.0030	4.5
	Terrestrial	Mt1	14	0.0648	0.0039	6.0
Relative CSA: CSA <sup>(1/2)</sup> /bone length	Arboreal	Mt3	15	0.0552	0.0015	2.7
	Terrestrial	Mt3	15	0.0561	0.0021	3.7
Relative Zpol: Zpol <sup>(1/3)</sup> /bone length	Arboreal	Mt3	15	0.0308	0.0013	4.2
	Terrestrial	Mt3	15	0.0314	0.0018	5.7
Relative Mt1 CSA/relative Mt3 CSA	Arboreal		15	1.6280	0.0920	5.7
	Terrestrial		14	1.4880	0.1770	11.9
Relative Mt1 Zpol/relative Mt3 Zpol	Arboreal		15	3.0410	0.3777	12.4
	Terrestrial		14	2.7390	0.4710	17.2

each group. Therefore, the cross-sectional geometry variables were not scaled as is normally done in studies across a large range of species/animals to body mass or the product of body mass and bone length (e.g., Polk et al., 2000). Instead, all data were scaled only by bone length as an affirmation of the signal detected from the raw data. This approach also follows other studies of cross-sectional geometry in mice (e.g., Carlson and Judex, 2007; Carlson et al., 2008). In addition to comparing scaled CSA and Zpol for each metatarsal, the scaled variables were compared in a ratio of Mt1 relative to Mt3. This ratio yields another mechanism to assess Mt1 morphology given a point of reference for a more lateral portion of that same individual's foot that would oppose the hallux during grasping (i.e., Mt3).

All data were normally distributed. Welch's 1-tailed *t*-tests (for use with samples of possibly unequal variances) were used to test the directional hypotheses that for each variable the climbers have relatively more robust morphology (i.e.,  $H_1 = \mu_{\text{climber}} > \mu_{\text{control}}$  vs.  $H_0 = \mu_{\text{climber}} \leq \mu_{\text{control}}$ ). This procedure allows for a doubling of the region under the statistical curve that will reject a false null hypothesis if in fact climbing mice demonstrate a more robust phenotype. R version 3.0.1 (The R Foundation for Statistical Computing) was used to analyze cross-sectional geometry variables.

## RESULTS

Descriptive statistics for relative (scaled to bone length) and ratio of relative Mt1/relative Mt3 CSA

and Zpol are presented in Table 1. For each treatment group, the Mt1 was found to be greater than the Mt3 in both cross-sectional properties (Fig. 3). Group means for relative Mt1 CSA and Zpol are significantly larger for the arboreal mice compared to those raised on flat substrates (Table 2,  $P < 0.05$ ). Conversely, relative Mt3 CSA and Zpol in the arboreal group are not significantly different than those of the terrestrial group. The ratio of scaled CSA and Zpol Mt1/Mt3 for arboreal mice was also significantly higher (Tables 1 and 2,  $P < 0.05$ ). Thus, mice trained to walk on narrow substrates tend to have more robust hallucal metatarsals.

## DISCUSSION

This study was conducted to see if the hallucal metatarsal (Mt1) is subject to use-dependent morphological plasticity in a hypothetical evolutionary model of Stages 1 and 2 euarchontogloran arboreal adaptation. As defined by Gebo (2004) and modified by Sargis et al. (2007), this multistaged evolutionary sequence depicts a nongrasping, small,

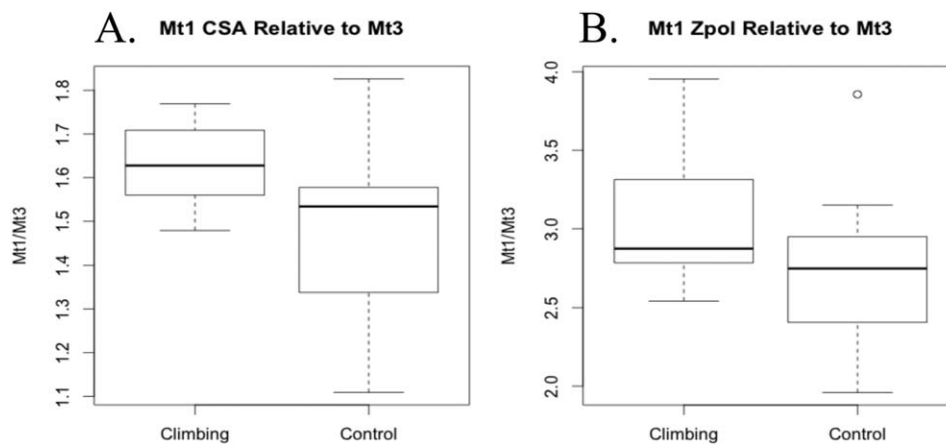


Fig. 3. Box plots comparing the arboreal (climbing) group to the terrestrial (control) group. (A) Mt1 CSA relative to Mt3 CSA. (B) Mt1 Zpol relative to Mt3 Zpol.

TABLE 2. Results of independent samples *t*-tests for Mt1 and Mt3 midshaft cross-sectional geometry variables. P-values at or below 0.05 are in bold and deemed statistically significant.

