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Adaptive Patterns and Processes in Mammalian Arboreality

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Adaptive Patterns and Processes in Mammalian Arboreality

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Table of Contents

Table of Contents	1
Abstract.....	4
Περίληψη	6
Acknowledgments	9
Chapter 1: Introduction	11
1.1. The Evolution of Arboreality in Mammals.....	11
1.2. Adaptations to Arboreality	16
Morphological adaptations	17
Gaits and their metrics in arboreal locomotion.....	20
1.3. The Effect of Substrates	26
Substrate size	26
Substrate inclination	27
1.4. Small Arboreal Mammals	29
1.5. Aims and Scope	31
Chapter 2: From the ground up to the trees.....	35
2.1. Introduction.....	35
2.2. Materials and Methods	40
Specimens.....	40
Experimental setup.....	42
Gait analyses.....	43
2.3. Results.....	44
Gait parameters and their interactions.....	45
Symmetrical and asymmetrical gaits.....	48
Comparisons between species	50
Effect of substrate size.....	52
Effect of direction of movement	52
2.4. Discussion.....	53
Chapter 3: Reaching up to the arboreal milieu.....	60
3.1. Introduction.....	60
3.2. Materials and Methods	65
Specimens.....	65

Experimental setup.....	66
Gait analyses.....	67
3.3. Results.....	69
Vertical ascents	71
Vertical descents.....	75
Vertical ascents vs. vertical descents.....	76
3.4. Discussion.....	76
Chapter 4: Arboreal locomotion in a tiny scansorial mammal.....	81
4.1. Introduction.....	81
4.2. Materials and Methods	85
Specimens.....	85
Experimental setup.....	86
Gait analyses.....	87
4.3. Results.....	90
Gait metrics and their interactions.....	92
Effects of substrate diameter	95
Effect of direction of movement	95
4.4. Discussion.....	96
Chapter 5: Arboreal gaits in a medium-sized rodent.....	103
5.1. Introduction.....	103
5.2. Materials and Methods	109
Specimens.....	109
Experimental setup.....	110
Gait analyses.....	111
5.3. Results.....	115
Gait metrics and their interactions.....	116
Effects of substrate diameter	117
Effect of direction of movement	117
Symmetrical and asymmetrical gaits.....	120
5.4. Discussion.....	120
Chapter 6: Effects of body size on specialized arboreal glirids: Gaits in Muscardinus avellanarius and Glis glis.....	125
6.1. Introduction.....	125

6.2. Materials and Methods	128
Specimens.....	128
Experimental setup.....	129
Gait analyses.....	130
6.3. Results.....	134
Gait metrics, their interaction, and comparisons between specimens.....	134
The effect of substrate size	137
6.4. Discussion.....	137
Chapter 7: Gaits in an arboreal specialist	141
7.1. Introduction.....	141
7.2. Materials and Methods	145
Specimens.....	145
Experimental setup.....	146
Gait analyses.....	147
7.3. Results.....	149
Diagonality and duty factor	149
Effects of substrate diameter and direction of movement on diagonality...	150
Duty factor index	150
Velocity and stride parameters.....	153
7.4. Discussion.....	158
Chapter 8: Conclusions	164
8.1. The Effect of Substrates on Gait Metrics.....	164
Substrate Size.....	164
Direction of Movement.....	169
8.2. Adaptive Patterns and Processes in Mammalian Arboreality.....	172
Literature Cited.....	184
Appendix	208

Abstract

The arboreal habitat is a complex mesh of intertwining elements, which make locomotion challenging. Nevertheless, mammals have managed to cope with its complexity. During above-branch arboreal locomotion, the necessity for safety and stability is greatly stressed. Towards this end, many mammals adjust their gait and related metrics in respect to substrate characteristics, such as substrate diameter and inclination. Moreover, as early eutherian mammals exhibit postcranial correlates for arboreality, it is important to assess the behavioural mechanisms that are related to competent arboreal locomotion. However, even though there is wealth of information on primate gaits, there is a general lack of research in other mammals, and especially at a small body size. This study utilizes an experimental approach of simulated arboreal settings, attempting to isolate substrate parameters and compare how different small mammals modify their gait for arboreal locomotion.

For the purposes of the current dissertation we examined eight small-bodied mammals: the acrobatid diprodont *Acrobates pygmaeus* (~12 g), the murid rodents *Micromys minutus* (~10 g), *Apodemus agrarius* (~20 g), *Apodemus flavicollis* (~30 g), and *Thallomys paedulus* (~70 g) the cricetid rodent *Myodes glareolus* (~20 g), and the glirid rodents *Muscardinus avellanarius* (~20 g) and *Glis glis* (~130 g). All the specimens were filmed walking on four different arboreal substrate diameters (2mm, 5mm, 10mm, 25mm) and three different inclinations (45° descending, horizontal, 45° ascending) at 240 fps in a specially designed terrarium. Our analyses concerned basic gait parameters such as diagonality, duty factor, duty factor index, velocity, stride length and stride frequency. Most of the examined small clawed scansorial mammals primarily opted for lateral-sequence gaits during arboreal locomotion. *Acrobates pygmaeus* was a notable exception, by displaying diagonal-sequence gaits, convergently to primates. Most small mammals reduced diagonality, the relative swing phases of the limbs, and velocity on narrower substrates. These locomotor adaptations, although not always similar, represent behavioural mechanisms that increase stability, and promote continuous navigation along arboreal substrates. On wider substrates, most small mammals significantly increased velocity, and relative swing phases, which in some cases were enhanced by the use of asymmetrical gaits. These behaviours assist in swift movements, obstacle avoidance, and energetic optimization, and may also minimize exposure to

predators. The effect of inclination on gait parameters and metrics was very dissimilar between the examined species, implying that there is probably much underlying complexity, and various mechanisms that affect it. In terms of velocity regulation, almost all small mammals increased velocity primarily by stride frequency, rather than stride length, which also promotes arboreal safety, by reducing body oscillations.

Moreover, some of the examined parameters, such as duty factor and stride frequency, were able to predict the degree of arboreality consistently. Increased duty factor and decreased stride frequency in more terrestrial species accommodated stability by reducing touchdown torques, increasing simultaneous contact points and contact periods with substrates for which they are not well adapted. On the other hand, decreased duty factor and increased stride frequency on more arboreal species facilitates obstacle avoidance and manoeuvrability. Diagonality, which has often been linked to arboreality, was not a good predictor of the level of arboreality, and does not seem to be necessary for successful arboreal locomotion, at least in small mammals.

In conclusion, despite some limited dissimilarities, small arboreal and scansorial mammals display behaviours that ultimately contribute to the successful exploitation of the arboreal milieu. More terrestrial species, which lack similar arboreal morphological adaptations, respond to arboreal substrates by adjusting their gaits to increase stability and safety, thus reducing the risk of falling. On the other hand, species that are more arboreally adapted in terms of morphology, also display gait adaptations to facilitate proficient use of arboreal substrates. Body size and morphological similarities between extant arboreal/scansorial small mammals and early eutherian ancestors (e.g. *Eomaia* and *Juramaia*) suggest that these early forms would have behaved in similar ways, displaying lateral-sequence gaits, relatively high duty factors, and increasing velocity mainly by increasing stride frequency. These adaptations, which would have assisted in the efficient use of both terrestrial and arboreal substrates, very likely contributed to their successful dominance and subsequent radiations.

Περίληψη

Το ενδιαίτημα των δέντρων είναι ένα περίπλοκο πλέγμα κορμών, κλαδιών και φυλλώματος, τα οποία καθιστούν την κίνηση και μετακίνηση απαιτητικές. Όμως, τα θηλαστικά έχουν καταφέρει να ανταπεξέλθουν στην πολυπλοκότητά του. Κατά την κίνηση επί των κλαδιών, η αναγκαιότητα για ασφάλεια και σταθερότητα εντείνεται. Για αυτό το λόγο, πολλά θηλαστικά τροποποιούν την βάδισή τους και τις σχετικές μετρικές παραμέτρους με βάση τα χαρακτηριστικά των υποστρωμάτων, όπως η διάμετρος και η κλίση. Επιπρόσθετα, καθώς τα πρώιμα ευθήρια θηλαστικά επιδεικνύουν μετακρανιακό σκελετό που σχετίζεται με τη διαβίωση στα δέντρα, είναι σημαντικό να μελετήσουμε τους συμπεριφορικούς μηχανισμούς που σχετίζονται με την αποδοτική δενδρόβια κίνηση. Ωστόσο, αν και υπάρχει πλούτος πληροφοριών σχετικά με την βάδιση των πρωτεύοντων, υπάρχει μία γενική έλλειψη ερευνών σε άλλα θηλαστικά και ιδιαίτερα τα πιο μικρόσωμα. Η παρούσα έρευνα χρησιμοποιεί μία πειραματική προσέγγιση προσομοιωμένων δενδρικών συνθηκών, επιχειρώντας να απομονώσει παραμέτρους των υποστρωμάτων και να συγκρίνει το πώς διαφορετικά μικρά θηλαστικά τροποποιούν τον βηματισμό τους για την δενδρόβια κίνηση.

Για τους σκοπούς της παρούσας διατριβής εξετάσαμε οκτώ μικρόσωμα θηλαστικά: το διπρωτοδόντιο μαρσιποφόρο *Acrobates pygmaeus* (~12 g), τα μυοειδή τρωκτικά *Micromys minutus* (~10 g), *Apodemus agrarius* (~20 g), *Apodemus flavicollis* (~30 g), και *Thallomys paedulus* (~70 g), το κρηκιτοειδές τρωκτικό *Myodes glareolus* (~20 g), και τα μυωξοειδή τρωκτικά *Muscardinus avellanarius* (~20 g) και *Glis glis* (~130 g). Όλα τα δείγματα κινηματογραφήθηκαν να βαδίζουν επί τεσσάρων διαφορετικών διαμετρών δενδρικών υποστρωμάτων (2mm, 5mm, 10mm, 25mm) και τριών διαφορετικών κλίσεων (κάθοδος 45°, οριζόντιο, άνοδος 45°) στα 240 καρέ ανά δευτερόλεπτο σε ένα ειδικά σχεδιασμένο τερράριο. Οι αναλύσεις μας αφορούσαν βασικές παραμέτρους βάδισης, όπως η διαγωνιότητα, ο παράγοντας φόρτου, ο δείκτης παράγοντα φόρτου, η ταχύτητα, το μήκος διασκελισμού και η συχνότητα διασκελισμού. Τα περισσότερα από τα εξεταζόμενα μικρά γαμψονυχοφόρα αναρριχητικά θηλαστικά κατά κύριο λόγο επέλεξαν βάδιση πλευρικής-αλληλουχίας κατά την δενδρόβια κίνηση. Το *Acrobates pygmaeus* ήταν μια αξιοσημείωτη εξαίρεση, επιδεικνύοντας βάδιση διαγώνιας-αλληλουχίας, σε σύγκλιση με τα πρωτεύοντα. Τα περισσότερα μικρά θηλαστικά μείωσαν την διαγωνιότητα, τις σχετικές φάσεις αιώρησης των άκρων, και την

ταχύτητα στα λεπτότερα υποστρώματα. Αυτές οι κινητικές προσαρμογές, αν και δεν ήταν πάντα όμοιες, αποτελούν συμπεριφορικούς μηχανισμούς που αυξάνουν την σταθερότητα και προωθούν την απρόσκοπτη πλοήγηση κατά μήκος δενδρικών υποστρωμάτων. Σε πλατύτερα υποστρώματα, τα περισσότερα μικρά θηλαστικά αύξησαν σημαντικά την ταχύτητα και τις σχετικές φάσεις αιώρησης, τα οποία σε κάποιες περιπτώσεις ενισχύθηκαν από την χρήση ασύμμετρης βάδισης. Αυτές οι συμπεριφορές βοηθούν σε ταχείες κινήσεις, στην αποφυγή εμποδίων και στην ενεργιακή βελτιστοποίηση, και μπορεί επίσης να ελαχιστοποιήσουν την έκθεση στους θηρευτές. Η επίδραση της κλίσης στις παραμέτρους βάδισης ήταν πολύ ανόμοια ανάμεσα στα εξεταζόμενα είδη, υπονοώντας ότι πιθανώς να υπάρχει κάποια ενδογενής περιπλοκότητα, και διάφοροι μηχανισμοί οι οποίοι να επιδρούν σε αυτή. Σε σχέση με την ρύθμιση της ταχύτητας, σχεδόν όλα τα μικρά θηλαστικά αύξησαν την ταχύτητα κυρίως μέσω της συχνότητας διασκελισμού, παρά μέσω του μήκους διασκελισμού, το οποίο επίσης προωθεί την δενδρόβια ασφάλεια, μειώνοντας τις σωματικές ταλαντώσεις.

Επιπροσθέτως, κάποιες από τις εξεταζόμενες παραμέτρους, όπως ο παράγοντας φόρτου και η συχνότητα διασκελισμού, προέβλεψαν με συνέπεια τον βαθμό δενδροδιαβίωσης. Ο αυξημένος παράγοντας φόρτου και η μειωμένη συχνότητα διασκελισμού στα περισσότερα εδαφώβια είδη διευκόλυναν την σταθερότητα της κίνησης, μειώνοντας τις ροπές κατά την επαφή των άκρων, αυξάνοντας τα ταυτόχρονα σημεία επαφής και τις περιόδους επαφής με υποστρώματα για τα οποία δεν είναι καλά προσαρμοσμένα. Αντίθετα, ο μειωμένος παράγοντας φόρτου και η αυξημένη συχνότητα διασκελισμού στα περισσότερα δενδρόβια είδη διευκολύνει την αποφυγή εμποδίων και την ικανότητα ελιγμών. Η διαγωνιότητα, η οποία έχει συχνά συνδεθεί με την δενδροδιαβίωση, δεν ήταν καλός παράγοντας πρόβλεψης του επιπέδου δενδροδιαβίωσης και δεν φαίνεται να είναι απαραίτητη για την επιτυχή δενδρόβια κίνηση, τουλάχιστον στα μικρά θηλαστικά.

Εν κατακλείδι, παρά κάποιες περιορισμένες ανομοιότητες, τα μικρά δενδρόβια και αναρριχητικά θηλαστικά επιδεικνύουν συμπεριφορές που εν τέλει συνεισφέρουν στην επιτυχημένη εκμετάλλευση του ενδιαιτήματος των δέντρων. Τα περισσότερα εδαφώβια είδη, τα οποία δεν κατέχουν όμοιες μορφολογικές προσαρμογές, αποκρίνονται στα δενδρικά υποστρώματα μεταβάλλοντας την βάδισή τους ώστε να αυξήσουν την σταθερότητα και ασφάλεια, ώστε να μειώσουν τον κίνδυνο πτώσης. Σε αντίθεση, τα είδη που είναι περισσότερο μορφολογικά προσαρμοσμένα στην δενδροδιαβίωση

επιδεικνύουν προσαρμογές βάρδισης που διευκολύνουν την ικανή χρήση δενδρικών υποστρωμάτων. Το σωματικό μέγεθος και οι μορφολογικές ομοιότητες μεταξύ σύγχρονων δενδρόβιων/αναρριχώμενων μικρών θηλαστικών και των πρώιμων προγόνων των ευθηρίων (π.χ. *Eomaia* και *Juramaia*) υπονοούν ότι αυτές οι πρώιμες μορφές θα συμπεριφέρονταν με παρόμοιους τρόπους, επιδεικνύοντας βάρδιση πλευρικής-αλληλουχίας, σχετικά υψηλούς παράγοντες φόρτου, και αύξηση της ταχύτητας με αύξηση της συχνότητας διασκελισμού. Αυτές οι προσαρμογές, οι οποίες θα βοηθούσαν στην αποδοτική χρήση τόσο εδαφικών όσο και δενδρικών υποστρωμάτων, πολύ πιθανόν να συνεισέφεραν στην επιτυχή επικράτησή τους και στην ακόλουθη ακτινωτή διαφορά τους.

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Chapter 1: Introduction

There is a vast multiplicity of animal forms on earth, occupying extremely diverse environments, from the ice caps of the poles to deserts and tropical rainforests. In contrast to plants, animals need to move in order to evade predators, secure food and mate and have managed to do so in very diverse ways. Swimming through water, quadrupedal locomotion on the ground or on trees and flight through the air are just some of them. Aristotle was the first to systematically record some of the diversity of these specialisations, characteristically commenting in the opening paragraphs of his book “The History of Animals”: “Animals differ from one another in their modes of subsistence, in their actions, in their habits, and in their parts” (Thompson, 1910). After a long and complex history of scientific research on animal locomotion, we not only know a great amount of detailed information about it, but we are also able to effectively simulate it in computer programs and animate it but have also recently started to replicate it in robotics. Even though terrestrial locomotion has been thoroughly investigated, there is still a lot to be learned.

In the study presented here, I will focus on quadrupedal gait adaptations involved in mammalian arboreality, with a special focus on the gaits and efficiency of quadrupedal locomotion of small mammals on arboreal supports and their links to different degrees of arboreality. Towards this end, the main corpus will be comprised of case studies on mammalian arboreal quadrupedal gaits, which will both offer answers to individual questions and complement each other towards connecting gaits to arboreality. In this way, both single-species studies as well as comparative studies will be presented and thoroughly discussed as individual units. Lastly, I will attempt an analysis and a comparison of the common and divergent patterns of arboreal gaits, and provide insights in the evolution of relevant adaptations to arboreality.

1.1. The Evolution of Arboreality in Mammals

The arboreal habitat is a complex mesh of intertwining structural elements with specific particularities. Nevertheless, many animals, mammals included, have managed to cope with its complexity, and negotiate any available arboreal structure starting from low vegetation, such as the dense network of slender branches of shrubs near the ground,

reaching up to tree trunks and the branches of all sizes and foliage in the topmost stories. The branches on a tree are limited in number and are highly variable in diameter and orientation. Pathways are interrupted where a branch ends and a new one begins, and can be oriented at any possible angle; even a single branch can change orientations numerous times along its length. Moreover, branches are unstable, subject to oscillations, bending, and even breaking. They can be set in motion in unpredictable trajectories by the wind, an animal's own movement, as well as the movement of other animals. Falling, therefore, is a very prominent risk. For an arboreal mammal, tumbling to the ground can be deadly, due to injury or exposure to predators. Even if it remains unscathed, it must spend significant amounts of energy to re-ascend. Thus, the successful exploitation of the arboreal habitat is likely related to behavioural and morphological adaptations that reduce the possibility of falling.

Arboreal mammals spend all or most of their lives in different vegetation layers, while scansorial mammals exploit both arboreal and terrestrial substrates (Hildebrand, 1995). In extant mammals, arboreality is widespread, and has evolved independently in numerous taxa (Alexander, 1992; Hildebrand, 1995; Nowak, 1999; Kemp, 2005; Rose, 2006). Among metatherians, the majority of opossums (Order Didelphimorphia) are adapted for arboreality, as well as many genera of diprotodonts (Order Diprotodontia), including a secondarily arboreal kangaroo (Hildebrand, 1995). Among extant eutherians, arboreality is present in most extant clades. Among the Xenarthra, the order Pilosa contains arboreal anteaters and sloths (Taylor, 1978, 1985). Both colugo species (Order Dermoptera) are arboreal climbing gliders (Panyutina, Korzun & Kuznetsov, 2015). Most Primates (Order Primates) are adept climbers or primarily arboreal, and quite possibly of arboreal ancestry (Cartmill, 1972, 1974a, 1992; Jenkins, 1974; Sussman, 1991; Soligo & Müller, 1999; Soligo & Martin, 2006; Cartmill, Lemelin & Schmitt, 2007). Even though few treeshrews (Order Scandentia) are arboreal and most are terrestrial (Emmons, 2000; Panyutina *et al.*, 2015), all possess morphological features associated with arboreality and most probably descend from arboreal ancestors (Emmons, 2000; Olson, Sargis & Martin, 2004). Among the order Chiroptera, many species roost in trees, while some climb them as well (Hildebrand, 1995; Panyutina *et al.*, 2015), and are most certainly of arboreal origin (Simmons, 1995). Many carnivorans (Order Carnivora) engage in arboreal or scansorial locomotion, and especially the procyonids (McClern, 1992) and viverrids (Taylor, 1989). Rodents (Order Rodentia) display a wide locomotor diversity,

with some families such as the Sciuridae (Thorington, Miller & Anderson, 1998) and Gliridae (Juškaitis, 2008; Kryštufek, 2010) being extensively arboreal (and of arboreal origin), and numerous arboreal representatives are present among the other families, such as the Anomaluridae, Criticidae, Erethizontidae, Muridae, Nesomyidae, Platacanthomyidae, etc. (Samuels & Van Valkenburgh, 2008).

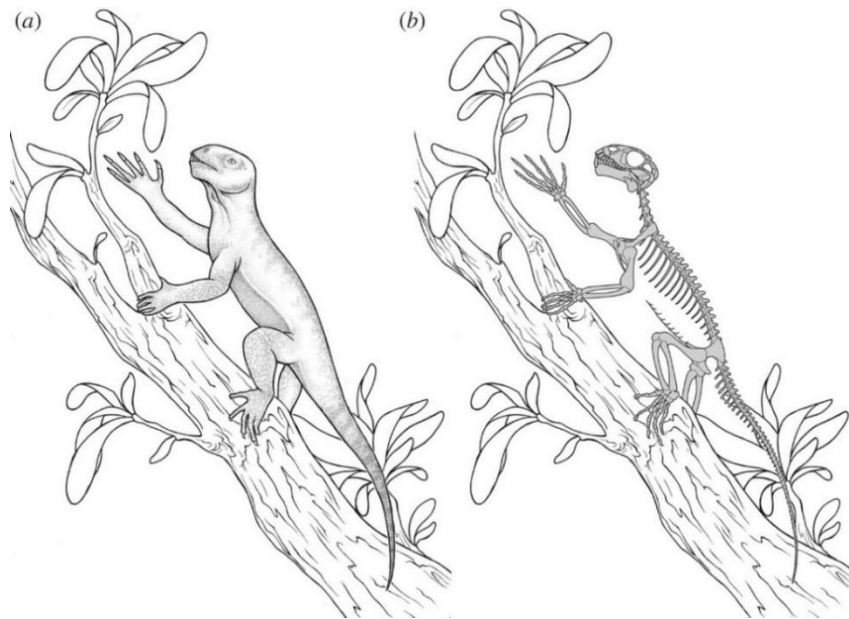


Figure 1.1. Reconstruction of *Suminia getmanovi* in both (a) flesh and (b) skeleton. From Fröbisch & Reisz (2009).

Apart from being widespread in extant mammals, arboreality has had a strong presence in mammalian evolutionary history. Plant-based life on the ground was not always as currently observed. The first plants started colonizing land at 470 to 425 mya (Bennici, 2008), fundamentally altering earth's land-based ecosystems (Algeo, Scheckler & Maynard, 2002; Driese & Mora, 2002). The earliest organisms resembling our idea of trees were tree ferns, horsetails and lycophytes, which formed the first forests. *Wattieza* is the oldest known tree from the Middle Devonian (~385 mya) (Stein *et al.*, 2007), followed by *Archaeopteris* (383-323 mya) (Beck, 1960). By the end of the Devonian period (417-354 mya), both invertebrate and vertebrate animals had also moved into terrestrial ecosystems. However, there is a significant gap (about 120 mya) in the fossil record separating the appearance of the first trees and the first recorded arboreal vertebrate. *Suminia getmanovi* (Fig. 1.1; Class Synapsida, Order Therapsida, Family Otsheridae), an herbivorous synapsid reptile (early mammalian ancestors), was the first

truly arboreal vertebrate encountered in the fossil record, and lived approximately 260 mya, within the Late Permian (Ivakhnenko, 1994; Rybczynski, 2000). The postcranial anatomy of *Suminia* contains skeletal evidence for prehension and arboreality as indicated by its elongate limbs, intrinsic phalangeal proportions, a divergent first digit and a possibly prehensile tail (Fröbisch & Reisz, 2009).

The first mammals and their sister taxa are encountered some 100 M years after *Suminia*, with a relatively rich record of fossil species showing scansorial and arboreal adaptations. Among them, the docodontan *Agilodocodon scansorius* was a small mammaliaform (27 g) from the Middle Jurassic (160 mya) possessing slender and gracile hands, feet, and digits, ending in curved claws (Fig. 1.2.a) indicating arboreal clinging and climbing activities (Meng *et al.*, 2015). From the same period, representatives of another early mammalian clade, the Allotherian Euharamiyidans, also display skeletal features related to arboreality: *Senshou lui* (300 g; Fig. 1.2.b), *Xianshou linklong* (83 g), *Xianshou songae* (40 g) and *Arboroharamiya jenkinsi* (354 g), all possess forelimb, hind limb and axial postcranial adaptations for arboreal locomotion, including tail prehensility (Zheng *et al.*, 2013; Bi *et al.*, 2014). Another Late Jurassic mammal, the cladotherium *Henkelotherium guimarotae* also possesses adaptations associated with climbing and was possibly scansorial (Vázquez-Molinero *et al.*, 2001). Moreover, around 125 mya, the multituberculate *Sinobataar sp.* bears morphological adaptations for arboreality (Hu *et al.*, 1997). Concurrently, the eutriconodontan *Volaticotherium antiquum* was the first gliding arboreal mammal, belonging to a sister taxon of the ancestors of all extant mammals (Gaetano & Rougier, 2011). With a body mass at ~70 g and a body length at 120-140 mm, it possessed a sizable furry patagium (flying membrane) for gliding flight, as well as elongated tail and limbs, clearly adapted for arboreal life (Meng *et al.*, 2006). To present, it represents the earliest gliding mammal, probably unrelated to extant gliders, and it stands as the first of the seven independent evolutionary lineages of mammalian gliding within the Diprotodontia (Petauridae, Acrobatidae, Pseudocheiridae), Dermoptera (Cynocephalidae) and Rodentia (Sciuridae, Anomaluridae) (Jackson & Thorington, 2012). More recent mammals of the early Cretaceous, the eutriconodontan *Jeholodens* (Chen & Wilson, 2015), multituberculate *Sinobaatar* (Hu & Wang, 2002), and symmetrodontan *Zhangotherium* (Chen & Wilson, 2015), all possess postcranial morphologies suggesting scansoriality or arboreality.

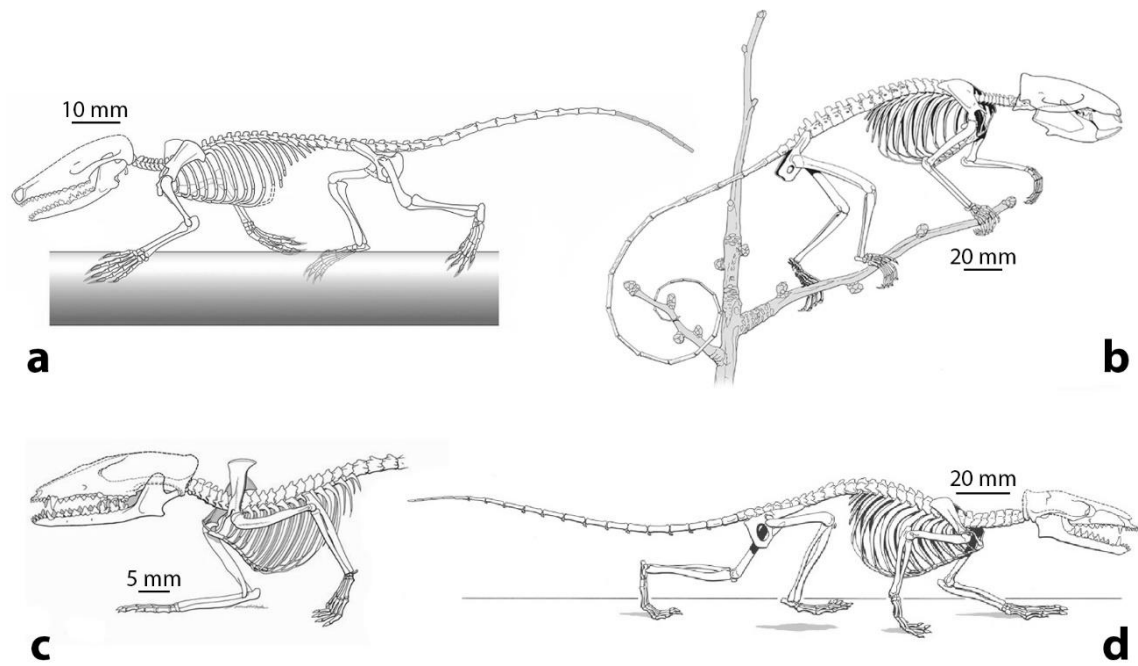


Figure 1.2. Skeletal reconstructions of: (a) *Agilodocodon scansorius* (adapted from Meng et al., 2015); (b) *Senshou lui* (adapted from Bi et al., 2014); (c) *Eomaia scansoria* (adapted from Ji et al., 2002); (d) *Juramaia sinensis* (Luo et al., 2011). Please note that each reconstruction is at a different scale.

Importantly, the two oldest known eutherian species have also been described as scansorial. *Juramaia sinensis* (Fig. 1.2.d; Class Mammalia, Infraclass Eutheria), the oldest known eutherian mammal is, dated at 160 mya, in the late Jurassic. *Juramaia* was a very small insectivore, weighing around 15-17 g. Their phalangeal indices suggest grasping capabilities similar to extant scansorial and arboreal mammals, and proposed paleohabitats involve small shrubs and medium to low height vegetation (Luo *et al.*, 2011). Arboreal adaptations have been also reported from another early eutherian mammal, *Eomaia scansoria* (Fig. 1.2.c) dating from the early Cretaceous (~125 mya), and weighing around 20-25 g. *Eomaia* bears fore- and hind feet adaptations that have been functionally related to scansorial activities and are reminiscent of the dormouse *Glis* (Ji *et al.*, 2002). More recent eutherians, from the Late Cretaceous, primarily include species adapted to terrestrial life (Hurum & Kielan-Jaworowska, 2008). However, the small basal euarchontan *Deccanolestes* appears to have postcranial adaptations related to scansoriality (Boyer *et al.*, 2010; Fabre *et al.*, 2014).

In the other major clade of extant therian mammals, the Metatheria, the oldest representative (early Cretaceous), *Sinodelphys szalayii*, was also a small (~30 g) arboreally adapted mammal, hinting towards a possible arboreal ancestry. *Sinodelphys* is

characterized by carpal, fore- and hind feet features that are linked to strong flexion and grasping, indicating climbing and scansoriality, as well as arboreal quadrupedalism (Luo *et al.*, 2003). Discoveries of metatherians from the Late Cretaceous reveal a radiation of locomotor forms, including terrestrial, and semiaquatic species (Williamson, Brusatte & Wilson, 2014). Moreover, descriptions of fragmented postcranial elements from Deltatheroid metatherians hint at scansorial and/or arboreal habits for some of the discovered representatives (Szalay & Sargis, 2006; Chester *et al.*, 2010, 2012).

Arboreal locomotion appears to be widespread among extant eutherians and metatherians, and has apparently played an important role in their evolution. Apart from a significant record of Mesozoic mammals (*sensu lato*) bearing characters suggestive of scansorial and arboreal activities (Luo *et al.*, 2003, 2011; Larson & Stern, 2006; Zheng *et al.*, 2013; Bi *et al.*, 2014; Meng *et al.*, 2015), the oldest fossil specimens from the two most successful extant mammalian clades, the Eutheria and Metatheria, were arboreal/scansorial (Ji *et al.*, 2002; Luo *et al.*, 2003, 2011; Larson & Stern, 2006; Zheng *et al.*, 2013; Bi *et al.*, 2014; Meng *et al.*, 2015). Taking this into account, the most parsimonious hypothesis is that the last common ancestor of these two clades (Eutheria and Metatheria) very likely involved a significant arboreal component in its locomotor repertoire. As most of these arboreal/scansorial Mesozoic mammals are generally small to very small (Jenkins, 1974; Ji *et al.*, 2002; Luo *et al.*, 2003, 2011; Larson & Stern, 2006; Kielan-Jaworowska, 2013; Zheng *et al.*, 2013; Bi *et al.*, 2014; Meng *et al.*, 2015), it is also important to understand locomotion at a small size. Towards this end, the comparative study of locomotor behaviour in extant arboreal and scansorial small mammals can reveal common and divergent adaptive patterns in arboreal locomotion, and their driving factors. These observations can then be used in conjunction with morphological characteristics to understand the arboreal adaptations of ancestral species, and infer patterns related to the evolution of mammalian arboreal locomotion.

1.2. Adaptations to Arboreality

Tree branches and trunks are the primary substrates for arboreal locomotion. They essentially differ from terrestrial substrates by being discontinuous, limited in length and variable in width and inclination, and subject to bending and oscillations (Grand, 1972; Cartmill, 1974b). Potentially, this variety holds a distinct possibility of falling, with potential injury or death and/or exposure to predators. Under this selective

pressure, arboreal adaptations (behavioural and morphological) evolved to facilitate safe and secure body displacement within the canopy, and to establish firm hold of arboreal substrates in order to avoid loss of balance or toppling over.

Contact with a substrate is usually achieved with the fore- and hind feet (autopodia), and in some cases the tail (Cartmill, 1974b; Alexander, 1977; Hildebrand, 1995). Animals walking on a horizontal or sub-horizontal branch are always at risk of toppling over; a risk which can be counteracted by: (i) possessing relatively short limbs, in order to lower the centre of mass closer to the substrate, as exemplified by dormice (Juškaitis, 2008; Kryštufek, 2010), (ii) having prehensile extremities to keep a secure grip on the branch, a common attribute in most arboreal tetrapods (mammals included) (Sustaita *et al.*, 2013), (iii) hanging below branch, as in sloths (Nyakatura *et al.*, 2007), colugos (Dzulhelmi & Abdullah, 2009), and many monkeys and apes (Hunt, 1991), and (iv) reducing body size or adopting a crouched posture, as observed in some small arboreal mammals in order to have the support points more widely spread on the branch surface, and to rapidly shift direction of movement in face of irregularities of the supports (Schmidt, 2008).

Most morphological adaptations of arboreal mammals stress the necessity for stability. Stability can be either static or dynamic (Lammers & Zurcher, 2011a). Static stability is the process when an animal remains motionless, with no forward or backward movements, nor any axial rotations; however, static stability may also apply to very slow body progression, as the underlying biomechanics remain similar. On the other hand, in faster locomotion, mass distribution on the limbs constantly changes, and so do the produced forces and torques (Lammers & Zurcher, 2011b). Combined with anatomical adaptations, gaits can be considered as a behavioural mechanism to further enhance dynamic stability during quadrupedal arboreal locomotion (Shapiro & Raichlen, 2005; Schilling & Hackert, 2006; Stevens, 2006; Young, 2009, 2012, Schmidt & Fischer, 2010, 2011, Lammers & Zurcher, 2011a, 2011b; Shapiro & Young, 2012).

Morphological adaptations

Even though the present research focuses mainly on behavioural adaptations to arboreal life, morphological and anatomical correlates are tightly linked to effective arboreal locomotion. No matter the specifics of locomotor patterns, both the forefeet and

hind feet of arboreal mammals bear adaptations which enhance friction, interlocking, and bonding at the molecular level with the surfaces they are moving on. These mechanisms assist in propelling the body upwards, or braking when moving downwards in the discontinuous three-dimensional arboreal milieu and avoid falling while moving or standing, especially under challenging conditions (Alexander, 1992; Hildebrand, 1995). To achieve interlocking, the digits of both fore- and hind feet of arboreal mammals often end in deep curved claws. These interlock with the crevices of branches and trunks and are particularly useful in climbing (Cartmill, 1974b, 1985; Hildebrand, 1995; Soligo & Müller, 1999). On the other hand, enhanced gripping by the pads of the feet (Cartmill, 1974a, 1974b; Sussman, 1991; Soligo & Müller, 1999) has led to the reduction of claws to flat nails in some mammals, such as primates and some marsupials (Cartmill, 1974b). Finally, apical pads bond with substrates at the molecular level, further assisted by the excretion of organic adhesives by glands on hands and feet (Hildebrand, 1995).

The hind feet of arboreal mammals are commonly characterised by powerful grasping, especially combined with the reduction or modification of claws. Pedal grasping is identified as the orientation and positioning of toes so as to apply functionally effective forces onto a substrate, wholly or partly surrounding it, in order to establish a static and/or dynamic posture (Sustaita *et al.*, 2013). Effective pedal grasping in arboreal mammals is achieved by robust musculature of the hind feet with powerful flexors with long tendons, independent movement of digits I, II and V, extended mobility of the upper and lower ankle, and inter-tarsal joints, as well as soft, sensitive and expanded foot pads with excretory glands at high densities, all contributing to an operative foot position that increases friction, interlocking and bonding with the substrate (Jones, 1953; Cartmill, 1974b, 1985; Jenkins & McClearn, 1984; Haffner, 1996, 1998; Meldrum, Dagosto & White, 1997; Smith & Smith, 2013; Sustaita *et al.*, 2013). Among different grasping modes, pedal grasping using a strong, divergent hallux is especially efficient for securing firm foot holds on most types of arboreal supports, as displayed in primates and a few other arboreal mammals (e.g. Szalay & Dagosto, 1988; Sargis *et al.*, 2007; Youlatos, 2008; Kingston *et al.*, 2010; Sustaita *et al.*, 2013; Urbani & Youlatos, 2013; Goodenberger *et al.*, 2015; Youlatos *et al.*, 2015; Youlatos, Karantanis & Panyutina, 2016). Kinematically, pedal grasping enables an animal to manipulate substrate reaction forces, i.e. the forces needed for propulsion (Preuschoft, 2002; Witte, Preuschoft & Fischer, 2002). By producing a counter-transfer of reaction moments with the substrate, pedal grasping assists in the

dynamic shift of the weight from the forelimbs to the hind limbs (Reynolds, 1985). Overall, the grasping ability of hind feet appears to be of major importance in achieving stability during arboreal locomotion, as is demonstrated in many mammalian lineages (i.e. primates, didelphimorphs, diprotodonts, scandentians) possessing efficient prehensile feet which probably evolved prior to grasping forefeet (Cartmill, 1974a; Szalay, 1984; Szalay & Dagosto, 1988; Sargis, 2001; Bloch *et al.*, 2007).

The musculature and bone morphology of the forelimbs of arboreal mammals varies depending on the locomotor modes employed (suspension, vertical climbing, leaping, or above-branch quadrupedalism; e.g. Taylor, 1985; Stalheim-Smith, 1989; Youlatos, 2000; Argot, 2001). Despite differences, arboreal mammals tend to have extremities which assist their ability to climb, stay on top of, or hang below substrates. This commonly leads to possessing longer proximal phalanges and shorter metacarpals than their terrestrial relatives (Kirk *et al.*, 2008). These are combined with the presence of overall long digits (Napier, 1967; Lemelin, 1999; Hamrick, 2001; Krattli, 2001; Zefferer, 2002; Kirk *et al.*, 2008), which enhance gripping by allowing the fingers to wrap around substrates (Lemelin, 1999; Lemelin & Schmitt, 2007). Especially in a fine-branch milieu, this enables countering of the pitching and rolling of the torso during locomotion (Napier, 1967; Cartmill, 1974b; Preuschoft, Witte & Fischer, 1995; Lemelin & Schmitt, 2007) and increases general prehensility (Lemelin, 1999; Hamrick, 2001). Moreover, elongation of the digits to the extreme is also related to gliding, i.e. in colugos, another form of arboreal locomotion (Beard, 1993; Hamrick, 2001; Panyutina *et al.*, 2015). In order to increase friction and interlocking with the substrate, forelimb digits form various grips, utilizing power exerted by strong flexors and extensors in the forearms. These muscles are differentially developed, depending on how digits apply power in each grip. Most primates habitually oppose the pollex to the lateral digits, thus gripping between digits I and II or I and III, when digit II is greatly reduced (Napier, 1961). However, arboreal metatherians and some primates form a zygodactylous or schizodactylous grasp (*sensu* Haines, 1958), establishing a powerful grip between digits II and III (Youlatos, 1999a, 2010). The grasping ability of the forelimbs can greatly contribute to stability during above-branch locomotion (Youlatos, 1999a, 2010), especially in a narrow-branch niche (Cartmill, 1992; but also see Orkin & Pontzer, 2011), as well as significantly assist the hind limbs in generating propulsion for vertical climbing (Youlatos, 2010; Reghem *et al.*, 2012).

Lastly, in some arboreal species, the tail is also used as a fifth limb, actively assisting in positional behaviour. When the tail alone can support the entire body, it is referred to as prehensile. Prehensile tails are curled at the end, and their caudal surface is often naked to increase friction (Lemelin, 1995; Youlatos, 2003; Organ, 2008). They are characterised by expanded sacroiliac joints and long proximal caudal regions or a high number of proximal caudal vertebrae, a powerful caudal flexor musculature and long flexor and extensor tendons, as well as developed mm. intertransversarii, and short, robust distal vertebrae with expanded transverse processes when present in both primates (Dor, 1937; Lemelin, 1995; Organ, 2008) and carnivores (Dor, 1937; Youlatos, 2003; Organ, 2008). Prehensile tails are more common in medium-sized (2-12 kg) species which mainly inhabit Neotropical forests, and may be evolutionarily linked to locomotion using weak branches, which are prone to downward deformation (Emmons & Gentry, 1983; Meldrum, 1998).

Gaits and their metrics in arboreal locomotion

Competent locomotion requires the effective combination of morphological and behavioural adaptations, to which they may be functionally related. During arboreal locomotion, gaits can serve as a mechanism to assure stability and consequent safe navigation in the arboreal milieu. A gait is the pattern, or sequence, of limb movements during locomotion over a solid substrate. Animals select a few different gaits on the basis of the velocity, the features of the substrate used, the need to manoeuvre, and the energetic efficiency (Hildebrand, 1967, 1968, 1976, 1995; Cartmill, Lemelin & Schmitt, 2002). A stride is the series of events between two consequent touchdowns of a foot. Stride period is the time interval during which a stride takes place. When velocity is kept constant, this interval remains the same for all feet. A gait cycle encompasses the sequence of fore- and hind limb displacements that take place within a stride period. Gaits can be either symmetrical, in which the movements of the left and right limbs of a girdle are evenly spaced in time, or asymmetrical, in which they move more or less concurrently. Asymmetrical gaits can be described by duty factor and duty factor index, whereas symmetrical gaits additionally include another important parameter, diagonality.

Diagonality, or forelimb-hind limb phase (Hildebrand, 1967; Cartmill *et al.*, 2007), is the percentage of the stride cycle the footfall of a forelimb follows behind the ipsilateral hind limb. It is measured as a scale variable ranging from 0 to 100, but is also often divided into ordinal classes: (a) Lateral Sequence Lateral Couplets ($0 \leq \text{LSLC} < 25$), (b) Lateral Sequence Diagonal Couplets ($25 \leq \text{LSDC} < 50$), (c) Trot ($=50$), (d) Diagonal Sequence Diagonal Couplets ($50 < \text{DSDC} \leq 75$), (e) Diagonal Sequence Lateral Couplets ($75 < \text{DSLCL} \leq 100$). Even more simplified, it can be divided into three categories: (a) Lateral Sequence ($0 \leq \text{LS} < 50$), (b) Trot ($=50$), (c) Diagonal Sequence ($50 < \text{DS} \leq 100$).

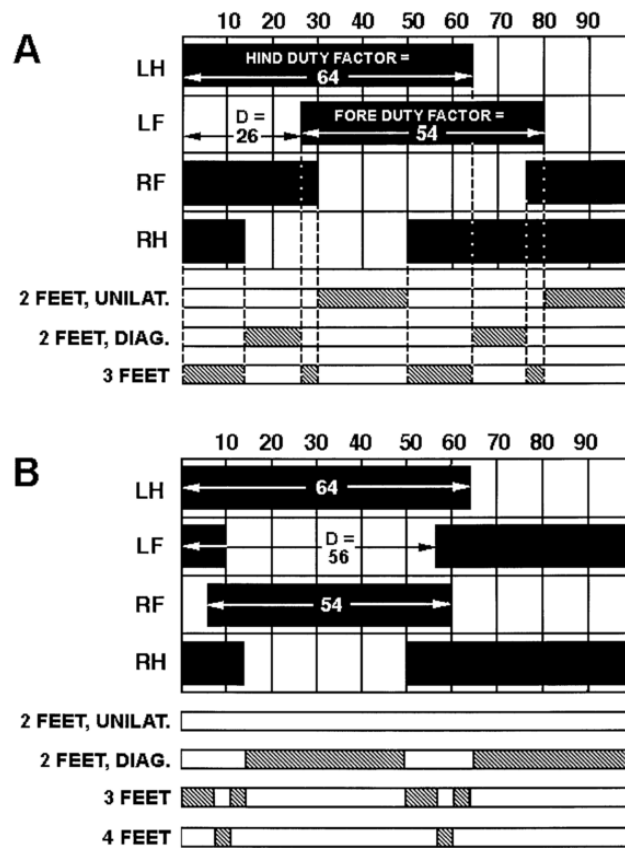


Figure 1.3. Support patterns in a lateral-sequence (A; Diagonality = 26) and a diagonal-sequence (B; Diagonality = 56) walk with a duty factor of 64. Reproduced from Cartmill *et al.* (2002).

Moreover, during a gait cycle, a foot can be either on the substrate (stance phase) or in the air (swing phase). A foot's duty factor (Hildebrand, 1967) is the percentage of the stride cycle during which it is anchored on the substrate (Fig. 1.3). If the duty factor of a gait is higher than 50, each foot will be on the substrate for most of the gait cycle. If the gait is symmetrical, it would be called a walk. On the other hand, a symmetrical gait with a duty factor below 50 is a running gait. Symmetrical gaits and their parameters are considered important in arboreal locomotion, as they have been linked to arboreal

adaptations (Prost, 1965; Tomita, 1967; Kimura, Okada & Ishida, 1979; Cartmill *et al.*, 2007). Nevertheless, recent results highlight that asymmetrical gaits are also common among arboreal mammals and should thus be further explored (Young, 2009; Schmidt & Fischer, 2010; Shapiro, Kemp & Young, 2016).

Lastly, the duty factor index (DFI) is calculated as the hundredfold ratio of the duty factor of the hind limbs divided by that of the forelimbs, and expresses the relative contact of the hind limbs compared to that of the forelimbs (Cartmill *et al.*, 2007). When the DFI is over 100, the hind limbs have a longer relative stance phase compared to the forelimbs, while this is inversed if the DFI is below 100. Consequently, the DFI can be insightful for detecting differences in the relative contribution of the forelimbs and hind limbs during locomotion (Cartmill *et al.*, 2002, 2007; Nyakatura, Fischer & Schmidt, 2008; Nyakatura & Heymann, 2010).

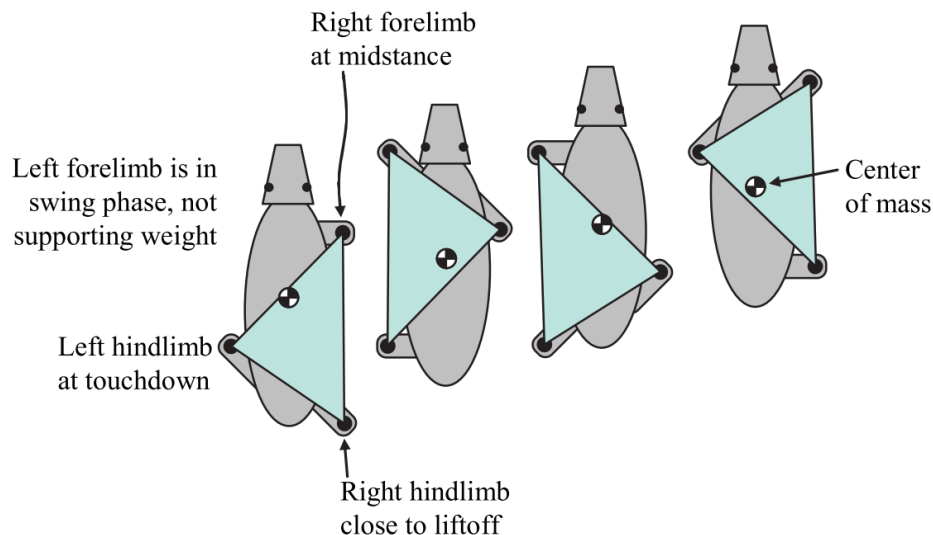


Figure 1.4. Support polygons during locomotion in a lateral-sequence gait. Note that 3 feet are in contact with the substrate at all times, hence a duty factor of 75. Reproduced from Lammers & Zurcher (2011a).

In symmetrical gaits, DS gaits (Diagonality > 50) involve a footfall pattern in which a forelimb touches down after more than half of the stride cycle following the touchdown of the ipsilateral hind limb. On the other hand, in LS gaits, the touchdown of a forelimb takes place after less than half of the cycle following the touchdown of the ipsilateral hind limb. Diagonal sequence (DS) gaits are particular by being prevalent among primates, while most other mammals primarily make use of LS gaits (Hildebrand, 1967; Cartmill *et al.*, 2007). Since DS gaits were initially only encountered in primates, they were thought to be superior to LS gaits in terms of stability during arboreal locomotion (Prost, 1965;

Tomita, 1967; Prost & Sussman, 1969). However, this has been disproven and attributed to a neurological reorganization in the primate brain (Vilensky & Larson, 1989). In recent years, DS gaits have been shown to have convergently evolved in a number of other taxa, including some arboreal metatherians (White, 1990; Schmitt & Lemelin, 2002; Karantanis, Youlatos & Rychlik, 2015) and the arboreal carnivoran *Potos flavus* (Lemelin & Cartmill, 2010). Consequently, it seems improbable that the convergent evolution of DS gaits is unrelated to any adaptive significance. Cartmill *et al.* (2007) proposed that DS gaits are beneficial in that they assist in the inspection of new, unknown and possibly unstable substrates, while moving on terminal branches. Essentially, DS gaits may allow for the safe anchoring of a hind limb, while the diagonally opposite forelimb moves and tests the substrate. If something goes wrong, the animal can fall back to the safety of the previous substrate, assisted by the anchored hind limb, while keeping its centre of mass within a support polygon generated by the limbs, thus preventing it from toppling over.

DS or LS gaits, with diagonality scores between 25 and 75, are referred to as diagonal couplet (DC) gaits. In these gaits, the footfalls of contralateral limbs are more concurrent than in lateral couplets gaits, with D scores >75 or < 25 . Diagonal couplets (DC) in DS (DSDC) and LS (LSDC) gaits (Cartmill *et al.*, 2002, 2007) may have different advantages for arboreality, relative to keeping the centre of mass within the support polygons produced by the limbs (Fig 1.4). This is of great importance as if the centre of mass leaves the support polygon, it may result in loss of balance (Fig. 1.5) (Cartmill *et al.*, 2002, 2007; Lammers & Biknevicius, 2004; Lammers & Zurcher, 2011a, 2011b). DSDC gaits are associated with dynamic stability, i.e. the control and transfer of moments and torques imposed on the body axes (Lammers & Zurcher, 2011a). In DS gaits, the contralateral footfall pattern promotes a dynamic weight shift from side to side at any given moment of a stride cycle leading to increased dynamic stability. Simultaneously, the other two diagonally paired fore- and hind limbs take off and move forward, offsetting the motion on the transverse body axis (Cartmill *et al.*, 2002, 2007). On the other hand, in arboreal locomotion, LSDC gaits may be preferable in terms of static stability during slower locomotion, as they enable the continuous placement of the centre of mass within the support polygon (Fig. 1.4) produced by the limbs (Gray, 1944; Tomita, 1967; Shapiro & Raichlen, 2005; Lammers & Zurcher, 2011a).



Figure 1.5. A male *Apodemus flavicollis* losing balance on an unstable, 2mm substrate.

Asymmetrical gaits (Fig. 1.6) are often used for achieving higher velocities (Hildebrand, 1977; Pridmore, 1994; Gasc, 2001; Young, 2009; Shapiro *et al.*, 2016), and confer a number of advantages in comparison to symmetrical gaits. They can optimize the metabolic cost of locomotion (Hoyt & Taylor, 1981), or decrease the strain borne by the musculoskeletal system by decreasing peak substrate reaction forces (Farley & Taylor, 1991). Specifically in small mammals, asymmetrical gaits may also contribute by an energetic input to locomotion through the sagittal movements of their flexible spines and related muscles (Schilling & Hackert, 2006). Moreover, in arboreal locomotion, the use of asymmetrical gaits reduces peak vertical forces, constrain the movement of the centre of mass, and bring about shorter and more frequent strides, all of which contribute to arboreal stability (Young, 2009; Schmidt & Fischer, 2010; Shapiro *et al.*, 2016).

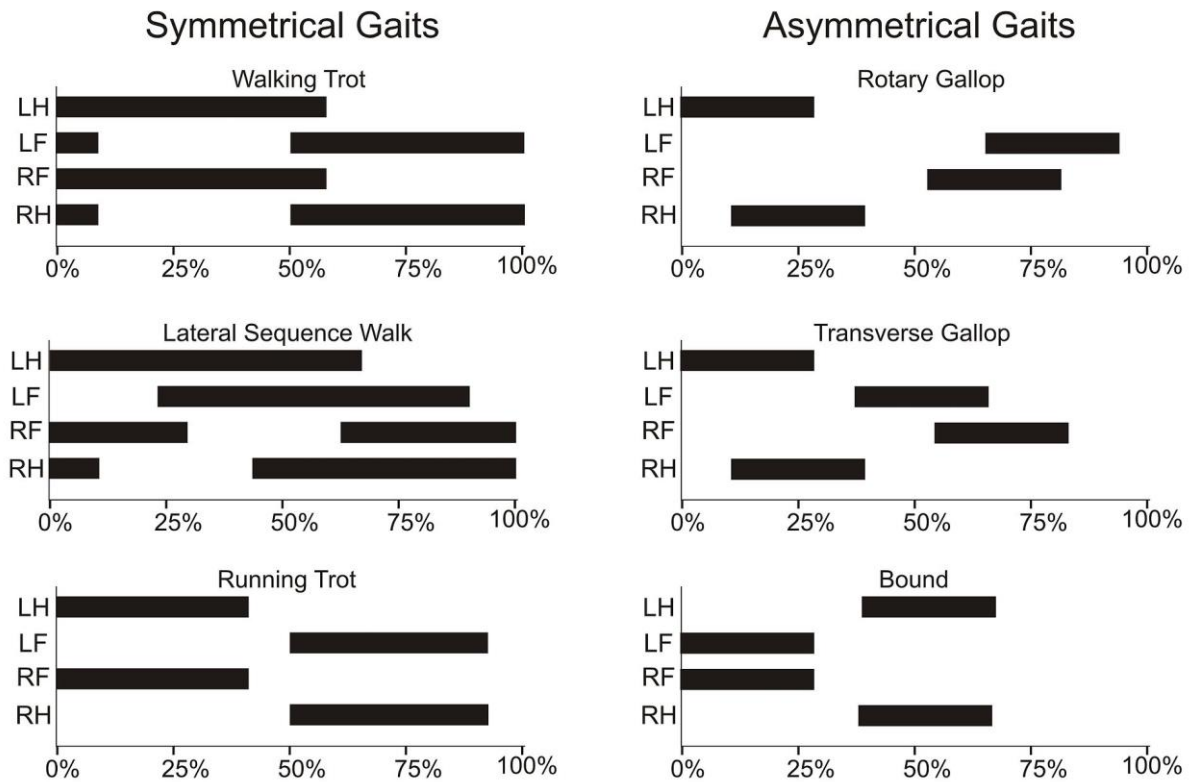


Figure 1.6. Support patterns in symmetrical and asymmetrical gaits. Reproduced from Wikipedia. Permanent link at https://en.wikipedia.org/wiki/Gait#/media/File:Gait_graphs.jpg

Locomotor speed may also serve as an indicator of locomotor efficiency on arboreal substrates. For example, in some neotropical marsupials and rodents, arboreal species tend to display higher velocities than terrestrial species when using arboreal substrates (Delciellos & Vieira, 2006, 2007; Camargo *et al.*, 2016), which may contribute to maintaining dynamic stability (Schmidt & Fischer, 2010). Velocity is a function of two parameters: stride frequency, i.e. number of strides per unit of time, and stride length, i.e. the distance covered within a single stride (Alexander, 1992; Hildebrand, 1995). Arboreal mammals may increase their velocity in a number of different ways: (i) increase stride frequency and decrease stride length (Delciellos & Vieira, 2006, 2007; Camargo *et al.*, 2016); (ii) decrease stride frequency and increase stride length (Larson *et al.*, 2000, 2001, Delciellos & Vieira, 2006, 2007); or (iii) increase primarily stride frequency, accompanied at a lesser rate by increase in stride length (Nyakatura *et al.*, 2008; Karantanis *et al.*, 2015). Increasing velocity through longer strides can be safer on arboreal substrates as they enable a farther reach by the forelimb and thus reduce involuntary branch sway (Demes *et al.*, 1994). This method of velocity increase is often encountered in medium-sized and larger arboreal mammals, which may cause significant branch movement under

their weight (Larson *et al.*, 2000, 2001, Delciellos & Vieira, 2006, 2007). In contrast, increasing velocity by increasing stride frequency, although energetically costly (Reilly, McElroy & Biknevicius, 2007), decreases body oscillations, allows continuous progression, and may thus be more advantageous for smaller mammals, for which, branch sway may be insignificant, but body oscillations may disrupt continuous progression (Strang & Steudel, 1990; Delciellos & Vieira, 2007).

In the arboreal niche, substrates are available at a great range of possible sizes, and are placed at any possible inclination, from horizontal (0°) to vertical (90°). Substrate size directly affects the width of the support polygons that can be obtained during locomotion (Cartmill *et al.*, 2002, 2007; Lammers & Biknevicius, 2004; Lammers & Zurcher, 2011a). Moreover, ascending and descending locomotion affects how the weight is distributed towards the forelimbs and the hind limbs and consequent reaction forces developed at the contact points with the substrate (Lammers, Earls & Biknevicius, 2006; Lammers, 2007; Nyakatura *et al.*, 2007; Schmidt, 2011; Schmidt & Fischer, 2011). Arboreal mammals, therefore, are expected to adjust their behaviour according to substrate properties, such as size and inclination, in ways that affect effectiveness and safety in the arboreal milieu.

1.3. The Effect of Substrates

Substrate size

The diameter of arboreal substrates may impose significant constraints on locomotion (Jenkins, 1974; Witte *et al.*, 2002), as it has an immediate impact on the size of the support polygons during quadrupedal locomotion (Cartmill *et al.*, 2002, 2007; Lammers & Biknevicius, 2004; Lammers & Zurcher, 2011a). DS gaits have been theorised to be useful towards achieving safe negotiation of smaller and potentially unstable substrates in the presence of grasping hind feet (Cartmill *et al.*, 2007), which translates into an increase in diagonality or occurrence of DS gaits in small substrate. Nevertheless, existing results are contrasting. The kinkajou *Potos flavus* (Lemelin & Cartmill, 2010) does display a higher frequency of DS gaits on smaller substrates. In contrast, DS gaits are in fact less frequent in the majority of strepsirrhine primates examined (Stevens, 2008), the tamarin *Saguinus fuscicollis* (Nyakatura & Heymann, 2010), and the arboreal metatherian

Petaurus breviceps (Shapiro & Young, 2010), and a lack of correlation between substrate size and DS gaits has been detected in free-ranging tamarins *Saguinus mystax* (Nyakatura & Heymann, 2010).

In most arboreal mammals, DF, velocity and its regulating factors, stride frequency and stride length, are often lower on smaller substrates but gradually increase on larger substrates (Lammers & Biknevicius, 2004; Delciellos & Vieira, 2006; Shapiro & Young, 2012). When a quadruped moves on an arboreal substrate, the support polygons produced by the limbs are very narrow (Lammers & Zurcher, 2011a) increasing the risk of the centre of mass being placed outside them. Furthermore, each time a limb touches down, the torque produced further enhances the probability of losing balance (Lammers & Zurcher, 2011b). A slow, controlled walk, especially on the smallest of substrates, should minimize this possibility by reducing the produced torques, increasing the simultaneous contact points on the substrate at any given time, and providing longer periods to accommodate a stable and secure dynamic posture (Lammers & Zurcher, 2011a). In contrast, some neotropical arboreal rodents seem to walk faster on smaller substrates (Camargo *et al.*, 2016), probably in order to achieve dynamic stability, which may be potentially related to lack of significant grasping abilities (Schmidt & Fischer, 2010).

These results indicate a lack of consensus on how substrate size influences gait metrics. More importantly, it seems that different species with different morphologies have adapted to arboreal locomotion on narrow arboreal substrates with contrasting behavioural strategies. It is, therefore, of importance, to further study how substrate size affects gaits, especially in the absence of primate-like powerful grasping.

Substrate inclination

Moving upwards or downwards on an inclined substrate has a different effect on gait parameters, due to a more posterior distribution of body weight in ascents and a more anterior one in descents (Lammers *et al.*, 2006; Lammers, 2007; Nyakatura *et al.*, 2007; Schmidt, 2011; Schmidt & Fischer, 2011). In ascending locomotion, diagonality and duty factor index increase, thus signifying more diagonal gaits and a higher dependence on the hind limbs (Prost & Sussman, 1969; Lammers, 2007; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Shapiro & Young, 2010; Shapiro, Young & VandeBerg,

2014). This functions twofold, reducing yawing and lateral torques, while enhancing the potential to propel the body upwards (Vilensky, Moore & Libii, 1994; Lammers & Biknevicius, 2004; Stevens, 2006; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010). Results regarding velocity in ascending locomotion are contradictory. European red squirrels (*Sciurus vulgaris*) move with a higher velocity during ascents than in horizontal locomotion, possibly in an effort to achieve dynamic stability, whereas rats (*Rattus norvegicus*) tend to move more slowly, probably in order to maintain static stability (Schmidt & Fischer, 2011).

During descents, diagonality and duty factor index are decreased in comparison to ascents, suggesting more lateral gaits and a reliance on forelimbs. This behavioural shift has been related to braking through a “stop-jolt” mechanism using the forelimbs (Rollinson & Martin, 1981; Nyakatura & Heymann, 2010). Furthermore, due to the anterior shift to the centre of mass, contact times between the forelimbs and the substrate are increased, resulting in a higher fraction of vertical impulse, which may enhance their supportive and regulatory role (Rollinson & Martin, 1981; Lee *et al.*, 2004; Nyakatura *et al.*, 2008; Young, 2012; Karantanis *et al.*, 2015). In arboreal mammals, descents are often also characterised by a lower velocity compared to ascents and horizontal locomotion (Vilensky *et al.*, 1994; Lammers *et al.*, 2006; Lammers, 2007; Karantanis *et al.*, 2015). A need for a higher control of speed for cautiousness brings about a higher frictional effect of the forefeet through pads and claws, combined with an anterior weight shift to the forelimbs and an emphasis on their braking role (Lammers *et al.*, 2006; Lammers, 2007). However, arboreal primates appear not to adjust velocity to direction of movement, which has also been suggested to be related to the maintenance of dynamic stability (Nyakatura *et al.*, 2008; Shapiro *et al.*, 2016).

To sum up, substrate inclination, and consequently, an ascending, descending, or horizontal direction of movement may affect gait parameters and metrics in various ways. It seems, though, that these effects are not similar in all species, and may depend on their individual needs for static or dynamic stability. However, there is both a general lack of data, as well as a disaccord on the causes of these differences. Therefore, the collection of more observations from different taxa is necessary in order to form more concise and clear hypotheses on how and why mammals modify gait metrics for different directions of movement.

1.4. Small Arboreal Mammals

The effect of body size on gaits has been involved in a long-standing debate. In terms of velocity, for instance, Hill (1950) constructed a model predicting that, due to geometric similarity, locomotor speed should be independent of body size. Nevertheless, other scaling models suggest that large animals move (run, swim or fly) faster than small animals, especially when converting velocity to dimensionless measures (Alexander & Jayes, 1983; Garland, 1983; Alexander & Maloiy, 1984; Alexander, 1992; Donovan & Gleeson, 2008). Unfortunately, the majority of studies on locomotor performance and efficiency have focused on terrestrial locomotion. However, arboreal locomotion may pertain to body size in different manners than in terrestrial species, as arboreal substrates are highly flexible and the risk of losing balance is increased (Grand, 1972, 1984).

Regardless of their phylogeny, small mammals perceive substrates as wider and flatter, distances as longer, and obstacles, such as small rocks on the ground or small protrusions on tree branches, as larger than large mammals (Cartmill, 1974b; Jenkins, 1974). Moreover, in an arboreal context, larger animals tend to display increased below-branch, suspensory locomotion, and cross discontinuities with bridging more often than leaping (Cartmill & Milton, 1977a; Fleagle & Mittermeier, 1980). On the other hand, smaller mammals view discontinuities in the canopy as larger (Cartmill & Milton, 1977b), therefore engaging in more frequent leaping (Fleagle & Mittermeier, 1980). In this way, compared to larger mammals, smaller mammals tend to be faced with similar locomotor constraints, regardless of the specific microhabitat they use (Jenkins, 1974). In order to cope with their size-related convergent challenges in locomotion, small mammals share features that differentiate them even from their larger phylogenetically close relatives. They usually walk and run with a crouched limb posture, while larger mammals run with a more upright posture (Biewener, 1989a, 1989b, 1990). Moreover, they often move in quick bursts of intermittent locomotion, likely contributing to vigilance and predator avoidance (McAdam & Kramer, 1998). Their kinematics, such as the increased contribution and combined operation of proximal limb segments, more flexible spine, pelvic movements, and overall locomotor performance are convergently similar, compared to those of their larger relatives (Biewener, 1989a; Fischer *et al.*, 2002; Iriarte-Díaz, 2002).

Studying small arboreal and scansorial extant mammals is of importance in discussions of mammalian evolution because of the central role of small body size therein. Many Mesozoic early mammals, the first known metatherian (Luo *et al.*, 2003) and the two oldest eutherians (Ji *et al.*, 2002; Luo *et al.*, 2011) were small, clawed, agile climbers, which likely utilized both terrestrial and arboreal substrates. Furthermore, the early ancestors of the order Primates, the mammalian order where humans are placed, have also been hypothesised to have been very small, clawed climbers, which shifted to a more confined use of a fine-branch niche (Cartmill, 1974a, 1992; Sussman, 1991; Gebo, 2004).

Even though the limited available data on the effects of body size on locomotion in arboreal mammals has centred primarily around primates and their origin, they show that body size may significantly impact locomotor preferences, substrate use, and interplays adaptively with morphology (Fleagle & Mittermeier, 1980; Fleagle, 1985). For instance, in terms of substrate use, larger monkeys tend to use larger substrates, which can better support their weight (Fleagle & Mittermeier, 1980). Yet, by shifting to suspensory locomotion, they can readily exploit smaller substrates (Cartmill & Milton, 1977b; Fleagle & Mittermeier, 1980; Cant, 1992). Furthermore, body size is especially relevant to how animals approach vertical locomotion. Large and smaller vertical substrates enable rapid vertical ranging within the canopy, permitting arboreal mammals to exploit a wider range of forest strata. Smaller mammals tend to encounter larger vertical substrates, but can easily negotiate them via clawed locomotion, by interlocking with irregularities or crevices on the surface of tree trunks (Cartmill, 1974b, 1985; Youlatos, 1999b; Samaras & Youlatos, 2010; Orkin & Pontzer, 2011). Conversely, larger mammals are more prone to use prehensile grasping, utilising friction via expanded apical pads, as vertical clawed locomotion is biomechanically and physiologically disproportionately costly (Soligo and Martin 2006). These morpho-behavioural differences indicate divergent responses of small and large mammals to arboreal constraints.

On the other hand, gait parameters related to arboreal stability, such as diagonality, seem to be independent of body size. Among smaller mammals, DS gaits are encountered in some arboreal primates (Shapiro *et al.*, 2016), and some arboreal metatherians (Schmitt & Lemelin, 2002), whereas LS gaits are exhibited by other arboreal primates (Nyakatura *et al.*, 2008; Stevens, 2008; Nyakatura & Heymann, 2010), few metatherians (Shapiro & Young, 2010), and scansorial rodents (Schmidt & Fischer, 2011).

In larger mammals, terrestrial, arboreal, and scansorial primates (Cartmill *et al.*, 2007) and an arboreal carnivore (Lemelin & Cartmill, 2010) exhibit DS gaits, whereas some arboreal metatherians (White, 1990) display LS gaits. Consequently, DS and LS gait distribution among arboreal mammals is particularly difficult to interpret, as many factors, such as phylogeny, habitat use, and substrate handling may play important roles. Apart from diagonality, data on other gait parameters, such as duty factor and duty factor index, are largely lacking. In terms of velocity, past research on both terrestrial and arboreal marsupials and rodents found only very weak to no correlations between body size and relative velocity (Delciellos & Vieira, 2006; Camargo *et al.*, 2016), leading to conjectures that locomotor competence is better predicted by the ecological correlates of a species, rather than body size or phylogeny (Camargo *et al.*, 2016). However, smaller mammals tend to regulate velocity primarily via stride frequency, which reduces disrupting body oscillations (Delciellos & Vieira, 2006; Nyakatura *et al.*, 2008). In contrast, larger mammals display a higher contribution of stride length in the increase of velocity, which reduces branch sway which poses a more significant problem at a larger body size (Larson *et al.*, 2001; Delciellos & Vieira, 2006).

Hence, to clarify how small body size relates to the utilization of arboreal substrates, studying both clawed and non-clawed arboreal and scansorial mammals is essential. This would help elucidate the locomotor constraints related to any transition between terrestriality, scansoriality and arboreality, which may be confounded at a small body size (Jenkins, 1974). Although many small to mid-size therians follow similar locomotor patterns regardless of evolutionary history and phylogeny (Jenkins, 1974; Fischer *et al.*, 2002; Schmidt, 2005), they can also differ in at least some aspects, such as gait patterns and the use of symmetrical and asymmetrical gaits (e.g. Schmitt & Lemelin, 2002; Shapiro & Young, 2010; Shapiro *et al.*, 2016). It is thus important to identify any similarities or divergences in locomotor behaviour of small scansorial and arboreal mammals, as well as their adaptive value in the relevant microhabitats they exploit.

1.5. Aims and Scope

Even though we possess extensive information on gait parameters used during arboreal locomotion, most studied species are primates, or have been examined in relation to primate evolution. Furthermore, there is a significant lack of observations at

the lower end of the mammalian size range. However, small clawed arboreal and scansorial mammals hold a special position, as they face much similar arboreal challenges (Jenkins, 1974). This leads to convergent locomotor responses that differ from those of their larger phylogenetically close relatives (Fischer *et al.*, 2002). As ancestral therians (Ji *et al.*, 2002; Luo *et al.*, 2011) and possibly ancestral primates (Gebo, 2004) were purportedly equally small-sized and scansorially/arboreally adapted, they would have faced comparable challenges in arboreal locomotion. This renders extant small arboreal and scansorial mammals suitable models for identifying behavioural adaptive patterns in the evolution of mammalian arboreality.

In order to achieve successful arboreal locomotion, mammals must cope with the properties of the arboreal milieu. This translates into moving on a mesh of branches ranging from very narrow to very wide, and placed at any possible angle. Mammals, therefore, must possess behavioural mechanisms which will increase arboreal stability and safety, and which will likely be related to the properties of the substrates they move on, such as substrate diameter and inclination, and the direction of locomotion (ascending, descending, or horizontal locomotion). However, previous research has demonstrated differential responses to substrate diameter or inclination, which may relate to the specific needs for arboreal locomotion. Nevertheless, it is likely that small body size may impose convergent behavioural effects, regardless of phylogeny. Regardless of convergences or dissimilarities, I expect to observe behaviours which will enhance stability and contribute to safe and secure body displacement. Towards this end, this study will address three goals:

The first research goal is to examine how small mammals adjust gait parameters in relation to substrate diameter. On narrower substrates, animals are expected to adjust their gaits to increase stability and safety. For enhanced safety, study subjects, at least those which possess grasping hind feet, are expected to display higher diagonality on narrower substrates. As narrow substrates represent dangerous, unstable situations in a naturalistic setting, this would theoretically allow the forelimb to test novel substrates, while the contralateral hind limb remains anchored on the previous, stable substrate. To achieve better arboreal stability, duty factor is expected to increase on narrow substrates and velocity to decrease, as a slow, controlled walk should minimize instability by reducing the produced torques, increasing the simultaneous contact points on the substrate at any given time, and providing longer time periods to accomplish a stable and

secure dynamic posture. On the other hand, subjects should be able to move faster on wider substrates, which would allow them to move swiftly to safety and avoid exposure to dangers in a natural setting. Hence, duty factor should decrease and velocity should increase as substrates become larger. Moreover, the use of asymmetrical gaits is expected to be more frequent on larger substrates, assisting in the increase of velocity.

The second goal is to assess if and how substrate inclination and consequent ascending, descending or horizontal directions of movement affect gaits. During descents, there is an increased requirement for control of locomotion for safety, further marked by an anterior shift of the weight. Thus, the breaking and supportive role of the forelimbs should be enhanced and this may be expressed by a lower duty factor index, which should also be accompanied by a decrease in gait diagonality to assist in breaking. Furthermore, to assist in maintaining control of progression and safety, the velocity is expected to be lower than any other direction of movement. During ascents, animals need to generate propulsion to carry their body upwards, while maintaining stability. This propulsion is expected to be mainly produced by the hind limbs, leading to a higher duty factor index, also assisted by higher diagonality. Moreover, in maintenance of dynamic stability, ascents are expected to be characterised by higher velocity than descents or horizontal locomotion.

The third goal is to diagnose whether gait metrics relate to any degree of arboreality in small mammals. More arboreal mammals are expected to be better adjusted to the challenges of arboreal locomotion and should be able to enhance locomotor stability and safety through their more specialised morphology and behaviour. On the other hand, less arboreal species are expected to be more cautious in their arboreal locomotion to minimise the chances of losing stability. In terms of gait parameters, this will likely lead to higher values of diagonality, higher velocity and possibly lower duty factor in more arboreal species.

To expand our knowledge on the interplay of substrate characteristics and gaits in the arboreal locomotion of small mammals, this study will use an experimental approach of simulated arboreal settings, attempting to isolate substrate parameters and compare how different small mammals approach arboreal locomotion. Gait parameters, such as diagonality, duty factor, duty factor index, velocity, stride length, and stride frequency, which possibly all relate to arboreality, will be recorded in relation to the properties of the substrates. This research is broken down into case studies, addressing

the behaviour of either a single, or comparing more than one species. Discovered trends, or lack thereof, similarities or divergences between species will be reviewed and will be linked to arboreal locomotion. Finally, I anticipate that this thesis will ultimately provide more insight into the arboreal behaviour of mammals, and serve as a valuable resource for future researchers.

Chapter 2: From the ground up to the trees

Arboreal gaits in three sympatric rodents *Apodemus agrarius*, *Apodemus flavicollis* (Rodentia, Muridae) and *Myodes glareolus* (Rodentia, Cricetidae)

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2.1. Introduction

Arboreal and scansorial mammals have evolved to exploit the vertical stratification of forests in order to gain shelter from predators and timely access to specific resources (Hildebrand, 1995). This vertical stratification appears to accommodate a variety of sympatric small mammals exploiting the diversity of available arboreal substrates and resources (Galetti *et al.*, 2016). The arboreal milieu is a complex, three-dimensional, discontinuous habitat with diverse substrates of variable size, orientation, fragility, stability, and length (Hildebrand, 1995). This complexity requires arboreal mammals to evolve diverse ways to grasp and move above, below, along, and across arboreal substrates in order to avoid potentially fatal falls. These constraints make arboreal locomotion and postures quite challenging (Grand, 1972; Cartmill, 1974b).

In terms of locomotion, arboreal gaits function as a behavioural mechanism to enhance stability on arboreal substrates of different orientations (Lammers & Zurcher, 2011a; Schmidt & Fischer, 2011). Gaits can be either symmetrical, when the left and right limbs of a pair (i.e. forelimbs or hind limbs) alternate, or asymmetrical, when they move more or less concurrently. As in terrestrial locomotion, arboreal symmetrical gaits are best described by diagonality and duty factor. Diagonality, or forelimb-hind limb phase (Hildebrand, 1967; Cartmill *et al.*, 2007) is the time between the footfall of a hind limb and the subsequent footfall of the ipsilateral forelimb measured in percentage of the stride cycle, and distinguishes diagonal sequence (DS) gaits ($D > 50$) from lateral sequence (LS) gaits ($D < 50$). Duty factor (DF) represents the percentage of a cycle during which a

foot is in contact with the substrate and separates walking ($DF > 50$) from running ($DF < 50$) (Hildebrand, 1967). The duty factor index (DFI) indicates the differing role of fore- and hind limbs. DFI values of > 100 indicates higher hind limb to forelimb stance duration, whereas DFI values of < 100 represents increased forelimb to hind limb contact times. These parameters are of high interest, as they may reflect behavioural adaptations for locomotion on arboreal substrates (Cartmill *et al.*, 2002, 2007).

Of the symmetrical gaits, DS gaits, which involve a contralateral footfall pattern, appear to promote dynamic stability, i.e. the control and transfer of moments and torques imposed on the body axes (Lammers & Zurcher, 2011a) and have been related to arboreal adaptations (Cartmill *et al.*, 2007). As the diagonally paired fore- and hind limbs make contact with the substrate concurrently, they produce a dynamic weight shift that leads to stability, while the opposite fore- and hind limbs, which swing forward synchronously, counterbalance the momentum on the transverse body axis (Cartmill *et al.*, 2002, 2007). DS gaits are prevalent among primates (Hildebrand, 1967; Cartmill *et al.*, 2007), some metatherians (White, 1990; Pridmore, 1994; Schmitt & Lemelin, 2002; Karantanis *et al.*, 2015), and the carnivoran *Potos flavus* (Lemelin & Cartmill, 2010). On the other hand, the coordinated movements of ipsilateral limbs of LS gaits, which have been functionally related to static stability (Lammers & Zurcher, 2011a), dominate in most terrestrial mammals. Nevertheless, they are used by some arboreal specialists, such as sugar gliders *Petaurus breviceps* (Shapiro & Young, 2010), callitrichid primates (Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010) and scansorial rodents (Schmidt & Fischer, 2010).

On the other hand, asymmetrical gaits are used by horses at the highest velocities (Hildebrand, 1977). Locomotion via an asymmetrical gait (gallop) is associated with a reduction of peak reaction forces, reduced strain on the musculoskeletal system, and a reduction in the energetic cost of locomotion, with respect to locomotion via a symmetrical gait (trot) at the same velocity (Hoyt & Taylor, 1981; Farley & Taylor, 1991). In small mammals, asymmetrical gaits induce large oscillations of the spine in the sagittal plane (Schilling & Hackert, 2006). In larger mammals, sagittal oscillations of the spine and associated muscles and tendons contribute elastic energy to galloping locomotion, substantially decreasing energetic cost of transport (Alexander, Dimery & Ker, 1985). In an arboreal setting, asymmetrical gaits also confer advantages in stability by reducing peak vertical forces, limiting centre of mass movements, and using shorter and more

frequent strides (Young, 2009; Schmidt & Fischer, 2011). Thus, they appear to be especially important for small arboreal mammals.

Apart from gaits, velocity also plays an important role in the negotiation of arboreal substrates. Velocity is regulated by stride frequency, i.e. number of strides per unit of time, and stride length, i.e. the distance covered within a single stride (Hildebrand, 1995). Arboreal species tend to exhibit higher velocities than terrestrial species on arboreal (Delciellos & Vieira, 2006; Camargo *et al.*, 2016) substrates, suggesting that arboreal species are capable of utilizing dynamic stability (Schmidt & Fischer, 2011). There are multiple mechanisms to increase velocity: increasing stride frequency and decreasing stride length (Delciellos & Vieira, 2006; Camargo *et al.*, 2016), reducing frequency and increasing stride length (Larson *et al.*, 2001; Delciellos & Vieira, 2006), or increasing primarily stride frequency and, at a lesser rate, stride length (Nyakatura *et al.*, 2008; Karantanis *et al.*, 2015). Increasing velocity through stride length enables a farther reach of the forelimb and reduces involuntary branch sway (Demes *et al.*, 1994). This mechanism promotes careful navigation in an arboreal setting and is often encountered in medium-sized and larger arboreal mammals (Larson *et al.*, 2001; Delciellos & Vieira, 2006). On the other hand, smaller arboreal mammals have a decreased body mass to substrate size ratio, and therefore produce low to negligible branch swaying despite significant body oscillations (Delciellos & Vieira, 2006). Thus, increasing stride frequency is a valid, though energetically costly (Reilly *et al.*, 2007), option for increasing velocity for smaller arboreal mammals.

Because arboreal substrates are rather variable, it is ecologically relevant to test for the effect of substrate size and inclination on arboreal locomotion. Substrate size may impose significant constraints on locomotor patterns (Jenkins, 1974; Witte *et al.*, 2002). Cartmill *et al.* (2007) proposed that DS gaits should be more frequent on narrower substrates, to enable safer arboreal navigation. Results are, however, conflicting. Most strepsirrhine primates (Stevens, 2008), the tamarin *Saguinus fuscicollis* (Nyakatura & Heymann, 2010), and the arboreal metatherian *Petaurus breviceps* (Shapiro & Young, 2010) do not exhibit a higher occurrence of DS gaits on narrower substrates. In contrast, the metatherian *Acrobates pygmaeus* (Karananis *et al.*, 2015) increases the frequency of DS gaits on narrower substrates, whereas no correlation between substrate size and DS gaits has been detected in free-ranging tamarins *Saguinus mystax* (Nyakatura &

Heymann, 2010). These findings imply no clear relationships between the use of DS or LS gaits and substrate size.

Substrate size also bears significant effects on velocity in different ways. Some arboreal rodents tend to increase velocity on narrower substrates (Camargo *et al.*, 2016). In contrast, most arboreal metatherians tend to use lower velocities on narrower substrates (Delciellos & Vieira, 2006; Karantanis *et al.*, 2015). Slow, controlled walking on narrow substrates appears to minimize the possibility of the animal's centre of mass being placed outside the relatively narrow support polygon of the limbs. This is realised by reducing touchdown torques, increasing the contact points on the substrate, and providing longer periods of time to accommodate a stable and secure dynamic posture (Lammers & Zurcher, 2011b).

Direction of movement (considered in the present study as ascent, descent or horizontal locomotion) may also affect gait parameters. Thus, DS gaits are usually more frequent in ascents (Lammers, 2007; Nyakatura & Heymann, 2010; Shapiro & Young, 2010; Shapiro *et al.*, 2014; Karantanis *et al.*, 2015). It has been suggested that DS gaits, coupled with increased hind limb stance phases, reduce yawing and lateral torques and may enhance the ability to propel the body upwards (Vilensky *et al.*, 1994; Nyakatura & Heymann, 2010). This implies that increased diagonality and duty factor index may be advantageous in ascents. In contrast, LS gaits are more frequent in descents, as they allow the forelimbs to provide braking, though a “stop-jolt” mechanism, conferring an advantage in downward locomotion (Rollinson & Martin, 1981; Nyakatura & Heymann, 2010). Additionally, the cranial/anterior weight shift generates increased contact times with the substrate and a higher fraction of vertical impulse on the forelimbs, which further enhance their regulatory and supportive role (Rollinson & Martin, 1981; Nyakatura & Heymann, 2010). Therefore, lower diagonality and DFI are usually functionally associated with descents.

Direction of movement also has a variable effect on velocity. In most arboreal mammals, descents are usually slower than ascents or horizontal locomotion (Vilensky *et al.*, 1994; Lammers *et al.*, 2006). It is very likely that the frictional adjustments of the extremities and the loading and braking role of the forelimbs may contribute to controlled and slower descents (Lammers *et al.*, 2006). On the other hand, response differ during ascents, with arboreal red squirrels increasing speeds during ascending locomotion, probably to achieve dynamic stability, whereas generalised rats decrease

velocity to maintain static stability (Schmidt & Fischer, 2011). Finally, some other arboreal mammals, such as some primates, appear not to adjust velocity to direction of movement (Nyakatura *et al.*, 2008; Shapiro *et al.*, 2016).

These different behavioural responses to the challenges of the arboreal substrates indicate that more arboreally adapted mammals should differentiate from more generalised ones. In order to test such differing patterns of arboreal behavioural adaptations we examined gait metrics (diagonality, duty factor, duty factor index, velocity, stride length, and stride frequency) on different arboreal substrates in three sympatric rodents, the striped field mouse *Apodemus agrarius*, the yellow-necked mouse *Apodemus flavicollis*, and the bank vole *Myodes glareolus*, living in a deciduous forest in Poznań, Poland. *Apodemus agrarius* (Pallas, 1771) are primarily granivorous mice, they supplement their diet with invertebrates (Holisova, 1967; Obrtel & Holisova, 1974), and inhabit crop-fields and open habitats, especially along rivers and streams, as well as forest ecotones (Zejda, 1967; Pucek, 1981; Karaseva, Tichonova & Bogomilov, 1992). They are primarily field-dwelling with an overall morphology associated with terrestrial burrowing activities, such as short ears, vibrissae, tail, forelimbs, ilia, tibiae, long olecrana and robust ulnae (Kuncová & Frynta, 2009). *Apodemus flavicollis* (Melchior, 1834) feed mainly on fruit and seeds (Holisova, 1967; Obrtel & Holisova, 1974), and are found primarily in forested areas (Montgomery, 1978; Marsh & Harris, 2000). The yellow-necked mice display significant arboreal behaviour and frequent use of tree branches (Borowski, 1962; Holisova, 1969; Montgomery, 1980; Juškaitis, 2002), in order to access food stores (Balát & Pelikán, 1959; Juškaitis, 2002), nesting holes (Czeszczewik, Walankiewicz & Stańska, 2008), and to avoid predators (Montgomery & Gurnell, 1985). Their long vibrissae, tail, distal limb elements, short olecrana and lumbar vertebrae, and partial grasping abilities are all functionally linked to climbing (Krattli, 2001; Zefferer, 2002; Kuncová & Frynta, 2009). *Myodes glareolus* (Schreber, 1780) are more omnivorous than other arvicolines, with diets in between arvicolines and murids (Watts, 1968; Butet & Delettre, 2011). The bank voles are habitat generalists, showing preference to woodlands with dense understorey cover (Gurnell, 1985; Mazurkiewicz, 1994), where they usually travel on the ground but also often engage in climbing activities (Nations & Olson, 2015), mainly to avoid predators (Jędrzejewska & Jędrzejewski, 1990; Jędrzejewski, Rychlik & Jędrzejewska, 1993). Their short limbs and tail, as well as the shape of the ungual phalanges relate functionally to terrestrial and burrowing activities,

rather than climbing (Zefferer, 2002; Nations & Olson, 2015). The three species often coexist and engage in interspecific competition for food sources, with *A. flavicollis* being the more arboreal, *M. glareolus* also engaging in arboreal activities, but less frequently, and *A. agrarius* exploiting mainly the ground (Andrzejewski & Wrocławek, 1961; Olszewski, 1968; Holisova, 1969; Gliwicz, 1981; Chełkowska, Walkowa & Adamczyk, 1985; Heroldova, 1994). Given these differences, the three rodents are expected to show variable locomotor responses on arboreal substrates.

In this context, the current study is the first report on gait metrics of *Apodemus agrarius*, *A. flavicollis*, and *Myodes glareolus*, carried out in an experimental context simulating the arboreal habitat. It aims to test whether these three species display differences in their arboreal locomotion that covary with their habitat preferences. More specifically, we expect diagonality, use of asymmetrical gaits, velocity, stride frequency, and, to a lesser extent, stride length to increase with arboreality. Moreover, within more arboreal species, we expect that diagonality will increase and velocity will decrease with substrate size, and we expect that diagonality and velocity will increase in ascents and decrease in descents. In contrast, we expect diagonality and velocity to decrease with terrestriality. Additionally, within terrestrial species we expect that diagonality will not be adjusted to substrate size and inclination, and that velocity will decrease with substrate size and remain unmodified by substrate inclination or increase in descents.

2.2. Materials and Methods

Specimens

For the purposes of the current study, we tested one wild-caught adult male and one wild-caught adult female of each of three sympatric rodent species: *Apodemus agrarius* and *Apodemus flavicollis* (Murinae, Muridae, Rodentia), and *Myodes glareolus* (Arvicolinae, Cricetidae, Rodentia) (Fig. 2.1). All individuals were trapped in a deciduous forest around Lake Moraskie, close to the Morasko campus of the Adam Mickiewicz University (AMU) in Poznań, during June 2013. The trapped individuals were subsequently transported to laboratory facilities at the School of Biology of AMU. The animals were housed for 20 days in glass enclosures enriched with natural materials for nesting and climbing, in an attempt to reduce stress, under a reversed day-night regime.

All specimens were healthy, handled with care and did not display any stereotypical or stress behaviours. Mean head-body length and mass was for *A. agrarius*, 10.2 cm (10.0 cm and 10.4 cm) and 19.2 g (19.0 g and 19.4 g), for *A. flavicollis*, 12.15 cm (12.0 cm and 12.3 cm) and 28.0 g (27.2 g and 29.2 g), and for *M. glareolus*, 11.1 cm (both 11.1 cm) and 20.7 g (20.5 g and 20.9 g).

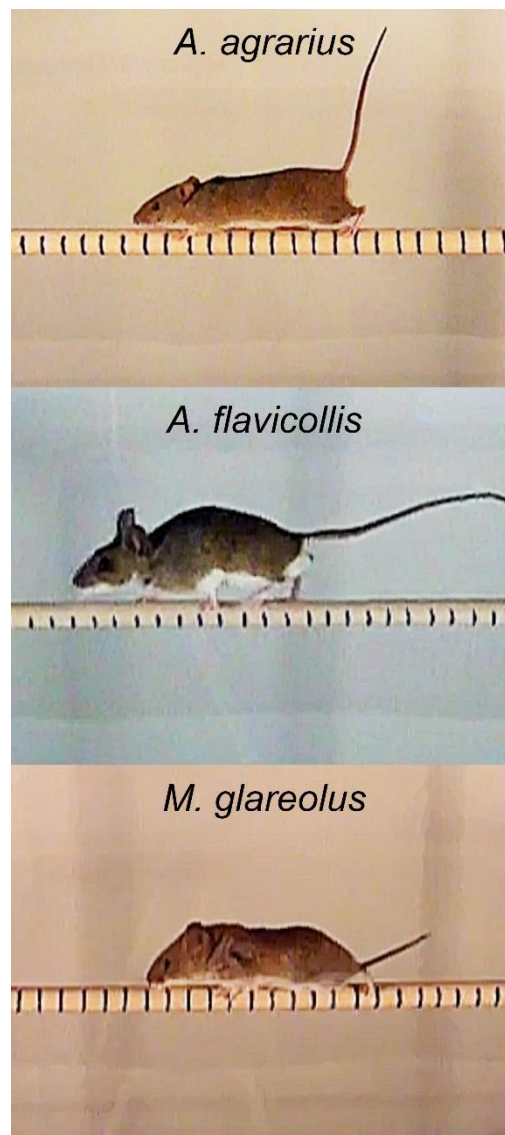


Figure 2.1. Stills from the videos used for data collection. From left to right, the striped field mouse (*Apodemus agrarius*), the yellow-necked mouse (*Apodemus flavicollis*), and the bank vole (*Myodes glareolus*). The images are on the same scale. In the stills, the animals are traversing a horizontal substrate of 10mm diameter, and the distance between two vertical lines on the substrate is 1 cm.