Dependent variable	Welch two-sample test statistic	df	P-value (1-tailed)
Body mass	-1.253	27.2	0.150
Relative CSA: Mt1 CSA <sup>(1/2)</sup> /bone length	2.242	19.2	<b>0.019</b>
Relative Zpol: Mt1 Zpol <sup>(1/3)</sup> /bone length	1.713	24.4	<b>0.050</b>
Relative CSA: Mt3 CSA <sup>(1/2)</sup> /bone length	-1.244	25.5	0.110
Relative Zpol: Mt3 Zpol <sup>(1/3)</sup> /bone length	-1.180	25.1	0.125
Relative Mt1 CSA/relative Mt3 CSA	2.661	19.3	<b>0.008</b>
Relative Mt1 Zpol/relative Mt3 Zpol	1.900	24.9	<b>0.035</b>

clawed euarchontoglires mammal (Stage 1) that transitioned to a clawed, pedal grasper-like *Ptilocercus* (Stage 2). The mouse system in this study attempts to model this Stage 1–2 transition that occurred prior to more specialized adaptations such as a nailed hallux for terminal branch feeding as seen in Sargis et al.'s (2007) Stage 3 Euprimateform (plesiadapoid and euprimate) ancestors. In fact, both Byron et al., (2009) and Urbani and Youlatos (2013) showed that rodents (and presumably taxa at the root of Euarchontoglires) are capable of pedal grasping and climbing on narrow supports. These mice can be considered rudimentary, facultative fine branch walkers, and thus, they reflect a minor shift away from Stage 1 euarchontoglires arboreality toward modestly more specialized fine branch locomotion. Our results support the research hypothesis presented earlier that mice trained to walk on simulated fine branch substrates would have relatively more robust Mt1s than those mice raised on terrestrial/flat substrates. This confirms that a small-bodied euarchontoglires using rudimentary hallucal grasps during arboreal locomotion can exhibit phenotypic changes in Mt1 bone strength.

In similar laboratory mouse (*Mus musculus*) experiments Siegel and Jones (1975) reported that a robusticity index for Mt1 did not show significant differences between climbing and control groups while Mts2–4 were more robust in terrestrial controls. They determined robusticity using Mt length/(Mt mass<sup>1/3</sup>) so that a lower index value was interpreted as greater Mt robusticity. Our methods differ because we include a morphomechanical assessment of mouse metatarsals by relating midshaft geometric properties to bone length. Of additional comparative interest, their dataset also included nonlaboratory murid rodents *Peromyscus floridanus* and the more arboreal *P. gossypinus*. The more arboreal *Peromyscus* species

only showed significantly greater Mt5 robusticity, and no difference for Mts1–4 (Siegel and Jones, 1975). Based on our results, a more comprehensive study of *Peromyscus* metatarsal mid-diaphyseal cross-sectional geometry would be informative.

In our experimental system, arboreality was associated with morphological plasticity related to hallucal grasping whereby the Mt1 was more robust in morphology by itself and relative to more lateral regions of the foot. A relatively greater Mt1 CSA and section modulus (Zpol), two biomechanical variables that estimate compressive and bending strength respectively, suggest that fine branch arboreality may stimulate the growth of mechanically stronger hallucal metatarsals. The terrestrial group of mice was not associated with these gains in Mt1 robusticity, most likely because they use a plantigrade posture with all toes pointing forward, instead of having a foot posture with a hallux draped over the substrate and in opposition with the other toes. Therefore, despite the categorization of this type of climbing by a nonspecialist mouse as rudimentary, we suggest it is associated with a morphological signal for hallucal grasping. However, we want to emphasize that the signal is qualitatively different from larger scale events such as the evolution of nails and hypothesized leaping specializations used by early euprimates in the fine branch niche.

Some aspects of Mt morphology from our mouse model are consistent with other taxa that show rudimentary adaptations for arboreality (compared to euprimates). For example, *Ptilocercus* has been qualitatively observed to have a robust Mt1 relative to other metatarsals in its foot (Le Gros Clark, 1926; Sargis, 2002). Moreover, when compared to terrestrially adapted tupaiines, *Ptilocercus* has a more abducted hallux that can oppose the lateral digits which is a morphology that actually may reduce stability of the entire foot during parasagittal motions. Also described by Sargis (2002) for *Ptilocercus* is the presence of a wide distal entocuneiform facet and a more globular proximal Mt1 articular facet. These are interpreted as adaptations that facilitate a greater range of abduction at the entocuneiform-Mt1 joint (i.e., it supports hallucal grasping) in *Ptilocercus*. In addition to *Ptilocercus*, short and robust Mt1s are observed in other noneuprimate grasp-climbing taxa like *Caluromys* (Argot, 2002). In *Caluromys*, the Mt1 and Mt5 are both robust relative to Mts2–4. With this condition the medial and lateral most metatarsals promote flexion and extension away from the parasagittal plane. This is not homologous with the grasping condition presumed to be at the base of Euarchonta, which is better represented by *Ptilocercus* (see Szalay and Dagosto, 1988; Sargis, 2001, 2002).

If Sargis (2001) is correct and the *Ptilocercus* (ancestral scandentian) condition is antecedent to

the primate condition, the plasticity exhibited by our model system may represent its most incipient form. We hypothesize that a slightly more robust Mt1 without entocuneiform modification should be the earliest feature associated with the type of prehensility without opposability that is described by Szalay and Dagosto (1988) for the euarchontan foot. Furthermore, this trait might even precede Euarchonta as the most rudimentary morphology associated with pedal grasping. These findings do not contradict the notion that euprimate pedal grasping morphology evolved as a later adaptive event. This morphology includes a significantly reinforced proximal hallux and associated distal entocuneiform joint facet that is uniquely derived for stability on arboreal substrates. This is consistent with the interpretation that euprimate origins signal an adaptive shift toward a more agile and demanding form of graspleaping locomotion (Szalay and Dagosto, 1988) rather than simply climbing and grasping among fine branch arboreal supports.

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