Experimental setup

For the locomotion experiments, we used a single, specially configured, filming glass terrarium (L: 90 cm x H: 40 cm x W: 40 cm), topped by a wooden cover. Inside the terrarium, we used poles of 80 cm long, composed of cylindrical semi-hardwood rods, which were supported by wooden frames on each end. All poles were marked with vertical blue lines every 1 cm for scaling purposes (Fig. 2.1). During the recordings, the visible length of the rod was 30-40 cm. Diameter and direction of movement accounted for the classification of the different substrate categories. Thus, we considered four different diameters (2 mm, 5 mm, 10 mm, 25 mm) and three different movement directions (45° descent, horizontal, 45° ascent) totalling 12 distinct substrate categories.

The locomotion experiments were recorded in several video sessions during June 2013. During each session, single individuals of each species were transferred from their enclosures to the filming terrarium. Initially, every individual was allowed to move freely within the filming terrarium in order to accommodate itself with the new environment. Subsequently, minimal or no stimulation was required for the subjects to walk on the poles. Despite the limited number of sampled animals, and in order to increase statistical robusticity, we tried to maximize the number of locomotion trials, by filming every subject several times on all 12 substrate categories. After each filming session, the animal was transported back to the temporary enclosure. During video recording, we used a Sanyo digital camcorder (VPC-HD 2000, Sanyo, Osaka, Japan), filming at 240 fps, which was positioned at 1 m from the filming terrarium to reduce image distortion. For our analyses, we only considered complete symmetrical gaits for diagonality and duty factor analysis, initiating with the touchdown of the left hind limb and ending at the subsequent touchdown of the same limb, including both lift-offs and touchdowns of all limbs (Hildebrand, 1976). In the analysis of velocity and its regulation, we incorporated asymmetrical gaits as well, always controlling for the type of gait. We discarded any cycles involving beginnings or endings of locomotor bouts or loss of balance. We only analysed continuous locomotion, regardless of velocity, as indicative of natural unbiased behaviour. Trapping, handling, housing of animals, and filming were performed with the permission of the Local Ethical Commission for the Animal Experiments in Poznań, following the guidelines for the treatment of animals in behavioural research and

teaching (ASAB/ABS, 2012), and complied with relative regulations and legislations of the Adam Mickiewicz University and the Aristotle University of Thessaloniki.

Gait analyses

Video analysis and data collection, distance and time calculations were made by importing videos and calibrating time and distance measurements using Tracker 4.92 (Brown, 2009). Microsoft Excel 2010 (Redmond, WA, USA), and SPSS 23 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

For our analyses, we considered the following gait parameters:

- (i) Diagonality (D) (Hildebrand, 1967; Cartmill *et al.*, 2007) as the percentage of the stride cycle interval the footfall of a forelimb follows behind the ipsilateral hind limb. Although it was measured as a scale variable, it was also divided into five ordinal classes: (a) Lateral Sequence Lateral Couplets ($0 \leq \text{LSLC} < 25$), (b) Lateral Sequence Diagonal Couplets ($25 \leq \text{LSDC} < 50$), (c) Trot ($=50$), (d) Diagonal Sequence Diagonal Couplets ($50 < \text{DSDC} \leq 75$), (e) Diagonal Sequence Lateral Couplets ($75 < \text{DSLCL} \leq 100$);
- (ii) Duty Factor (DF) is the mean of duty factors of all limbs, defined as the percentage of a cycle during which a limb is in contact with the substrate. Walking gaits correspond to values > 50 , whereas running gaits score < 50 ;
- (iii) Duty Factor Index (DFI) is the ratio of hind limb duty factor (DF_h) to forelimb duty factor (DF_f), calculated as $100 \cdot \text{DF}_h / \text{DF}_f$ (Cartmill *et al.* 2007a). Values > 100 indicate longer hind limb than forelimb stance durations, whereas values < 100 specify shorter hind limb than forelimb stance durations;
- (iv) Stride Duration (t) is total duration of a single stride in seconds;
- (v) Stride Length (l) is the distance covered during a single stride cycle, in meters;
- (vi) Velocity (v) is the speed at which the subjects moved, calculated by dividing stride length by stride duration, in meters/second (ms^{-1});
- (vii) Stride Frequency (f) is the number of strides per second;

As these four last parameters are size-dependent, they were standardized using the effective hind limb length (Pontzer, 2005; 2007a,b). The effective hind limb length corresponds to the distance between the hip joint and the substrate, parallel to the axis of gravity, and is usually shorter than the actual limb length. In the case of our study

animals, it was 2.4 cm and 2.6 cm for *A. agrarius*, 3.0 cm and 3.1 cm for *A. flavicollis*, and 2.8 cm for both specimens of *M. glareolus*. The effective hind limb length is used to calculate the dimensionless measures of stride duration, stride length, velocity and stride frequency which provide comparative measures across different species (Alexander, 1977; Alexander & Jayes, 1983; Hof, 1996). The dimensionless measures were calculated as follows:

$$\text{Dimensionless Stride Duration (t}_D) = \frac{t}{\sqrt{l_0/g}}$$

$$\text{Dimensionless Stride Length (l}_D) = \frac{l}{l_0}$$

$$\text{Dimensionless Velocity (v}_D) = \frac{v}{\sqrt{gl_0}}$$

$$\text{Dimensionless Stride Frequency (f}_D) = \frac{f}{\sqrt{g/l_0}}$$

where l_0 is the effective hind limb length of each animal and g is the acceleration of gravity ($g=9.81 \text{ m s}^{-2}$). All measures of time and length were converted to SI units.

For statistical analyses, we opted for Analysis of Covariance (ANCOVA) to explore relationships between variables, including individuals as random factors, while controlling for other possible co-variables. When necessary, comparisons between categories or across species were performed by Bonferroni Post-Hoc tests (Bonferroni Mean Difference, BMD) based on value estimates of the univariate ANCOVA tests. Finally, we constructed stepwise regression models to examine the impact of both dimensionless stride frequency and stride length on velocity. The impact of each parameter was expressed with the R value of partial correlations, using the best fitting model (Harrell, 2001).

2.3. Results

Despite the limited number of sampled animals, we recorded 273 complete gait cycles for all three species (*A. agrarius*, $N=93$; *A. flavicollis*, $N=123$; *M. glareolus*, $N=54$). Out of this sample, we identified 226 symmetrical walking gaits (*A. agrarius*, $N=93$; *A. flavicollis*, $N=79$; *M. glareolus*, $N=54$). Asymmetrical half-bounding gaits in which the hind limbs touched down simultaneously while one forelimb was trailing the other were recorded only for *A. flavicollis* ($N=44$). Table 2.1 summarizes the breakdown of the absolute frequencies of these observations. We detected no significant differences

between individuals for all parameters ($p>0.05$), and therefore results were grouped together for each species.

Gait parameters and their interactions

Diagonality was calculated only for symmetrical gaits (Hildebrand, 1967), and therefore analysis was restricted to these gaits. Interestingly, diagonality covaried with duty factor in all species (Fig. 2.2), controlling for the effect of both the direction of movement and substrate diameter (*A. agrarius*, $N=93$, $F_{(1,92)}=3.83$, $p<0.001$; *A. flavicollis*, $N=79$, $F_{(1,78)}=2.54$, $p=0.010$; *M. glareolus*, $N=54$, $F_{(1,53)}=2.04$, $p=0.047$). Consequently, whenever testing for variation in diagonality or duty factor, we controlled for one or the other. Diagonality did not covary with the DFI in any species (Fig. 2.2), controlling for the effect of both the direction of movement and substrate diameter (*A. agrarius*: $N=93$, $F_{(1,92)}=1.74$, $p=0.070$; *A. flavicollis*, $N=79$, $F_{(1,78)}=1.18$, $p=0.314$; *M. glareolus*, $N=54$, $F_{(1,53)}=1.06$, $p=0.425$). Dimensionless velocity demonstrated a correlation with dimensionless stride length and frequency (Fig. 2.3). Our stepwise regression model showed that stride frequency was a better predictor of velocity than stride length in both *A. agrarius* (stride frequency, $N=93$, $R_{\text{Part}}=0.60$, $F_{(1,90)}=297.57$, $p<0.001$; stride length, $N=93$, $R_{\text{Part}}=0.47$, $F_{(1,91)}=1590.58$, $p<0.001$) and *A. flavicollis* (stride frequency, $N=123$, $R_{\text{Part}}=0.70$, $F_{(1,121)}=652.38$, $p<0.001$; stride length, $R_{\text{Part}}=0.38$, $F_{(1,122)}=5066.25$, $p<0.001$). On the other hand, the effect was shared between stride frequency ($N=54$, $R_{\text{Part}}=0.79$, $F_{(1,52)}=17.15$, $p<0.001$) and stride length ($N=54$, $R_{\text{Part}}=0.79$, $F_{(1,51)}=193.23$, $p<0.001$) in *M. glareolus*.

Table 2.1. Breakdown of symmetrical and asymmetrical gaits of *Apodemus agrarius*, *A. flavicollis* and *Myodes glareolus* recorded per substrate category in absolute frequencies of observations.

	Species	Gait Type	Descent				Horizontal				Ascent				Table Means
			2mm	5mm	10mm	25mm	2mm	5mm	10mm	25mm	2mm	5mm	10mm	25mm	
2.2. and	<i>A. agrarius</i>	Symmetrical	-	15	10	9	-	24	17	18	-	-	-	-	
	<i>A. flavicollis</i>	Symmetrical	8	10	9	-	-	15	11	-	9	17	-	-	
		Asymmetrical	-	-	-	6	-	-	14	7	-	-	11	6	
	<i>M. glareolus</i>	Symmetrical	-	3	13	9	-	10	12	7	-	-	-	-	

standard deviations (in brackets) of both symmetrical and asymmetrical gait parameters of *Apodemus agrarius*, *A. flavicollis* and *Myodes glareolus*.

Species	Gait Type	N	Diagonality	Duty Factor	Hind limb Duty Factor	Forelimb Duty Factor	Duty Factor Index	Velocity (m s ⁻¹)	Stride Length (m)	Stride Frequency (s ⁻¹)	Stride Duration (s)	Dimensionless Velocity	Dimensionless Stride Length	Dimensionless Stride Frequency	Dimensionless Stride Duration
<i>A. agrarius</i>	Symmetrical	93	32.52 (8.04)	74.83 (4.74)	74.01 (5.27)	75.64 (5.55)	98.10 (6.87)	0.244 (0.074)	0.054 (0.008)	4.428 (0.915)	0.236 (0.053)	0.490 (0.148)	2.151 (0.328)	0.225 (0.047)	4.652 (1.034)
<i>A. flavicollis</i>	Symmetrical	79	28.77 (7.75)	73.77 (5.93)	72.41 (6.53)	75.13 (6.91)	96.78 (8.62)	0.306 (0.141)	0.069 (0.010)	4.365 (1.830)	0.264 (0.093)	0.582 (0.271)	2.464 (0.374)	0.234 (0.097)	4.915 (1.711)
	Asymmetrical	44	- (6.31)	58.84 (6.31)	60.99 (7.69)	56.69 (7.07)	108.42 (13.60)	0.582 (0.143)	0.089 (0.011)	6.558 (1.458)	0.159 (0.034)	1.096 (0.261)	3.098 (0.391)	0.355 (0.082)	2.958 (0.656)
<i>M. glareolus</i>	Symmetrical	54	25.86 (7.98)	67.40 (9.47)	68.35 (10.97)	66.45 (9.31)	103.22 (11.64)	0.370 (0.149)	0.063 (0.010)	5.784 (2.006)	0.198 (0.080)	0.630 (0.275)	2.423 (0.385)	0.260 (0.111)	3.846 (1.554)

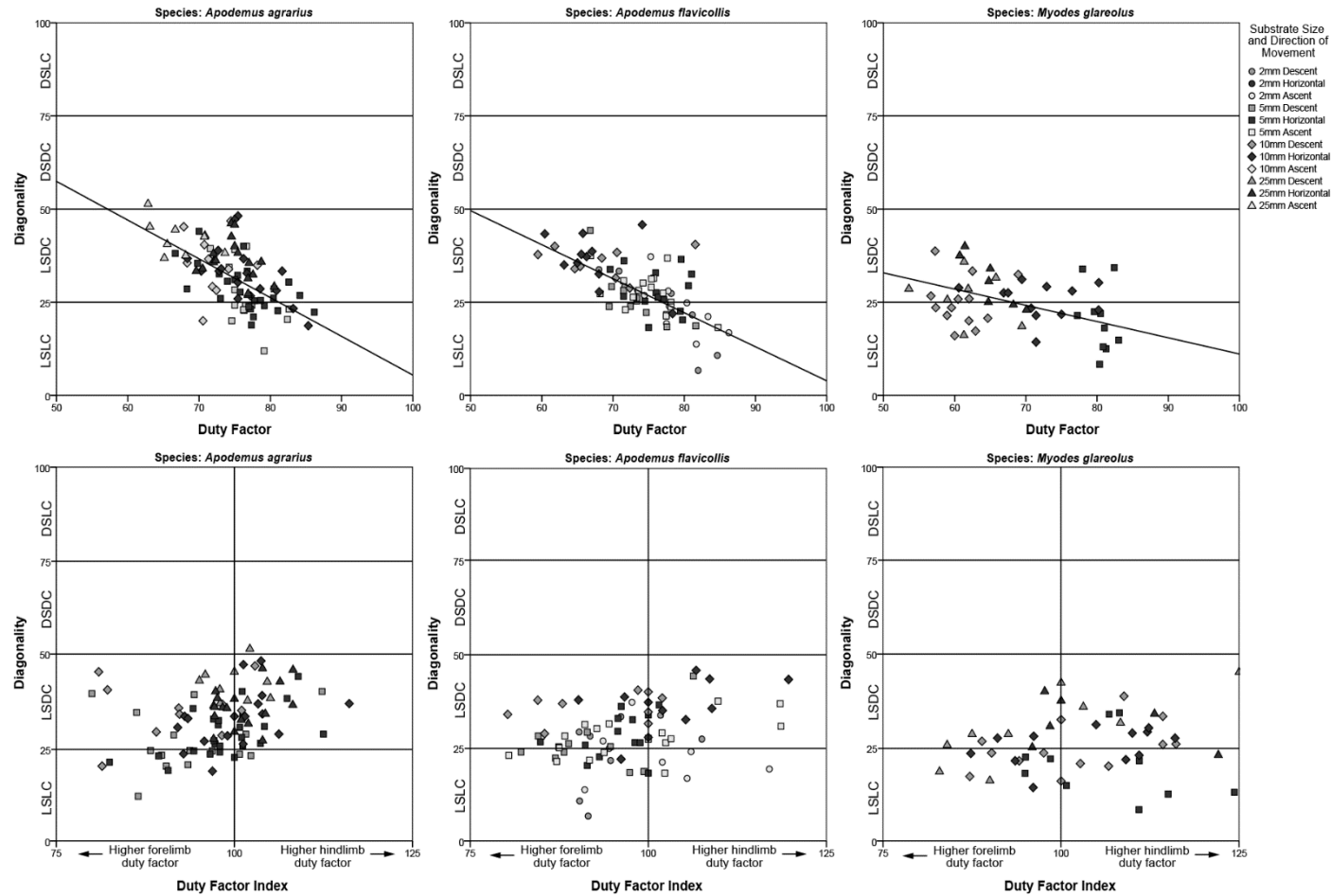


Figure 2.2. Scatterplots of diagonality as a function of duty factor (top) and duty factor index (bottom) for symmetrical gaits in all species in the dataset. The horizontal lines split the sample in quadrants of decreasing diagonality (DSL, DSLC, DSLC, DSLC, from top to bottom, acronyms explained in text). The trend line equations reported are significant at $p < 0.05$, and their functions are: *A. agrarius*, $D = 1.07E-1 \cdot DF$; *A. flavicollis*, $D = 93.51 - 0.88 \cdot DF$; *M. glareolus*, $D = 53.83 - 0.42 \cdot DF$. The vertical line in the duty factor index graphs stands at DFI=100, denoting an equilibrium between the duty factors of the forelimbs and hind limbs.

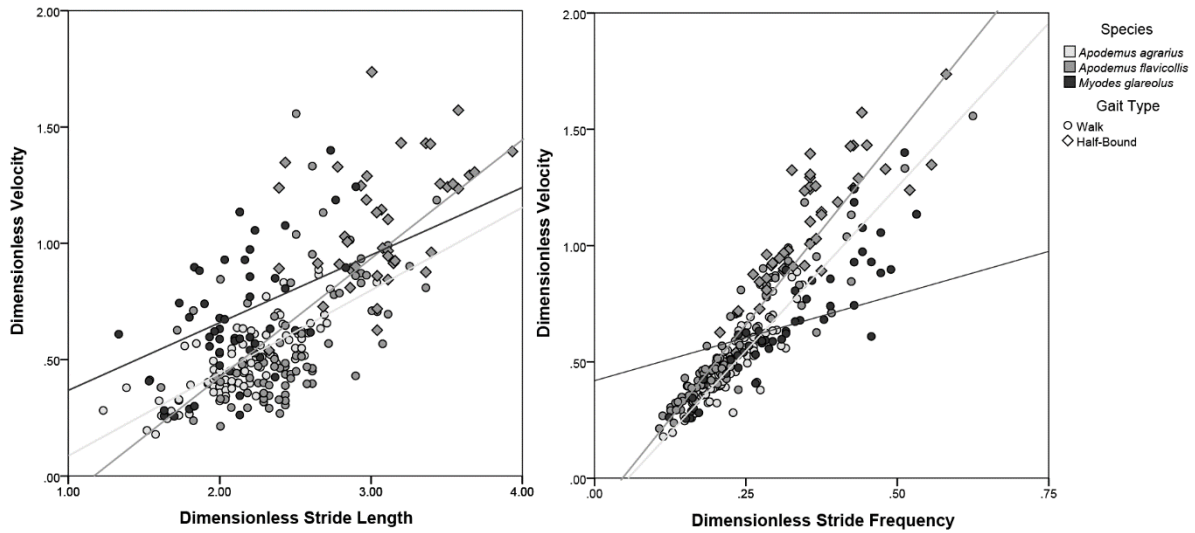


Figure 2.3. Scatterplot of dimensionless velocity (V) as a function of dimensionless stride length (SL) (left) and dimensionless stride frequency (SF) (right) for all substrate sizes and directions of movement. Walks are denoted by a circle, while in asymmetrical gaits, half-bounds are represented by a diamond. The trend line equations reported are significant at $p < 0.05$. In the left graph, functions are: *A. agrarius*, $V = -0.28 + 0.36 \cdot SL$; *A. flavicollis*, $V = -0.65 + 0.53 \cdot SL$; *M. glareolus*, $V = 0.1 + 0.28 \cdot SL$. In the right graph, functions are: *A. agrarius*, $V = -0.13 + 2.76 \cdot SF$; *A. flavicollis*, $V = -0.09 + 3.07 \cdot SF$; *M. glareolus*, $V = 0.41 + 0.75 \cdot SF$.

Symmetrical and asymmetrical gaits

Apodemus flavicollis was the only species that used asymmetrical gaits. All were half-bounds, in which the hind limbs came into contact with the substrate simultaneously (± 1 frame or $1/240$ sec) and one forelimb was trailing the other at touchdown. *Apodemus flavicollis* used walking gaits on the smallest substrates (2 mm and 5 mm), gradually shifting to half-bounds on the larger ones (Table 2.1). On 10 mm substrates, the animals were descending exclusively by symmetrical walks, ascending only by half-bounds, and sharing both gaits during horizontal locomotion. Half-bounds also dominate on 25mm substrates. When controlling for substrate size and direction of movement, half-bounds demonstrated significantly lower overall duty factor ($N=123$, $F_{(1,122)}=24.20$, $p < 0.001$), higher velocity ($N=123$, $F_{(1,122)}=29.96$, $p < 0.001$) and stride frequency ($N=123$, $F_{(1,122)}=30.22$, $p < 0.001$) than symmetrical gaits, but similar stride length ($N=123$, $F_{(1,122)}=2.06$, $p=0.154$). Even when velocity is added as a covariate to the duty factor and gait type model, half-bounds still demonstrate lower duty factors than walks ($N=123$, $F_{(1,122)}=9.06$, $p=0.003$; $BMD=4.59$, $p=0.003$), implying a means for increasing speed by increasing the aerial phase of the limbs.

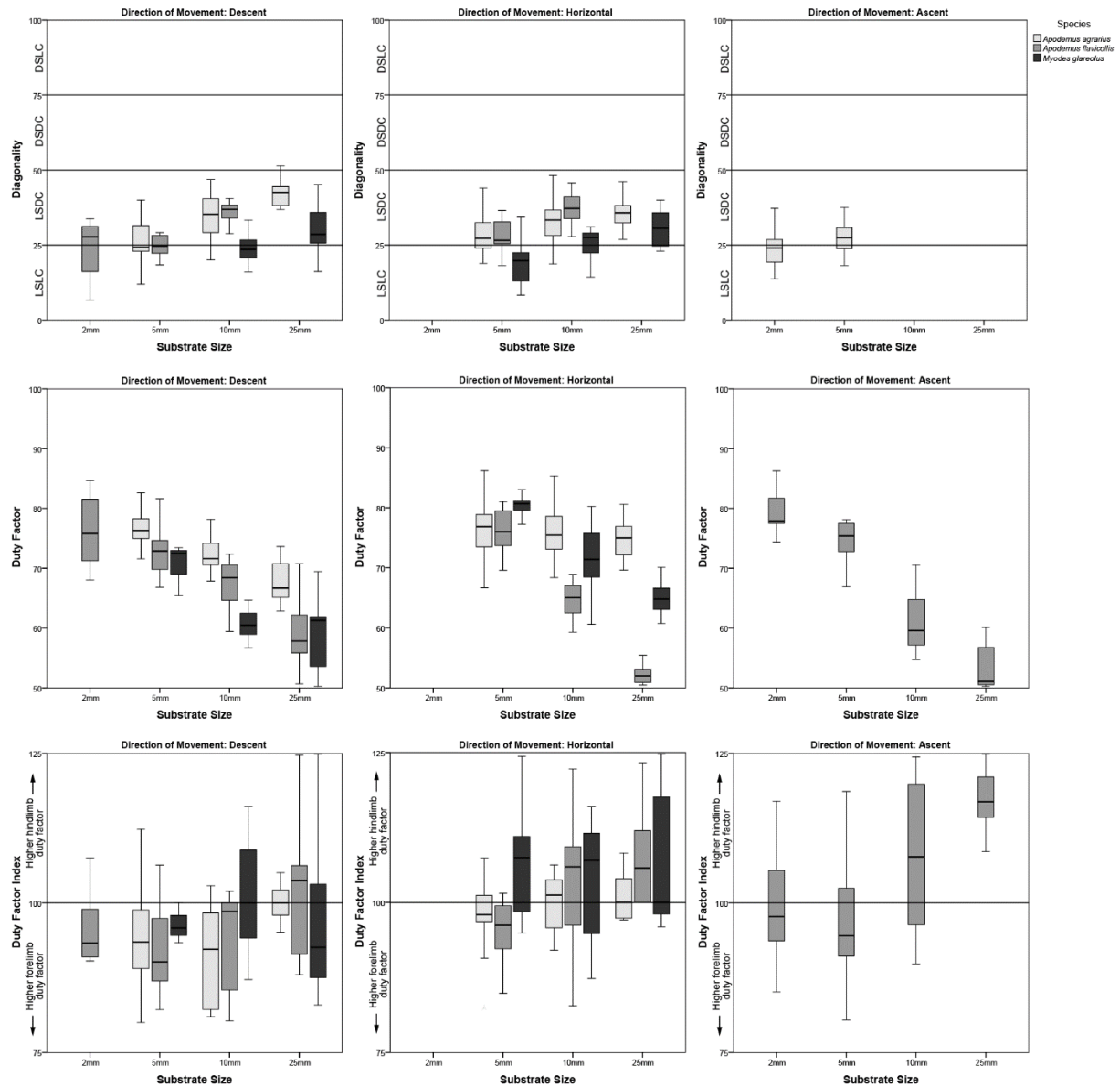


Figure 2.4. Boxplots of the distribution of gait diagonality (top), duty factors (middle) and duty factor indices (bottom) in the substrate size categories examined, split into three graphs for each direction of movement. In *Apodemus flavicollis* both symmetrical and asymmetrical gaits have been included. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the top and bottom whiskers represent the maximum and minimum values obtained. The horizontal lines split the sample in quadrants of decreasing diagonality (DSLDC, DSDC, LSDC, LSLC, from top to bottom, acronyms explained in text), or at DFI=100, denoting an equilibrium between the duty factors of the forelimbs and hind limbs.

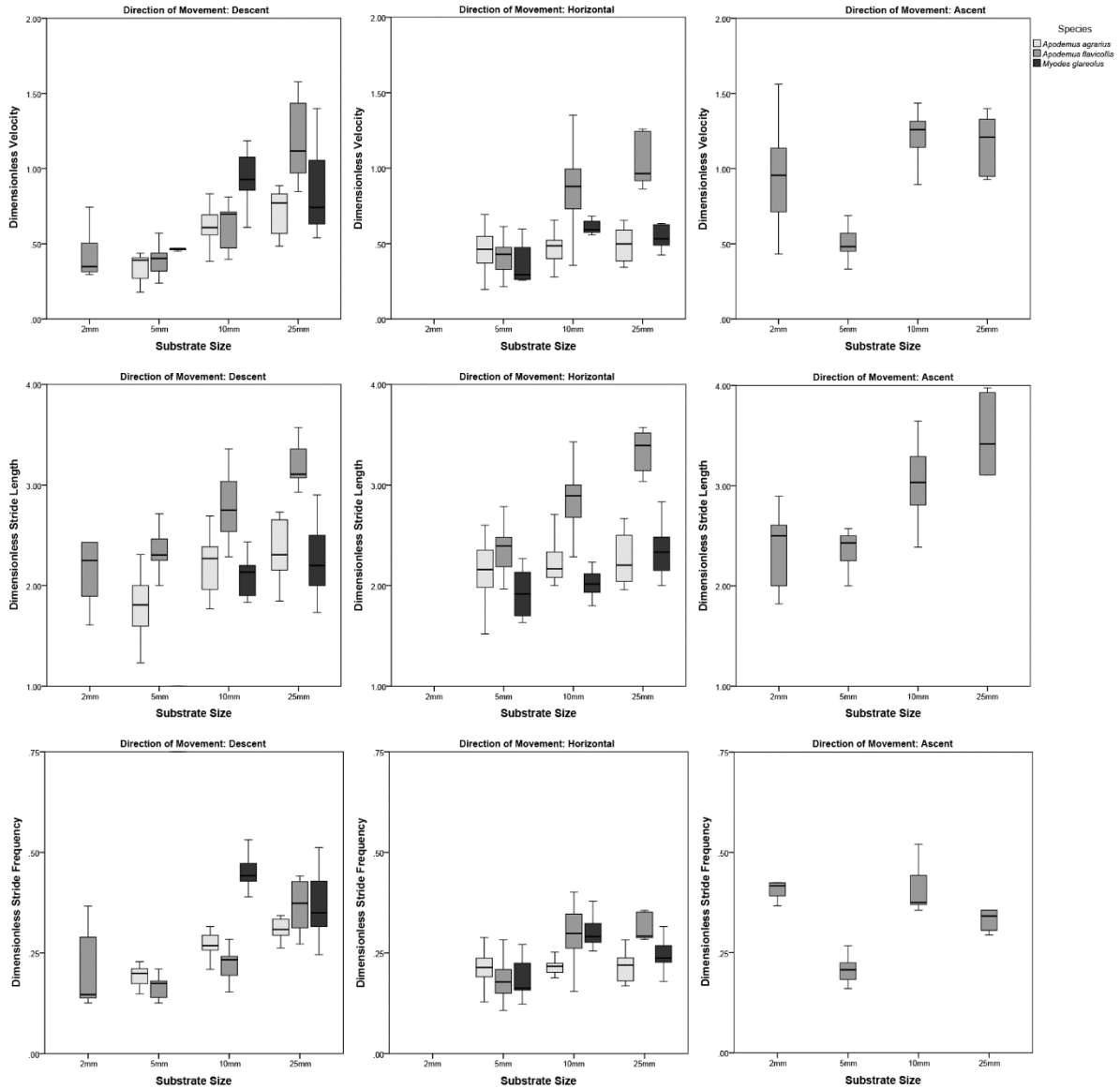


Figure 2.5. Boxplots of the distribution of dimensionless velocity (top), dimensionless stride length (middle) and dimensionless stride frequency (bottom) in the substrate size categories examined, split into three graphs for each direction of movement. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the top and bottom whiskers represent the maximum and minimum values obtained.

Comparisons between species

In all species, mean diagonality was well below 50.00 (*A. agrarius*, $N=93$, $D=32.52$; *A. flavicollis*, $N=79$, $D=28.77$; *M. glareolus*, $N=54$, $D=25.86$; Table 2.2, Fig. 2.2, 2.4), with only a single gait being recorded over the 50.00 threshold for *A. agrarius* (Fig. 2.2). Thus, all species habitually adopted LS gaits, regardless of substrate categories, and were significantly different between them ($N=226$, $F_{(2,225)}=45.30$, $p<0.001$; controlling for duty factor, substrate size and inclination). Post-hoc tests showed that *A. agrarius* and *A.*

flavicollis did not differ significantly (BMD=1.93, $p=0.297$), whereas *M. glareolus* scored significantly lower than both *A. agrarius* (BMD=11.17, $p<0.001$) and *A. flavicollis* (BMD=9.23, $p<0.001$). All recorded symmetrical gaits fell within the walking category, with duty factor values between 67 and 75 (*A. agrarius*, $N=93$, $DF=74.83$; *A. flavicollis*, $N=79$, $DF=73.77$; *M. glareolus*, $N=54$, $DF=67.40$; Table 2.2, Fig. 2.2, 2.4). Duty factor differed significantly between the three species ($N=270$, $F_{(2,269)}=53.54$, $p<0.001$; controlling for diagonality, substrate size, direction of movement and the effect of gait type). *Apodemus agrarius* scored significantly higher than both *A. flavicollis* (BMD=5.26, $p<0.001$) and *M. glareolus* (BMD=8.78, $p<0.001$). Additionally, duty factor in *A. flavicollis* was significantly higher than *M. glareolus* (BMD=3.52, $p=0.003$), the latter engaging in less cautious walking.

Overall, mean duty factor indices (DFI) for all species were close to 100.00 (Table 2.2, Fig. 2.2, 2.4), counting both symmetrical and asymmetrical gaits, implying an overall equilibrium in hind limb and forelimb use (*A. agrarius*, $N=93$, $DFI = 98.10$; *A. flavicollis*, $N=133$, $DFI = 96.78$; *M. glareolus*, $N=54$, $DFI = 103.22$). Differences between species were observed ($F_{(2,269)}=8.41$; $p<0.001$; controlling for substrate size, direction of movement and gait type). DFI was comparable between *A. agrarius* and *A. flavicollis* (BMD=1.67, $p=0.757$), but was significantly higher in *M. glareolus* than both *A. flavicollis* (BMD=6.75, $p<0.001$) and *A. agrarius* (BMD=5.07, $p=0.002$), implying longer stance durations of the hind limbs than the forelimbs, compared to the other two species.

Overall, dimensionless velocities of the three species (Fig. 2.3, 2.5) differed significantly ($N=270$, $F_{(2,269)}=12.56$, $p<0.001$; controlling for the effect of gait type, substrate size and orientation). Velocity was significantly higher in *M. glareolus* than in *A. flavicollis* (BMD=0.02, $p=0.001$), and both species were significantly faster than *A. agrarius* (BMD=0.17, $p<0.001$; BMD= 0.15, $p<0.001$, respectively). Dimensionless stride length (Fig. 2.3, 2.5) also differed significantly between species ($N=270$, $F_{(2,269)}=54.49$, $p<0.001$; controlling for gait type, substrate size and inclination). Strides were significantly longer in *A. flavicollis* than in both *M. glareolus* (BMD=0.50, $p<0.001$) and *A. agrarius* (BMD=0.36, $p<0.001$). Additionally, *A. agrarius* used significantly longer strides than *M. glareolus* (BMD=0.16, $p=0.017$). Finally, dimensionless stride frequency (Fig. 2.3, 2.5) varied between species ($N=270$, $F_{(2,269)}=28.16$, $p<0.001$; controlling for gait type, substrate size and inclination). Strides were as frequent in *A. flavicollis* as in *A. agrarius*

(BMD=0.09, $p=1.000$), but *M. glareolus* exhibited a greater stride frequency than both *A. flavicollis* (BMD=0.12, $p<0.001$) and *A. agrarius* (BMD=0.12, $p<0.001$).

Effect of substrate size

Results on the effect of substrate size on diagonality were variable (Fig. 2.4). Diagonality increased on larger substrates, controlling for direction of movement and duty factor, for both *A. agrarius* ($N=93$, $F_{(2,92)}=13.08$, $p<0.001$) and *A. flavicollis* ($N=79$, $F_{(3,78)}=6.47$, $p=0.003$). *Myodes glareolus*, however, did not seem to adjust diagonality for substrate size ($N=54$, $F_{(2,53)}=1.14$, $p=0.329$), even though Fig. 2.4 implies some interaction. Duty factor decreased with substrate size increase (Fig. 2.4) in *A. flavicollis* ($N=123$, $F_{(3,122)}=5.27$, $p=0.008$) and *M. glareolus* ($N=54$, $F_{(2,53)}=5.62$, $p=0.010$). In contrast, substrate size had no impact on duty factor in *A. agrarius* ($N=93$, $F_{(2,92)}=0.60$, $p=0.699$). Finally, DFI showed no dependence on substrate size in any species (*Apodemus agrarius*, $N=93$, $F_{(2,92)}=2.89$, $p=0.061$; *Apodemus flavicollis*, $N=123$, $F_{(3,122)}=2.29$, $p=0.108$; *Myodes glareolus*, $N=54$, $F_{(2,53)}=0.03$, $p=0.972$).

In all species, velocity increased on larger substrates (Fig. 2.5) when controlling for gait type and substrate orientation (*A. agrarius*, $N=93$, $F_{(2,92)}=10.90$, $p<0.001$; *A. flavicollis*, $N=123$, $F_{(2,122)}=12.38$, $p<0.001$; *M. glareolus*, $N=54$, $F_{(2,53)}=13.75$, $p<0.001$). Stride length also increased with substrate size increase (Fig. 2.5; *A. agrarius*, $N=93$, $F_{(2,92)}=7.32$, $p=0.001$; *A. flavicollis*, $N=123$, $F_{(3,122)}=23.02$, $p<0.001$; *M. glareolus*, $N=54$, $F_{(2,53)}=12.93$, $p<0.001$, controlling for gait type and substrate orientation). Stride frequency tended to increase with substrate size in *A. agrarius* ($N=93$, $F_{(2,92)}=8.13$, $p=0.001$) and *A. flavicollis* ($N=123$, $F_{(3,122)}=17.42$, $p<0.001$), but not in *M. glareolus* ($N=54$, $F_{(2,53)}=2.76$, $p=0.073$).

Effect of direction of movement

Regarding direction of movement, only *A. flavicollis* used all the inclination categories. In contrast, *A. agrarius* and *M. glareolus* used only descents, but strongly avoided ascending upon inclined substrates. Diagonality did not co-vary with direction of movement in any species (Fig. 2.4; *A. agrarius*, $N=93$, $F_{(1,92)}=0.12$, $p=0.732$; *A. flavicollis*, $N=79$, $F_{(2,79)}=0.14$, $p=0.871$; *M. glareolus*, $N=54$, $F_{(1,53)}=3.09$, $p=0.061$) when controlling

for duty factor and substrate size. Duty factor (Fig. 2.4), increased from descent to horizontal locomotion in both *A. agrarius* (N=93, $F_{(1,92)}=8.79$, $p=0.004$) and *M. glareolus* (N=54, $F_{(1,53)}=38.64$, $p<0.001$) when controlling for diagonality and substrate size. No comparable trend was detected in *A. flavicollis* (N=123, $F_{(2,122)}=1.61$, $p=0.211$). Despite its great variability (Table 2.2, Fig. 2.4), DFI increased from descent to horizontal locomotion in *A. agrarius* (N=93, $F_{(1,92)}=12.76$, $p=0.001$) and from descent to horizontal locomotion to ascent in *A. flavicollis* (N=123, $F_{(2,122)}=3.24$, $p=0.045$) when controlling for the effect of substrate diameter. No such trend was found in *M. glareolus* (N=54, $F_{(1,53)}=2.93$, $p=0.093$).

Tests on the effect of the direction of movement on velocity, when controlling for substrate size and gait type (Fig. 2.5), revealed three different patterns. In *A. agrarius*, velocity between descent and horizontal locomotion differed only marginally (N=93, $F_{(1,92)}=3.74$, $p=0.056$). On the other hand, in *A. flavicollis*, there was an increase of velocity from descent to horizontal to ascent (N=123, $F_{(2,122)}=7.22$, $p=0.001$). In contrast, *M. glareolus* was faster in descents than in horizontal locomotion (N=54, $F_{(1,53)}=28.42$, $p<0.001$). We found no significant covariance between stride length and direction of movement (Fig. 2.5; *A. agrarius*, $F_{(1,92)}=2.18$, $p=0.143$; *A. flavicollis*, N=123, $F_{(2,122)}=2.57$, $p=0.081$; *M. glareolus*, N=54, $F_{(1,53)}=3.73$, $p=0.059$; controlling for gait type and substrate size). Controlling for gait type and substrate size, *A. agrarius* and *M. glareolus* used higher stride frequencies in descents than in horizontal locomotion (N=93, $F_{(1,92)}=5.99$, $p=0.003$; and N=54, $F_{(1,53)}=54.67$, $p<0.001$, respectively). In contrast, in *Apodemus flavicollis*, stride frequency increased from descents to horizontal locomotion to ascents (N=123, $F_{(2,122)}=13.64$, $p<0.001$).

2.4. Discussion

The species examined display morphological differences that are functionally linked to differential use of the habitat, with *A. flavicollis* being more arboreal, *M. glareolus* using both terrestrial and arboreal substrates, and *A. agrarius* being a mainly burrowing species. The vertical stratification of their activity is further marked when they engage in sympatric interspecific competition (Olszewski, 1968; Holisova, 1969; Tattersall & Whitbread, 1994; Buesching *et al.*, 2008), implying that some may be more adept at exploiting arboreal pathways than others. In this context, gait metrics represent a useful tool to estimate arboreal locomotor capabilities (Delciellos & Vieira, 2006; Schmidt &

Fischer, 2011; Camargo *et al.*, 2016). In effect, our results demonstrated differences between the species, but relationships appeared to be more complex than expected. However, as our results were based on a small number of sampled individuals, there might be a risk for false-positive or negative trends among the discussed variables. Nevertheless, the number of recorded trials partly overcomes this flaw.

Our findings showed that the studied sympatric rodents displayed overall low diagonality (i.e. used LS gaits), much like many arboreal mammals without prehensile extremities, as well as terrestrial ones (Stevens, 2006; Lammers, 2007; Nyakatura *et al.*, 2008; Schmidt & Fischer, 2010; Shapiro & Young, 2012). Upon arboreal substrates, the coordinated movements of ipsilateral limbs of LS gaits appear to provide more static locomotor stability than DS gaits (Lammers & Zurcher, 2011a). Therefore, they might be more advantageous for mammals without grasping extremities in a challenging arboreal environment. However, the differing degrees of diagonality across species only partially supported our initial predictions, where the more arboreally adapted species (*A. flavicollis* and *M. glareolus*) would have used more DS (or less LS) gaits, than the burrowing *A. agrarius*. More precisely, diagonality scores in *Apodemus* spp. did not differ significantly between the more arboreal *A. flavicollis* and the burrowing *A. agrarius*. This remains puzzling, if one further considers the divergent morphologies between the two species (Kuncová & Frynta, 2009). On the other hand, the arvicoline *M. glareolus* displayed the lowest diagonality, indicating increased use of LS gaits. However, when sympatric, both *M. glareolus* and *A. flavicollis* tend to exploit more arboreal pathways to access food sources than the terrestrial *A. agrarius* (Andrzejewski & Wrocławek, 1961; Gliwicz, 1981; Heroldova, 1994). It is very likely that the morphology of bank voles, and arvicolines in general, characterized by a short compact body, short limbs and lack of prehensile extremities (Zefferer, 2002; Nations & Olson, 2015) benefits from increased static locomotor stability, provided by the LS gaits, in order to balance and navigate along arboreal substrates. This strategy was further substantiated by the result that diagonality in bank voles did not vary with either substrate size or direction of movement (see Table 2.1). This may imply that overall gait use can be driven by phylogenetic rather than ecological constraints.

Considering the effects of substrate size, diagonality was lower on narrower substrates and increased on the larger ones in both *A. flavicollis* and *A. agrarius*, but remained unaffected in *M. glareolus*. This pattern in part contrasts our predictions for the

more arboreal *A. flavicollis*. In effect, this observation appears opposite to earlier findings in a small arboreal marsupial, which tends to use DS gaits on narrower substrates (Karantanis *et al.*, 2015). DS gaits, characteristic of many arboreal mammals, provide more dynamic locomotor stability with the contralateral footfall patterns, and would be advantageous upon slender substrates (Cartmill *et al.*, 2007). Nevertheless, the possession of grasping extremities may be a prerequisite for the efficient use of DS gaits in challenging arboreal settings (Cartmill *et al.*, 2007). As *A. flavicollis* possess relatively limited grasping capacities (Krattli, 2001; Zefferer, 2002), DS gaits may confer much advantage on slender substrates, underscoring the importance of the static locomotor stability of LS gaits (Lammers & Zurcher, 2011a).

The possible association of grasping extremities with DS gaits may further explain the consistent use of LS gaits by all species during the challenging activities of descents and ascents. However, we did not record any ascents in two of the three studied species (*A. agrarius*, *M. glareolus*) potentially biasing any trends. The consistent use of LS gaits differs from arboreal locomotion in other small mammals, in which LS is thought to provide braking assistance during descent (Lammers *et al.*, 2006; Nyakatura *et al.*, 2008; Karantanis *et al.*, 2015). However, mammals which lack prehensile extremities and already employ LS gaits, may not need to shift to LS gaits of even lower diagonality during descents, conforming to our findings. The interplay between grasping extremities and DS gaits use on different substrate sizes and inclinations requires more in depth investigations. Additionally, during descents, arboreal mammals face a cranial/anterior weight shift, which produces increased contact times with the substrate and a higher fraction of vertical impulse on the forelimbs, enhancing their regulatory and supportive role (Rollinson & Martin, 1981; Nyakatura *et al.*, 2008). Actually, in *Apodemus* spp., the enhanced role of the forelimbs was emphasised by the lower duty factor indices (DFI) in descents, indicating an increased forelimb duty factor compared to that of the hind limb, potentially emphasizing the controlling and braking role of the forelimbs (Nyakatura *et al.*, 2008). Once more, the arvicoline *M. glareolus* did not follow the trend, characterised by an increased hind limb duty factor compared to that of the forelimb, which was not affected by descending or horizontal locomotion.

All the studied rodents displayed relatively low velocities, indicating cautious walking. Interspecific differences showed that the burrowing *A. agrarius* exhibited the lowest velocities, whereas *M. glareolus* was significantly faster than the more arboreal *A.*

flavicollis. This partially contradicted our predictions, as increased velocity may be a significant mechanism to maintain dynamic stability on arboreal substrates (Schmidt & Fischer, 2011), and is usually linked to arboreal adaptations (Delciellos & Vieira, 2006; Camargo *et al.*, 2016). Moreover, in all three species, velocity decreased on narrower substrates. A similar pattern characterises the terrestrially adapted sigmodontine rodents, whereas arboreal species demonstrate an inverse relation between velocity and substrate size (Camargo *et al.*, 2016). Thus, our sampled species showed locomotor similarities to terrestrial rodents, as may have been expected for the more terrestrial *A. agrarius* and, to a lesser extent, *M. glareolus*. On the other hand, this pattern resembles that of arboreal metatherians, which tend to use lower velocities on finer substrates (Delciellos & Vieira, 2006; Karantanis *et al.*, 2015). Cautious walking on slender substrates appears to provide a functional advantage for generalist species. When a quadruped moves on an arboreal substrate, the support polygons produced by the limbs are much narrower than those on larger substrates or on the ground (Lammers & Zurcher, 2011a). During locomotion, each time a limb touches down, it produces a torque which needs to be counteracted, or else the centre of mass is likely to be placed outside the support polygon (Lammers & Zurcher, 2011b). A slow controlled walk, especially on narrow substrates, should minimize this possibility, by reducing torques, increasing the contact points on the substrate and providing longer time for accommodating a stable and secure dynamic posture (Lammers & Zurcher, 2011a). This profile better suits the more arboreal yellow-necked mice, which engage in intense arboreal activities, especially under sympatric competition (Andrzejewski & Wrocławek, 1961; Holisova, 1969; Gliwicz, 1981; Heroldova, 1994; Tattersall & Whitbread, 1994). The arboreal capacity of *A. flavicollis* is further supported by velocity increase from descending towards ascending locomotion. The decelerating and braking role of the forelimbs in descents, that provide a controlled downwards progression (*vide supra*), and the prevailing propulsive role of the hind limbs, providing agile dynamic upward stability during ascents (Nyakatura *et al.*, 2008), was different from the pattern observed in the less arboreal *M. glareolus* and *A. agrarius*, in which descents were faster than horizontal locomotion.

Velocity regulation further differentiated between the three species, in manner different from that predicted for the more arboreally and the more terrestrially adapted. Thus, in the scansorial arvicoline *M. glareolus*, velocity depended almost equally on stride length and stride frequency, a pattern common in most small mammals (Strang & Steudel,

1990). On the other hand, both *A. flavicollis* and *A. agrarius* regulated velocity primarily by stride frequency, whereas stride length also played a lesser, though significant role. The latter pattern is observed in some terrestrial mammals during walking and running (Biewener, 2005), as well as in a few arboreal neotropical rodents (Camargo *et al.*, 2016), and some arboreal marsupials (Delciellos & Vieira, 2006; Karantanis *et al.*, 2015). In contrast, primates (Larson *et al.*, 2000, 2001) and medium and large-bodied marsupials (Delciellos & Vieira, 2006) increase velocity primarily by increasing stride length. This option enables a farther reach of the forelimb and reduces involuntary branch swaying promoting safe arboreal navigation (Demes *et al.*, 1994). On the other hand, smaller arboreal mammals induce low to negligible branch swaying but experience significant body oscillations, which may disrupt regular progression (Delciellos & Vieira, 2006). Thus, for smaller mammals, increasing velocity by increasing stride frequency, although energetically costly (Reilly *et al.*, 2007), stands as a suitable mechanism for secure negotiation of arboreal substrates (Delciellos & Vieira, 2006).

This strategy was further demonstrated by the fact that both murids increased velocities through stride frequency and length when substrate size increased. However, only the more arboreal *A. flavicollis* shifted to asymmetrical locomotion at higher velocities on larger substrates, leading to an increase in stride frequency. The use of asymmetrical gaits has been reported in other relatively small arboreal mammals, such as squirrels and mouse lemurs (Schmidt & Fischer, 2011; Shapiro *et al.*, 2016). In mammals, asymmetrical gaits assist in increase of velocity, enhance manoeuvrability, and minimize the energetic cost of locomotion (Hoyt & Taylor, 1981; Farley & Taylor, 1991; Gasc, 2001). They also confer advantages in stability by reducing peak vertical forces, limiting centre of mass movements, and using shorter and more frequent strides (Young, 2009; Schmidt & Fischer, 2011; Shapiro *et al.*, 2016). This may further corroborate the arboreal capabilities of the yellow-necked mice. In contrast, *M. glareolus* kept on using symmetrical gaits and tended to only increase length when moving upon larger substrates, in an analogous manner to some arboreally adapted sigmodontine rodents (Camargo *et al.*, 2016). To understand the variable responses of velocity increase upon different substrate sizes, further investigations are required, as we lack data on the contribution of both stride frequency and length for other arboreal mammals.

The present study is among the few that deal with gait parameters during arboreal locomotion in sympatric rodents. Previous work on some of these related parameters

across sympatric neotropical metatherians and sigmodontine rodents managed to partly differentiate arboreal from terrestrial species (Camargo *et al.*, 2016). In contrast, our results demonstrated that, when considering more generalised rodents, there were no clear differences between more arboreal and more terrestrial species and that locomotor behavioural adaptations were much more complex than expected. Among the three studied rodents, only *A. flavicollis* demonstrated some features of locomotion related to arboreality: use of asymmetrical locomotion at higher velocity, velocity regulated by stride frequency and to a lesser, but significant extent, stride length, lower velocity on narrower substrates, increased velocity and duty factor index in ascents, and decreased velocity and duty factor index in descents. These findings corroborate ecological observations that the species is a relatively skilled arborealist, and able to exploit the arboreal milieu, especially under sympatric competition (Holisova, 1969; Montgomery, 1980). On the other hand, the other two species revealed a more confusing locomotor profile, only partly related to our initial hypotheses. Interestingly, and in contrast to our predictions, the more arboreal *A. flavicollis* and the more terrestrial *A. agrarius* shared many gait features: similar diagonality scores, the equal relative use of both fore- hind limbs in locomotion, similar stride frequencies, which increased on larger substrates, increased role of forelimbs in descents, and velocity regulation by stride frequency and, to a lesser extent, stride length. Despite their divergent morphological adaptations, and differential habitat use (Zejda, 1967; Montgomery, 1980; Chełkowska *et al.*, 1985), they appear to employ comparable strategies when they negotiate different arboreal substrates. This indicates the possibility of a phylogenetic signal in the regulation of similar locomotor mechanisms, in contrast to previous findings, where ecology predicted common locomotor patterns (Camargo *et al.*, 2016).

Lastly, the arvicoline *M. glareolus*, although an adept climber, exhibited locomotor features common to the burrowing *A. agrarius* (reduced limb contact times, increased velocity, and stride frequency in descents), common to the arboreal *A. flavicollis* (increased limb contact on narrower substrates), but mainly diverged by its different behavioural responses. In effect, bank voles differed in their increased LS gaits, independent of substrate size, highest hind limb to forelimb relative contact times, increased velocities and stride frequency, and regulation of speed by both stride frequency and length. These strategies may represent a compromise of arboreal capacities with a morphology mainly adapted to a more terrestrial and burrowing way of

life (Zefferer, 2002; Nations & Olson, 2015). Despite the fact that our results were based on a small number of sampled subjects and sampled sympatric species, we believe that they actually demonstrate that gait parameters may partly categorize generalised small mammals. As the interactions between morphology, behaviour and ecology are usually very complex, it is particularly difficult to establish robust links between them. Nevertheless, as the present study is the first of its kind, it may lay a foundation for further research on the locomotor ecology of these species and a good starting point for investigating the arboreal locomotor abilities of these rodents in more detail in future studies.

Chapter 3: Reaching up to the arboreal milieu

Vertical locomotion in *Micromys minutus* (Rodentia: Muridae): insights into the evolution of eutherian climbing

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3.1. Introduction

Climbing is the locomotor mode in which an animal moves upwards or downwards on vertical or very steep substrates (Cartmill 1985; Hunt et al. 1996). During climbing, the body is kept orthograde and more or less parallel to the substrate (Hunt et al. 1996). Besides being characteristic of arboreal species, climbing is also particularly important for scansorial species, which exploit both the terrestrial and arboreal habitat. For these animals, vertical climbing enables the entry to the arboreal milieu with significant ecological and evolutionary advantages: timely access to arboreal food sources (e.g., flush leaves, buds, flowers, ripe fruit, arboreal arthropods), enhanced vigilance and predator avoidance, secure resting and nesting sites (Hildebrand 1995), and overall increased longevity (Shattuck and Williams 2010).

It is not surprising that an arboreal lifestyle has played a fundamental role in the evolution of early mammals. Paleontological evidence has traced climbing in the fossil record back to 160 mya (Gaetano and Rougier 2011; Zheng et al. 2013; Bi et al. 2014). Importantly, both oldest known eutherians, *Juramaia sinensis* (160 mya) and *Eomaia scansoria* (125 mya) possess postcrania that show possible climbing adaptations, such as the morphology and proportions of manual and pedal proximal, intermediate and ungual phalanges, the morphology of the scapula, the long tail, and the elongation of mid-caudal vertebrae (Ji et al. 2002; Luo et al. 2011; Bonnan et al. 2016). Fossil remains of other early mammalian radiations, such as the Docodonta (Meng et al. 2015), Euharamiyida (Zheng et al. 2013), and Eutriconodonta (Meng et al. 2006), have also been functionally linked to climbing and arboreal activities. This demonstrates that arboreality and climbing are integral patterns of early mammalian evolutionary history. Due to biomechanical,

physiological, and energetic constraints, climbing up and down arboreal substrates appears to be more effective at a smaller body size, allowing for the successful transition between the ground and the arboreal milieu (Cartmill 1974a; Jenkins 1974; Preuschoft et al. 1995; Preuschoft 2002; Soligo and Martin 2006; Hanna and Schmitt 2011; Soligo and Smaers 2016). Moreover, all known early eutherians are relatively small, with body mass estimates for *Juramaia* ranging at 15-17g (Luo et al. 2011) and for *Eomaia* at 20-25g (Ji et al. 2002), and have morphological features predisposing them for a climbing life-style.

Climbing up and down a vertical or sub-vertical substrate can be carried out with gaits similar to those used in above-branch locomotion (Hunt et al. 1996). Therefore, they can be translated into comparable metrics, such as diagonality, duty factor, duty factor index, velocity, stride length, and stride frequency. Diagonality (D), or forelimb-hind limb phase (Hildebrand 1967; Cartmill et al. 2007), is the percentage of the stride cycle interval the footfall of a forelimb follows the footfall of the ipsilateral hind limb. In diagonal sequence (DS) gaits ($D > 50$), the contralateral limbs move forward more in sync, whereas in lateral sequence (LS) gaits ($D < 50$), the ipsilateral limbs are more synchronized. The duty factor (DF) represents the percentage of the cycle during which a foot (fore or hind) is in contact with the substrate (Hildebrand 1967) and separates walking ($DF > 50$) from running ($DF < 50$). Finally, the duty factor index (DFI), a ratio derived by the DF of the hind limbs and forelimbs (Cartmill et al. 2007), infers the differing role of forelimbs compared to that of the hind limbs. All these parameters are of interest, as they have been linked to locomotor adaptations on different substrates (Cartmill et al. 2002, 2007).

In an arboreal context, gaits are a behavioral mechanism to enhance stability and safety (Cartmill et al. 2002; Lammers and Gauntner 2008; Lemelin and Cartmill 2010; Lammers and Zurcher 2011). Either DS or LS gaits may confer different advantages in the context of arboreal locomotion (Lammers and Zurcher 2011). LS gaits promote static stability (as in slow walking), in relation to the location of the centre of mass relative to the support polygon of the limbs (Lammers and Zurcher 2011). They are more often encountered in terrestrial mammals, but are also used by some arboreal specialists, such as sugar gliders *Petaurus breviceps* (Shapiro and Young 2010), callitrichid primates (Stevens 2006, 2008; Nyakatura et al. 2008; Nyakatura and Heymann 2010) and scansorial rodents (Schmidt and Fischer 2010). On the other hand, DS gaits seem to provide dynamic stability, i.e. secure control and transfer of moments and torques

imposed on the body axes in faster locomotion (Lammers and Zurcher 2011). Habitual use of DS gaits is common among primates (Hildebrand 1967; Cartmill et al. 2007), metatherian didelphimorphs and phalangeroids (White 1990; Pridmore 1994; Cartmill et al. 2002; Schmitt and Lemelin 2002; Karantanis et al. 2015), as well as the carnivoran *Potos flavus* (Lemelin and Cartmill 2010), and are hypothesized to be related to arboreal adaptations (Cartmill et al. 2007).

Velocity on arboreal substrates is also a significant parameter that can be related to arboreal competence (Arnold 1983; Delciellos and Vieira 2006, 2009; Camargo et al. 2016). Increased velocity is a significant mechanism to maintain dynamic stability (Schmidt and Fischer 2010) and has been related to arboreal adaptations (Delciellos and Vieira 2006, 2007; Camargo et al. 2016). Velocity, being a function of stride length by stride frequency, is regulated by variations in these two parameters (Alexander 1992; Hildebrand 1995). Climbing primates typically take longer strides compared to generalized species (Hirasaki et al. 1992, 1993, 2000; Isler 2005; Hanna 2006; Hanna and Schmitt 2011) as it is considered to provide energetic advantages (Isler 2005; Hanna 2006; Hanna and Schmitt 2011). This contrasts with observations for didelphid and acrobatid marsupials, in which velocities are generally determined by the combination of stride length and frequency (Delciellos and Vieira 2009; Karantanis et al. 2015). However, the energetics of force generation at a small scale, along with the crouched limb posture of small mammals may render vertical climbing easier than at a larger body mass (Cartmill 1974a; Demes et al. 1994; Preuschoft et al. 1995; Hanna 2006; Hanna and Schmitt 2011), with consequent implications to velocity regulation.

All gait parameters are not static intrinsic factors, but are adjusted according to properties of the used substrates, such as substrate inclination and size (Schmitt 2003; Franz et al. 2005; Nyakatura et al. 2008; Wallace and Demes 2008; Young 2009; Lemelin and Cartmill 2010; Schmidt and Fischer 2011; Karantanis et al. 2015). Climbing upwards requires the most effort, next to leaping, while in downward climbing less force production is necessary (Preuschoft et al. 1995; Preuschoft 2002). In ascents, upward progression is based on the generation of propulsion by the hind limbs (Hirasaki et al. 1993; Hanna and Schmitt 2011), and is related to increased D (i.e., more DS gaits) and DFI (increased duration of contact of the hind limbs relative to the forelimbs) (Preuschoft et al. 1995; Preuschoft 2002; Isler 2004; Nyakatura et al. 2008; Nyakatura and Heymann 2010; Karantanis et al. 2015). Moreover, in ascents, velocity increases in order to achieve

dynamic stability, as in the agile European red squirrels, or decreases in order to maintain static stability as in rats (Schmidt and Fischer 2011). In descents, more frequent LS gaits and increased forelimb contact occur to promote safe and controlled descent by enhancing the regulative and braking role of the forelimbs (Hirasaki et al. 1993; Preuschoft et al. 1995; Preuschoft 2002; Nyakatura et al. 2008; Nyakatura and Heymann 2010; Hesse et al. 2014). Furthermore, in descents, lower velocities are observed in comparison to ascents or horizontal locomotion, at least at intermediate inclinations (Vilensky et al. 1994; Lammers et al. 2006; Lammers 2007; Karantanis et al. 2015) possibly brought about by the enhanced role of the load-bearing forelimbs and the frictional adjustment of the extremities.

Apart from direction of movement, substrate size also imposes constraints on locomotion (Jenkins 1974; Witte et al. 2002). However, behavioral responses vary across taxa. DS gaits, with the contralateral movement of limbs, are supposed to enhance safe progression along unstable, slender branches (Cartmill et al. 2007), and some arboreal mammals tend to increase gait diagonality on smaller substrates (Lemelin and Cartmill 2010; Karantanis et al. 2015). In contrast, many strepsirrhine primates (Stevens 2008), some callitrichids (Nyakatura et al. 2008; Nyakatura and Heymann 2010), and the arboreal metatherian *Petaurus breviceps* (Shapiro and Young 2010) exhibit negative to no correlation between substrate size and diagonality. Moreover, in both horizontal and vertical locomotion arboreal specialists tend to reduce their velocity on larger substrates, with an opposite pattern observed in terrestrial species (Isler 2004; Delciellos and Vieira 2006, 2009; Camargo et al. 2016).

In mammals, the majority of available data on gait parameters and their relation to the properties of arboreal substrates mainly derive from horizontal and inclined locomotion, but not vertical upwards and downwards climbing. Apart from some primates (i.e., Isler 2004), it is unknown if and how gait parameters are modified during vertical locomotion and how substrate properties could relate to any modifications. This is of particular importance in arboreal locomotion, as one of the major challenges is the effective negotiation of vertical substrates of variable size. This can be even more demanding, when considering that most arboreal mammals lack an increased grasping ability, similar to that of primates. In order to investigate relations between gait parameters and substrate properties during vertical upward and downward climbing in small-bodied arboreal mammals, we experimentally tested the climbing competence of

one of the smallest rodents, the Eurasian harvest mice, *Micromys minutus*. Eurasian harvest mice are known to exploit arboreal and terrestrial substrates alike (Ylönen 1990). Thus they occupy any available low vegetation, such as short trees, shrubs, vines, hedgerows, long grass or even reed beds, making them specialists in climbing and moving on very fine substrates (Harris 1970; Nordvig et al. 2001; Surmacki et al. 2005). The use of arboreal substrates increases during the breeding season, in order to build arboreal nests and avoid interspecific competition (Ylönen 1990), to keep away from potential predators (Jędrzejewska and Jędrzejewski 1990; Jędrzejewski et al. 1993), as well as to forage for fruits, seeds, and invertebrates on terminal twigs and stems (Leach 1990; Nowak 1999). Eurasian harvest mice are adept arborealists and their small size, semi-prehensile tail, grasping extremities, diffuse bowing of the lumbodorsal region, along with a diverse arboreal positional behaviour, increased rates of pedal grasping and significant use of vertical climbing and of vertical substrates (Leach 1990; Ylönen 1990; Haffner 1996, 1998; Ishiwaka and Mori 1999; Krattli 2001; Vázquez-Molinero et al. 2001; Urbani and Youlatos 2013) allow them to efficiently and swiftly move vertically from the ground to arboreal vegetation and back down. While accessing the arboreal niche, they can further acquire novel food sources, such as fruit, seeds, and invertebrates on the terminal twigs and stems increasing their fitness (Leach 1990; Nowak 1999). Moreover, proficient head-first downward climbing allows for constant scanning of the ground, enhancing detection of predators, competitors, and potential food sources (Cartmill 1974b; Preuschoft et al. 1995; Preuschoft 2002).

In this study, we aim to identify the locomotor mechanisms underlying vertical climbing in a small-bodied arboreal mammal, and if and how they differentiate in relation to substrate size and direction of movement (ascending or descending). More precisely, considering that *M. minutus* are adept climbers, we hypothesize that they should increase D, DFI and velocity during ascending locomotion. Furthermore, velocity is expected to be regulated mainly by stride length, followed by stride frequency in ascents. As substrate size decreases, we expect D and velocity to increase, DF to decrease, and DFI to remain constant. On the other hand, we expect harvest mice to decrease D, DF, and velocity in descents. Furthermore, as the earliest eutherians were equally small and likely displayed comparable arboreal behaviours, our findings may provide insights into understanding the adaptive context of the successful exploitation of the arboreal habitat.



Figure 3.1. Still image from a video recording taken during the habituation of the Eurasian harvest mice in the experimental set-up. The support diameter is 2mm, and the vertical markings every 1 cm are clearly visible.

3.2. Materials and Methods

Specimens

Six adult male *Micromys minutus* (Fig. 3.1; Muridae, Rodentia) were tested under experimental conditions. All subjects were captive born and permanently housed in large naturalistic enclosures, in the collections of the Nowe Zoo, Poznań, Poland. The original housings contained a large variety of available substrates of diverse sizes and orientations, enabling the harvest mice to move freely both arboreally and terrestrially in an enriched environment. In their regular enclosures, the Eurasian harvest mice made extensive use of any available arboreal substrate inside the enclosures and were also naturally inclined to use the substrates provided during the experimental procedures. Prior to the experiments, all participating Eurasian harvest mice were transported to a laboratory at the Faculty of Biology, Adam Mickiewicz University (AMU), Poznań, Poland where they were jointly housed in a glass terrarium on a reversed day-night regime. The terrarium was completed with natural materials for nesting and climbing, to reduce

stress. All specimens were fully habituated to human presence and did not display any stereotypical or stressful behaviours. Mean total head-body length of the subjects was 5.9 cm (SD=0.12, range=5.7-6.1 cm, N=6) and mean body mass was 6.8 g (SD=1.02, range=5.8-7.9g, N=6). Mean effective hind limb length (see below), i.e., the distance between the hip joint and the substrate, parallel to the axis of gravity (Pontzer 2007), was 1.73 cm (SD=0.02, range=1.68-1.74 cm, N=6).

Experimental setup

A single, specially configured, filming terrarium (L: 90 cm x H: 40 cm x W: 40 cm) was used for the experiments. Its sides and base were transparent glass windows and it was topped by a wooden frame, with a lid door and wire mesh for ventilation. Within the terrarium, we established a wooden frame to support the poles. The poles were 80 cm long, cylindrical, semi-hardwood rods, set vertically within the terrarium. They were marked with vertical blue lines every 1 cm for a reliable estimation of absolute lengths. During the recordings, the length of the rod in view of the camera was approximately 30 cm. Diameter and the direction of movement accounted for the classification of different substrate categories. Thus, four diameters (2 mm, 5 mm, 10 mm, and 25 mm), and two different directions of movement (descent and ascent) were used to define a total of eight distinct substrate categories.

Data collection was completed in a total of six recording sessions, during June 2013. During each recording session, the individuals were transferred from their enclosure to the experimental terrarium. Each individual was allowed to move freely within the filming terrarium for habituation. Minimal or no stimulation was required for the subjects to walk on the poles. After each filming session, the animal was transported to another accommodation terrarium, to ensure that no individual was tested twice during a particular session.

For video recording, we used a Sanyo digital camcorder (VPC-HD 2000, Sanyo, Osaka, Japan), filming at 240 fps, which was positioned at a distance of 1 m from the filming terrarium to reduce image distortion. For our analyses, we considered only symmetrical gaits (in which the left and right limbs of a pair alternate) as we observed no occurrence of asymmetrical gaits (in which the limbs move together), initiating with the touchdown of the left hind limb and ending at the subsequent touchdown of the same

limb, including both lift-offs and touchdowns of all limbs (Hildebrand 1967, 1968, 1976). Cycles involving beginnings or endings of locomotor bouts, in which significant acceleration or deceleration was involved, or loss of balance, in which footfalls were irregular such as continuous lift offs and touchdowns of a single limb, were discarded. Overall, only cycles of stable symmetrical locomotion, regardless of velocity, were retained and regarded as indicative of natural, unbiased behaviour. The present research strictly adhered to the guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS 2012) and complied with relevant regulations and legislations of the Nowe Zoo and the Adam Mickiewicz University in Poznan and the relevant legislation of the Aristotle University of Thessaloniki. Handling, housing of animals and behavioural tests were carried out with permission by the Local Ethical Commission for the Animal Experiments in Poznan.

Gait analyses

Video analysis and data collection, distance and time calculations were made by importing videos and appropriately calibrating time and distance measurements using Tracker 4.87 (Brown 2009). Microsoft Excel 2016 (Redmond, WA, USA), and SPSS 20 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

For our analyses, we considered the following gait parameters:

- (i) Diagonality (D) (Cartmill et al. 2007), the percentage of the stride cycle interval the footfall of a forelimb follows behind that of the ipsilateral hind limb. Although it was measured as a scale variable, it was also divided into five ordinal classes (following Cartmill et al. 2002): (a) Lateral Sequence Lateral Couplets ($0 \leq \text{LSLC} < 25$), (b) Lateral Sequence Diagonal Couplets ($25 \leq \text{LSDC} < 50$), (c) Trot ($= 50$), (d) Diagonal Sequence Diagonal Couplets ($50 < \text{DSDC} \leq 75$), (d) Diagonal Sequence Lateral Couplets ($75 < \text{DSLC} \leq 100$);
- (ii) Duty Factor (DF), the mean of DFs of all limbs, defined as the percentage of a cycle during which a limb is in contact with the substrate;
- (iii) Duty Factor Index (DFI), the ratio of forelimb duty factor (DF_f) and hind limb duty factor (DF_h), calculated as $100 \cdot \text{DF}_h / \text{DF}_f$ (Cartmill et al. 2007). Values > 100 indicate higher hind limb than forelimb duty factors, whereas values < 100 specify lower hind limb than forelimb duty factors;

- (iv) Stride Duration (t), total duration of a single stride in seconds, measured from the frame where a stride cycle began until the frame the same stride cycle ended (a stride cycle encompassed all events between two consecutive touchdowns of the left hind limb);
- (v) Stride Length (l), the corresponding distance covered during a single stride cycle, in meters;
- (vi) Velocity (v), the speed in which the subjects moved, calculated by dividing stride length with stride duration, and measured in meters/second (m/s);
- (vii) Stride Frequency (f), the number of strides per second;

As these parameters are size-dependent, we used the effective hind limb length of Eurasian harvest mice to calculate the dimensionless measures of stride duration, stride length, velocity and stride frequency (Hof, 1996). These calibrated absolute measurements are useful for estimating competence during locomotion (Alexander 1977; Alexander and Jayes 1983; Hof 1996):

$$\text{Dimensionless Stride Duration (} t_D \text{)} = \frac{t}{\sqrt{l_0/g}}$$

$$\text{Dimensionless Stride Length (} l_D \text{)} = \frac{l}{l_0}$$

$$\text{Dimensionless Velocity (} v_D \text{)} = \frac{v}{\sqrt{gl_0}}$$

$$\text{Dimensionless Stride Frequency (} f_D \text{)} = \frac{f}{\sqrt{g/l_0}}$$

where l_0 is the effective hind limb length of each animal and g is the acceleration of gravity ($g=9.81 \text{ m/s}^2$). When overall values for parameters are reported, they represent the sum of means of each category, divided by the number of categories with observations.

We tested for statistically significant discrepancies in the utilization of diagonal and lateral gaits using binomial testing, but also incorporated the trot category into the analysis by using a chi-square test (χ^2). These analyses were carried out using two-tailed Monte Carlo procedures for enhanced p estimation accuracy (Kalos and Whitlock 2009).

Analysis of covariance (ANCOVA) was selected in order to explore the relationships of variables, including individuals as random factors, while controlling for other possible covariates. When needed, a Bonferroni Post-Hoc test (BMD) was used for pairwise category comparisons, using estimates of means, as calculated by ANCOVA.

Stepwise regression models were constructed to examine the impact of both stride frequency and stride length on velocity, using their dimensionless counterparts. The impact of each parameter was calculated using the partial correlation coefficient, i.e. the correlation between a dependent variable and its covariate, after the impact of other covariates is removed (Harrell 2001).

3.3. Results

Overall trends in gait parameters

We recorded a total of 157 complete stride cycles across all categories (Table 3.1). We detected no significant differences between individuals for all parameters ($p > 0.05$), and therefore their individual results were grouped together. All recorded gaits were symmetrical, lateral-sequence ($D < 50$) walks ($DF > 50$), where LSDC gaits dominated (Table 3.2; $n = 135$).

Overall, D significantly increased as DF increased, controlling for the effect of substrate size (Fig. 3.2) in both descents ($N = 79$, $F_{(77,78)} = 3.818$, $p < 0.007$) and ascents ($N = 78$, $F_{(76,77)} = 2.514$, $p = 0.037$). Moreover, DFI had an overall positive relation with D (Fig. 3.2; Descent: $N = 79$, $F_{(77,78)} = 3.916$, $p = 0.012$; Ascent: $N = 78$, $F_{(76,77)} = 2.742$, $p = 0.049$; controlling for the effect of substrate size). Essentially, as the DF of the hind limbs became higher than those of the forelimbs, gaits became more diagonal (Fig. 3.2).

Table 3.1. Summary of means and standard deviations (in brackets) of the gait statistics for *Micromys minutus* (N indicates the number of analyzed cycles for each substrate size and direction).

		N	Diagonality	Duty Factor	Hind limb Duty Factor	Forelimb Duty Factor	Duty Factor Index	Velocity (m s ⁻¹)	Stride Length (m)	Stride Frequency (s ⁻¹)	Stride Duration (s)	Dimensionless Velocity	Dimensionless Stride Length	Dimensionless Stride Frequency	Dimensionless Stride Duration
2mm	Ascent	32	36.69 (4.44)	71.97 (2.69)	73.39 (4.35)	70.55 (2.63)	104.13 (6.81)	0.164 (0.035)	0.037 (0.003)	4.400 (0.748)	0.234 (0.042)	0.402 (0.086)	2.178 (0.161)	0.183 (0.031)	5.625 (1.013)
	Descent	23	29.81 (4.30)	76.75 (2.11)	75.45 (3.24)	78.04 (2.21)	96.74 (4.59)	0.16 (0.044)	0.035 (0.003)	4.520 (1.043)	0.232 (0.050)	0.391 (0.109)	2.064 (0.165)	0.188 (0.043)	5.572 (1.195)
5mm	Ascent	17	31.4 (4.75)	75.51 (4.24)	74.68 (5.73)	76.35 (3.94)	97.86 (6.52)	0.135 (0.040)	0.031 (0.004)	4.249 (0.823)	0.243 (0.045)	0.330 (0.099)	1.837 (0.249)	0.177 (0.034)	5.850 (1.082)
	Descent	23	31.31 (5.14)	78.96 (2.78)	78.17 (3.08)	79.74 (3.34)	98.13 (4.04)	0.158 (0.028)	0.034 (0.002)	4.677 (0.641)	0.218 (0.033)	0.386 (0.068)	1.977 (0.137)	0.195 (0.027)	5.241 (0.806)
10mm	Ascent	22	29.29 (5.57)	81.83 (3.30)	80.91 (4.52)	82.75 (2.74)	97.77 (4.25)	0.107 (0.026)	0.025 (0.004)	4.140 (0.530)	0.245 (0.031)	0.262 (0.064)	1.508 (0.249)	0.172 (0.022)	5.892 (0.739)
	Descent	15	34.38 (5.42)	77.53 (2.71)	75.73 (3.48)	79.32 (3.01)	95.54 (4.61)	0.157 (0.057)	0.032 (0.006)	4.858 (1.108)	0.217 (0.054)	0.384 (0.139)	1.859 (0.365)	0.202 (0.046)	5.213 (1.306)
25mm	Ascent	7	35.64 (8.91)	83.21 (5.80)	85.81 (5.30)	80.62 (11.33)	108.78 (21.42)	0.09 (0.038)	0.023 (0.005)	3.777 (1.097)	0.284 (0.078)	0.221 (0.092)	1.378 (0.315)	0.157 (0.046)	6.815 (1.866)
	Descent	18	26.09 (6.31)	87.65 (2.47)	86.56 (3.72)	88.73 (1.73)	97.54 (3.44)	0.063 (0.013)	0.026 (0.002)	2.477 (0.480)	0.417 (0.076)	0.155 (0.032)	1.500 (0.115)	0.103 (0.020)	10.025 (1.823)
Overall	Ascent	78	33.26 (5.92)	78.13 (4.01)	78.70 (4.98)	77.57 (5.16)	102.14 (9.75)	0.124 (0.035)	0.029 (0.004)	4.142 (0.800)	0.252 (0.049)	0.304 (0.085)	1.725 (0.244)	0.172 (0.033)	6.046 (1.175)
	Descent	79	30.40 (5.29)	80.22 (2.52)	78.98 (3.38)	81.46 (2.57)	96.99 (4.17)	0.135 (0.036)	0.032 (0.003)	4.133 (0.818)	0.271 (0.053)	0.329 (0.087)	1.850 (0.196)	0.172 (0.034)	6.513 (1.283)

Table 3.2. Frequencies and percentages (in brackets) of lateral-sequence lateral couplets (LSLC) and lateral-sequence diagonal couplets (LSDC) gaits during ascents and descents of *Micromys minutus*.

Substrate Size	Direction of Movement	Classification of Strides	
		LSLC	LSDC
2mm	Ascent	0 (0.0%)	19 (82.6%)
	Descent	4 (17.4%)	32 (100%)
5mm	Ascent	7 (38.9%)	21 (91.3%)
	Descent	2 (8.7%)	11 (61.1%)
10mm	Ascent	5 (22.7%)	15 (100%)
	Descent	0 (0.0%)	17 (77.3%)
25mm	Ascent	2 (28.6%)	11 (61.1%)
	Descent	7 (38.9%)	5 (71.4%)
All sizes	Ascent	9 (11.5%)	66 (83.5%)
	Descent	13 (16.5%)	69 (88.5%)

Vertical ascents

Ascending on the largest substrates (25 mm) was difficult for *M. minutus*, which resulted in a low sample size (n=7). Therefore, any results regarding ascents on 25mm substrates should be regarded with caution. Overall mean D was 33.26 (SD=5.92, N=78) for ascents. The majority of recorded strides were LSDC (N=69), with only a small sample falling into the LSLC category (N=9; Table 3.2; Binomial Exact $p < 0.001$). Diagonality (D) decreased gradually on larger substrates (N=78, $F_{(3,77)} = 5.116$, $p = 0.003$), with the exception of the largest (25 mm) substrate (Table 3.1; Fig. 3.3). Duty factor (DF) was high, averaging 78.13 (SD=4.01, N=79) in ascents (Table 3.1). This translates into long contact times of the limbs on the substrates, as in quadrupedal walking. Moreover, DF increased significantly from smaller to larger substrates (Fig. 3.3; N=78, $F_{(3,77)} = 34.34$, $p < 0.001$), controlling for the effect of D. Mean DFI was close to 100 (Table 3.1). DFI varied with substrate size and was significantly higher on 2mm and 25mm substrates (Fig. 3.3; N=78, $F_{(3,77)} = 5.28$, $p = 0.002$). Interestingly, the mean DFI values on these size categories scored >100 (Table 3.1), denoting a shift towards the hind limbs. However, the 25mm value should be treated with caution due to the low small sample.

In ascents, dimensionless velocity and dimensionless stride length showed a strong trend towards decreasing as substrate size increased (Fig. 3.4; Velocity: N=78, $F_{(3,77)} = 16.53$, $p < 0.001$; stride length: N=78, $F_{(3,77)} = 50.55$, $p < 0.001$). In contrast, there was no covariance between substrate size and dimensionless stride frequency (Fig. 3.4; N=78,

$F_{(3,77)}=1.51$, $p=0.218$). Moreover, stride length explained a large part of variation in velocity (Fig. 3.5; $N=78$, $R_{\text{part}}=0.558$, $F_{(1,77)}=224.87$, $p<0.001$), closely followed by stride frequency ($N=78$, $R_{\text{part}}=0.490$, $F_{(2,77)}=2992.82$, $p<0.001$).

Throughout our experiments, we also recorded tail use by *M. minutus*, but it was never used during ascents (0.0%, $n=78$).

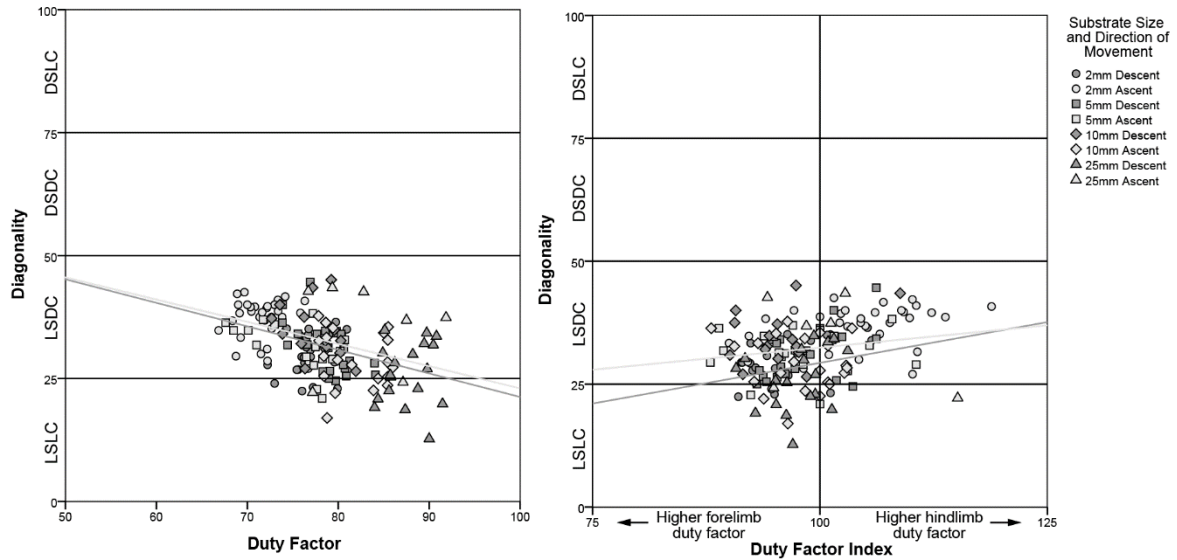


Figure 3.2. Scatterplot of D as a function of DF (left) and the DFI (right) for all recorded cycles. The horizontal lines in the first graph split the sample in quadrants of decreasing D (DSDC, DSLC, LSDC, LSLC, from top to bottom). In the second graph, horizontal lines are drawn similarly to the former and a vertical line is drawn at a DFI of 100, where the DFs of the forelimbs and the hind limbs are equal.

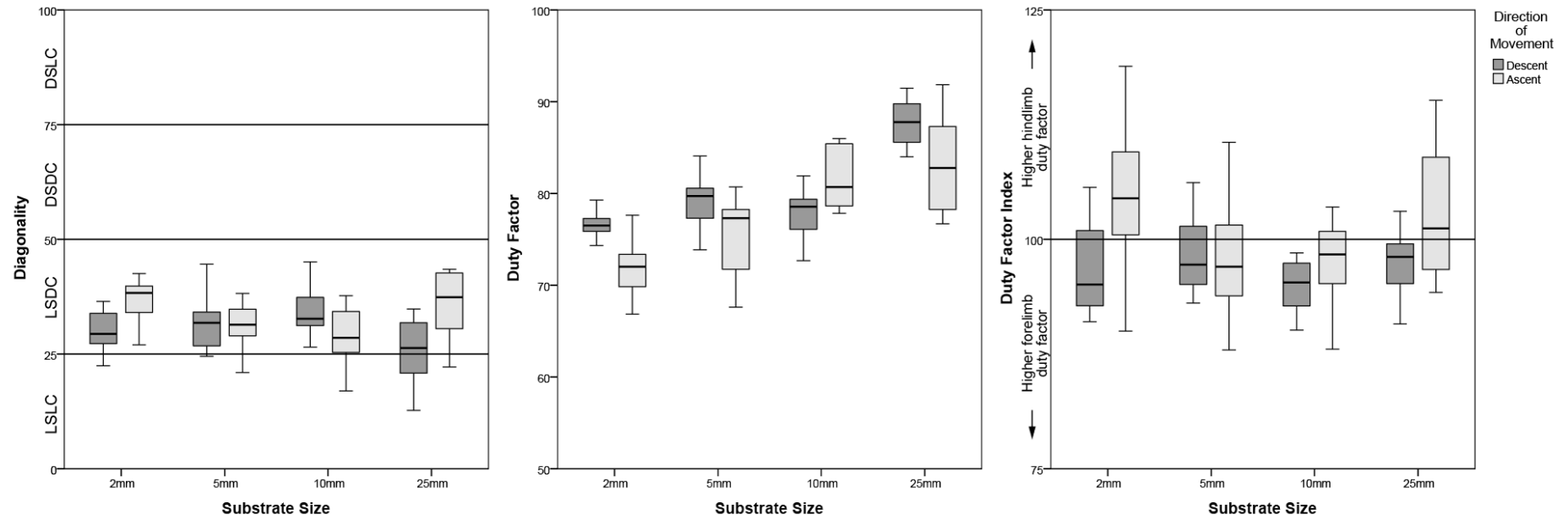


Figure 3.3. Boxplots of the distribution of gait D, DF, and DFI for the substrate sizes examined. The horizontal lines in the first graph splits the sample in quadrants of decreasing D (DSDC, DSLC, LSLC, LSLC, from top to bottom). In the last graph, the horizontal line is drawn at a DFI of 100, where the DFs of the forelimbs and the hind limbs are equal. Dark grey boxes indicate values for descents; light grey boxes values for ascents.

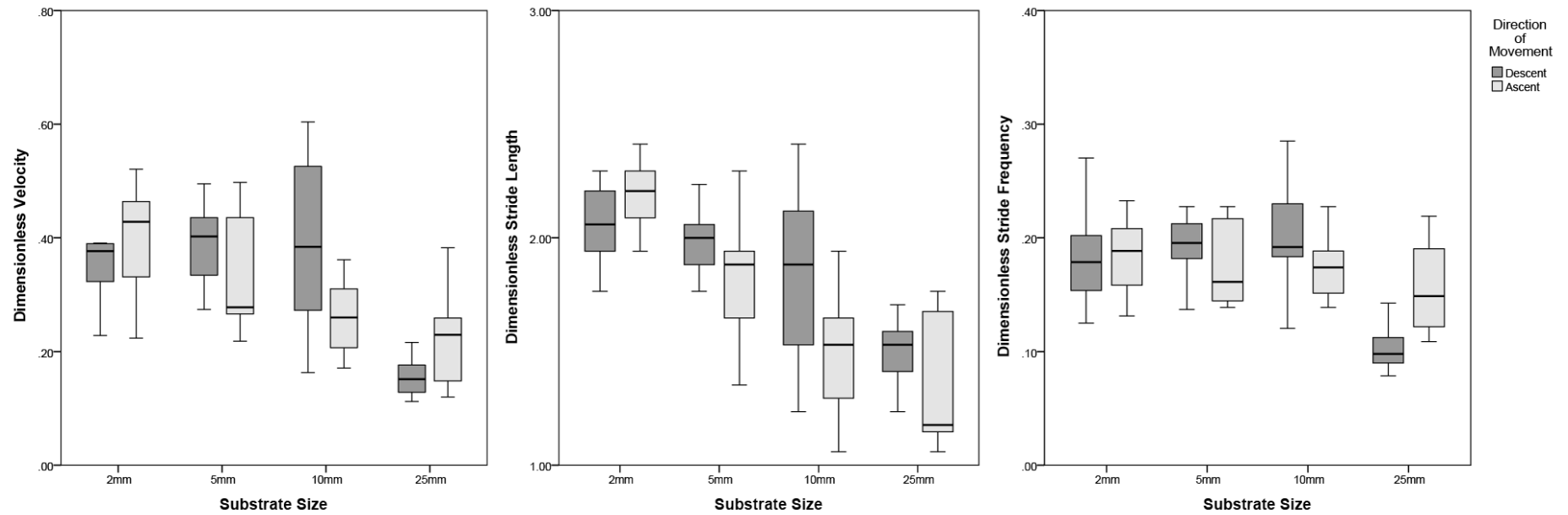


Figure 3.4. Boxplots of the distribution of dimensionless velocity, dimensionless stride length and dimensionless stride frequency for the substrate sizes examined. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the

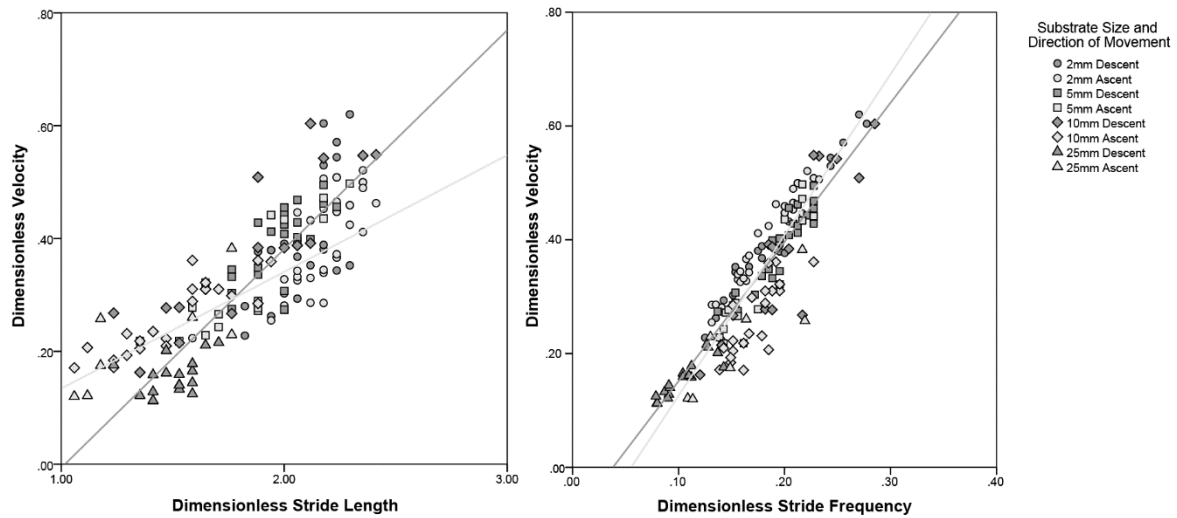


Figure 3.5. Scatterplot of dimensionless velocity as a function of dimensionless stride length (left) and as a function of dimensionless stride frequency (right).

Vertical descents

Overall mean D was 30.40 (SD=5.29, N=79) for descents. The majority of recorded strides were LSDC (N=66), with only a small sample falling into the LSLC category (N=13; Table 3.2; Binomial Exact $p < 0.001$). Regarding the effect of substrate size, gaits became more diagonal (D increased) as substrates became larger (N=79, $F_{(3,78)} = 3.45$, $p = 0.021$), with the exception of the largest substrates (25mm) (Fig. 3.3; Table 3.1). Duty factor (DF) was very high, averaging 80.22 (SD=2.52, N=78) during descents (Table 3.1) and significantly increased from smaller to larger substrates (Fig. 3.3; N=79, $F_{(3,78)} = 60.49$, $p < 0.001$), controlling for the effect of D. Mean DFI was slightly below 100. In contrast to the other gait metrics, substrate size had no overall effect on DFI (Fig. 3.3; N=79, $F_{(3,78)} = 1.27$, $p = 0.290$) in descents.

As in ascents, dimensionless velocity also showed a strong trend towards decreasing with substrate size increase (Fig. 3.4; N=79, $F_{(3,78)} = 29.05$, $p < 0.001$). The same trend was observed for dimensionless stride length and dimensionless stride frequency (Fig. 3.4; stride length: N=79, $F_{(3,78)} = 28.93$, $p < 0.001$); stride frequency: N=79, $F_{(3,78)} = 31.14$, $p < 0.001$). In terms of velocity, the stepwise regression model for velocity (Fig. 3.5) revealed that stride frequency was its primary regulator (N=79, $R_{\text{part}} = 0.521$, $F_{(1,78)} = 854.54$, $p < 0.001$), followed by stride length (N=79, $R_{\text{part}} = 0.268$, $F_{(2,78)} = 3411.31$, $p < 0.001$).

In contrast to ascending locomotion, the tail was wrapped around the substrate in all descents (100.0%, n=79).

Vertical ascents vs. vertical descents

Diagonality was similar between ascents and descents (Fig. 3.3; $N=157$, $F_{(1,156)}=1.812$, $p=0.180$), controlling for the effect of substrate diameter and DF. However, post-hoc tests revealed significantly lower mean D in ascents than descents, both on the 2mm substrates (BMD=1.39, $p<0.001$) and the 25mm substrates (BMD=1.87, $p=0.021$). Duty factor (DF) was significantly lower in ascents than descents (Fig. 3.3; $F_{(1,156)}=10.68$, $p=0.001$; controlling for the effect of substrate size and D). On the other hand, DFI was significantly higher in ascents than descents (Table 3.1; Fig. 3.3; $N=177$, $F_{(1,156)}=13.41$, $p<0.001$).

Ascents were carried out at approximately similar velocities with descents (Fig. 3.5; $N=157$, $F_{(1,156)}=2.25$, $p=0.114$; controlling for the effect of substrate size). However, post-hoc tests between categories, showed that even though velocity was similar between both directions at the smallest substrates (BMD=0.01, $p=0.690$), ascents were slightly, but significantly, slower at 5mm (BMD=0.06, $p=0.038$) and 10mm (BMD=0.12, $p=0.001$) than descents. In contrast, ascents were significantly faster than descents at 25mm (BMD=0.066, $p=0.012$), despite the sample limitations mentioned earlier.

3.4. Discussion

The results presented here show that *M. minutus*, one of the smallest rodents, are highly adept at vertical locomotion. They effectively climbed up and down, using LSDC symmetrical gaits with extended limb contact times on the different substrate sizes. Competent climbing skills are essential for these tiny rodents which are especially dependent on arboreal substrates during the reproductive season (Harris 1970, 1979b, 1979a; Nordvig et al. 2001; Bence et al. 2003; Özkan et al. 2003; Surmacki et al. 2005; Kuroe et al. 2007).

Compared to descents, ascending gaits of Eurasian harvest mice were characterized by a similar D, a lower DF, a higher DFI, and a complete lack of tail use. These findings partly support our initial expectations. In general, the relatively reduced

DF, and hence the increase of swing phases, accompanied by the disengagement of the tail, promoted faster upward progression, by reducing contact duration with the substrate, probably counteracting the opposing pull of gravity (Cartmill 1974a; Hildebrand 1995; Preuschoft et al. 1995). Furthermore, as expressed by the increased DFI, the hind limbs were primarily responsible for generating force for the upward propulsion in *M. minutus* (Preuschoft et al. 1995; Preuschoft 2002). During vertical ascents, harvest mice face the challenge of negotiating both very fine and very large substrates. Fine substrates can be effectively and securely grasped by the prehensile feet of *M. minutus* (Urbani and Youlatos 2013), allowing for better control of progression. Furthermore, on fine substrates, hind limb upward propulsion was accompanied by increased D, within the LSDC category, a pattern encountered in other mammals as well (Cartmill et al. 2007; Nyakatura et al. 2008). Diagonal couplets gaits, when combined with increased hind limb stance phases, may enhance the ability to propel the body upwards for mammals that lack powerful primate-like grasping (Lammers and Biknevicius 2004), in a manner analogous to the DSDC gaits of primates (Stevens 2006; Nyakatura et al. 2008; Nyakatura and Heymann 2010). In this way, fine branch vertical ascents appeared to be effectively negotiated by behavioural adaptations promoting dynamic stability (Lammers and Zurcher 2011). As substrates became larger, D decreased, DF increased, and DFI remained statistically constant, despite lower mean values for 5 mm and 10 mm substrates. The progressive increase of stance duration compared to swing duration (increase in DF) on larger substrates, accompanied by a shift to more LS gaits (as suggested by lower D) implies cautious locomotor displacement and behavioural adaptations to more static than dynamic stability (Schmidt and Fischer 2011). Additionally, hind limbs cannot effectively grasp the largest (25 mm) substrates. On such substrates, DF and hind limb dominance (high DFI) are increased, in comparison to intermediately sized substrates. This shift suggests an emphasis on high force generation for upward propulsion by the hind limbs, despite the lack of grasping. Moreover, during ascents, velocity was regulated mainly by stride length, followed by stride frequency, corroborating our initial hypothesis that regulation by stride length is favourable in climbing. A similar pattern is observed in most climbing primates (Hirasaki et al. 1992; Isler and Thorpe 2003; Isler 2005; Hanna 2006; Delciellos and Vieira 2009; Hanna and Schmitt 2011). Longer strides probably save more energy than more frequent strides in

ascending vertical locomotion (Hanna and Schmitt 2011), as stride length is inversely proportional to cost of transport (Kram and Taylor 1990).

On the other hand, when *M. minutus* was climbing down, they showed a similar D, but a higher DF and lower DFI compared to ascents. These findings are in accordance with our expectations. In effect, downward locomotion imposes a cranial/anterior shift of weight, which causes behavioural adaptations such as increased contact times and a higher fraction of the vertical impulse transferred to the forelimbs, enhancing their regulative and supportive role (Rollinson and Martin 1981; Lee et al. 2004; Nyakatura et al. 2008; Nyakatura and Heymann 2010; Shapiro and Young 2012). As our data on downward progression of *M. minutus* demonstrate, the potential risk of losing control because of the effect of gravity is counteracted by a higher reliance on the forelimbs, an increase in stance phase duration (i.e., substrate contact) of all limbs, and assistance in braking by tail anchoring in the posterior part of the body. Moreover, D was lowest (i.e., very lateral gaits) on the 2mm and 25mm substrates. As lateral-sequence gaits have been related to providing assistance in controlling and braking downward progression (Rollinson and Martin 1981; Nyakatura et al. 2008; Nyakatura and Heymann 2010), they may be indeed favourable for the most challenging substrates, such as the very fine (e.g., 2mm) and very large (e.g., 25mm) ones. Contrary to ascents, in descents velocity was regulated primarily by stride frequency. Despite being more energetically costly than regulation by stride length, stride frequency may confer enhanced safety and control of body progression. This is brought by a more frequent contact with the substrate, which allows for a higher resolution control of progression and the ability to halt or change direction swiftly (Kram and Taylor 1990; Hoyt et al. 2000; Isler 2005; Hanna 2006; Hanna and Schmitt 2011). Even though Eurasian harvest mice cannot invert their ankles, as squirrels can (pers. obs.), the combination of these gait parameters seems to guarantee secure and efficient downward progression, enabling the animals to exploit different arboreal layers and reach the ground.

The fact that Eurasian harvest mice used increased velocities and stride lengths, and reduced DF on the 2mm substrates suggests that they are well adapted to climbing vertically both upwards and downwards on slender twigs. This is in accordance with previous findings indicating that they show an overall preference towards fine substrates (Urbani and Youtalos 2013) and is further in line with ecological data indicating a specialization in this particular niche during arboreal activities (Harris 1970; Nordvig et

al. 2001; Bence et al. 2003; Surmacki et al. 2005; Kuroe et al. 2007). Their small size, along with their morphological and behavioural specializations, such as grasping extremities, a semi-prehensile tail, a diffuse bowing of the lumbodorsal region, diverse arboreal positional modes, and increased rates of pedal grasping (Ylönen 1990; Haffner 1996, 1998; Krattli 2001; Vázquez-Molinero et al. 2001; Urbani and Youlatos 2013) allow them to efficiently and swiftly move vertically from the ground to arboreal vegetation and back down. In this way, they can exploit challenging substrates including short trees, shrubs, vines, hedgerows, long grass, and even reed beds (Harris 1970, 1979b, 1979a; Nordvig et al. 2001; Bence et al. 2003; Özkan et al. 2003; Surmacki et al. 2005; Kuroe et al. 2007). Their proficiency in vertical climbing is integral to their ecology. Climbing is more pronounced during reproduction, when they build arboreal nests to avoid interspecific competition for nesting spaces (Ylönen 1990), as well as potential terrestrial predators (Jędrzejewska and Jędrzejewski 1990; Jędrzejewski et al. 1993). While accessing the arboreal niche, they can further acquire novel food sources, such as fruit, seeds, and invertebrates on the terminal twigs and stems increasing their fitness (Leach 1990; Nowak 1999). Moreover, secure and competent head-first downward climbing allows for constant scanning of the ground, enhancing detection of predators, competitors and potential food sources (Cartmill 1974b; Preuschoft et al. 1995; Preuschoft 2002). To do so, *M. minutus* can readily select the smallest substrates for moving downwards to the lower vegetation strata and the ground. In this way, they are able to reap the advantages of arboreality, while also efficiently utilizing the ground.

This ease of movement between terrestrial and arboreal habitats may have been an important ecological advantage in early eutherian evolution. In effect, two of the oldest eutherian mammals, *Juramaia sinensis* and *Eomaia scansoria*, were very small (15-17 g and 20-25 g, respectively) and have been functionally reconstructed as adept scansorialists (Ji et al. 2002; Luo et al. 2011). Reliable postcranial markers for arboreality, such as the phalangeal index (PI) and the proximal phalangeal index (PPI), are remarkably comparable between Eurasian harvest mice and the two fossil species [*M. minutus*: PI=131, PPI=68 (N=3, pers. obs.); *J. sinensis*: PI=121, PPI=65 (Luo et al. 2011); *E. scansoria*: PI=118, PPI=68 (Ji et al. 2002)], and all fall within the range of arboreal or generalized extant eutherians (Kirk et al. 2008). Despite the fact, that both fossils are relatively larger than *M. minutus*, their overall small size (<30 g) suggests that they would presumably be confronted with comparable challenges when facing uneven terrain and

obstacles on both arboreal and terrestrial substrates (Jenkins 1974; Gasc 2001). Therefore, these features, i.e., overall small size and comparable branch seizing ability of the extremities, would likely induce them to employ comparable locomotor and postural solutions in the negotiation of available substrates (Gasc 2001; Fischer et al. 2002; Schmidt 2005; Bonnan et al. 2016). Additionally, the consistent use of LS gaits, and the ecological advantages they confer in the use of unstable arboreal substrates, might have also been employed by both *Juramaia* and *Eomaia*. Moreover, this is the most common gait pattern among extant eutherians, metatherians, and reptiles (Hildebrand 1976; Cartmill et al. 2007; McElroy et al. 2008). It is very likely that early eutherians also exploited arboreal substrates to negotiate ecological pressures, such as predation and inter- and intraspecific competition on a seasonally or yearly basis, in a way analogous to *M. minutus* (Jędrzejewska and Jędrzejewski 1990; Ylönen 1990; Jędrzejewski et al. 1993).

Although access to the arboreal milieu by climbing is accompanied by increased energetic costs, they are not as important at this small size (Hanna and Schmitt 2011). Apart from morphological adaptations, such as functional claws and pedal grasping (Cartmill 1974a; Preuschoft et al. 1995; Preuschoft 2002), upward climbing could be facilitated by behavioural mechanisms such as higher velocity, mainly regulated by stride length, and increased reliance on the hind limbs, as in *M. minutus*. Additionally, return to the ground by downward climbing would be further promoted by employing more lateral gaits for static stability, increased contact with the substrate, and a subtler regulation of velocity by stride frequency, assuring a swift and secure displacement (Cartmill 1974b; Preuschoft et al. 1995; Preuschoft 2002). The long tail of *Juramaia* (Luo et al. 2011) may have had a similar anchoring role as in *M. minutus*. Unfortunately, the lack of hind limb or tail remains from *Eomaia* need to be treated with caution in making behavioural inferences. In both ascents and descents, relatively slender substrates could have been preferentially used to maximize the competence of vertical locomotion and grasping. This combination of fine-branch arboreal and terrestrial habitats would have very likely increased the fitness of early eutherians and eutherians allowing them to subsequently evolve into the diverse Cenozoic radiations. Extant small arboreal mammals can thus constitute good models for elucidating and understanding the functional and behavioural mechanisms that may have promoted the evolution of early mammals and we would strongly encourage further research along this avenue.

Chapter 4: Arboreal locomotion in a tiny scansorial mammal

Arboreal locomotion in Eurasian harvest mice *Micromys minutus* (Rodentia: Muridae): the gaits of small mammals

This chapter has been accepted, pending revision, as:

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4.1. Introduction

Small mammals walk and run in markedly different ways compared to larger mammals, as body size and mass impose significant constraints on locomotion (Alexander & Jayes, 1983). In this way, they share some common locomotor features that differentiate them from their larger relatives. They usually run with a crouched limb posture, while larger animals run with more extended limbs (Biewener, 1989a, 1989b, 1990) and often move in quick bursts of intermittent locomotion (McAdam & Kramer, 1998). Their limb kinematics, such as the increased contribution and combined operation of proximal limb segments, spine and pelvic movements, as well as locomotor performance are convergent to one another, though different from those of their larger relatives (Biewener, 1989b; Fischer *et al.*, 2002; Iriarte-Díaz, 2002). In an arboreal context, small mammals perceive substrates as wider, longer and flatter, where they move with continuous slow and fast quadrupedal gaits and cross gaps mainly by leaping rather than bridging (Cartmill, 1985). Moreover, the three-dimensional, discontinuous arboreal milieu, with substrates of variable size, orientation, length and robustness compels arboreal mammals to diverse morpho-behavioural solutions to avoid losing balance and falling (Cartmill, 1974b). Larger arboreal mammals are more prone to branch fracture, and face difficulties balancing on top of supports (Cartmill, 1985). Nevertheless, for very small mammals, arboreal and terrestrial locomotion may be a false dichotomy, as they face similar challenges when coping with obstacles on both terrestrial and arboreal substrates (Jenkins, 1974). Although studies of locomotion of small

mammals have increased in recent years (e.g. Fischer *et al.*, 2002; Lammers, 2007; Schmidt, 2008; Hanna & Schmitt, 2011a; Schmidt & Fischer, 2011; Karantanis *et al.*, 2015; Shapiro *et al.*, 2016), data at the very low end of mammalian body mass spectrum are still lacking.

Despite their locomotor similarities and convergences, small mammals are characterized by differing gait patterns and metrics, and respond to changes in substrate properties in different ways. Thus, scansorial/arboreal rodents and some scansorial/arboreal metatherians employ lateral-sequence (LS) gaits, where the footfall of a forelimb takes place at a time interval less than half of the gait cycle, following the footfall of the ipsilateral hind limb (Cartmill *et al.*, 2002; Schmidt & Fischer, 2010; Shapiro & Young, 2010). Lateral-sequence (LS) gaits have been frequently related to arboreal static stability, as they favour the placement of the centre of mass within the support polygon of the limbs during slower locomotion (Lammers & Zurcher, 2011a). On the other hand, many arboreal metatherians and primates display primarily diagonal-sequence (DS) gaits, in which the footfall of a forelimb takes place at a time interval more than half of the gait cycle, following the footfall of the ipsilateral hind limb (Pridmore, 1994; Lemelin, Schmitt & Cartmill, 2003; Karantanis *et al.*, 2015; Shapiro *et al.*, 2016). Diagonal-sequence gaits have been related to dynamic stability, allowing for a more refined control and transfer of moments and torques imposed on the body axes (Lammers & Zurcher, 2011b), and appears to facilitate the exploration of unstable substrates with the forelimbs while the protracted grasping hind limb allows for safe anchoring of the body (Cartmill *et al.*, 2007).

Apart from gaits, velocity and its regulation through stride length and stride frequency also differs among different small mammals. High arboreal velocities contribute to dynamic stability (Schmidt & Fischer, 2010), and have been linked to efficient arboreal capacities (Delciellos & Vieira, 2006; Flaherty, Ben-David & Smith, 2010; Camargo *et al.*, 2016). Velocity can increase either by increasing primarily stride frequency and, at a lesser rate, stride length (Nyakatura *et al.*, 2008; Karantanis *et al.*, 2015, 2017a), by increasing stride frequency and decreasing stride length (Delciellos & Vieira, 2006; Camargo *et al.*, 2016), or by reducing stride frequency and increasing stride length (Larson *et al.*, 2000, 2001; Delciellos & Vieira, 2006). The increase of velocity through an increase in stride length is possibly safer in an arboreal setting, allowing for a longer reach of the forelimbs and reducing involuntary branch sway (Demes *et al.*, 1994),

and is often encountered in medium-sized and larger arboreal mammals (Larson *et al.*, 2000, 2001; Delciellos & Vieira, 2006). On the other hand, increasing velocity by stride frequency, although more energetically costly (Reilly *et al.*, 2007), probably reduces body oscillations and facilitates continuous progression, making it a better option for smaller mammals, subject to negligible branch sway (Delciellos & Vieira, 2006).

The responses of small mammals to substrate properties also seem to vary significantly. Some small mammals negotiate narrower substrates by increasing diagonality (i.e. the relative time interval between ipsilateral forelimb and hind limb footfalls) (Pridmore, 1994; Shapiro & Young, 2010; Karantanis *et al.*, 2015; Shapiro *et al.*, 2016), whereas others may shift to more LS gaits (Karananis *et al.*, 2017a). Moreover, some mammals with prehensile extremities, appear to increase the fractions of stance phases in stride cycles (i.e. duty factor) and decrease velocity on narrower substrates (Delciellos & Vieira, 2006; Shapiro & Young, 2010; Karantanis *et al.*, 2015), promoting a stable and secure locomotion by decreasing torques produced at limb touchdowns (Lammers & Zurcher, 2011b). Some neotropical rodents seem to have opted for higher speeds on smaller substrates (Camargo *et al.*, 2016), possibly compensating for the lack of grasping abilities to maintain dynamic stability (Schmidt & Fischer, 2010; Shapiro *et al.*, 2016). Finally, velocity in others remains unaffected by substrate size (Shapiro *et al.*, 2016).

Substrate inclination also seems to influence the arboreal locomotion of small mammals. During ascents, both diagonality and duty factor index (i.e. ratio of the duty factor of the hind limbs to that of forelimbs) increase, denoting more diagonal-sequence gaits and a higher reliance on the hind limbs compared to the forelimbs (Shapiro & Young, 2010; Schmidt & Fischer, 2011; Karantanis *et al.*, 2015; Shapiro *et al.*, 2016). This reduces yawing and lateral torques and enhances the ability to propel the body upwards (Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010). On the other hand, available data on velocity show contrasting behaviours, with European red squirrels increasing speed during ascents, to obtain dynamic stability, and rats decreasing speed, to maintain static stability (Schmidt & Fischer, 2011). During descents, diagonality and duty factor index are lower compared to ascents, suggesting more lateral gaits and forelimb control, allowing the forelimbs to provide braking through a “stop-jolt” mechanism and to enhance their regulatory and supportive role (Rollinson & Martin, 1981; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Karantanis *et al.*, 2015).

Despite the differences in locomotor responses of small mammals to the properties of arboreal substrates, all these behavioural mechanisms appear to enhance stability and safety that apparently contribute to the effective and successful exploitation of the arboreal milieu. But how are these achieved at a very small size? In this context, we studied the arboreal locomotion of one of the smallest scansorial rodents, the Eurasian harvest mice *Micromys minutus* (Pallas, 1771). Eurasian harvest mice weigh about 10g (Trout, 1978a, 1978b) and use both terrestrial and arboreal substrates, but increase arboreal activities during the breeding season (Ylönen, 1990). *Micromys minutus* habitually use low vegetation, including long grass or even reed beds, short trees, shrubs, vines and hedgerows (Harris, 1970; Nordvig, Reddersen & Jensen, 2001; Bence, Stander & Griffiths, 2003; Özkan, Yigit & Colak, 2003; Surmacki, Gołdyn & Tryjanowski, 2005; Kuroe *et al.*, 2007). Arboreality enables safe nest construction, avoidance of interspecific competition (Ylönen, 1990) and of potential predators (Jędrzejewska & Jędrzejewski, 1990; Jędrzejewski *et al.*, 1993), and promotes foraging for seeds, fruits, and invertebrates on terminal twigs and stems (Leach, 1990). Additionally, *M. minutus* exhibit a diverse arboreal locomotor and postural behaviour (Urbani & Youlatos, 2013), facilitated by the semi-prehensile tail, which aids in descents, enables suspensory postures, and assists in bridging gaps, and the grasping feet, which allow firm hold of the substrates for suspensory behaviour, habitual vertical climbing, and enable walking and clambering over twigs (Leach, 1990; Haffner, 1996, 1998; Ishiwaka & Mori, 1999; Krattli, 2001; Zefferer, 2002).

Considering the arboreal capacities of Eurasian harvest mice, the current study aims to elucidate behavioural adaptations related to arboreal quadrupedal locomotion at a very small size scale in mammals. We expect Eurasian harvest mice to display behavioural adaptations related to increased stability and successful utilization of the smallest substrates, analogous to other specialized arboreal mammals. On the narrowest substrates, we anticipate *M. minutus* to use more DS gaits, shorter aerial phases, and low velocity. More LS gaits, comparatively increased forelimb duty factor, and lower velocity were also expected during descending locomotion. On the other hand, during ascents, we expect more DS, comparatively higher hind limb duty factor and higher velocity. Finally, similarly to other small mammals, we also expected velocity to be regulated primarily by stride frequency, rather than stride length.



Figure 4.1. Still from the videos used for data collection. The support diameter is 2mm, and the vertical markings every 1 cm are clearly visible.

4.2. Materials and Methods

Specimens

For the purposes of the current study, we tested 6 male adult *Micromys minutus* (Muridae, Rodentia) (Fig. 4.1). All specimens were captive-born and permanently housed in the collections of the Nowe Zoo, Poznań, Poland, in large naturalistic enclosures. Their housing contained a large variety of available substrates of diverse sizes and orientations, enabling the harvest mice to move freely in an enriched environment. In their regular enclosures, the Eurasian harvest mice made extensive use of any available arboreal substrate inside the enclosures and were naturally inclined to use the provided substrates during the experimental procedures. Before the experiments, the Eurasian harvest mice were transported to a laboratory in the Faculty of Biology (Adam Mickiewicz University, Poznań, Poland), where they were housed all together in a glass terrarium filled with natural materials for nesting and climbing, in an attempt to reduce stress. Since the animals are nocturnal, lighting was configured to operate in a reversed day-night regime. All specimens were fully habituated to human presence and did not display any stereotypical or stressful behaviour. Mean total head-body length of the subjects was 5.9 cm (SD=0.12, range=5.7-6.1 cm, $n=6$), mean body mass was 6.8 g (SD=1.02, range=5.8-7.9

g, $n=6$), and mean effective hind limb length, i.e. the length of the leg as a strut (Pontzer, 2005, 2007a, 2007b), was 1.73 cm (SD=0.02, range=1.68-1.74 cm, $n=6$). All measurements were taken directly on the subject animals.

Experimental setup

A single, specially configured glass terrarium (L: 90 cm x H: 40 cm x W: 40 cm) was used for filming. It was surrounded by transparent glass windows and it was topped by a wooden frame, with a lid door and wire mesh for ventilation. Within the terrarium, we established a wooden frame to support the poles. The poles were 80 cm long, cylindrical, semi-hardwood rods. They were marked with vertical blue lines every 1 cm for a reliable estimation of absolute lengths. During filming, the visible rod length was approximately 30 cm. Diameter and direction of movement accounted for the classification of different substrate categories. Thus, four diameters (2 mm, 5 mm, 10 mm, and 25 mm), and three movement directions (descent at 45°, horizontal, and ascent at 45°) defined a total of 12 distinct substrate categories.

Data collection was completed in six recording sessions, during June 2013. During each recording session, the animals were transferred from their enclosure to the filming terrarium. Prior to filming, each individual was allowed free movement within the filming terrarium. Minimal or no stimulation was required for the subjects to walk on the poles. After each filming session, the animal was transported to another accommodation terrarium, to ensure that no individual was tested twice during a session.

For video recording, we used a Sanyo digital camcorder (VPC-HD 2000, Sanyo, Osaka, Japan), filming at 240 fps, which was positioned at 1 m from the filming terrarium to reduce image distortion. For our analyses, we considered only complete gaits, as indicative of natural, unbiased behaviour, initiating with the touchdown of the left hind limb and ending at the subsequent touchdown of the same limb (Hildebrand, 1967, 1976, 1985). Cycles involving beginnings or endings of locomotor bouts (action of significant acceleration or deceleration) or loss of balance (irregular footfalls) were discarded, due to not being able to measure metrics reliably. The present research followed the guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS, 2012) and complied with relevant regulations and legislations of the Nowe Zoo and the Adam Mickiewicz University in Poznan and the relevant legislation of the Aristotle University of Thessaloniki. Handling, housing of animals and behavioural tests were done

with the permission of the Local Ethical Commission for the Animal Experiments in Poznan.

Gait analyses

Video analysis and data collection, distance and time calculations were made by importing videos and calibrating time and distance measurements using Tracker 4.87 (Brown, 2009). Microsoft Excel 2010 (Redmond, WA, USA), and SPSS 20 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

Asymmetrical cycles were diagnosed by whether the time from the first fore- or hind footfall to the next fore- or hind footfall was greater than 60% or less than 40% of the total stride period ($\pm 10\%$ deviation from symmetry) (Lemelin and Cartmill, 2010). For our analyses, we considered the following gait parameters:

- (i) Diagonality (D) (Cartmill *et al.*, 2002), the percentage of the stride cycle by which the footfall of a forelimb follows behind the ipsilateral hind limb, and applies only at symmetrical gaits. Although it was measured as a scale variable, it was also divided into five ordinal classes (*sensu* Cartmill *et al.*, 2002): (a) Lateral Sequence Lateral Couplets ($0 \leq \text{LSLC} < 25$), (b) Lateral Sequence Diagonal Couplets ($25 \leq \text{LSDC} < 50$), (c) Trot ($= 50$), (d) Diagonal Sequence Diagonal Couplets ($50 < \text{DSDC} \leq 75$), (e) Diagonal Sequence Lateral Couplets ($75 < \text{DSLCL} \leq 100$);
- (ii) Duty Factor (DF), the mean of duty factors of all limbs, defined as the percentage of a cycle during which a limb is in contact with the substrate;
- (iii) Duty Factor Index (DFI), the ratio of forelimb duty factor (DF_f) and hind limb duty factor (DF_h), calculated as $100 \cdot \text{DF}_h / \text{DF}_f$ (Cartmill *et al.*, 2002). Values > 100 indicate longer hind limb than forelimb relative stance durations, whereas values < 100 specify shorter hind limb than forelimb relative stance durations.
- (iv) Stride Duration (t), total duration of a single stride in seconds, measured from the frame where a stride cycle began until the frame the same stride cycle ended;
- (v) Stride Length (l), the corresponding distance covered during a single stride cycle, in meters;
- (vi) Velocity (v), the speed in which the subjects moved, calculated by dividing stride length with stride duration, and measured in meters/second (m/s);
- (vii) Stride Frequency (f), the number of strides per second;

As these parameters are size-dependent, we used the effective hind limb length to calculate the dimensionless measures of stride duration, stride length, velocity and stride frequency (Hof, 1996). These calibrated absolute measurements are useful for estimating competence during locomotion (Alexander, 1977; Alexander & Jayes, 1983; Hof, 1996):

$$\text{Dimensionless Stride Duration (t}_D) = \frac{t}{\sqrt{\frac{l_0}{g}}}$$

$$\text{Dimensionless Stride Length (l}_D) = \frac{l}{l_0}$$

$$\text{Dimensionless Velocity (v}_D) = \frac{v}{\sqrt{g \times l_0}}$$

$$\text{Dimensionless Stride Frequency (f}_D) = \frac{f}{\sqrt{\frac{g}{l_0}}}$$

where l_0 is the effective hind limb length of each animal and g is the acceleration of gravity ($g=9.81 \text{ m/s}^2$).

Where overall values are reported, they represent the sum of means of each recorded category, divided by the number of categories with observations. We tested for statistically significant discrepancies in the utilization of D and LS gaits using binomial testing, but also incorporated the trot category into the analysis by using a chi-square test (χ^2). All of these analyses were carried out using two-tailed Monte Carlo procedures for enhanced p estimation accuracy (Kalos & Whitlock, 2009).

Table 4.1. Summary of means and standard deviations (in brackets) of the gait statistics for *Micromys minutus*. N indicates the number of analyzed cycles for each substrate size and direction of movement category.

Substrate Size	Direction of Movement	N	Diagonality	Duty Factor	Hindlimb Duty Factor	Forelimb Duty Factor	Duty Factor Index	Velocity (m s ⁻¹)	Stride Length (m)	Stride Frequency (s ⁻¹)	Stride Duration (s)	Dimensionless Velocity	Dimensionless Stride Length	Dimensionless Stride Frequency	Dimensionless Stride Duration
2mm	Descent	14	40.69 (5.73)	65.06 (3.31)	63.77 (4.24)	66.35 (3.88)	96.31 (7.01)	0.294 (0.067)	0.042 (0.004)	7.014 (1.212)	0.147 (0.026)	0.712 (0.164)	2.396 (0.214)	0.295 (0.051)	3.487 (0.622)
	Horizontal	22	23.88 (5.29)	79.61 (4.25)	76.88 (5.29)	82.34 (4.40)	93.46 (5.91)	0.13 (0.046)	0.03 (0.006)	4.309 (1.153)	0.248 (0.065)	0.318 (0.114)	1.747 (0.338)	0.18 (0.047)	5.942 (1.560)
	Ascent	13	31.63 (6.68)	73.58 (8.79)	72.67 (8.14)	74.48 (9.99)	98.01 (6.47)	0.155 (0.070)	0.033 (0.006)	4.573 (1.478)	0.242 (0.079)	0.377 (0.171)	1.899 (0.361)	0.192 (0.062)	5.765 (1.877)
5mm	Descent	15	44.2 (10.75)	59.64 (3.31)	58.89 (3.52)	60.38 (4.87)	97.96 (7.75)	0.431 (0.344)	0.048 (0.004)	8.853 (5.911)	0.147 (0.092)	1.047 (0.833)	2.758 (0.249)	0.372 (0.252)	3.51 (2.186)
	Horizontal	18	37.14 (4.79)	62.86 (2.79)	59.93 (3.23)	65.79 (2.94)	91.13 (3.85)	0.309 (0.056)	0.041 (0.003)	7.571 (1.172)	0.135 (0.023)	0.755 (0.139)	2.39 (0.211)	0.315 (0.049)	3.251 (0.558)
	Ascent	9	45.03 (2.89)	58.66 (1.66)	58.98 (2.72)	58.34 (2.29)	101.25 (6.41)	0.405 (0.094)	0.051 (0.008)	7.904 (1.111)	0.129 (0.021)	0.982 (0.227)	2.934 (0.434)	0.332 (0.047)	3.072 (0.511)
10mm	Descent	11	42.83 (5.40)	59.15 (2.66)	58.87 (2.45)	59.44 (3.20)	99.15 (3.44)	0.313 (0.110)	0.049 (0.004)	6.382 (2.095)	0.185 (0.100)	0.757 (0.267)	2.8 (0.219)	0.269 (0.088)	4.4 (2.373)
	Horizontal	17	34.72 (5.35)	65.95 (4.21)	63.29 (5.46)	68.62 (5.42)	92.66 (10.00)	0.268 (0.080)	0.038 (0.003)	6.954 (1.742)	0.153 (0.040)	0.652 (0.193)	2.212 (0.197)	0.292 (0.073)	3.642 (0.947)
	Ascent	16	46.79 (3.73)	59.33 (2.19)	59.41 (2.83)	59.26 (1.89)	100.24 (3.23)	0.377 (0.150)	0.055 (0.005)	6.858 (2.624)	0.193 (0.145)	0.913 (0.362)	3.176 (0.288)	0.288 (0.111)	4.611 (3.525)
25mm	Descent	10	42.55 (6.98)	59.07 (1.47)	57.92 (0.93)	60.22 (2.48)	96.3 (3.58)	0.421 (0.145)	0.048 (0.007)	8.684 (2.609)	0.145 (0.120)	1.023 (0.353)	2.791 (0.388)	0.364 (0.109)	3.465 (2.850)
	Horizontal	9	41.48 (5.98)	64.48 (4.40)	62.42 (5.57)	66.54 (6.08)	94.43 (11.44)	0.268 (0.162)	0.041 (0.007)	6.21 (2.975)	0.198 (0.088)	0.65 (0.393)	2.352 (0.398)	0.261 (0.126)	4.702 (2.099)
	Ascent	13	51.43 (3.99)	60.03 (1.84)	60.31 (2.93)	59.76 (1.29)	100.92 (4.43)	0.4 (0.174)	0.055 (0.006)	7.373 (3.245)	0.187 (0.136)	0.972 (0.423)	3.192 (0.355)	0.31 (0.136)	4.459 (3.225)
Total		164	40.20 (5.63)	63.95 (3.41)	62.78 (3.94)	65.13 (4.06)	96.82 (6.13)	0.314 (0.125)	0.044 (0.005)	6.890 (2.277)	0.176 (0.078)	0.763 (0.303)	2.554 (0.304)	0.289 (0.096)	4.192 (1.861)

Analysis of covariance (ANCOVA) was selected to explore variable relationships while controlling for other possible covariants, when both scale and ordinal variables were involved. When comparisons between categories were necessary, a Bonferroni Mean Difference (BMD) was calculated using ANCOVA estimates. Stepwise regression models were constructed to examine the impact of both stride frequency and stride length on velocity, using their dimensionless counterparts. The impact of each parameter was calculated with the R^2 of partial correlations, i.e. the correlation between a dependent variable and its covariate, after the impact of other covariates is removed (Harrell, 2001).

Table 4.2. Summarized statistics of gait diagonality classifications for *Micromys minutus*, reporting frequencies and per row percentages.

Substrate Size	Direction of Movement	Classification of Strides				
		LSLC	LSDC	Trot	DSDC	DSLCL
2mm	Descent	0 (0.0%)	12 (85.7%)	1 (7.1%)	1 (7.1%)	0 (0.0%)
	Horizontal	16 (72.7%)	6 (27.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Ascent	1 (7.7%)	12 (92.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
5mm	Descent	1 (6.7%)	9 (60.0%)	1 (6.7%)	4 (26.7%)	0 (0.0%)
	Horizontal	0 (0.0%)	18 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Ascent	0 (0.0%)	9 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
10mm	Descent	0 (0.0%)	10 (90.9%)	0 (0.0%)	1 (9.1%)	0 (0.0%)
	Horizontal	1 (5.9%)	16 (94.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Ascent	0 (0.0%)	9 (69.2%)	1 (7.7%)	3 (23.1%)	0 (0.0%)
25mm	Descent	0 (0.0%)	8 (80%)	1 (10%)	1 (10%)	0 (0.0%)
	Horizontal	0 (0.0%)	8 (88.9%)	1 (11.1%)	0 (0.0%)	0 (0.0%)
	Ascent	0 (0.0%)	3 (23.1%)	3 (23.1%)	7 (14.0%)	0 (0.0%)
All sizes	Descent	1 (2.0%)	39 (78.0%)	3 (6.0%)	7 (14.0%)	0 (0.0%)
	Horizontal	17 (25.8%)	48 (72.7%)	1 (1.5%)	0 (0.0%)	0 (0.0%)
	Ascent	1 (2.1%)	33 (68.8%)	4 (8.3%)	10 (20.8%)	0 (0.0%)
Total		19 (11.6%)	120 (73.2%)	8 (4.9%)	17 (10.4%)	0 (0.0%)

4.3. Results

We recorded a total of 164 valid, walking stride cycles during the experiments. Overall, the walks of harvest mice were LS gaits (Table 4.1), with moderate velocities. No asymmetrical gaits were observed in any substrate category or direction of movement. We found no statistical differences between individuals in any of the metrics ($p>0.05$), hence all data were lumped together for subsequent analyses.

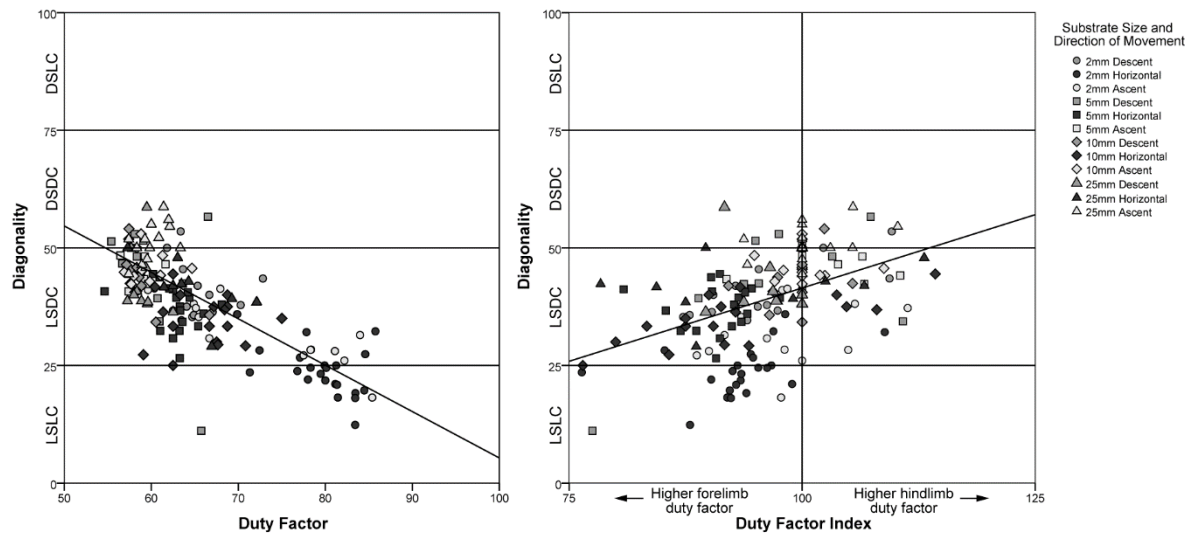


Figure 4.2. Scatterplot of D as a function of DF and DFI for all recorded cycles. The horizontal lines in both graphs split the sample in quadrants of decreasing D (DSDC, DSLC, LSDC, LSLC, from top to bottom). In the second graph, a vertical line is drawn at a duty factor index of 100, where the duty factors of the forelimbs and the hind limbs are equal. The regression lines are significant at $p < 0.05$, with functions $D = 99.43 - 0.93 \cdot DF$ (left graph) and $D = -22.75 + 0.64 \cdot DFI$ (right graph).

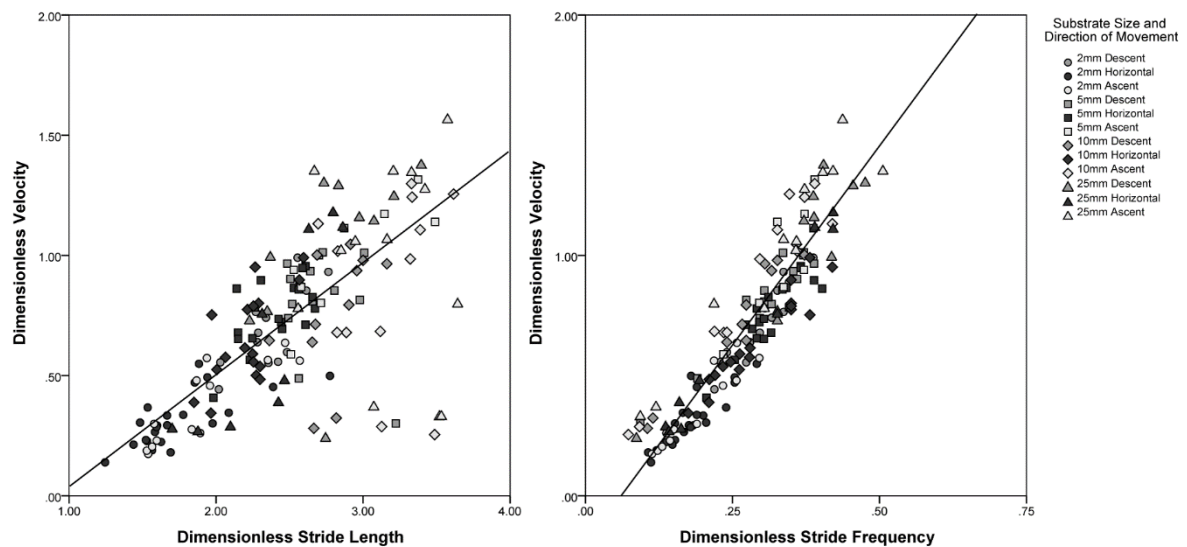


Figure 4.3. Scatterplot of dimensionless velocity (V) first as a function of dimensionless stride length (SL) and then as a function of dimensionless stride frequency (SF). The regression lines are significant at $p < 0.05$, with functions $V = 0.43 + 0.47 \cdot SL$ (left graph) and $V = -0.2 + 3.29 \cdot SF$ (right graph).

Gait metrics and their interactions

The mean diagonality (D) of *M. minutus* strides was 38.90 (Table 4.1, Fig. 2). LS gaits dominated (LSDC and LSLC, combined $n=139$), with few DSDC gaits ($n=17$), and even fewer trots ($n=8$) (lateral vs. diagonal: binomial test, $p<0.001$; lateral vs. trot vs. diagonal, $\chi^2=204.634$, $p<0.001$; Table 2).

Duty factor (DF) ranged from 54.63 to 85.75, with individual values and category means consistently higher than 50.00 (Table 4.1, Fig. 4.2), indicating that Eurasian harvest mice only walked. Furthermore, we found a significant negative effect of DF on D ($N=156$, $F_{(120,163)}=2.518$, $p=0.001$, controlling for substrate size, direction of movement, and velocity). Consequently, all subsequent tests, regarding either D or DF, were also controlled for the other parameter.

The duty factor index (DFI) averaged 96.30 (Table 4.1, Fig. 4.2), indicating a generally lower DF of the hind limbs compared to that of forelimbs. Diagonality and the DFI had a strong positive relation, controlling for the effect of substrate size, and direction of movement ($N=164$, $F_{(117,163)}=2.728$, $p<0.001$).

The construction of a stepwise regression model on the effects of stride frequency and stride length on velocity (Fig. 4.3) showed that stride frequency was the main factor for velocity regulation ($N=164$, $R_{\text{part}}=0.772$, $F_{(1,162)}=1833.757$, $p<0.001$). Stride length also played a significant role, but explained a much smaller part of variation in velocity ($N=164$, $R_{\text{part}}=0.260$, $F_{(1,161)}=484.695$, $p<0.001$).

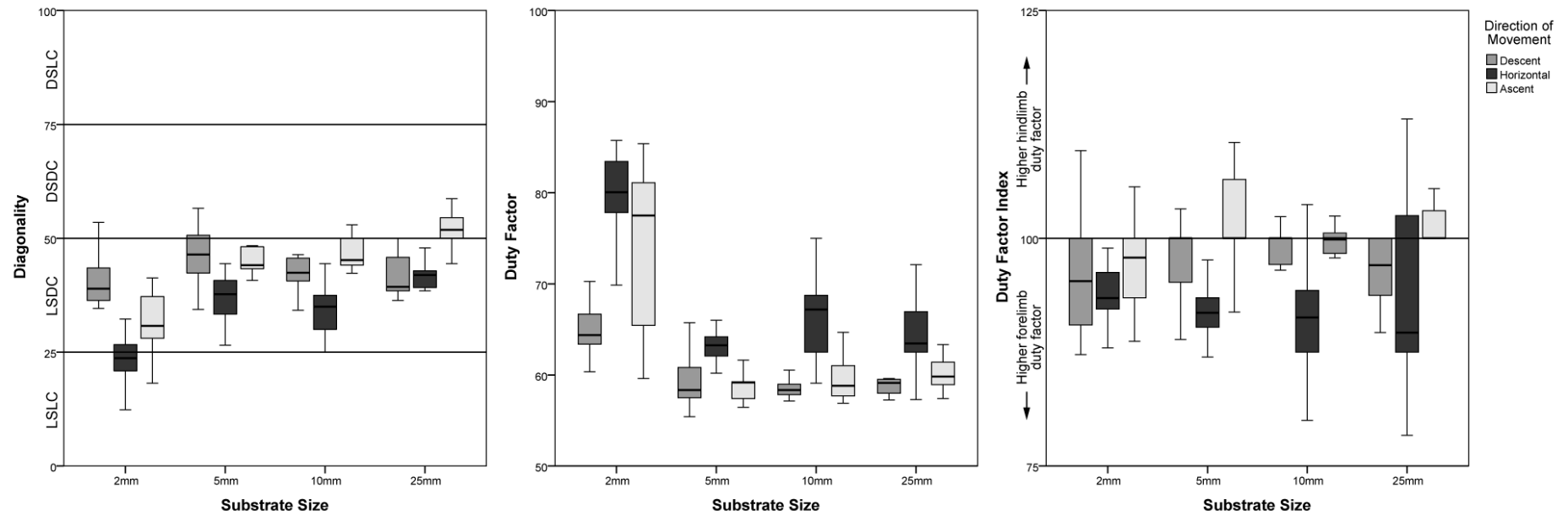


Figure 4.4. Boxplots of the distribution of gaits' D, DF and DFI in the substrate size categories examined, split into three graphs for each direction of movement. The horizontal lines in the first graph split the sample in quadrants of decreasing D (DSDC, DSLC, LSLC, LSLC, from top to bottom). In the last graph, the horizontal line is drawn at a duty factor index of 100, where the duty factors of the forelimbs and the hind limbs are equal.

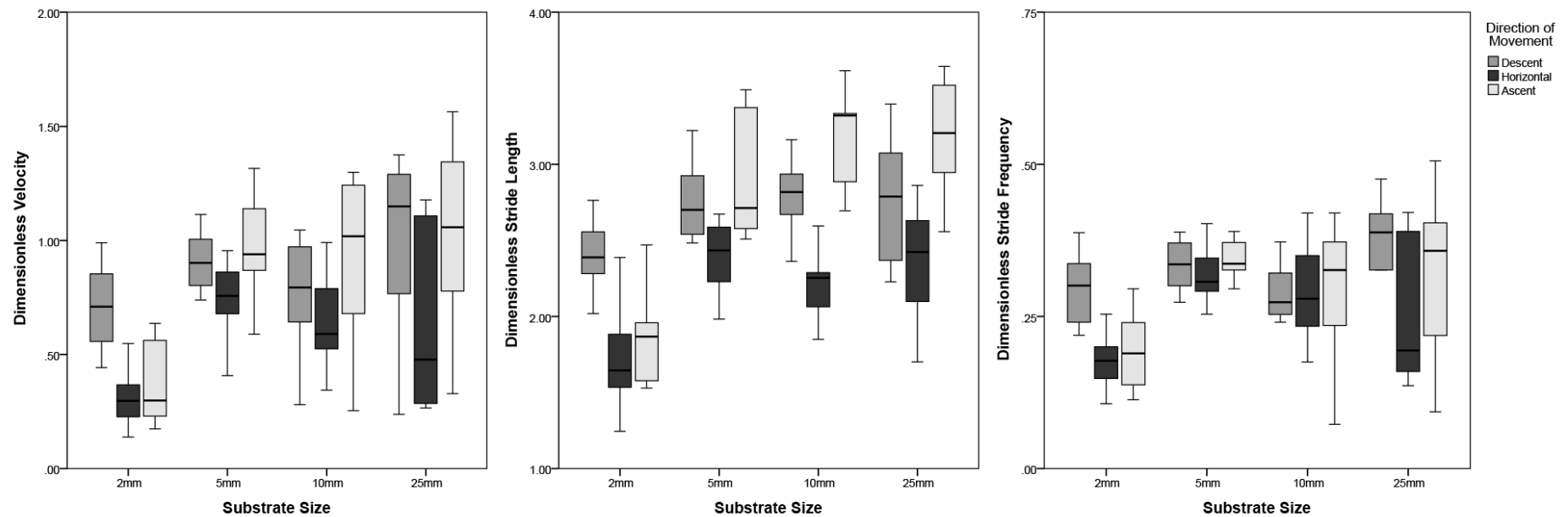


Figure 4.5. Boxplots of the distribution of dimensionless velocity, dimensionless stride length and dimensionless stride frequency in the substrate size categories examined, split into three graphs for each direction of movement. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the top and bottom whiskers represent the maximum and minimum values obtained.

Effects of substrate diameter

Overall, there was a significant increase of D with substrate size increase (Fig. 4.4; $N=164$, $F_{(3,163)}=3.719$, $p=0.013$; controlling for DF and direction of movement). On the contrary, DF tended to decrease on larger substrates (Fig. 4.4; $N=164$, $F_{(3,163)}=36.658$, $p<0.001$; controlling for the direction of movement). Essentially, even though the subjects used the smallest substrates (2mm) without loss of balance, they were more cautious, with an increased relative stance phase of all limbs. DFI was not affected by substrate size (Fig. 4.4; $N=164$, $F_{(3,163)}=2.839$, $p=0.094$, controlling for direction of movement and D).

Substrate size had a significant effect on velocity (Fig. 4.5; $N=164$, $F_{(3,163)}=15.391$, $p<0.001$; controlling for direction of movement). Furthermore, stride frequency (controlling for the effect of stride length, $N=164$, $F_{(3,163)}=3.083$, $p=0.029$) and stride length (controlling for the effect of stride frequency, $N=164$, $F_{(3,163)}=23.624$, $p<0.001$) also increased from smaller to larger substrates (Fig. 4.5). However, the velocity and stride frequency increases from 5mm to 10mm substrates were small to negligible (Fig. 4.5).

Effect of direction of movement

The direction of movement had a significant impact on D (Fig. 4.4; $N=164$, $F_{(2,163)}=13.705$, $p<0.001$; controlling for substrate size and DF). Diagonality on horizontal supports was significantly lower than on both descents (BMD=3.853, $p=0.008$) and ascents (BMD=6.154, $p<0.001$). However, ascents and descents did not differ significantly (BMD=2.301, $p=0.173$). The direction of movement also had a significant influence on DF (Fig. 4.4; $N=164$, $F_{(2,163)}=6.093$, $p=0.003$; controlling for substrate size and velocity), with overall shorter relative contact periods of the limbs on descents than in both horizontal locomotion (BMD=2.959, $p=0.008$) and ascents (BMD=2.653, $p=0.012$). Nevertheless, on both the 5 mm and 10 mm substrates the values of DF in all directions of movement were similar, and were most dissimilar on 2 mm and 25 mm substrates. Direction of movement had an impact on DFI as well (Fig. 4; $N=164$, $F_{(2,163)}=4.794$, $p=0.010$, controlling for substrate diameter and D), with ascents scoring overall higher DFIs than horizontal locomotion (BMD=4.074, $p=0.008$). However, there were no significant differences between ascents and descents (BMD=2.444, $p=0.150$), or descents and horizontal locomotion (BMD=1.663, $p=0.649$).

Velocity (Fig. 4.5; $N=164$, $F_{(2,163)}=8.791$, $p<0.001$) was lower in horizontal locomotion than in descents ($BMD=0.027$, $p<0.001$) and ascents ($BMD=0.017$, $p=0.045$). No statistically significant difference was recorded between ascents and descents ($BMD=0.011$, $p=0.406$). Stride length (Fig. 4.5; $N=164$, $F_{(2,163)}=27.110$, $p<0.001$) during horizontal locomotion was lower than descents ($BMD=0.568$, $p<0.001$) and ascents ($BMD=0.582$, $p<0.001$), but was very similar between ascents and descents ($BMD=0.164$, $p=1.000$). Stride frequency (Fig. 4.5; $N=164$, $F_{(2,163)}=5.566$, $p=0.005$) was significantly higher in descents compared to horizontal locomotion ($BMD=0.063$, $p=0.014$), but only marginally, and not significantly, compared to ascents ($BMD=0.053$, $p=0.079$), and similar between ascents and horizontal locomotion ($BMD=0.010$, $p=1.000$).

4.4. Discussion

The present findings show that *Micromys minutus* are capable of using a wide range of substrate sizes and inclinations, employing predominantly LS walking gaits (high duty factor, low velocity, and diagonality < 50). On smaller substrates, gaits became less diagonal and more cautious (higher DF, lower velocity). Diagonality (D) and duty factor index (DFI) were significantly higher in non-horizontal (ascent, descent) locomotion compared to horizontal. Stride frequency was the main regulator of velocity, with stride length playing a secondary, but significant, role. Although, these behavioural responses conform to the arboreal capacities of Eurasian harvest mice (Urbani & Youlatos, 2013), our initial predictions were only partly supported.

The Eurasian harvest mice gradually reduce D and increase DF on narrow substrates (i.e. 2mm and 5mm in our study), with the most extreme values on the narrowest substrates (2mm). Essentially, this combination leads to primarily slow lateral-sequence, diagonal-couplets (LSDC) walks, with extensive relative stance phases of the limbs. Additionally, the inverse correlation of D with DF at LS gaits helps keep the centre of mass within the support polygons (Cartmill *et al.*, 2002), resulting in a continuous, non-disrupted locomotion along arboreal substrates. Even though DS gaits coupled with hind foot grasping have been functionally linked to the safe negotiation of unstable substrates, as they allow for testing novel substrates (Cartmill *et al.*, 2007), *M. minutus* used exclusively LS gaits on all types of substrates. In fact, LS gaits are also common in other scansorial mammals, such as rats, *Rattus norvegicus* (Schmidt & Fischer,

2011), woodmice, *Apodemus agrarius*, *Apodemus flavicollis*, voles, *Myodes glareolus* (Karantanis *et al.*, 2017a), and the metatherians *Monodelphis domestica* (Lemelin *et al.*, 2003; Lammers & Biknevicius, 2004) and *Dasyurus hallucatus* (White, 1990). Moreover, LS gaits are also common among some arboreal specialists, such as the rodents *Sciurus vulgaris* (Schmidt & Fischer, 2011) and *Thallomys paedulus* (Karantanis *et al.*, In Prep.), the metatherians *Petaurus breviceps* (Shapiro & Young, 2010) and *Didelphis virginiana* (White, 1990), and callitrichid primates (Stevens, 2006, 2008; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010). On the other hand, many arboreal methatherians with prehensile extremities, from the small *Acrobates pygmaeus* (Karantanis *et al.*, 2015) and *Caluromys philander* (Schmitt & Lemelin, 2002; Lemelin *et al.*, 2003) to the larger *Trichosurus vulpecula* (White, 1990), most primates, from the small *Microcebus murinus* (Shapiro *et al.*, 2016) to the larger strepsirrhines and catarrhines (Hildebrand, 1967; Stevens, 2006, 2008; Cartmill *et al.*, 2007), and the carnivoran *Potos flavus* (Lemelin & Cartmill, 2010) habitually employ DS gaits. The distribution of DS and LS gaits among arboreal mammals is particularly difficult to interpret. Phylogeny (Primates, Didelphidae, Phalangerioidea, Procyonidae), microhabitat use (tree crown extremities), and substrate handling (prehensile hind- and/or forefeet) may possibly play important roles.

Velocity and its regulating factors, stride frequency and stride length, were low on smaller substrates but gradually increased on larger substrates. However, in a study on sigmodontine rodents (Camargo *et al.*, 2016), velocities of arboreal species decreased as substrate size increased, while the opposite was true for terrestrial species. On narrower substrates, arboreal species moved faster, increased stride frequency and decreased stride length, but terrestrial species decreased both stride frequency and stride length or maintained stride frequency and decreased stride length (Camargo *et al.*, 2016). Higher walking velocity on narrow substrates may, in some cases, denote a better adaptation to arboreal locomotion (Delciellos & Vieira, 2006; Camargo *et al.*, 2016), and promote the maintenance of dynamic stability (Schmidt & Fischer, 2011). On the other hand, when a quadruped moves on an arboreal substrate, the support polygons are very narrow (Lammers & Zurcher, 2011a), increasing the risk of the centre of mass being placed outside them. Furthermore, each time a limb touches down, the torque produced further enhances the possibility to lose balance and locomotor flow (Lammers & Zurcher, 2011b). These torques may be relatively reduced in a tiny arboreal mammal, but are, nevertheless, present. A slow, controlled walk, especially on the narrowest of substrates,

would reduce the produced torques, increase the simultaneous contact points on the substrate at any given time, and provide longer time periods to accommodate a dynamic continuous locomotor pattern, which is essential for successful arboreal navigation (Lammers & Zurcher, 2011a).

Regardless of the direction of movement, *M. minutus* maintained LS gaits. Moreover, even though D did not differ between oblique ascents and descents, as was also the case for vertical ascents and descents in *M. minutus* (Karantanis *et al.*, 2017b), it was significantly higher in non-horizontal locomotion than in horizontal locomotion, resulting in more LSDC gaits. The DFI was also higher in ascents and descents than during horizontal locomotion, but no significant difference was detected between them, although the vertical ascents of the Eurasian harvest mice were characterized by higher DFI than vertical descents (Karantanis *et al.*, 2017b). In a natural setting, downwards angled substrates are usually rather unstable and would require secure anchoring of the limbs (Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Karantanis *et al.*, 2015). On declines, arboreal mammals face a cranial/anterior shift of the centre of mass, which is associated with increasing contact time length and a higher fraction of vertical impulse on the forelimbs, enhancing their regulative and supportive role (Rollinson & Martin, 1981; Nyakatura *et al.*, 2008). Lateral sequence (LS) gaits are thought to allow the forelimbs to provide retardation through a “stop-jolt” before the hind limbs contact the substrate (Rollinson & Martin, 1981). The increase in D in *M. minutus* can be viewed as a shift towards diagonal couplet gaits, which, whether LSDC, trot, or DSDC, appear to confer advantages in arboreal stability (Cartmill *et al.*, 2002, 2007). The more contralaterally paired limbs of diagonal couplets gaits exert opposing substrate reaction forces when in contact with the substrate, and reduce cranio-caudal torsional moments, thus assisting in stabilizing the torso (Lammers & Gauntner, 2008; Nyakatura *et al.*, 2008). On the other hand, the increased DFI of descents suggests a key role of the hind limbs, contrary to predictions (Preuschoft, 2002; Nyakatura *et al.*, 2008). It is very likely, that the efficiently grasping feet of *M. minutus* (Haffner, 1996, 1998; Krattli, 2001; Zefferer, 2002) assist the controlling and braking role of the forelimbs in descents. Furthermore, an increase in D and DFI was also observed during ascending locomotion. In ascents, more diagonal gaits assist in generating additional propulsion by limiting the retarding role of the forelimbs in the first part of the stance phase when the autopodium is anterior to the extremities’ pivot (Nyakatura *et al.*, 2008). Although *M. minutus* did not shift to DS gaits, LSDC gaits

may have a similar effect on locomotor competence, especially in the presence of grasping hindfeet (Preuschoft, 2002). LSDC gaits are equally stable to DSDC (Cartmill *et al.*, 2002, 2007), and when coupled with higher hind limb than forelimb duty factors (higher DFI), may enable a more controlled, faster upward progression, with a similar effect to DSDC gaits.

Successful negation of the arboreal milieu, and especially of the finest, unstable substrates, is usually achieved by behavioural mechanisms that contribute to stable, safe and continuous uninterrupted body progression along branches. These constraints appear to be similar even at a small body scale, as demonstrated by our observations on the Eurasian harvest mice. In this context, regarding velocity regulation in *M. minutus*, stride frequency was the main regulating mechanism, with stride length playing a secondary, although significant, role. This way of increasing velocity is different from that of arboreal sigmodontine rodents, which increase velocity by increasing stride length and reducing stride frequency (Camargo *et al.*, 2016). However, two small-bodied metatherians, *Gracilinanus microtarsus* (Delciellos & Vieira, 2006), *A. pygmaeus* (Karantanis *et al.*, 2015), and some scansorial rodents (Karantanis *et al.*, 2017a) adjust velocity in a similar manner to Eurasian harvest mice. Primates, larger arboreal marsupials (Larson *et al.*, 2000, 2001; Delciellos & Vieira, 2006), and a few terrestrial neotropical rodents (Camargo *et al.*, 2016) regulate velocity by increasing stride length instead of frequency. The increase of stride length enables a farther reach of the forelimbs which, coupled with secure grasping, when available, ultimately reduces involuntary branch sway (Demes *et al.*, 1994). On the other hand, an increase in stride frequency, although relatively costly in energy (Strang & Steudel, 1990; Reilly *et al.*, 2007), may be better suited for small-bodied arboreal mammals, for which branch swaying may be negligible, but not body oscillations. Body oscillations produce moments that disturb regular progression and may cause loss of stability, which may be particularly important when negotiating small, slender substrates. Regulation by stride frequency reduces these involuntary oscillations and could thus promote a more continuous and safer progression along arboreal substrates (Delciellos & Vieira, 2006).

The habitual use of LS gaits by a small narrow-branch dweller, along with a further reduction of D in narrower substrates, can only be adequately explained by comparing this strategy to other small mammals that use DS gaits. Especially at the lower end of the mammalian size scale, it is interesting to compare the behavioural strategies adopted by

the similarly sized *M. minutus* and the metatherian feathertail glider *Acrobates pygmaeus*. In contrast to Eurasian harvest mice, feathertail gliders utilize fast DS gaits, and increase D on narrow substrates (Karantanis *et al.*, 2015). Both species are adept arboreal specialists and tend to habitually use fine branches (Urbani & Youlatos, 2013; Youlatos *et al.*, In Prep.). Both species possess morphological adaptations, such as broad apical pads, dermatoglyphs, a relatively divergent hallux, and deep curved claws for effective hind foot grasping (Haffner, 1996, 1998; Rosenberg & Rose, 1999; Krattli, 2001; Zefferer, 2002). However, the hind and forefeet of *A. pygmaeus* possess gland-rich plantar and palmar pads that further increase friction and resist substrate reaction forces enhancing their grasping ability (Rosenberg & Rose, 1999). Finally, in contrast to Eurasian harvest mice, feathertail gliders are much faster (Karantanis *et al.*, 2015). The advantage of DS gaits to provide safe arboreal progression, by testing new unstable new substrates, seems improbable during high-speed locomotion (Nyakatura *et al.*, 2008). Nevertheless, DS gaits may confer better dynamic stability (i.e. during fast locomotion), possibly allowing a better control and transfer of moments and torques across the body axes (Lammers & Zurcher, 2011a). Increased D on narrow branches, as exemplified by *A. pygmaeus*, but not *M. minutus*, produces medially-oriented forces (Schmitt, 2003a; Lammers & Biknevicus, 2004; Schmidt & Fischer, 2010). This allows animals to seize substrates and increase friction, which reduces the possibility of slipping (Lammers & Zurcher, 2011a). Moreover, opposing mediolateral and angular impulses generated by contralateral limbs may reduce mediolateral deviations from the long axis of the branch (Shapiro & Raichlen, 2007). These mechanisms assure continuous uninterrupted progression and minimize any risks of toppling over, both of which contribute to successful branch negotiation.

Although the interpretation of the advantages of DS gaits has functionally focused on grasping hind feet (Cartmill *et al.*, 2007), we believe that increased forelimb grasping capacity may be integral for effective DS gaits, as the combined grasping function of fore- and hind limbs would contribute to increasing stability. In contrast, mammals that do not possess strong hind and/or forefoot grasping abilities may not be able to apply equally strong contralateral forces during arboreal locomotion. In this case, the habitual use of LS gaits enables keeping the centre of mass within the support polygon throughout the gait cycle, provided that three or four limbs are in contact with the substrate (i.e. high DF) (Lammers & Zurcher, 2011a). The combination of lower D with a higher DF on narrow substrates in some scansorial rodents with similar grasping capabilities (Karantanis *et*

al., 2017a), as well as in *M. minutus*, implies behavioural adaptations for increased static stability (slow locomotion) on arboreal substrates. It is very likely that these differences account for the variation in the functional use of DS and LS gaits in arboreal locomotion, therefore calling for further investigations in arboreal and semi-arboreal taxa.

The use of relatively cautious locomotion by Eurasian harvest mice is further denoted by the lack of use of any asymmetrical gaits, regardless of substrate diameter and inclination. In effect, many small arboreal and scansorial mammals tend to switch to asymmetrical gaits at higher velocities (Young, 2009; Schmidt & Fischer, 2011; Shapiro *et al.*, 2016; Karantanis *et al.*, 2017a). Extended aerial phases in gaits may be associated with bounding and leaping and likely facilitate obstacle avoidance and enhanced manoeuvring (Hildebrand, 1985). This is particularly highlighted by one of the smallest primates, *Microcebus murinus*, which display asymmetrical gaits with extended aerial phases, especially on larger substrates, which maximize acceleration, manoeuvrability, and energetic economy (Shapiro *et al.*, 2016). Although it may appear uneconomical for *M. minutus* to not use asymmetrical gaits, it may be relevant to its locomotor ecology. Eurasian harvest mice are characterized by the predominant use of very narrow, flexible substrates and the lack of airborne or bounding locomotion (Urbani & Youlatos, 2013). The sparsity of leaping, as well as the need for increased cautiousness on narrow substrates, by lowering velocity and increasing DF, possibly constitute long aerial phases, and therefore asymmetrical gaits, unnecessary in their locomotor behaviour.

The findings of the present study demonstrate that, in contrast to kinematics (Fischer, 1994; Fischer *et al.*, 2002; Schmidt, 2005; Schilling & Hackert, 2006), gaits and associated metrics may not be size dependent. Only velocity regulation, with a higher input of stride frequency to stride length was functionally related to small size, possibly linked to safer and uninterrupted arboreal locomotion (Delciellos & Vieira, 2006). This lack of differences may imply a certain behavioural plasticity across body mass range and the complex interaction of mechanisms that regulate locomotion. They might further underscore the common constraints that all mammals face when negotiating arboreal substrates. Despite of this, our results further indicate that *M. minutus* invest on safe, secure continuous locomotion contributing to an effective negotiation of arboreal substrates of a variety of inclinations and sizes. Although, they almost exclusively used LS gaits, they adjusted gait parameters per substrate constraints to enhance stability. Despite their miniscule size, they did not differ from other arboreal mammals without

grasping forefeet, in the ways they adjusted gaits for terminal branch locomotion (i.e. decrease of D, DF and increase of velocity), thus promoting uninterrupted, stable progression (Pridmore, 1994; Cartmill *et al.*, 2002; Shapiro & Raichlen, 2005; Shapiro & Young, 2010; Lammers & Zurcher, 2011a). At such a small body size, we may wonder why arboreal stability is important. After all, such tiny animals are far less likely to be injured or killed after falling off. However, reassuming their previous position on an elevated arboreal layer would be both energetically and time consuming, and would very likely decrease fitness. But more importantly, falling from a tree, even though it may not be physically damaging, would severely expose the animal to potential terrestrial predators. Considering this significant risk, it is of no surprise that *M. minutus*, despite its miniscule size, displays behavioural gait adaptations that optimize arboreal stability, in similar ways to larger arboreal and scansorial mammals, and ensure a safe, uninterrupted, continuous progression along arboreal substrates.

Finally, it is important to highlight the significant role of the grasping hind feet in ascending and descending locomotion where they enable upwards body propulsion and facilitated finer braking and control over declines. In fact, the enhanced stability provided by the hind feet allows successful negotiation and effective climbing on the narrowest, most unstable arboreal substrates (Urbani & Youlatos, 2013; Karantanis *et al.*, 2017b). These substrates correspond to long grass and reeds, and fine twigs on short trees, shrubs, vines and hedgerows that abound in their natural habitat. These locomotor adjustments enable *M. minutus* to successfully exploit the arboreal habitat, facilitating nest construction during reproduction, reducing interspecific competition, avoiding predation, and providing timely access to valuable arboreal food sources (Harris, 1970, 1979; Nordvig *et al.*, 2001; Bence *et al.*, 2003; Özkan *et al.*, 2003; Surmacki *et al.*, 2005; Kuroe *et al.*, 2007). As locomotion plays an important part in the ecology and evolution of arboreal mammals, we hope that the present study provides a basis for similar studies investigating the locomotor abilities of arboreal rodents, as well as other small arboreal mammals. This will ultimately help elucidate the evolution and adaptive significance of gaits and their parameters within the mammalian radiations.

Chapter 5: Arboreal gaits in a medium-sized rodent

Arboreality in acacia rats (*Thallomys paedulcus*; Rodentia, Muridae): gaits and gait metrics

This chapter is under review by the Journal of Zoology.

5.1. Introduction

Arboreal locomotion, in its many forms, has characterized mammals from their early radiations and has repeatedly and independently evolved over their history in a number of taxa (Bensley, 1901; Haines, 1958; Cartmill, 1974b; Jenkins, 1974; Ji *et al.*, 2002; Meng *et al.*, 2015, 2006; Szalay, 2007; Gaetano & Rougier, 2011; Goswami *et al.*, 2011; Luo *et al.*, 2011; Zheng *et al.*, 2013; Bi *et al.*, 2014; Williamson *et al.*, 2014). Rodents are no exception (Krattli, 2001; Zefferer, 2002; Samuels & Van Valkenburgh, 2008). Through arboreal activities, rodents gain access to specific food sources and stores, and can move, rest, and eventually nest and raise offspring away from potential predators (Balát & Pelikán, 1959; De Graaff, 1978; Montgomery, 1980; Murúa & González, 1982; Kingdon, 1984; Ylönen, 1990; Jędrzejewska & Jędrzejewski, 1990; Jędrzejewski *et al.*, 1993; Wagner, Feldhamer & Newman, 2000; Juškaitis, 2002; Buesching *et al.*, 2008; Czeszczewik *et al.*, 2008; Dickman & Doncaster, 2009). In order to reap the advantages of arboreality, rodents (as other arboreal mammals) must safely and skilfully negotiate substrates of variable size, inclination, length and flexibility, in the complex, three-dimensional and discontinuous habitat of the different forest layers (Cartmill, 1974b; Hildebrand, 1995). Towards this end, the morphology and locomotor and postural behaviour are accordingly specialized (Cartmill, 1974b; Essner, 2003; Kirk *et al.*, 2008; Samuels & Van Valkenburgh, 2008; Schmidt & Fischer, 2011; Camargo *et al.*, 2012, 2016; Youlatos *et al.*, 2015). Regarding locomotion, gaits can function as a mechanism that promotes stable and safe displacement along arboreal substrates (Cartmill *et al.*, 2002, 2007; Lammers & Gauntner, 2008; Lemelin & Cartmill, 2010; Lammers & Zurcher, 2011a, 2011b).

Gaits are related to the sequence of forelimb and hind limb swing and stance phases within a stride period, i.e. two consecutive touchdowns of a reference limb, often

the left hind limb (Hildebrand, 1967, 1976, 1977, Alexander, 1977, 1992). Gaits can be analysed on the basis of limb synchronicity, as either symmetrical (Hildebrand, 1976), when the footfalls of a girdle are evenly spaced in time, or asymmetrical (Hildebrand, 1977), when the footfalls of a girdle move more or less concurrently. In both gait types, duty factor (DF) represents the percentage of a cycle during which a foot is on a substrate. Duty factor separates walking ($DF > 50$) from running ($DF < 50$) (Hildebrand, 1967). Duty factor index (DFI) is the hundredfold ratio of the duty factor of the hind limbs divided by that of the forelimbs, and expresses the relative contact of the hind limbs compared to that of the forelimbs (Cartmill *et al.*, 2007). Finally, diagonality (D) or forelimb-hind limb phase (Hildebrand, 1967, 1976, 1977; Cartmill *et al.*, 2007) is applied exclusively to symmetrical gaits and represents the percentage of the stride cycle interval the footfall of a forelimb follows the footfall of the ipsilateral hind limb. Diagonality separates diagonal sequence (DS) gaits ($D > 50$), in which the footfall of a forelimb takes place at a time interval more than half of the gait cycle, following the footfall of the ipsilateral hind limb, from a lateral sequence (LS) gaits ($D < 50$), in which the footfall of a forelimb takes place at a time interval less than half of the gait cycle, following the footfall of the ipsilateral hind limb (Hildebrand, 1967). Moreover, in diagonal couplets gaits, either diagonal-sequence (DSDC; $50 < D < 75$) or lateral-sequence (LSDC; $25 < D < 75$), contralateral limbs move more concurrently. On the other hand, during lateral-couplets gaits, either diagonal-sequence (DSLc; $75 < D < 100$) or lateral-sequence (LSLc; $0 < D < 25$), ipsilateral limbs are more synchronised.

Symmetrical gaits are of special interest in arboreal locomotion, as their metrics have been linked to arboreal adaptations (Prost, 1965; Prost & Sussman, 1969; Cartmill *et al.*, 2002, 2007). DSDC gaits are associated with dynamic stability, allowing the control and transfer of moments and torques imposed on the body axes (Lammers & Zurcher, 2011a). DSDC gaits have been linked to arboreality, as they are common in many arboreal taxa, such as primates (Hildebrand, 1967; Cartmill *et al.*, 2007), a few metatherian didelphimorphs and phalangeroids (White, 1990; Pridmore, 1994; Cartmill *et al.*, 2002; Schmitt & Lemelin, 2002; Karantanis *et al.*, 2015), and the carnivoran *Potos flavus* (Lemelin & Cartmill, 2010). DSDC gaits produce medially-oriented forces (Schmitt, 2003; Lammers and Biknevicius, 2004; Schmidt and Fischer, 2010). This allows animals to seize substrates and increase the applied force, and consequent friction, which reduces the possibility of slipping (Lammers and Zurcher, 2011a). Moreover, opposing mediolateral

and angular impulses generated by contralateral limbs may reduce mediolateral deviations from the long axis of the branch (Shapiro and Raichlen, 2007). Moreover, in DSDC gaits, the forelimb moves onto new, untested substrates after the contralateral hind limb has already been safely anchored on the previously used substrate. This may allow for safe withdrawal to safety if the new substrate is proven to be unstable, while keeping the centre of mass within the support polygon (Cartmill *et al.*, 2007). However in arboreal locomotion, LSDC gaits may be preferable in terms of static stability, as they allow an animal to keep its centre of mass within the support polygons, throughout the gait cycle, provided at three or four limbs are anchored on the substrate, hence at high DF (Lammers & Zurcher, 2011a). Even though LS gaits are more common among terrestrial mammals, LSDC are also habitually used by a number of arboreal taxa, including callitrichid primates (Stevens, 2006, 2008, Nyakatura *et al.*, 2007, 2008; Nyakatura & Heymann, 2010), sugar gliders *Petaurus breviceps* (Shapiro & Young, 2010), as well as scansorial and arboreal rodents (Schmidt & Fischer, 2010, 2011; Karantanis *et al.*, 2017a).

On the other hand, asymmetrical gaits allow reaching higher speeds of locomotion (Hildebrand, 1977; Pridmore, 1994; Gasc, 2001; Young, 2009; Shapiro *et al.*, 2016). In this context, they are favourable over symmetrical gaits as they produce a decrease in the peak forces on the animal, hence decreasing strain on the musculoskeletal system (Farley & Taylor, 1991), or optimizing metabolic costs (Hoyt & Taylor, 1981). In small mammals, asymmetrical gaits may also provide an energetic input to locomotion through the sagittal movements of their flexible spine and related muscles (Schilling & Hackert, 2006). In an arboreal setting, asymmetrical gaits also confer advantages in stability by reducing peak vertical forces, limiting centre of mass movements, and resulting in shorter and more frequent strides (Young, 2009; Schmidt & Fischer, 2010; Shapiro *et al.*, 2016).

Other locomotor metrics, such as velocity, may also be useful as indicators of locomotor competence on arboreal substrates. Velocity is a function of stride frequency, i.e. number of strides per unit of time, and stride length, i.e. the distance covered within a single stride (Alexander, 1992; Hildebrand, 1995). Interestingly, among some neotropical rodents and marsupials, arboreal species usually exhibit overall higher velocities than terrestrial species on arboreal substrates (Delciellos & Vieira, 2006, 2007; Camargo *et al.*, 2016). Higher velocities on narrow, arboreal substrates may contribute to maintenance of dynamic stability (Schmidt & Fischer, 2010). In arboreal mammals, an increase in velocity is achieved in different ways: stride frequency increase and stride length

decrease (Delciellos & Vieira, 2006, 2007; Camargo *et al.*, 2016); stride frequency decrease and stride length increase (Larson *et al.*, 2000, 2001, Delciellos & Vieira, 2006, 2007); and stride frequency may primarily increase, followed, at a lesser rate, by stride length (Nyakatura *et al.*, 2008; Karantanis *et al.*, 2015). Increasing velocity through longer strides can be safer on arboreal substrates as it enables the forelimb to reach farther and reduces involuntary branch sway (Demes *et al.*, 1994). It is often encountered in medium-sized and larger arboreal mammals (Larson *et al.*, 2000, 2001, Delciellos & Vieira, 2006, 2007). In contrast, increasing velocity by increasing stride frequency, although energetically costly (Reilly *et al.*, 2007), decreases body oscillations, allows continuous progression, and may thus be more advantageous for smaller mammals, which face insignificant branch sway (Strang & Steudel, 1990; Delciellos & Vieira, 2007).

As arboreal substrates are variable, differences in substrate size and inclination can influence arboreal locomotion and its parameters. Substrate size may impose significant constraints on locomotor patterns (Jenkins, 1974; Witte *et al.*, 2002), and DS gaits may be especially useful on smaller, unstable substrates, assisting safe locomotion (Cartmill *et al.*, 2007). However, results from previous studies are equivocal. A lower frequency of DS gaits has been observed in the majority of strepsirrhine primates examined (Stevens, 2008), the tamarin *Saguinus fuscicollis* (Nyakatura & Heymann, 2010), and the arboreal metatherian *Petaurus breviceps* (Shapiro & Young, 2010), while there is no correlation between substrate size and DS gaits in free-ranging tamarins *Saguinus mystax* (Nyakatura & Heymann, 2010). On the other hand, the arboreal metatherian *Acrobates pygmaeus* (Karananis *et al.*, 2015) and the kinkajou *Potos flavus* (Lemelin & Cartmill, 2010) display a higher occurrence of DS gaits on smaller substrates. Regarding velocity, results are also divergent. Some neotropical arboreal rodents walk faster on smaller substrates (Camargo *et al.*, 2016), very likely to maintain stability while lacking substantial grasping abilities (Schmidt & Fischer, 2010). On the other hand, some arboreal metatherians move more slowly (Delciellos & Vieira, 2006, 2007; Karantanis *et al.*, 2015). Slow, cautious walking probably promotes a stable dynamic progression by reducing the torques produced at limb touchdown, increasing the simultaneous contact points on the substrate at any part of a gait cycle, and lengthening the relative duration of contact with the substrate (Hildebrand, 1976; Cartmill *et al.*, 2002, 2007, Lammers & Zurcher, 2011a, 2011b).

Apart from substrate size, substrate inclination, and more specifically ascents and descents, also influence gait parameters. In ascents, the occurrence of DSDC gaits (increased D) (Prost & Sussman, 1969; Lammers, 2007; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Shapiro & Young, 2010; Shapiro *et al.*, 2014; Karantanis *et al.*, 2015), coupled with increased hind limb stance phase durations (higher DFI), reduce yawing and lateral torques, enhancing the capacity to drive the body upwards (Vilensky *et al.*, 1994; Lammers & Biknevicius, 2004; Stevens, 2006; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010). On the other hand, LSDC gaits may be advantageous in descents, allowing increased braking by the forelimbs, though a “stop-jolt” mechanism (Rollinson & Martin, 1981; Nyakatura & Heymann, 2010). Additionally, as the centre of mass is shifted cranially/anteriorly, the longer contact times (lower DFI) and the higher fraction of vertical impulse on the forelimbs further augment their regulatory and supportive importance (Rollinson & Martin, 1981; Lee *et al.*, 2004; Nyakatura *et al.*, 2008; Young, 2012; Karantanis *et al.*, 2015). In terms of velocity, descents are slower, compared to ascents and horizontal locomotion (Vilensky *et al.*, 1994; Lammers *et al.*, 2006; Lammers, 2007; Karantanis *et al.*, 2015), likely because of the frictional adjustments of the extremities, and the loading and braking role of the forelimbs (Lammers *et al.*, 2006; Lammers, 2007). Ascents may be faster than horizontal locomotion, as in the agile European red squirrels, as they help achieve dynamic stability (Schmidt & Fischer, 2011), but can also be slower, as in the more generalised rats (Schmidt & Fischer, 2010, 2011) and larger primates (Nakano, 2002). On the other hand, some smaller arboreal primates do not adjust velocity relative to their direction of movement (Nyakatura *et al.*, 2008; Shapiro *et al.*, 2016).

Considering the divergent responses of different arboreal mammals to the constraints imposed by the properties of the arboreal substrates on locomotor parameters, we examined arboreal gait patterns in an African arboreal, nocturnal rodent, the acacia rat *Thallomys paedulcus* (Sundevall, 1846). As is the case for most nocturnal African small mammals, there is limited published information for the species and almost nothing is known about its locomotion. *Thallomys paedulcus* is a murid rodent encountered in the African Acacia bushland, from Ethiopia, south to the east coast of South Africa (De Graaff, 1978; Kingdon, 1984; Mills & Hes, 1997; Monadjem, 1999; Skinner & Chimimba, 2005; Taylor & Boitani, 2008). Acacia trees play an important role on the ecology and biology of the species. Acacia rats exploit heavily the trunks and

branches of Acacia trees, where they build their nests at heights around 4m. The thick thorns of the trees provide them protection from predators, such as owls and arboreal snakes (Delany, 1972; De Graaff, 1978; Kingdon, 1984; Dean, Milton & Jeltsch, 1999). Their morphology is adapted to an arboreal lifestyle with a long tail covered with long tactile hairs, relatively short limbs, curved claws on the digits, and broad hind feet, with short toes and raised pads and a well-developed, long, partially opposable fifth digit (De Graaff, 1978; Reed, 2003). As this morphology makes acacia rats able and committed arborealists (Earl & Nel, 1976), it is also important to identify the behavioural locomotor adaptations they have evolved in order to successfully exploit the arboreal habitat. In this context, we here investigate the relation between gait parameters, i.e. diagonality, duty factor, duty factor index, velocity, stride length and stride frequency, and substrate size and direction of movement of *T. paedulus*. In our experiments, acacia rats negotiated inclining, declining and horizontal arboreal substrates from small to relatively larger diameters, simulating the branch diversity encountered in acacia forests, the natural habitat of the species. On small substrates, we expect acacia rats to display higher diagonality (D), in a manner to promote secure locomotion, unmodified duty factor index (DFI), higher velocity, and lower duty factor (DF). During ascents, acacia rats should demonstrate increased diagonality (D) and duty factor index (DFI), with an opposite trend in descents, as well as lower velocity, compared to horizontal locomotion. This is the first study that explores gait metrics in these arboreal rodents, and similar data are valuable for elucidating the different locomotor adaptations that rodents have evolved in relation to arboreality.

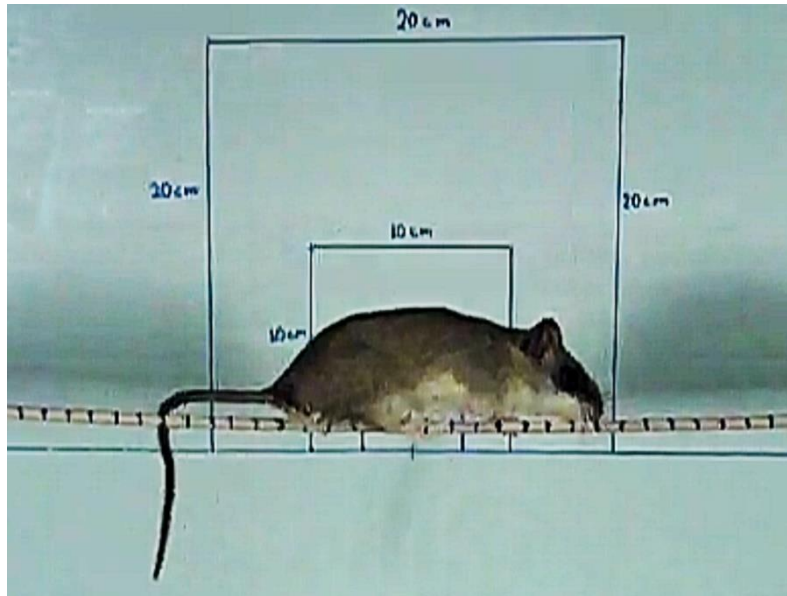


Figure 5.1. Still image of a male *Thallomys paedulus* from the video recordings, walking on a 5 mm substrate (the vertical markings on every 1 cm are clearly visible).

5.2. Materials and Methods

Specimens

For the purposes of the current study, we tested 12 adult male acacia rats *Thallomys paedulus* (Muridae, Rodentia; Fig. 5.1). All specimens were captive-born and are housed in the permanent collection of Nowe Zoo, Poznan, Poland. The animals inhabited a large enclosure (H: 120 cm x W: 240 cm x 120 cm) under a reversed day-night regime. The enclosure contained natural materials for nesting and a wide variety of arboreal substrates of different sizes and inclinations that simulate their natural habitat. The animals moved freely within this enriched environment and showed no indications of stress or stereotypies. During the experiments, all specimens were healthy and active, were handled with care, and did not display any stereotypical or stressful behaviour. The zoo keeps no data on any relatedness between the different animals, therefore it was unknown whether the subjects were related or not. Mean head-body length of the specimens used was 13.4 cm (SD=1.6 cm, range: 12.6-14.0 cm), mean mass was 70.2 g (SD=11 g, range: 62 -74 g), and mean effective hind limb length, i.e. the distance between the hip joint and the substrate, parallel to the axis of gravity, was 3.5 cm (SD=0.69 cm, range: 2.9-3.7cm). All measurements were taken directly on the study subjects.

Experimental setup

We used a single, specially configured, glass terrarium (L: 90 cm x H: 40 cm x W: 40 cm), topped by a wooden cover, for filming. Within the terrarium, we set up 80 cm long poles, made of cylindrical semi-hardwood, which were supported by wooden frames on each end. All poles were marked with vertical blue lines every 1 cm for reliable absolute length estimation (Fig. 5.1). During the recording, the visible length of the rod on the videos was between 30-40 cm. Diameter and direction of movement accounted for the classification of the different substrate categories. Following previous experimental study on a series of small bodied mammals, we considered four classes of diameters (2 mm, 5 mm, 10 mm, 25 mm) (Karantanis *et al.*, 2015, 2017a, 2017b). These classes simulate a range of branches, from the finest terminal twigs to boughs, which impose differential locomotor constraints in the natural habitat of the studied species. In a similar manner, we also used three different directions of movement (descent at 45°, horizontal, ascent at 45°), which in combination with the four size classes, totalled to 12 distinct substrate categories.

The experiments occurred in three video recording sessions, during June 2013. All experiments occurred under special lighting conditions, during daytime hours, which corresponded to the nocturnal active period of the subjects. During each session, two subjects were individually transferred from their enclosures to the filming terrarium. Initially, every individual was allowed to move freely within the filming terrarium in order to accommodate itself with the new environment. Minimal or no stimulation was required for the subjects to walk on the poles. During each filming session, subjects were tested several times on all 12 substrate categories. After each filming session, the animal was transported back to a temporary enclosure, to ensure that no individual was tested twice during a particular session. For video recording, we used a Sanyo digital camcorder (VPC-HD 2000, Sanyo, Osaka, Japan), set at 240 fps, and positioned at a distance of 1 m from the filming terrarium in order to reduce image distortion. For our analyses on diagonality we considered only symmetrical gaits [i.e. initiating with the touchdown of the left hind limb and ending at the subsequent touchdown of the same limb, including both lift-offs and touchdowns of all limbs (Hildebrand, 1967; 1968; 1976)]. For all other parameters, we considered both complete symmetrical and asymmetrical gaits. We discarded any cycles involving beginnings or endings of locomotor bouts or loss of

balance. We only analysed continuous locomotion, regardless of velocity, as indicative of natural unbiased behaviour. Handling of the animals, and filming were performed with the permission of the Local Ethical Commission for Animal Experiments in Poznan, followed the guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS, 2012), and complied with relative regulations and legislations of the Adam Mickiewicz University and the Aristotle University of Thessaloniki.

Gait analyses

Video analysis and data collection, distance and time calculations were made by importing videos and calibrating time and distance measurements using Tracker 4.92 (Brown, 2009). Time was calibrated taking into account the 240 fps of the videos, and distance was calibrated using the reference marks on the substrates. Using Tracker, we digitized lift-offs and touchdowns of each limb when they took place. Microsoft Excel 2010 (Redmond, WA, USA), and SPSS 23 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

For our analyses, we considered the following gait parameters:

- (i) Diagonality (D) (Hildebrand, 1967; Cartmill *et al.*, 2007) is the percentage of the stride cycle interval the footfall of a forelimb follows behind the ipsilateral hind limb. Although it was measured as a scale variable, it was also divided into five ordinal classes: (a) Lateral Sequence Lateral Couplets ($0 \leq \text{LSLC} < 25$), (b) Lateral Sequence Diagonal Couplets ($25 \leq \text{LSDC} < 50$), (c) Trot ($=50$), (d) Diagonal Sequence Diagonal Couplets ($50 < \text{DSDC} \leq 75$), (e) Diagonal Sequence Lateral Couplets ($75 < \text{DSLC} \leq 100$);
- (ii) Duty Factor (DF) is the mean of duty factors of all limbs, defined as the percentage of a cycle during which a limb is in contact with the substrate. Walking gaits correspond to $\text{DF} > 50$, whereas running gaits score < 50 ;
- (iii) Duty Factor Index (DFI) is the ratio of hind limb duty factor (DF_h) to forelimb duty factor (DF_f), calculated as $100 \cdot \text{DF}_h / \text{DF}_f$ (Cartmill *et al.* 2007a). $\text{DFI} > 100$ indicate longer hind limb than forelimb stance durations, whereas $\text{DFI} < 100$ specify shorter hind limb than forelimb stance durations;
- (iv) Stride Duration (t) is total duration of a single stride in seconds;
- (v) Stride Length (l) is the distance covered during a single stride cycle, in meters;

- (vi) Velocity (v) is the speed at which the subjects moved, calculated by dividing stride length with stride duration, in meters/second (ms^{-1});
- (vii) Stride Frequency (f) is the number of strides per second;

As these four last parameters are size-dependent, we standardized them using the effective hind limb length (Pontzer, 2005; 2007a,b). The effective hind limb length is used to calculate the dimensionless measures of stride duration, stride length, velocity and stride frequency which provide comparative measures of locomotor competence across different species (Alexander, 1977; Alexander & Jayes, 1983; Hof, 1996). The dimensionless measures were calculated as follows:

$$\text{Dimensionless Stride Duration (t}_D) = \frac{t}{\sqrt{l_0/g}}$$

$$\text{Dimensionless Stride Length (l}_D) = \frac{l}{l_0}$$

$$\text{Dimensionless Velocity (v}_D) = \frac{v}{\sqrt{gl_0}}$$

$$\text{Dimensionless Stride Frequency (f}_D) = \frac{f}{\sqrt{g/l_0}}$$

where l_0 is the effective hind limb length of each animal and g is the acceleration of gravity ($g=9.81 \text{ m s}^{-2}$). All measures of time and length were converted to the SI system. All overall values reported represent the sum of mean values per substrate category, divided by the number of substrate categories in which gaits were recorded.

For statistical analyses, we opted for Analysis of Covariance (ANCOVA) to explore relationships between variables while controlling for other possible covariates. When necessary, comparisons between categories or across species were performed by Bonferroni Post-Hoc tests, calculating Bonferroni Mean Difference (BMD) values, based on estimates by the univariate ANCOVA models. Finally, we constructed stepwise regression models to examine the impact of both dimensionless stride frequency and stride length on velocity. The impact of each parameter was estimated using the R calculated by the model (Harrell, 2001). Spearman's rank correlation was used to test for relations between stride length and stride frequency.

Table 5.1. Summary of means and standard deviations (in brackets) of the gait statistics for symmetrical, walking gaits in *Thallomys paedulus*. N of observations indicates the number of analyzed cycles for each substrate size and direction of movement category. N of individuals indicates the number of individuals for which valid gait cycles were recorded in each category.

		N of Observations	N of Individuals	Diagonality	Duty Factor	Hindlimb Duty Factor	Forelimb Duty Factor	Duty Factor Index	Velocity (m s ⁻¹)	Stride Length (m)	Stride Frequency (s ⁻¹)	Stride Duration (s)	Dimensionless Velocity	Dimensionless Stride Length	Dimensionless Stride Frequency	Dimensionless Stride Duration
Horizontal	2mm	0	0	No data												
	5mm	14	8	27.99 (6.14)	76.45 (7.13)	76.96 (7.36)	75.94 (7.28)	101.4 (4.18)	0.264 (0.107)	0.073 (0.007)	3.583 (1.305)	0.312 (0.101)	0.444 (0.182)	2.024 (0.187)	0.217 (0.078)	5.131 (1.628)
	10mm	12	6	35.78 (8.95)	67.3 (7.80)	67.12 (6.48)	67.49 (10.01)	100.35 (8.61)	0.480 (0.216)	0.088 (0.014)	5.301 (1.884)	0.210 (0.069)	0.801 (0.360)	2.406 (0.367)	0.324 (0.115)	3.452 (1.131)
	25mm	0	0	Exclusively asymmetrical gaits												
	Overall Horizontal			31.89 (7.55)	71.88 (7.47)	72.04 (6.92)	71.72 (8.65)	100.88 (6.40)	0.372 (0.162)	0.081 (0.011)	4.442 (1.595)	0.261 (0.085)	0.623 (0.271)	2.215 (0.277)	0.271 (0.097)	4.292 (1.380)
Ascent	2mm	0	0	No data												
	5mm	6	5	29.28 (9.28)	75.97 (3.10)	75.53 (4.21)	76.4 (4.27)	99.08 (7.45)	0.371 (0.099)	0.075 (0.009)	4.887 (0.851)	0.210 (0.039)	0.639 (0.170)	2.180 (0.280)	0.289 (0.050)	3.546 (0.653)
	10mm	16	6	40.25 (4.78)	63.26 (3.25)	62.2 (3.77)	64.33 (3.65)	96.82 (5.68)	0.672 (0.132)	0.086 (0.007)	7.803 (1.283)	0.132 (0.023)	1.151 (0.228)	2.474 (0.194)	0.464 (0.075)	2.210 (0.384)
	25mm	9	4	41.17 (4.62)	61.68 (2.87)	61.53 (2.93)	61.83 (3.24)	99.6 (3.67)	0.778 (0.244)	0.096 (0.010)	8.025 (1.817)	0.13 (0.028)	1.339 (0.419)	2.778 (0.287)	0.475 (0.108)	2.197 (0.466)
	Overall Ascent			36.90 (6.23)	66.97 (3.07)	66.42 (3.64)	67.52 (3.72)	98.50 (5.60)	0.607 (0.158)	0.086 (0.009)	6.905 (1.317)	0.157 (0.030)	1.043 (0.272)	2.477 (0.254)	0.409 (0.078)	2.651 (0.501)
Overall				34.89 (6.75)	68.93 (4.83)	68.67 (4.95)	69.20 (5.69)	99.45 (5.92)	0.513 (0.160)	0.084 (0.009)	5.920 (1.428)	0.199 (0.052)	0.875 (0.272)	2.372 (0.263)	0.354 (0.085)	3.307 (0.852)

Table 5.2. Summary of means and standard deviations (in brackets) of the gait statistics for asymmetrical, half-bounding gaits in *Thallomys paeulcus*. N indicates the number of analyzed cycles for each substrate size and direction of movement category. N of individuals indicates the number of individuals for which valid gait cycles were recorded in each category.

		N of Observations	N of Individuals	Duty Factor	Hindlimb Duty Factor	Forelimb Duty Factor	Duty Factor Index	Velocity (m s ⁻¹)	Stride Length (m)	Stride Frequency (s ⁻¹)	Stride Duration (s)	Dimensionless Velocity	Dimensionless Stride Length	Dimensionless Stride Frequency	Dimensionless Stride Duration
Horizontal	2mm	0	0	No data											
	5mm	0	0	Exclusively symmetrical gaits											
	10mm	12	7	58.84 (7.12)	60.01 (7.89)	57.68 (8.60)	105.60 (16.77)	0.999 (0.335)	0.104 (0.016)	9.384 (2.067)	0.113 (0.030)	1.770 (0.617)	3.191 (0.635)	0.542 (0.115)	1.934 (0.467)
	25mm	9	5	55.56 (3.40)	52.08 (5.14)	59.05 (5.75)	89.14 (14.63)	1.26 (0.323)	0.110 (0.016)	11.354 (1.758)	0.090 (0.014)	2.175 (0.557)	3.202 (0.471)	0.672 (0.105)	1.521 (0.244)
	Overall Horizontal			57.20 (5.26)	56.04 (6.52)	58.36 (7.17)	97.37 (15.70)	1.131 (0.329)	0.107 (0.016)	10.369 (1.913)	0.101 (0.022)	1.973 (0.587)	3.197 (0.553)	0.607 (0.110)	1.727 (0.355)
	2mm	0	0	No data											
Ascent	5mm	0	0	Exclusively symmetrical gaits											
	10mm	0	0	Exclusively symmetrical gaits											
	25mm	4	4	55.12 (1.92)	56.75 (2.32)	53.50 (5.49)	107.30 (15.98)	1.011 (0.110)	0.108 (0.010)	9.474 (1.447)	0.108 (0.017)	1.731 (0.162)	3.102 (0.320)	0.564 (0.093)	1.811 (0.311)
	Overall Ascent			55.12 (1.92)	56.75 (2.32)	53.50 (5.49)	107.30 (15.98)	1.011 (0.110)	0.108 (0.010)	9.474 (1.447)	0.108 (0.017)	1.731 (0.162)	3.102 (0.320)	0.564 (0.093)	1.811 (0.311)
	Overall			56.51 (4.15)	56.28 (5.12)	56.74 (6.61)	100.68 (15.79)	1.091 (0.256)	0.107 (0.014)	10.071 (1.757)	0.103 (0.020)	1.892 (0.445)	3.165 (0.475)	0.593 (0.104)	1.755 (0.340)

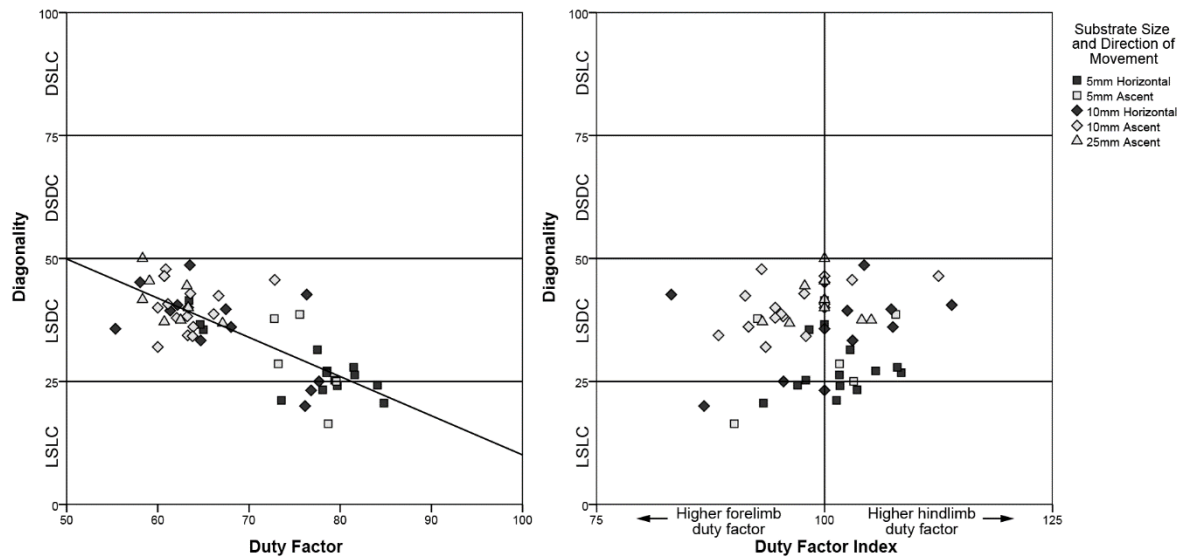


Figure 5.2. Scatterplot of diagonality as a function of duty factor and the duty factor index for all symmetrical gait cycles. The horizontal lines in the left graph split the sample in quadrants of decreasing diagonality (DSLCL, DSDCL, LSDCL, LSLCL, from top to bottom). The regression line has a function of $D=89.79-0.8*DF$, significant at $p<0.05$. In the right graph, horizontal lines are drawn similarly to the former and a vertical line at a duty factor index of 100, where the duty factors of the forelimbs and the hind limbs are equal.

5.3. Results

We recorded a total of 81 gait cycles over a total of six substrate categories: 56 symmetrical walks (70.6%) and 25 asymmetrical half-bounding gaits (29.4%), in which the hind limbs touched down simultaneously while one forelimb was trailing the other. The animals failed to negotiate the 2mm substrates, so no data on this category are presented. Additionally, we failed to obtain data on descents, as the animals constantly preferred to climb down the vertical support at the tallest end of the setup. In order to avoid stressing the animals, we opted not to force them further. Tables 1 and 2 present the summary of observations per substrate and direction of movement.

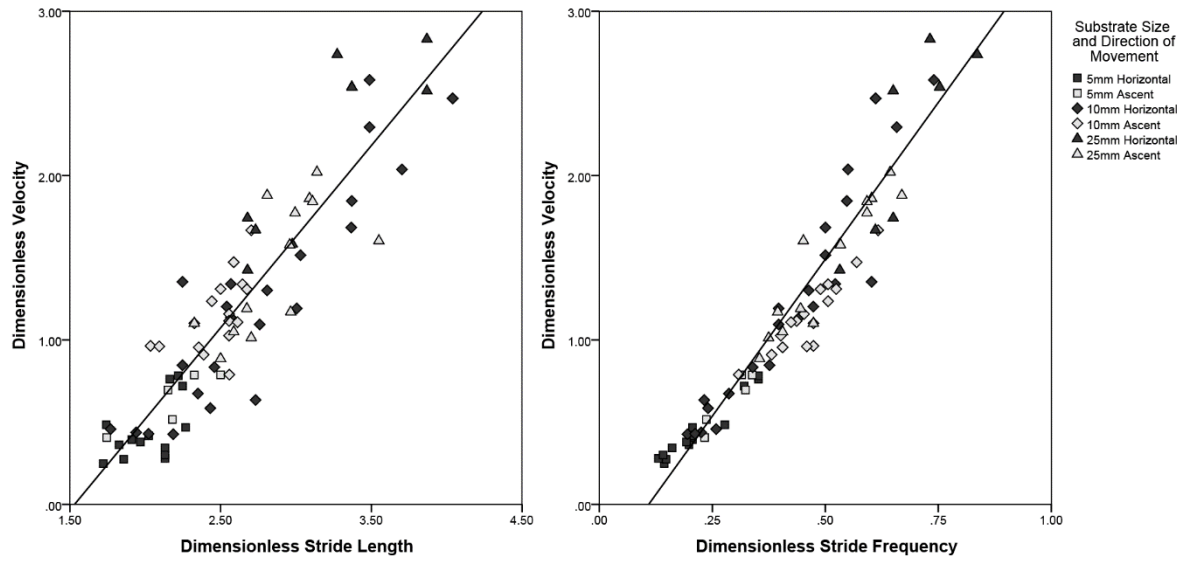


Figure 5.3. Scatterplot of dimensionless velocity (V) first as a function of dimensionless stride length (SL) and then as a function of dimensionless stride frequency (SF). Both symmetrical and asymmetrical gaits are included. Regression lines are significant at $p < 0.05$, with functions $V = -1.66 + 1.09 \cdot SL$ (left graph) and $V = -0.42 + 3.76 \cdot SF$ (right graph).

Gait metrics and their interactions

Diagonality and its correlations to other gait parameters were calculated only for symmetrical gaits (Hildebrand, 1967, 1968, 1976; Cartmill *et al.*, 2002). A breakdown of all recorded parameters is shown in Table 5.1 for symmetrical gaits and Table 5.2 for asymmetrical gaits. Overall diagonality for symmetrical gaits was 34.89 ($n=5$, $SD=6.75$) indicating lateral-sequence diagonal couplets (LSDC) gaits. Mean duty factor was 68.93 ($n=5$, $SD=4.83$) indicating slow walking. The duty factor index (DFI) was 99.45 ($n=5$, $SD=5.92$), implying an equilibrium in the stance phases of the fore- and hind limbs. We found an overall significant covariance between diagonality and duty factor (Fig. 5.2; $N=57$, $F(1,56)=9.225$, $p=0.004$; controlling for substrate size and direction of movement), but not DFI (Fig. 5.2; $N=57$, $F(1,56)=1.287$, $p=0.289$; controlling for substrate size and direction of movement). Overall duty factor for asymmetrical gaits was 56.51 ($n=3$, $SD=4.15$) and DFI was 100.68 ($n=3$, $SD=15.79$).

Our regression model showed that, during horizontal locomotion, stride frequency had a bigger impact ($N=47$, $F(1,45)=755.627$, $R_{\text{part}}=0.386$, $p<0.001$) on velocity than stride length (Fig. 5.3; $N=47$, $F(1,44)=112.869$, $R_{\text{part}}=0.201$, $p<0.001$). The same pattern held in ascents (Stride frequency: $N=81$, $F(1,32)=247.588$, $R_{\text{part}}=0.587$, $p<0.001$;

Stride length: $N=81$, $F(1,31)=261.720$, $R_{\text{part}}=0.320$). Stride frequency and stride length also correlated in both horizontal ($N=81$, $R=0.848$, $p<0.001$) and ascending ($N=81$, $R=0.534$, $p=0.001$) locomotion.

Effects of substrate diameter

Diagonality in symmetrical gaits showed no covariance with substrate size, controlling for the effect of DF (horizontal: $N=26$, $F(2,25)=0.389$, $p=0.535$; ascent; $N=34$, $N=31$, $F(3,30)$, $p=0.108$). DF decreased significantly on larger substrates (Fig. 5.4) during ascents ($N=34$, $F(3,33)=10.405$, $p<0.001$) and horizontal locomotion ($N=47$, $F(2,46)=5.386$, $p=0.026$). DFI did not covary with substrate size (Fig. 5.4) in either horizontal locomotion ($N=47$, $F(2,46)=3.342$, $p=0.086$) or ascents ($N=34$, $F(3,33)=1.689$, $p=0.201$).

Dimensionless velocity increased on larger substrates (Fig. 5.5), in both horizontal ($N=47$, $F(2,46)=26.017$, $p<0.001$) or ascending ($N=34$, $F(3,33)=13.704$, $p<0.001$) locomotion. The same pattern (Fig. 5.5) was observed for stride length (horizontal: $N=47$, $F(2,46)=16.270$, $p<0.001$; ascent: $N=34$, $F(3,33)=15.446$, $p<0.001$) and stride frequency (horizontal: $N=47$, $F(2,46)=34.133$, $p<0.001$; ascent: $N=34$, $F(3,33)=11.113$, $p<0.001$).

Effect of direction of movement

Direction of movement had no effect on D ($F=0.837$, $p=0.365$; controlling for DF and substrate size), DF ($F=0.002$; $p=0.963$; controlling for D and substrate size) or DFI ($F=0.000$, $p=0.994$; controlling for substrate size).

Velocity was higher in horizontal compared to ascending locomotion (BMD=0.238; $N=81$, $F(2,80)=4.293$, $p=0.042$). We obtained a similar result for stride length (BMD=0.233; $N=81$, $F(2,80)=5.261$, $p=0.025$), but there was no effect of direction of movement on stride frequency (BMD=0.019; $N=81$, $F(2,80)=0.440$, $p=0.509$).

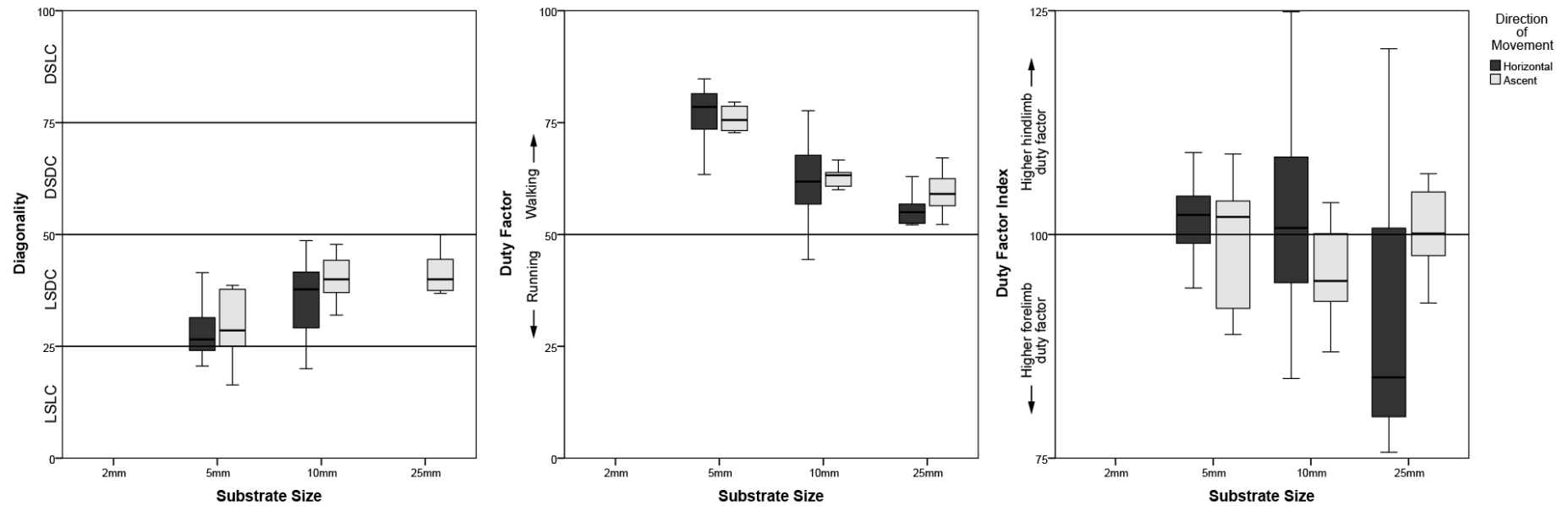


Figure 5.4. Boxplots of the distribution of gaits' diagonality, duty factor and duty factor index in the substrate size categories examined, split into two graphs, one for each direction of movement. The first (diagonality) graph includes only symmetrical gait values. The second (duty factor) and third (duty factor index) graphs include values from both symmetrical and asymmetrical gaits. The horizontal lines in the first graph split the sample in quadrants of decreasing diagonality (DSL_C, DSD_C, LSD_C, LSL_C, from top to bottom). In the last graph, the horizontal line is drawn at a duty factor index of 100, where the duty factors of the forelimbs and the hind limbs are equal.

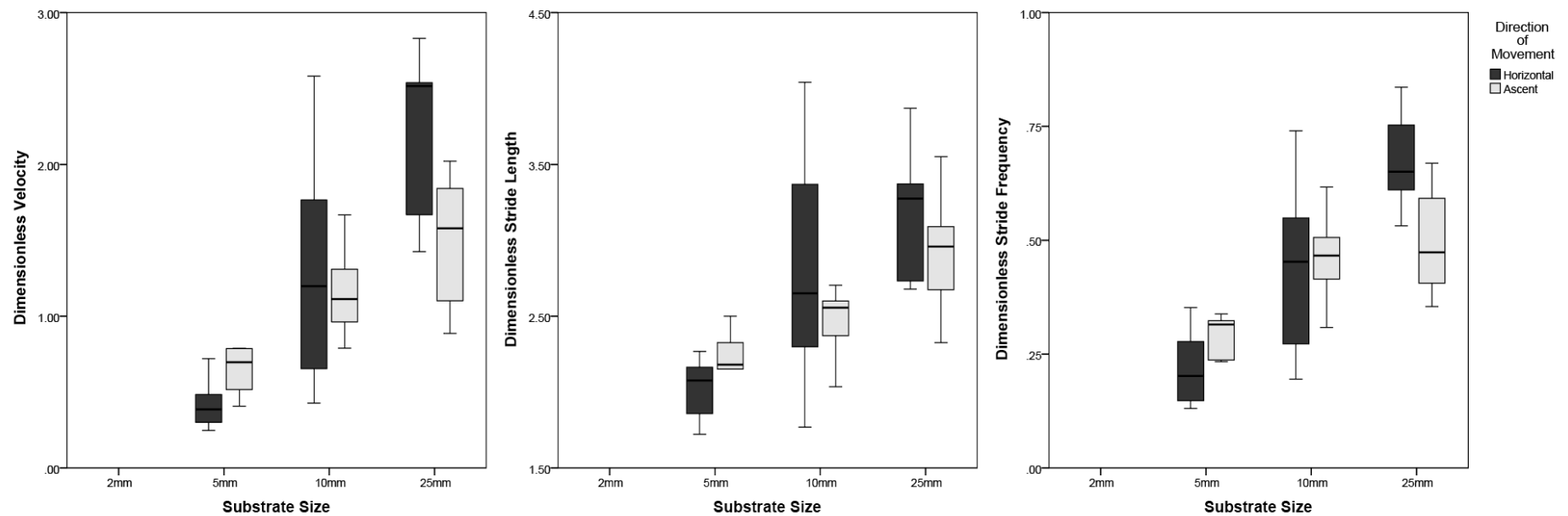


Figure 5.5. Boxplots of the distribution of dimensionless velocity, dimensionless stride length and dimensionless stride frequency in the substrate size categories examined, split into two graphs for each direction of movement. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the top and bottom whiskers represent the maximum and minimum values obtained. Both symmetrical and asymmetrical gaits are included.

Symmetrical and asymmetrical gaits

Half-bounding asymmetrical gaits were characterized by lower DF (BMD=7.545, N=81, $F(2,80)=16.457$, $p<0.001$), similar DFI (BMD=3.556, N=81, $F(2,80)=1.078$, $p=0.301$), and higher velocity (BMD=0.829, N=81, $F(2,80)=41.986$, $P<0.001$), stride frequency (BMD=0.190, N=81, $F(2,80)=32.932$, $p<0.001$) and stride length (BMD=0.649, N=81, $F(2,80)=29.598$, $p<0.001$) than symmetrical walks, controlling for substrate size and direction of movement. Half-bounding became more frequent on larger substrates (Tables 1, 2), and appeared to be the preferred method for faster progression.

5.4. Discussion

Overall, arboreal locomotion of *Thallomys paedulus* was confined to the 5mm, 10mm, 25mm substrates. However, unlike other similarly-sized rodents, acacia rats were not able to utilize the 2mm substrates (Karantanis *et al.*, 2017a). Their symmetrical gaits were characterised by lateral-sequence diagonal couplets (LSDC) gaits, of relatively high duty factor (DF), and balanced fore- and hind limbs DFs, as indicated by the duty factor index (DFI). Diagonality (D) and DFI did not covary with substrate size, though DF, velocity, stride length and stride frequency decreased in smaller substrates. Additionally, the occurrence of asymmetrical gaits increased on larger substrates. D, DF, and DFI remained unaffected by direction of movement, however velocity and stride length, but not stride frequency, significantly decreased in ascents compared to horizontal locomotion. Therefore, our results were not entirely in line with our a priori hypotheses. This first report on the locomotion of acacia rats confirms ecological observations of its skilled and effective use of arboreal substrates. Unfortunately, we failed to record descents on sloped substrates. Nevertheless, this failure hints at the possible importance of vertical climbing in *T. paedulus*, which requires further investigations.

Contrary to our expectations, D decreased on smaller substrates. It has been suggested that D increases (i.e. more diagonal gait) on narrow substrates, enhancing safety by allowing the test of new substrates by the forelimbs, while keeping the contralateral hind limb anchored on a stable substrate (Cartmill *et al.*, 2007). However, observations on a number of arboreal mammals provide equivocal evidence for

(Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Shapiro *et al.*, 2014) and against this conjecture (Cartmill *et al.*, 2007; Lemelin & Cartmill, 2010; Karantanis *et al.*, 2015). Acacia rats were able to negotiate narrow substrates by employing less diagonal gaits in comparison to more diagonal gaits on larger substrates. In this way, it appears that LSDC gaits enhance stability for arboreal mammals without strongly prehensile forelimbs (Lammers & Biknevicius, 2004), in a way analogous to the DSDC gaits of the prehensile-footed primates (Stevens, 2006; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010). Moreover, the overall reduction of relative swing phases (increased DF), along with a decrease in velocity imply cautious walking. This contrasts our predictions for faster gaits on narrow substrates, based on observations on some arboreal neotropical rodents (Camargo *et al.*, 2016). Faster gaits may be favourable for achieving dynamic stability (Schmidt & Fischer, 2011), but there may be other advantages of lower velocity in fine-branch locomotion. Small substrates induce a more mid-sagittal limb placement producing narrower support polygons (Hildebrand, 1976; Cartmill *et al.*, 2002, 2007; Lammers & Zurcher, 2011a). Narrow support polygons amplify the risk of the centre of mass being placed outside, which is further accentuated by the laterally-directed, destabilising torques produced every time a limb touches down (Lammers & Zurcher, 2011b). A lower speed of progression and short aerial phases increase the number of contact points at any part of a gait cycle, and provide more time to counteract the destabilising torques (Lammers & Gauntner, 2008; Lammers & Zurcher, 2011a, 2011b). In this way, these gait adjustments, relative to substrate size, most probably assist acacia rats in negotiating a wide array of substrates.

Regarding direction of movement, we did not observe any significant correlations with most of the examined gait parameters, excluding velocity and stride length, which were lower in ascents. This contrasts previous findings, where DFI often increases and D decreases in ascending locomotion, to assist in generating additional propulsion (Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Karantanis *et al.*, 2015). Considering that the hind limbs carry a higher load and exhibit higher stance ratios than forelimbs in ascents (Preuschoft, 2002), the balanced use of fore- and hind limbs in *T. paedulus* could be related to its limited powerful grasping abilities. In this way both fore- and hind limbs possibly accomplish equally significant roles during ascents (Schmidt, 2011; Schmidt & Fischer, 2011). However, further investigations on grasping ability and kinematics may be more informative on the absence of such differences. On the other

hand, ascending velocity and stride length were lower than those of horizontal locomotion. These adjustments are similar to brown rats (Schmidt & Fischer, 2010, 2011) and some primates (Nakano, 2002). In contrast, European red squirrels, *Sciurus vulgaris*, display higher velocity in ascents. Functionally, reduced velocity during ascent promotes safe progression through static stability, contrary to the dynamic stability obtained through higher velocity (Schmidt & Fischer, 2011). In this way, these adjustments very likely contribute to safe, stable and continuous upward progression.

Thallomys paedulus were able to competently negotiate relatively narrow substrates (5mm). On larger substrates, velocity, stride length and stride frequency gradually increased, as expected. This pattern was further accompanied by a higher occurrence of asymmetrical gaits, which completely replaced symmetrical gaits on the largest substrates (25mm). In *T. paedulus*, asymmetrical half-bounding gaits were characterized by lower DF, similar DFI, and higher velocity, stride frequency and stride length, than symmetrical walks. The extended suspension phases of the limbs (lower DF), brought about by asymmetrical gaits, optimise the ability to avoid obstacles, such as bumps and stubs on a branch, and may enhance energetic economy (Bertram & Gutmann, 2009). Moreover, asymmetrical gaits may contribute to reduced strain on the musculoskeletal system (Farley & Taylor, 1991) or to increased energetic economy at higher speeds (Hoyt & Taylor, 1981; Schilling & Hackert, 2006), in comparison to symmetrical running. For small arboreal mammals, such as *T. paedulus*, asymmetrical gaits may also assist in dynamic stability, by reducing overall peak vertical forces, restraining the movement of the centre of mass within the support polygons, and using shorter and more frequent strides which allows for a greater control of locomotion (Young, 2009; Schmidt & Fischer, 2010; Shapiro *et al.*, 2016). In acacia rats this is further underscored by the regulation of velocity mainly by stride frequency, with a secondary, though significant, role by stride length. This pattern is often observed in other small mammals (Delciellos & Vieira, 2006, 2007; Karantanis *et al.*, 2015; Camargo *et al.*, 2016). On one hand, velocity increase by stride frequency reduces body oscillations in arboreal locomotion, increasing arboreal stability (Strang & Steudel, 1990; Delciellos & Vieira, 2006). On the other hand, it is energetically costly (Reilly *et al.*, 2007) and causes more branch sway compared to velocity regulation by stride length (Demes *et al.*, 1994), which is more common for larger mammals (Larson *et al.*, 2000, 2001). However, body oscillations may be more disrupting than branch sway, for small-bodied arboreal

mammals, rendering velocity increase by stride frequency more advantageous (Strang & Steudel, 1990; Delciellos & Vieira, 2007).

The current study showed that *T. paedulcus* used and adjusted gaits in a way to maximize the effectiveness of locomotion on arboreal substrates. The arboreal capacities of acacia rats are further supported by their morphology, and more precisely, the long tail, covered with long tactile hair, the relatively short limbs, the curved claws on the digits, and the broad hind feet, with short toes and raised pads and a well-developed, long, partially opposable fifth digit (De Graaff, 1978; Reed, 2003). Similar characters have been functionally related to arboreal adaptations in rodents (Krattli, 2001; Zefferer, 2002; Samuels & Van Valkenburgh, 2008; Camargo *et al.*, 2012; Carrizo *et al.*, 2014). The functional interplay between these morphological adaptations and the related gait modifications, allows *T. paedulcus* to successfully negotiate the diversity of sizes and inclinations of the available substrates of Acacia trees. Acacia rats respond to narrow substrates by reducing their velocity and the aerial phase of their strides, and by using more lateral gaits. This strategy, assisted by their partly grasping hind feet (De Graaff, 1978), possibly enables access to fruit, seeds and arthropods, which are often found on terminal branches. When on larger substrates, they maximize their locomotor competence by increasing velocity and aerial phases through asymmetrical half-bounding gaits. As a result, they are able to move swiftly, minimizing exposure and maximizing vigilance for predators (McAdam & Kramer, 1998). Acacia rats are also able to successfully ascend moderately inclined substrates by reducing their velocity and achieving static stability. In this way, they can travel securely upwards from their ground burrows along up to the terminal branches, taking advantage of the inclined branch architecture of Acacia trees (Delany, 1972; Earl & Nel, 1976; De Graaff, 1978; Kingdon, 1984; Mills & Hes, 1997; Dean *et al.*, 1999; Monadjem, 1999; Reed, 2003; Skinner & Chimimba, 2005).

These locomotor adaptations are very likely crucial for the survival of species, such as *T. paedulcus*, which concentrate in scattered tree formations within the dry and nutrient deprived environment of the African savannah. These agglomerations provide high accumulation of nutrients from plant materials and animal waste (Belsky, 1994) and richer water concentration compared to their surroundings (Vetaas, 1992). These features attract a high diversity of savannah animals, which are subject to increased competition (Dean *et al.*, 1999). The morpho-behavioural adaptations enable acacia rats

to exploit the available microhabitats provided by Acacia trees for nesting, protection from predators among the tree thorns, burrowing among roots, and feeding on tree products and arthropods found on or near the trees (Delany, 1972; Earl & Nel, 1976; De Graaff, 1978; Kingdon, 1984; Mills & Hes, 1997; Dean *et al.*, 1999; Monadjem, 1999; Reed, 2003; Skinner & Chimimba, 2005). The successful utilization of their environment underscores the important role of Acacia rats, as prey, parasite hosts (Kingdon, 1984; Linzey & Kesner, 1997) and seed dispersers (Howe & Smallwood, 1982). Consequently, *T. paedulcus* may be a key species for the conservation of Acacia formations, which are of great importance for the African savannah (Lindenmayer & Fischer, 2006). Therefore, it is vital to identify and comprehend the ways acacia rats successfully respond to the challenges imposed by their demanding habitat. The present research provides information on the adaptive significance of behavioural mechanisms of acacia rats and we hope that it will serve as a launch pad for further research on an understudied species with a special ecological role.

Chapter 6: Effects of body size on specialized arboreal glirids: Gaits in *Muscardinus avellanarius* and *Glis glis*

This chapter is under review by Mammal Study.

6.1. Introduction

Throughout mammalian history, a great number of taxa have evolved an arboreal lifestyle (Cartmill, 1974b; Jenkins, 1974). The exploitation of the arboreal milieu, despite its constant risk of lethal falling, provides timely access to diverse resources, minimizes risk from mainly terrestrial predators and enables complex and variable ways of body displacement (Cartmill, 1974b), which ultimately contribute to significant evolutionary advantages, such as a reduction in extrinsic mortality, delay in senescence, and overall increased longevity (Shattuck & Williams, 2010). The diversity of available arboreal substrates (i.e. variable size, inclination, length, fragility, etc.) composes a demanding, discontinuous and unpredictable three-dimensional environment that imposes important and variable constraints to arboreal mammals of different sizes. Larger and heavier animals are challenged in staying on top of substrates and are faced with branch bending or breakage (Grand, 1972). On the other hand, smaller mammals perceive smaller substrates as wider, longer and flatter (Jenkins, 1974), but have difficulty in crossing gaps in the canopy (Cartmill, 1974b).

In order to overcome locomotor challenges, mammals have developed both behavioural and morphological adaptations, which contribute to stability and safety on arboreal substrates (Cartmill, 1974b, 1985). Towards this end, arboreal gaits serve as a behavioural mechanism to promote safe and constant navigation (Cartmill *et al.*, 2002; Lammers & Zurcher, 2011a). These gaits can be either symmetrical, when the limbs of a girdle are evenly spaced in time, or asymmetrical, when they move more concurrently. Both symmetrical and asymmetrical gaits can be described by duty factor (DF) and the duty factor index (DFI). The duty factor (DF) represents the percentage of the total cycle during which a foot (fore or hind) is in contact (in stance phase) with the substrate (Hildebrand, 1967) and separates walks (DF>50) from runs (DF<50). The DFI is the hundredfold ratio of the duty factor of the hind limbs, divided by that of the forelimbs and

expresses the relative contact of the hind limbs ($DFI > 100$) compared to that of the forelimbs ($DFI < 100$) (Cartmill *et al.*, 2007). Symmetrical gaits are further characterised by diagonality (D) or forelimb-hind limb phase (Hildebrand, 1967; Cartmill *et al.*, 2007), which represents the percentage of the stride cycle interval the footfall of a forelimb follows the footfall of the ipsilateral hind limb, and identifies diagonal sequence (DS) gaits ($D > 50$), which involve a contralateral footfall pattern, from lateral sequence (LS) gaits ($D < 50$), which involve ipsilateral footfall patterns. All these parameters are of high interest, as they may reflect behavioural adaptations for locomotion on arboreal substrates (Cartmill *et al.*, 2002, 2007).

Mammals adjust gait parameters according to substrate properties, especially substrate size (Lammers & Biknevicius, 2004; Stevens, 2008; Lemelin & Cartmill, 2010; Nyakatura & Heymann, 2010; Karantanis *et al.*, 2015). Narrower substrates are more prone to bending or breakage (Jenkins, 1974) and impose a more midsagittal limb placement resulting in narrower support polygons, compromising stability (Lammers & Zurcher, 2011a). Arboreal mammals either increase (Lemelin & Cartmill, 2010; Karantanis *et al.*, 2015), decrease (Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Shapiro & Young, 2010; Shapiro *et al.*, 2014), or do not modify (Nyakatura & Heymann, 2010) diagonality upon such narrow substrates. Increased diagonality (i.e. DS gaits) contributes to dynamic stability in substrate navigation, whereas decreased diagonality (i.e. LS gaits) has been functionally linked to static stability in substrate negotiation (Lammers & Zurcher, 2011a). On the other hand, the duty factor index does not seem to be influenced by substrate size (Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Karantanis *et al.*, 2015). Finally, the duty factor usually decreases on larger substrates (i.e. more swing phases) along with an increase in the frequency of asymmetrical gaits (Young, 2009; Shapiro *et al.*, 2016).

It has been proposed that asymmetrical gaits may be preferable over symmetrical gaits during faster locomotion as they confer a reduction in the peak ground reaction forces, decrease the strain on the musculoskeletal system (Farley & Taylor, 1991), and may optimize metabolic costs, at least at the trot-gallop transition (Hoyt & Taylor, 1981). For small arboreal mammals, asymmetrical gaits further confer advantages in stability by limiting centre of mass oscillations (Young, 2009; Schmidt & Fischer, 2011; Shapiro *et al.*, 2016). However, data on the use of asymmetrical gaits during arboreal locomotion in small mammals are limited.

Apart from diagonality, duty factor, and duty factor index, gaits are also characterised by velocity (Delciellos & Vieira, 2006; Camargo *et al.*, 2016), which is a function of stride frequency (i.e. number of strides per second) and stride length (i.e. the distance covered within a single stride) (Alexander, 1992). Arboreal neotropical rodents and metatherians exhibit overall higher velocities than terrestrial species (Delciellos & Vieira, 2006; Camargo *et al.*, 2016) as a mechanism to maintain dynamic stability (Schmidt & Fischer, 2010). Increased velocity during arboreal locomotion can be achieved either by increasing stride frequency and decreasing stride length (Delciellos & Vieira, 2006; Camargo *et al.*, 2016), by increasing primarily stride frequency and, at a lesser rate, stride length (Nyakatura *et al.*, 2008; Karantanis *et al.*, 2015) or by reducing stride frequency while increasing stride length (Delciellos & Vieira, 2006; Camargo *et al.*, 2016). Increasing velocity through increased stride length is often encountered in medium-sized and larger arboreal mammals (Larson *et al.*, 2000, 2001; Delciellos & Vieira, 2006) and enhances safety during arboreal locomotion as the longer reach of the forelimbs minimizes branch sway (Demes *et al.*, 1994). On the other hand, increasing velocity by stride frequency may be better suited for smaller arboreal mammals, which are subject to insignificant branch sway, as it decreases body oscillations at limb touchdowns (Strang & Steudel, 1990; Delciellos & Vieira, 2006).

Regarding arboreal behavioural adaptations, small-sized mammals tend to convergently use similar gaits and kinematics (Biewener, 1989a, 1989b, 1990; McAdam & Kramer, 1998; Fischer *et al.*, 2002; Iriarte-Díaz, 2002). Consequently, the study of arboreal locomotor behaviour in closely related mammals differing in body size may be especially insightful. In this context, we examined arboreal gaits in two dormice (Rodentia: Gliridae), the hazel dormouse (*Muscardinus avellanarius*; Linnaeus, 1758) and the edible or fat dormouse (*Glis glis*; Linnaeus, 1766). *Muscardinus avellanarius* are the smallest European dormice (HBL: 60-90 mm, BW: 15-30 g) (Corbet & Ovenden, 1980) and heavily depend on trees and bushes, except during torpor, when they descend into burrows (Juškaitis, 2008). The hazel dormice are a woodland species, primarily encountered in deciduous or mixed deciduous coniferous forests with a well-developed understory (Juškaitis & Šiožinyte, 2008). *Glis glis*, on the other hand, are the largest dormice [mean HBL: 152 mm, BW: 120-150g to 250 g (prior to hibernation)] (Kryštufek, 2010). Their ecology and microhabitats are similar to those of *M. avellanarius*, but they can be also found in more open or scarcely wooded environments, although in terms of

microhabitat utilization they prefer tree canopies (Kryštufek, 2010). Both species appear well adapted to an arboreal lifestyle possessing relatively long hairy tails, short forelimbs, long tibiae, relatively long manual and pedal digits, well-padded extremities with an abundance of glands, and well-developed claws and relevant flexing tendons which enable effective balancing and climbing on arboreal substrates (Haffner, 1996, 1998; Zefferer, 2002; Storch & Seiffert, 2007; Juškaitis, 2008; Kryštufek, 2010). However, data on substrate use and arboreal locomotor behaviour for these species are lacking. Therefore, considering their morphological adaptations to arboreality, body mass difference and comparable ecologies, we expect differences in their ability to negotiate arboreal substrates of different diameters. For these purposes, we investigated the differential responses of wild-caught specimens of both species on simulated arboreal substrates of different sizes. We expect *M. avellanarius* to be more effective in navigating on narrower substrates compared to *G. glis*. We anticipate that both species would go faster, with significantly shorter stance phases, as substrate size increases, and that they would shift to more asymmetrical gaits on larger substrates. Finally, we hypothesise that velocity would most probably be regulated by stride frequency in both species, but as *G. glis* is larger, we would further expect a greater contribution of stride length to the overall velocity increase.

6.2. Materials and Methods

Specimens

For the purposes of the current study, we tested one male adult hazel dormouse [*Muscardinus avellanarius*, HBL: 87 mm, BW: 17 g, effective hind limb length (Pontzer, 2007a): 23 mm] and one male adult edible dormouse [*Glis glis*, HBL: 178 mm, BW: 132 g, effective hind limb length (Pontzer, 2007a): 55 mm]. Both specimens were originally wild-caught individuals, captured in October 2012 in the Sudety Mts., Southern Poland and were transported to overwinter in specially designed group enclosures at the Adam Mickiewicz University Field Station at Jeziory, Wielkopolski National Park.

In May 2013, we transferred the specimens to the laboratory of the Institute of Environmental Biology of the Faculty of Biology AMU (Poznań), where they were housed in individual temporary enclosures under inverted day-night conditions. Prior to any

experimental procedure, both specimens were habituated to human presence and did not display any stereotypical or other stress-related behaviour. After our tests, the animals were transferred back to the enclosures of the Jeziory Field Station, used for another study, and finally returned to the wild. Unfortunately, because of technical and logistic limitations, we were not able to test more animals.

Experimental setup

A specially configured, filming terrarium (L: 90 cm x H: 40 cm x W: 40 cm) was used for the experiments. Its sides and base were transparent glass windows and was topped by a removable wooden lid. Within the terrarium, we established 2 wooden vertical stands to support the poles. The poles were 80 cm long, cylindrical, semi-hardwood rods. They were marked with vertical blue lines every 1 cm for a reliable estimation of absolute lengths. During the recordings, the visible length of the rod was approximately 30 cm for *M. avellanarius* and 50 cm for *G. glis*. Four different diameter substrates (2 mm, 5 mm, 10 mm and 25 mm) were set horizontally and the animals were allowed to move freely on them.

Data collection was carried out during June 2013. During each recording session, the individuals were transferred from their enclosure to the experimental terrarium. Each individual was allowed free movement within the filming terrarium for habituation. Minimal or no stimulation was required for the subjects to walk on the poles.

For the video recordings we used a Sanyo digital camcorder (VPC-HD 2000, Sanyo, Osaka, Japan) filming at 240 fps, which was positioned at 1 m from the filming terrarium to reduce image distortion. For the analyses, we considered only complete gaits (symmetrical or asymmetrical), initiating with the touchdown of the left hind limb and ending at the subsequent touchdown of the same limb, including both lift offs and touchdowns of all limbs. Cycles involving beginnings or endings of locomotor bouts or loss of balance were discarded, due to irregularities in footfalls (e.g. multiple consequent lift offs and touch downs of the same limb). Overall, only cycles of stable locomotion, regardless of velocity, were retained and regarded as indicative of natural, unbiased behaviour. The present research followed the guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS, 2012) and complied with relevant regulations and legislations of the Adam Mickiewicz University in Poznań and the

relevant legislation of the Aristotle University of Thessaloniki. Handling, housing of animals and behavioural tests were done with permission from the Local Ethical Commission for the Animal Experiments in Poznań.

Gait analyses

Video analysis and data collection, distance and time calculations were made by importing videos and calibrating time and distance measurements using Tracker 4.87 (Brown, 2009). Microsoft Excel 2010 (Redmond, WA, USA), and SPSS 20 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

For the analyses, we considered the following gait parameters:

- (i) Diagonality (D) (Cartmill *et al.*, 2007), the percentage of the stride cycle interval the footfall of a forelimb follows behind the ipsilateral hind limb. Although it was measured as a scale variable, it was also divided into five ordinal classes (following Cartmill *et al.*, 2002): (a) Lateral Sequence Lateral Couplets ($0 \leq \text{LSLC} < 25$), (b) Lateral Sequence Diagonal Couplets ($25 \leq \text{LSDC} < 50$), (c) Trot ($= 50$), (d) Diagonal Sequence Diagonal Couplets ($50 < \text{DSDC} \leq 75$), (e) Diagonal Sequence Lateral Couplets ($75 < \text{DSLC} \leq 100$);
- (ii) Duty Factor (DF), the mean of duty factors of all limbs, defined as the percentage of a cycle during which a limb is in contact with the substrate (a gait cycle encompasses a sequence of fore- and hind limb swing (moving) and stance (contact) phases that occur within a stride period, beginning and ending with the touchdown of a reference hind limb, often the left);
- (iii) Duty Factor Index (DFI), the ratio of forelimb duty factor (DF_f) and hind limb duty factor (DF_h), calculated as $100 * \text{DF}_h / \text{DF}_f$ (Cartmill *et al.*, 2007). Values > 100 indicate longer hind limb than forelimb stance durations, whereas values < 100 specify shorter hind limb than forelimb stance durations;
- (iv) Stride Duration (t), total duration of a single stride in seconds, measured from the frame where a stride cycle began until the frame the same stride cycle ended;
- (v) Stride Length (l), the corresponding distance covered during a single stride cycle, in meters;
- (vi) Velocity (v), the speed in which the subjects moved, calculated by dividing stride length with stride duration, and measured in meters/second (m/s);

(vii) Stride Frequency (f), the number of strides per second;

As these parameters are size-dependent, we used the effective hind limb length, as in the length of the hind limb at a strut (Pontzer, 2007a), of each specimen to calculate the dimensionless measures of stride duration, stride length, velocity and stride frequency. These calibrated absolute measurements are useful for estimating efficiency during locomotion (Alexander, 1977; Alexander & Jayes, 1983; Hof, 1996):

$$\text{Dimensionless Stride Duration (tD)} = \frac{t}{\sqrt{\frac{l_0}{g}}}$$

$$\text{Dimensionless Stride Length (lD)} = \frac{l}{l_0}$$

$$\text{Dimensionless Velocity (vD)} = \frac{v}{\sqrt{g \times l_0}}$$

$$\text{Dimensionless Stride Frequency (fD)} = \frac{f}{\sqrt{\frac{g}{l_0}}}$$

where l_0 is effective hind limb length of each animal and g is the acceleration of gravity ($g=9.81 \text{ m/s}^2$).

Analyses of covariance (ANCOVA) were selected in order to explore the relationships between variables, including individual as a random factor, while controlling for other possible covariates. When needed, a Bonferroni Post-Hoc test (BMD) was used for pair-wise category comparisons, using estimates of means, as calculated by the ANCOVA. Stepwise regression models were constructed to examine the impact of both stride frequency and stride length on velocity, using their dimensionless counterparts. The impact of each parameter was calculated using the partial correlation coefficient, i.e. the correlation between a dependent variable and its covariate, after the impact of other covariates is removed (Harrell, 2001).

Table 6.1. Summary of means and standard deviations (in brackets) of gait statistics for *Muscardinus avellanarius* and *Glis glis* (Gliridae: Rodentia) (N indicates the number of analysed gait cycles for each substrate size).

Species	Substrate size	N	Diagonality	Duty Factor	Hindlimb Duty Factor	Forelimb Duty Factor	Duty Factor Index	Velocity (m s ⁻¹)	Stride Length (m)	Stride Frequency (s ⁻¹)	Stride Duration (s)	Dimensionless Velocity	Dimensionless Stride Length	Dimensionless Stride Frequency	Dimensionless Stride Duration
<i>M. avellanarius</i>	2mm	10	36.74 (8.45)	66.48 (8.43)	64.25 (7.90)	68.71 (9.25)	93.76 (4.90)	0.491 (0.251)	0.055 (0.010)	8.401 (3.274)	0.145 (0.081)	1.033 (0.529)	2.400 (0.419)	0.407 (0.159)	3.001 (1.665)
	5mm	10	-	39.06 (3.16)	40.87 (4.61)	37.26 (5.80)	112.51 (23.19)	1.095 (0.186)	0.081 (0.009)	13.441 (1.269)	0.075 (0.007)	2.306 (0.391)	3.535 (0.402)	0.651 (0.061)	1.549 (0.146)
	10mm	12	-	35.22 (4.57)	37.30 (3.40)	33.13 (8.84)	119.50 (31.84)	1.237 (0.223)	0.091 (0.015)	13.571 (1.365)	0.074 (0.008)	2.605 (0.469)	3.978 (0.666)	0.657 (0.066)	1.537 (0.165)
	25mm	6	-	35.56 (4.44)	35.99 (4.23)	35.13 (9.74)	110.28 (36.61)	1.167 (0.201)	0.084 (0.022)	14.297 (1.887)	0.071 (0.010)	2.457 (0.423)	3.645 (0.977)	0.692 (0.091)	1.466 (0.201)
	Overall		-	44.08 (5.15)	44.60 (5.04)	43.56 (8.41)	109.01 (24.13)	0.998 (0.215)	0.078 (0.014)	12.427 (1.949)	0.091 (0.026)	2.100 (0.453)	3.390 (0.616)	0.602 (0.094)	1.888 (0.544)
<i>G. glis</i>	10mm	22	-	58.04 (3.85)	60.62 (3.65)	55.46 (6.39)	110.68 (14.73)	0.492 (0.254)	0.100 (0.013)	4.705 (2.175)	0.325 (0.263)	0.670 (0.346)	1.826 (0.231)	0.352 (0.163)	4.345 (3.509)
	25mm	7	-	54.19 (8.52)	56.46 (6.13)	51.93 (11.51)	111.10 (13.96)	0.673 (0.168)	0.111 (0.022)	6.014 (0.639)	0.168 (0.019)	0.916 (0.229)	2.018 (0.405)	0.450 (0.048)	2.244 (0.252)
	Overall		-	56.12 (6.18)	58.54 (4.89)	53.70 (8.95)	110.89 (14.35)	0.582 (0.211)	0.106 (0.017)	5.359 (1.407)	0.247 (0.141)	0.793 (0.287)	1.922 (0.318)	0.401 (0.105)	3.295 (1.881)

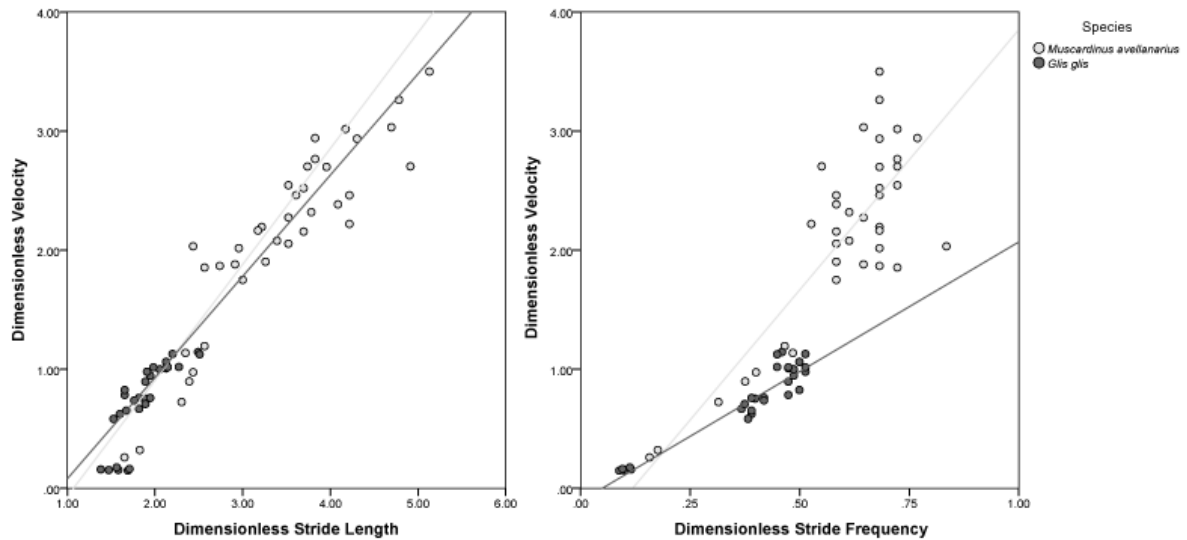


Figure 6.1. Scatterplots of dimensionless velocity (V) first as a function of dimensionless stride length (SL) and then as a function of dimensionless stride frequency (SF). The regression lines on both graphs are significant at $p < 0.05$. Their functions are, for the left graph: *M. avellanarius*: $V = -0.75 + 0.84 \cdot SL$, *G. glis*: $V = -1.07 + 0.96 \cdot SL$; for the right graph: *M. avellanarius*: $V = -0.47 + 4.3 \cdot SF$, *G. glis*: $V = -0.08 + 2.14 \cdot SF$.

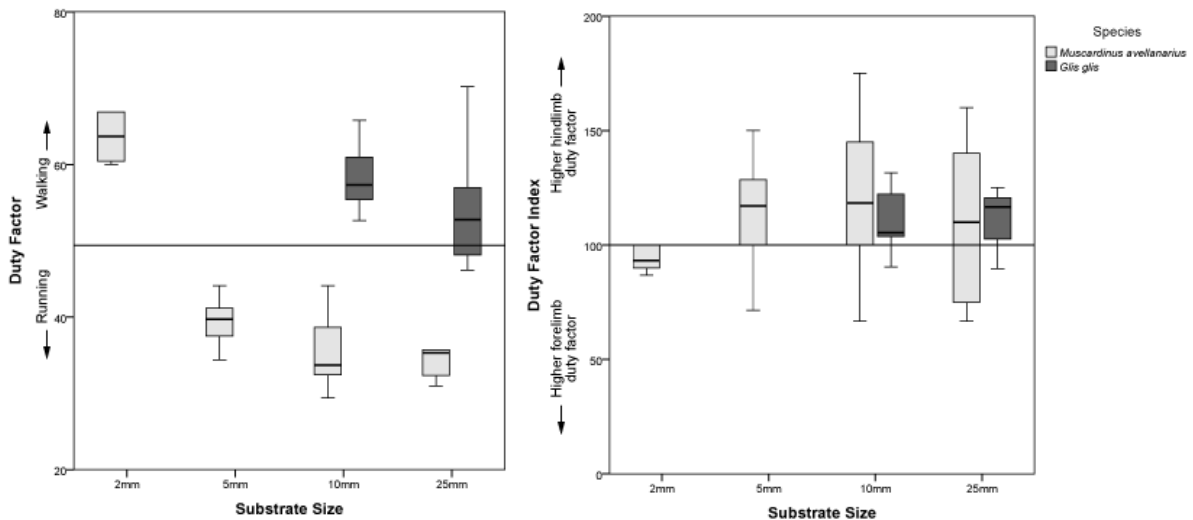


Figure 6.2. Boxplots of the distribution of gaits' DF and DFI in the substrate size categories examined. In the DF graph, a horizontal line is drawn at $DF = 50$, at which the stance and swing phases are equal. In the DFI graph, the horizontal line is drawn at a DFI value of 100, where the duty factors of the forelimbs and the hind limbs are equal. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the top and bottom whiskers represent the maximum and minimum values obtained.

6.3. Results

Muscardinus avellanarius used all provided substrate size categories (N=38 gait cycles), whereas *G. glis* was able to navigate only on the 10 mm and 25 mm substrates (N=29 gait cycles). Only the hazel dormouse used symmetrical gaits and exclusively on the 2 mm category, excluding any comparisons thereof between species. All other gaits for both species were asymmetrical, and all were half-bounds, in which the hind limbs touched down simultaneously, while one forelimb lagged behind the other.

Gait metrics, their interaction, and comparisons between specimens

The symmetrical gaits of *M. avellanarius* on 2 mm substrates scored an overall diagonality (D) of 36.74 (N=10, SD=8.45), with moderately high duty factors (DF) (Mean=66.48, SD=8.43), and higher forelimb than hind limb contact times (Mean=93.76, SD=24.13). These gaits fall in the lateral-sequence diagonal couplet (LSDC) walks category. Otherwise, all the other gaits of the hazel dormouse were asymmetrical half-bounds, with increased aerial phases (Table 6.1). Overall the DF was 44.08 (SD=5.15), implying frequent total body aerial phases among recorded gaits and DFI was 109.01 (SD=24.13), suggesting increased hind limb contact times compared to those of the forelimbs.

No symmetrical gaits were recorded for *G. glis*, which was also unable to move on the smaller substrate categories (2mm, 5mm). In the remaining categories (10mm, 25mm), all gaits were half-bounds, with mean DF=56.12 (SD=6.18) and mean DFI=110.89 (SD=14.35). Although the edible dormouse used gaits with whole-body aerial phases on the 25mm substrates, its locomotion was not overall characterised by them.

Duty factor was significantly lower in *M. avellanarius* than in *G. glis* (Table 6.1; N=67, BMD=21.284, $F_{(1,66)}=94.784$, $p<0.001$; controlling for substrate size), hence aerial phases were more prolonged and more frequent in the hazel dormouse than in the edible dormouse. However, when DF was controlled for dimensionless velocity, there were no differences between the two species (N=67, BMD=1.649, $F_{(1,66)}=0.402$, $p=0.529$). This likely indicates that, if they moved with the same velocity on proportionally similar substrates, their stance phases relative to swing phases would be very similar. On the

other hand, there were no significant differences in DFI between the two species (N=67, BMD=4.135, $F_{(1,66)}=0.463$, $p=0.499$; controlling for substrate size), implying a comparable use of forelimbs and hind limbs.

Muscardinus avellanarius displayed a higher overall dimensionless velocity (N=67, BMD=1.785, $F_{(1,66)}=175.437$, $p<0.001$), dimensionless stride length (N=67, BMD=1.919, $F_{(1,66)}=152.374$, $p<0.001$) and dimensionless stride frequency (N=67, BMD=0.301, $F_{(1,66)}=70.841$, $p<0.001$) than *G. glis*, when controlling for the effect of substrate size. When dimensionless velocity was added as a covariate, the applied ANCOVA displayed no significant differences between the two species in either dimensionless stride frequency (N=67, BMD=-0.037, $F_{(1,66)}=0.562$, $p=0.456$), or dimensionless stride length (N=67, BMD=0.163, $F_{(1,66)}=1.061$, $p=0.307$). These results suggest that if the two specimens moved at similar velocities, dimensionless stride frequency and stride length would be very similar.

In *M. avellanarius*, velocity was regulated primarily by stride frequency (N=38, $F_{(1,37)}=183.166$, $R_{\text{part}}=0.549$, $p<0.001$) followed by stride length (N=38, $F_{(1,37)}=288.752$, $R_{\text{part}}=0.383$, $p<0.001$) (Fig. 6.1). In *G. glis*, variation in velocity was also explained mainly by stride frequency (N=29, $F_{(1,28)}=268.605$, $R_{\text{part}}=0.568$, $p<0.001$) and less, though significantly also by stride length (N=29, $F_{(1,28)}=489.654$, $R_{\text{part}}=0.295$, $p<0.001$), suggesting a strikingly similar pattern in the two specimens.

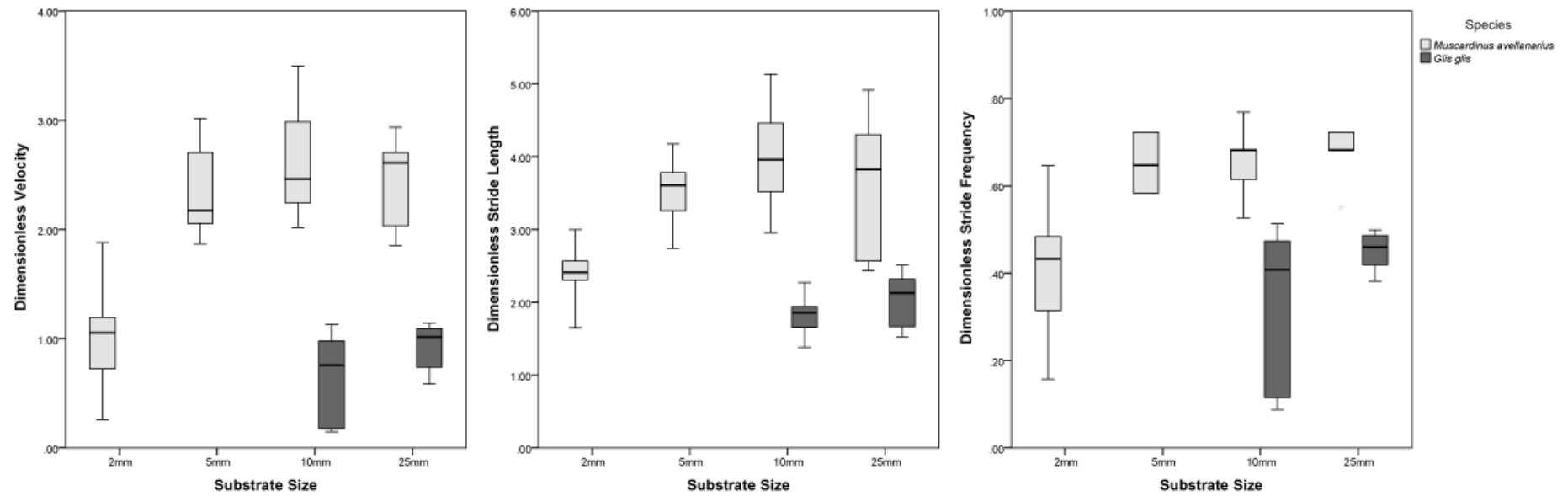


Figure 6.3. Boxplots of the distribution of dimensionless velocity, dimensionless stride length and dimensionless stride frequency in the substrate size categories examined. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the top and bottom whiskers represent the maximum and minimum values obtained.

The effect of substrate size

In the hazel dormouse, DF decreased significantly on smaller substrates (Fig. 6.2; $N=38$, $F_{(3,37)}=71.142$, $p<0.001$), but substrate size had no impact on DFI (Fig. 6.2; $N=38$, $F_{(3,37)}=1.866$, $p=0.154$). On the other hand, substrate size had no impact on DF ($N=29$, $F_{(1,28)}=2.848$, $p=0.103$) or DFI ($N=29$, $F_{(1,28)}=0.005$, $p=0.947$) in *G. glis*. However, the overall lack of statistical significance should be treated with caution, considering that observations in the edible dormouse derived from only two substrate sizes, one of which had a low sample size (Table 6.1).

In *M. avellanarius*, velocity ($N=38$, $F_{(3,37)}=24.560$, $p<0.001$), stride length ($N=38$, $F_{(3,37)}=13.000$, $p<0.001$) and stride frequency ($N=38$, $F_{(3,37)}=15.822$, $p<0.000$) increased significantly on larger substrates (Fig. 6.3). This was not the case in *G. glis* (Fig. 6.3; Velocity: $N=29$, $F_{(1,28)}=3.065$, $p=0.091$; Stride length: $N=29$, $F_{(1,28)}=2.524$, $p=0.124$; Stride frequency: $N=29$, $F_{(1,28)}=2.412$, $p=0.132$). Nevertheless, as in DF, the low sample for the edible dormouse may be responsible for possible false-negative statistical results (Fig. 6.3).

6.4. Discussion

The examination of only one specimen from each species imposes significant restrictions on the present research, especially regarding the statistical significance of trends and differences of the examined variables between species. This may lead to less robust conclusions, and therefore, our results should be treated with caution. However, given the lack of existing data on the locomotion of these dormice species, this study aims to serve as a first report providing important quantitative data, rather than a thorough and conclusive analysis. Restrictions aside, the current study identified some trends that provide first insights into the differences and similarities between these two specimens. The small *M. avellanarius* was able to use the smallest substrates provided (2 mm), whereas the much larger *G. glis* failed to move on the 2 mm and 5 mm substrates. Moreover, *M. avellanarius* moved significantly faster on larger substrates and showed increased aerial phases. However no similar trends were found for *G. glis*, although this may be due to sample size limitations. On the other hand, both species were similar in

using primarily asymmetrical gaits (although *M. avellanarius* used symmetrical gaits in the narrowest substrates) and in mean gait metrics.

Despite an overall preference for asymmetrical gaits, *M. avellanarius* exclusively used lateral-sequence diagonal couplet (LSDC) symmetrical gaits on 2 mm substrates, with a high DF (i.e. shorter swing phases relative to stance phases), and lower dimensionless velocity compared to larger substrates. These LSDC slow walks in which the limbs are anchored on the substrate for extensive parts of the gait cycle, assist in enhancing static stability by securing the placement of the centre of mass within the support polygons (Lammers & Zurcher, 2011a). This is critical in a fine-branch setting, where support polygons are especially narrow (Cartmill *et al.*, 2007; Lammers & Zurcher, 2011a). Interestingly, *G. glis* used exclusively asymmetrical gaits, even on the comparably narrowest substrates (10mm) for its size. Comparable exclusive use of asymmetrical gaits is also common in other arboreal rodents, such as squirrels (Flaherty *et al.*, 2010; Schmidt & Fischer, 2011).

On larger substrates, *M. avellanarius* used exclusively asymmetrical half-bounding gaits. These asymmetrical gaits were characterised by a low duty factor ($DF < 50$) which translates into extended whole-body aerial phases during a stride cycle. Additionally, velocity, stride length and stride frequency of the hazel dormouse progressively increased on larger substrates. *Glis glis* displayed analogous increases in these parameters on larger substrates, albeit not statistically significant, yet these were significantly lower than those of the hazel dormouse. Moreover, the asymmetrical gaits of the edible dormouse exhibited a high duty factor ($DF > 50$) indicating the lack of whole-body aerial phases. This is not uncommon, as, at such relatively low velocities, asymmetrical gaits are often characterised by relatively long stance phases (Schmidt & Fischer, 2011). Overall, asymmetrical gaits may be of great importance for improving arboreal locomotion (Flaherty *et al.*, 2010). Compared to symmetrical gaits, the asymmetrical ones assist in obtaining higher velocities (Hildebrand, 1977; Gasc, 2001; Young, 2009; Shapiro *et al.*, 2016) and may contribute to a decrease in peak substrate reaction forces, strain reduction on the musculoskeletal system, (Farley & Taylor, 1991), and the optimisation of metabolic costs (Hoyt & Taylor, 1981).

In both species, stride frequency was the major contributor of velocity regulation, whereas stride length had a less important, although statistically significant, role. This finding basically supports our predictions for both species, even though we expected a

higher stride length contribution in *G. glis*, due to its larger size. This way of velocity increase is advantageous in asymmetrical gaits as it enhances stability, manoeuvrability and acceleration (Shapiro *et al.*, 2016). This pattern differs from that of most arboreal primates, larger arboreal marsupials (Larson *et al.*, 2001; Delciellos & Vieira, 2006), and some arboreal and terrestrial neotropical rodents (Camargo *et al.*, 2016), which increase velocity by increasing stride length, instead of frequency. However, it is similar to some scansorial murid and arvicoline rodents (Karantanis *et al.*, 2017a), a few small-bodied arboreal marsupials (Delciellos & Vieira, 2006; Karantanis *et al.*, 2015), and cotton-top tamarins (Nyakatura *et al.*, 2008). Although, a stride length increase allows for a longer reach, and may reduce involuntary branch swaying (Demes *et al.*, 1994), stride frequency increase may be more appropriate for small-bodied arboreal mammals, which are subject to disrupting body oscillations, and not branch sway (Delciellos & Vieira, 2006). Body oscillations produce moments which interrupt continuous locomotion and can cause loss of stability, but frequent strides reduce involuntary body oscillations and contribute to required stability and agility for successful branch navigation (Delciellos & Vieira, 2006).

Essentially, *G. glis* and *M. avellanarius* differed in most gait metrics, as the latter utilised symmetrical gaits on narrow substrates, was overall faster, and used asymmetrical gaits with an aerial phase. These differences may be related to the lower mass of the hazel dormouse (17 g), compared to that of the edible dormouse (132 g). Body mass does bear a significant impact on gait metrics, such as velocity (Alexander, 1977; Heglund & Taylor, 1988), stride frequency (Heglund, Taylor & McMahon, 1974; Heglund & Taylor, 1988), and stride length (Heglund & Taylor, 1988). Furthermore, body mass also relates to differential substrate use, as a small mass reduces the possibility for smaller branches to bend or break, decreases the body size-branch size ratio, lowers the centre of mass increasing stability, and allows movement through more cluttered habitats (Cartmill, 1985). In this context, the present results, despite their limitations, provide some insight into the ecological interactions between *M. avellanarius* and *G. glis*. Both species are often encountered syntopically, where they exploit similar food sources and use similar nesting sites (Juškaitis, 2002, 2008; Juškaitis & Šiožinyte, 2008; Kryštufek, 2010), probably engaging in significant interspecific competition. The larger size of *G. glis* and its ability to move more cautiously on larger substrates, likely enables edible dormice to exploit successfully the architectural network of mature forests and top canopy layers (Juškaitis & Šiožinyte, 2008). This may render edible dormice better competitors against

M. avellanarius (Smith & Brown, 1986), compelling the latter to exploit different microhabitats and confine their activities in the shrubby layer of the understory, characterised by a lush network of finer branches (Juškaitis & Šiožinyte, 2008). The small size of hazel dormice, along with the LSDC symmetrical walks, higher velocities and whole-body aerial phase asymmetrical gait adaptations, would promote the effective and successful use of this environment. This would ultimately enable the two species to diminish interspecific competition by partial separation of microhabitat niches.

Even though the present report provides some preliminary results and information on *G. glis* and *M. avellanarius*, the statistical limitations of this study do not allow for definitive conclusions, or species-wide generalisations. However, the obtained results hint at possible interactions between body size and arboreal gaits, and their consequent effect on mammalian locomotor ecology. More extensive research is required for elucidating the effects of body size on gaits and their relation to the locomotor preferences of the two species. We hope that the current study serves as a platform for further investigations towards this end.

Chapter 7: Gaits in an arboreal specialist

Diagonal gaits in the feathertail glider *Acrobates pygmaeus* (Acrobatidae, Diprotodontia): Insights for the evolution of primate quadrupedalism

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7.1. Introduction

Understanding the origin of primates has always been one of the major research goals of biological anthropology. The quest for these origins has focused on the ecological and behavioural correlates of the unique morphological package of the order, primarily concerning the grasping nailed feet and hands, and orbital convergence (Cartmill, 1974a, 1974b, 1992). Regarding the adaptive significance of these evolutionary novelties, research on primate origins has revolved around arboreality, and more especially the habitual use of the fine-branch niche, whether it be foraging for arthropods, fleshy fruit or both (Cartmill, 1974a, 1974b; Szalay & Dagosto, 1980; Dagosto, 1988, 2007; Sussman, 1991; Rasmussen & Sussman, 2007). Whatever the case, negotiation of such small branches requires a small body size and, apart from Soligo and Martin's (2006) scenario, in which a larger body size was proposed as the basis for early primate adaptations, a small ancestral body size has been emphasized as part of a series of stages leading to primate evolution (Cartmill, 1974a; Larson *et al.*, 2000; Gebo, 2004; Sargis, 2007). More particularly, these stages include (a) a scansorial non-grasping clawed small mammal, (b) an arboreal, clawed mammal with non-opposable 'non-powerful' pedal grasping, (c) an arboreal clawed mammal with a clawless hallux, capable of opposable 'powerful' pedal grasping that facilitates the efficient use of terminal branches, and (d) a true arboreal primate with nails on all digits and grasping feet and hands (Gebo, 2004; Sargis, 2007). Stages b and c are of primary importance in the evolutionary history of primates as they attempt to reconstruct the acquisition of grasping nailed extremities and primate-like quadrupedal gait mechanics, and recent investigations have used extant analogs, mainly

marsupials, to model them (i.e. Rasmussen & Sussman, 1990; Cartmill, 1992; Lemelin, 1999; Schmitt & Lemelin, 2002; Lemelin, Schmitt & Cartmill, 2003; Youlatos, 2008; Shapiro, Young & VandeBerg, 2014).

In this context, woolly opossums (*Caluromys philander*) have been extensively used as models due to their primate-like gait, limb kinematics, morphological (long grasping digits, clawless hallux), as well as ecological and behavioural similarities to primates (Schmitt & Lemelin, 2002; Lemelin *et al.*, 2003; Cartmill *et al.*, 2007; Youlatos, 2008, 2010). However, woolly opossums are comparably large, weighing between 200-400 g, contrasting predictions of small body masses (at least < 100 g) in early primate ancestors. Another marsupial analogue is the sugar glider (*Petaurus breviceps*), which also bears ecological, morphological and behavioural similarities to the alleged early stages of primate evolution [see Shapiro & Young (2010) for a summary of morphological and eco-behavioural adaptations of sugar gliders], but, albeit smaller than *Caluromys*, they too are comparably large, ranging between 90 and 160 g. On the other hand, the use of juvenile subjects of *P. breviceps* and gray short-tailed opossums, *Monodelphis domestica*, for testing small body size effects on gait kinematics over narrow substrates (Shapiro *et al.*, 2014), is hindered by the effect of ontogeny on locomotion, which has proven to be substantial across a great number of taxa (Altman & Sudarshan, 1975; Peters, 1983; Berkum *et al.*, 1989; Carrier, 1996; Doran, 1997; Eilam, 1997; Irschick, 2000; Wells & Turnquist, 2001; Niemitz, 2002; Shapiro & Raichlen, 2006; Young, 2012), with its effects surpassing those predicted by body size (Vilensky, Gankiewicz & Townsend, 1990). As small size appears to be of vital importance in inferring early primate behavioural and morphological adaptations, other small mammals, such as shrews (Gebo, 2004) and treeshrews (Jenkins, 1974; Sargis, 2001) have been also proposed as models for early primate evolutionary stages. However, shrews are far from typically arboreal, with only a few species classified as scansorial (Churchfield, 1990), and the Tupaiidae do not seem suitable candidates, due to their increased terrestriality and low maternal care (Emmons, 2000). Recently, Eurasian harvest mice *Micromys minutus*, with a body mass at 10 g, flexible locomotion, foot grasping skills, and extensive use of fine branches were suggested as models for finer scaled evolutionary stages (Urbani & Youlatos, 2013), underscoring the significance of tiny size during early primate evolution.

Feathertail gliders *Acrobates pygmaeus* (Fig. 7.1) weigh between 10 and 15 g and are among the smallest extant marsupials (Nowak, 1999). They are primarily nocturnal arborealists, feeding and foraging mainly on arthropods, fruit, honeydew and pollen (Goldingay & Kavanagh, 1995). Feathertail gliders possess an opposable clawless hallux, capable of efficient grasping, and volar pads with extensive sweat gland complexes in all digits that enable increased adhesion on all types of surfaces, even glass panes (Rosenberg & Rose, 1999). These adaptations facilitate the use of both tree trunks and the finer branches of upper foliage and lower shrubs of subtropical, temperate and mature woodlands in their habitats in eastern Australia (Ward & Woodside, 2008). Furthermore, *A. pygmaeus* exhibits slow growth, and high maternal investment, which continues after weaning (Ward, 1990). All these morpho-behavioural features could fit well within suggested scenarios of early primate evolution (Cartmill, 1974a, 1974b; Sussman, 1991) and could render feathertail gliders a useful extant model to test some of the unique aspects of primate arboreal quadrupedalism (such as the frequent use of diagonal-sequence [DS] gaits, ultimately related to fine branch exploitation).

The frequent use of DS gaits is a general trait of primates, with only a few exceptions (Hildebrand, 1967; Cartmill *et al.*, 2002, 2007; Shapiro & Raichlen, 2005; Nyakatura & Heymann, 2010). Primates commonly use DS gaits, in which the forelimbs touch down soon after the contralateral hind limbs, while most other mammals commonly use lateral sequence (LS) gaits, in which the forelimbs land right after their ipsilateral hind limbs (Hildebrand, 1967). Diagonal sequence gaits have likely evolved to promote inspection of new unknown and unstable substrates, while moving on terminal branches (Cartmill *et al.*, 2007). This is partially supported by the common use of DS gaits by some arboreal marsupials (White, 1990; Pridmore, 1994; Cartmill *et al.*, 2002; Schmitt & Lemelin, 2002), and kinkajous (Lemelin & Cartmill, 2010). On the other hand, Vilensky and Larson (1989) proposed that gait selection is not related to locomotor stability, and the dominance of DS gaits in primates is a by-product of neurological reorganizations related to forelimb dexterity. The use of DS or LS gaits is, nevertheless, influenced by substrate properties, but if DS gaits were related to unstable substrate use, as Cartmill *et al.* (2007) have proposed, they should be more frequent on narrower and upwards or downwards inclined substrates. However, findings are conflicting. Most strepsirrhines do not exhibit a higher rate of DS gaits on narrower substrates (Stevens, 2008), and *Petaurus breviceps* does not increase frequencies of DS gaits with decreasing substrate size

(Shapiro & Young, 2010). Furthermore, no correlation between substrate size and DS gait use was detected in free-ranging *Saguinus mystax*, and DS gaits were less common on smaller substrates in *Saguinus fuscicollis* (Nyakatura & Heymann, 2010). Regarding substrate inclination, past research on a number of arboreal mammals has shown that ascents are characterized by more frequent use of DS gaits than descents (Prost & Sussman, 1969; Lammers, 2007; Nyakatura *et al.*, 2008; Nyakatura & Heymann, 2010; Shapiro & Young, 2010; Shapiro *et al.*, 2014). It has thus been argued that DS gaits reduce the retarding effect of the forelimbs during ascents (Nyakatura *et al.*, 2007), whereas LS gaits allow the forelimbs to provide retardation through a “stop-jolt” before the hind limbs contact the substrate (Rollinson & Martin, 1981). These findings indicate that there is a link between diagonality and the control of locomotion over substrate inclination and size.

Another feature unique to primate arboreal quadrupedalism is that primates control locomotion on fine arboreal substrates by relative stride length to a greater extent than stride frequency (Larson *et al.*, 2000, 2001). Increased stride length, rather than stride frequency, is regularly employed by primates to reach higher velocities without increasing involuntary branch sway (Demes, Jungers & Nieschalk, 1990). In contrast, most other arboreal mammals, marsupials included, tend to increase their velocity by higher stride frequencies, possibly reducing body oscillations, despite its relatively high energetic cost (Strang & Steudel, 1990). These different strategies, strongly related to the mechanically and energetically efficient use of small and unstable branches, are important for understanding the adaptive significance of early primate quadrupedal adaptations.

All these assumptions on the putative adaptive significance of primate-like quadrupedal gaits and their interplay with specific features of habitat structure have derived from observations on extant euprimates and tests on relatively large-sized extant analogues. However, body mass plays an essential role in the interactions between an animal and its environment and is considered significant during early primate evolution. Therefore, testing subjects of small adult body masses would provide a more realistic view of the mechanisms that likely promoted similar adaptations during early primate evolution. In this context, the current study aims to test whether early arboreal primate ancestors with a clawless grasping hallux, as modeled by the small-sized *A. pygmaeus*, could demonstrate key features related to primate-like arboreal quadrupedalism. More

specifically we aim to examine whether feathertail gliders would demonstrate an overall frequent use of DS gaits, an increased use of DS gaits with decreasing substrate size and ascending locomotion, and velocity regulation by either stride length or stride frequency and to what extent, and whether, such features could be linked to small body size quadrupedalism on fine branches.

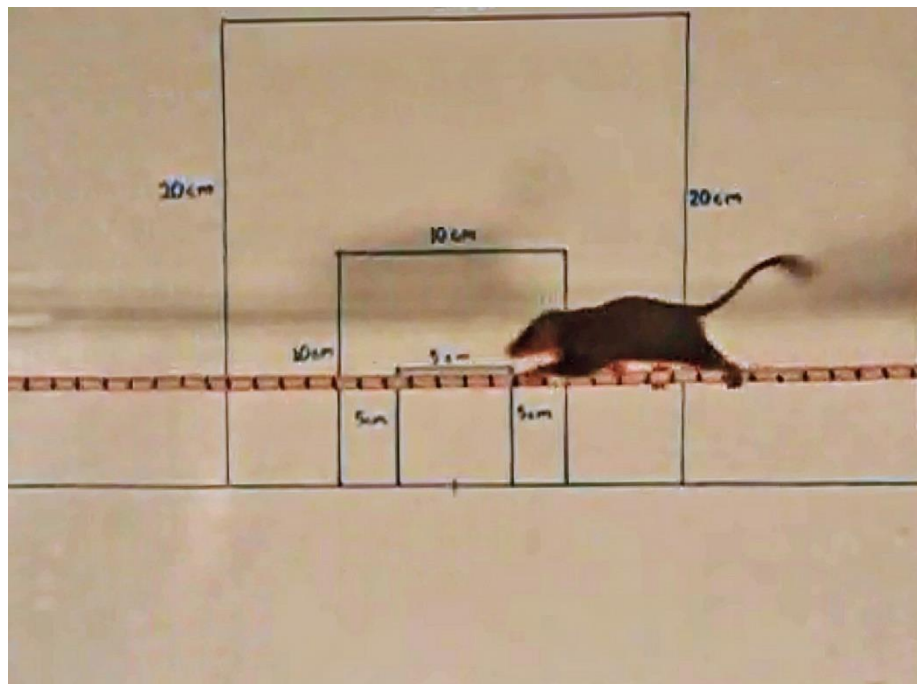


Figure 7.1. Feathertail glider (*Acrobates pygmaeus*) walking diagonally on a 2 mm pole (the pole is marked with vertical blue lines per 1 cm for a reliable estimation of absolute lengths; squares on the background represent different marked dimensions for video software calibration).

7.2. Materials and Methods

Specimens

For the purposes of the current study, we tested nine female and nine male adult *Acrobates pygmaeus* (Acrobatidae, Diprotodontia). All specimens were captive-born and permanently housed in the collections of the Nowe Zoo, Poznań, Poland. The animals inhabited large enclosures under a reversed day-night regime. The enclosures contained a large variety of available substrates of diverse sizes and orientations, enabling the gliders to move freely in an enriched environment. Feathertail gliders made extensive use of all available arboreal substrates within the enclosures and were inclined to willingly

use the provided substrates during the experimental procedure. All the specimens were handled entirely by the specialized zoo staff, were fully habituated to human presence and did not display any stereotypical or stressful behaviours. Mean total head-body length of the subjects was 7.4 cm ($n=18$, 7.0-7.7 cm, $SD=0.16$) and mean body mass was 12 g ($n=18$, 10-15g, $SD=1.23$).

Experimental setup

A single, specially configured, filming terrarium (L: 90 cm x H: 40 cm x W: 40 cm) was used for the experimental procedure. Its sides and base were transparent glass windows and it was topped by a wooden frame, with a lid door and wire mesh for ventilation. Within the terrarium, we established a wooden frame to support the poles. The poles were 80 cm long, cylindrical, semi-hardwood rods. They were marked with vertical blue lines every 1 cm for a reliable estimation of absolute lengths. During the recordings, the visible length of the rod was approximately 30 cm. Diameter and inclination and direction of movement accounted for the classification of different substrate categories. Thus, four diameters (2 mm, 5 mm, 10 mm and 25 mm), and two different inclinations set at 0° (horizontal) and at 45° (oblique), combined with three movement directions (horizontal, descent and ascent) were used to define a total of 12 distinct substrate categories.

Data collection was completed in a total of three recording sessions, during April 2013. During each session, six individuals were transferred from their enclosure to a temporary accommodation terrarium by specialized zoo staff. Subsequently, each individual was transported to the filming terrarium. Each single individual was allowed free movement within the filming terrarium in order to familiarize itself with the new environment. Minimal or no stimulation was required for the subjects to walk on poles. During each filming session, every subject was tested on all 12 substrate categories. After each filming session, the animal was transported to another accommodation terrarium, to ensure that no individual was tested twice during a particular session.

For video recording, we used a Sanyo digital camcorder (VPC-HD 2000, Sanyo, Osaka, Japan), filming at 240 fps, which was positioned at a distance of 1 m from the filming terrarium to reduce image distortion. For our analyses, we considered only complete symmetrical gaits (those in which the left and right limbs of a girdle were evenly

spaced in time), initiating with the touchdown of the left hind limb and ending at the subsequent touchdown of the same limb, including both liftoffs and touchdowns of all limbs (Hildebrand, 1967, 1968, 1976). Cycles involving beginnings or endings of locomotor bouts or loss of balance were discarded. Overall, only cycles of stable symmetrical locomotion, regardless of velocity, were retained and regarded as indicative of natural, unbiased behaviour. The present research followed the guidelines for the treatment of animals in behavioural research and teaching (ASAB/ABS, 2012) and complied with relevant regulations and legislations of the Nowe Zoo and the Adam Mickiewicz University in Poznan and the relevant legislation of the Aristotle University of Thessaloniki.

Gait analyses

Video analysis and data collection, distance and time calculations were made by importing videos and calibrating time and distance measurements using Tracker 4.84 (Brown, 2009). Microsoft Excel 2010 (Redmond, WA, USA), and SPSS 21 (SPSS Inc., Chicago, IL, USA) were used for all statistical analyses.

For our analyses we considered the following gait parameters:

- (i) Diagonality (D) (Cartmill *et al.*, 2007), the percentage of the stride cycle interval the footfall of a forelimb follows behind the ipsilateral hind limb. Although it was measured as a scale variable, it was also divided into five ordinal classes: (a) Lateral Sequence Lateral Couplets ($0 \leq \text{LSLC} < 25$), (b) Lateral Sequence Diagonal Couplets ($25 \leq \text{LSDC} < 50$), (c) Trot ($=50$), (d) Diagonal Sequence Diagonal Couplets ($50 < \text{DSDC} \leq 75$), (e) Diagonal Sequence Lateral Couplets ($75 < \text{DSLCL} \leq 100$);
- (ii) Duty Factor (DF), the mean of duty factors of all limbs, defined as the percentage of a cycle during which a limb is in contact with the substrate;
- (iii) Duty Factor Index (DFI), the ratio of forelimb duty factor (DF_f) and hind limb duty factor (DF_h), calculated as $100 \cdot \text{DF}_h / \text{DF}_f$ (Cartmill *et al.* (2007a). Values > 100 indicate longer hind limb than forelimb stance durations, whereas values < 100 specify shorter hind limb than forelimb stance durations;
- (iv) Stride Duration (t), total duration of a single stride in seconds, measured from the frame where a stride cycle began until the frame the same stride cycle ended;

- (v) Stride Length (l), the corresponding distance covered during a single stride cycle, in meters;
- (vi) Velocity (v), the speed in which the subjects moved, calculated by dividing stride length with stride duration, and measured in meters/second (m/s);
- (vii) Stride Frequency (f), the number of strides per second;

As these parameters are size-dependent, we used the hind limb length of the feathertail glider (Mean=3.21 cm, 3.0-3.4 cm, SD=0.09) to calculate the dimensionless measures of stride duration, stride length, velocity and stride frequency (Hof, 1996). These calibrated absolute measurements are useful for estimating efficiency during locomotion:

$$\text{Dimensionless Stride Duration (tD)} = \frac{t}{\sqrt{\frac{l_0}{g}}}$$

$$\text{Dimensionless Stride Length (lD)} = \frac{l}{l_0}$$

$$\text{Dimensionless Velocity (vD)} = \frac{v}{\sqrt{g \times l_0}}$$

$$\text{Dimensionless Stride Frequency (fD)} = \frac{f}{\sqrt{\frac{g}{l_0}}}$$

where l_0 is hind limb length of each animal and g is the acceleration of gravity ($g=9.81$ m/s²).

We tested for statistically significant discrepancies in the utilization of diagonal and lateral gaits using binomial testing, but also incorporated the trot category into the analysis by using a chi-square test (χ^2). All of these analyses were carried out using two-tailed Monte Carlo procedures for enhanced p estimation accuracy (Kalos & Whitlock, 2009).

Analysis of covariance (ANCOVA) was selected in order to explore variable relationships while controlling for other possible covariants, when both scale and ordinal variables were involved. Spearman, two-tailed tests were used to explore correlations between diagonality and duty factor index, as well as stride frequency and stride length.

Stepwise regression models were constructed to examine the impact of both stride frequency and stride length on velocity, using their dimensionless counterparts. The impact of each parameter was calculated with the R^2 of partial correlations, i.e. the correlation between a dependent variable and its covariate, after the impact of other covariates is removed (Harrell, 2001).

7.3. Results

Throughout our experiments, a total of 181 valid symmetrical gait stride cycles was recorded. Overall, the symmetrical gaits of *A. pygmaeus* were characterized by fast velocities, low duty factors, and mainly diagonal-sequence gaits (Table 7.1, Fig. 7.2).

Diagonality and duty factor

Feathertail gliders consistently exhibited mean diagonality over 50.00 (Table 7.1) and adopted diagonal sequence gaits (DSDC and DSLC, combined $n=100$) much more often than lateral sequence gaits (LSDC and LSLC, combined $n=49$) (lateral vs. diagonal: binomial test, $p<0.001$; lateral vs. trot vs. diagonal, $\chi^2=41.514$, $p<0.001$; Table 7.2, Fig. 7.2). However, the vast majority of gaits varied from LSDC to DSDC (98.34%, $n=178$), with LSLC and DSLC seldom encountered; DSDC was by far the most frequent gait ($\chi^2=179.193$, $p<0.001$; Table 7.2).

Duty factors ranged from 16.90 to 66.91, with means for each category consistently lower than 50.00 (Table 7.1). This indicates that feathertail gliders ambled [running at intermediate speed, without a whole-body aerial phase (Schmitt *et al.*, 2006)] and ran [gaits in which each foot is on the ground for less than half their duration (Hildebrand, 1967)], rather than walked (gaits in which each foot is on the ground for more than half their duration (Hildebrand, 1967)). In addition, the duty factor was significantly higher in narrower substrates ($F=27.304$, $p<0.001$, controlling for the direction of movement and velocity), indicating lower speeds ($F=8.842$, $p<0.001$, controlling for the direction of movement and duty factor) and a more cautious body displacement. There was also no significant covariance between diagonality and duty factor when controlling for substrate size, direction of movement and velocity ($F=1.087$, $p=0.348$). Nevertheless, as past research has demonstrated correlations between duty

factor and diagonality (Cartmill *et al.*, 2002), all subsequent tests, regarding gait parameters, were also controlled for duty factor.

Effects of substrate diameter and direction of movement on diagonality

In our gait sample, there was an overall significant effect of substrate diameter on diagonality, controlling for mean duty factor and direction of movement ($F=3.395$, $p=0.019$). More specifically, adjusted mean diagonality (i.e. after controlling for mean duty factor and direction of movement) tended to decrease as substrate size increased (Table 7.3a). However, differences were only significant between the 2mm and the 25 mm size classes (Bonferroni Post-Hoc: $p=0.012$). To test for covariance between diagonality and direction of movement, we controlled for the effects of mean duty factor and substrate size. Overall, adjusted mean diagonality (i.e. after controlling for mean duty factor and substrate size) increased from descending towards horizontal locomotion and decreased once more towards ascending locomotion (Table 7.3b; $F=7.936$, $p=0.001$). However, differences were significant only between descents and horizontal locomotion (Bonferroni Post-Hoc: $p<0.001$).

Duty factor index

The duty factor index averaged around 100, with variation among different categories (Table 7.1). The index increased significantly, though with moderate strength, with diagonality ($R=0.422$, $p<0.001$; Fig. 7.3). This indicates that hind limb stance phase increased in more diagonal gaits. Furthermore, the duty factor index and substrate diameter did not covary ($F=0.368$, $p=0.776$, controlling for direction of movement and diagonality; Fig. 7.4a), but increased along with the direction of movement ($F=3.851$, $p=0.023$, controlling for substrate diameter and diagonality; Fig. 7.4b). Consequently, the hind limb stance phase increased and the forelimb stance phase decreased, when moving from declining towards inclining substrates.

Table 7.1. Summary of means and standard deviations (in brackets) of the gait statistics for *Acrobates pygmaeus*. N indicates the number of analyzed cycles for each substrate size and orientation category.

		N	Diagonality (D)	Duty Factor (DF)	Duty Factor Index (DFI)	Stride Duration (t, s)	Stride Length (l, cm)	Velocity (v, m/s)	Stride Frequency (f, strides/s)	Dimensionless Stride Duration (t ₀)	Dimensionless Stride Length (f ₀)	Dimensionless Velocity (v ₀)	Dimensionless Stride Frequency (f ₀)
2mm	Descent	8	53.13 (7.95)	42.24 (3.53)	90.63 (16.44)	0.086 (0.009)	7.14 (0.45)	0.84 (0.12)	11.72 (1.32)	0.15 (0.02)	2.18 (0.15)	0.15 (0.02)	6.77 (0.74)
	Horizontal	22	50.19 (12.16)	55.35 (5.84)	99.08 (11.71)	0.144 (0.086)	6.45 (0.90)	0.52 (0.17)	8.2 (2.45)	0.25 (0.15)	2.05 (0.31)	0.09 (0.03)	4.65 (1.39)
	Ascent	12	56.75 (10.73)	43.75 (4.87)	112.2 (20.29)	0.148 (0.115)	6.97 (0.88)	0.66 (0.28)	9.71 (4.3)	0.26 (0.20)	2.19 (0.29)	0.12 (0.05)	5.55 (2.47)
5mm	Descent	7	50.93 (4.24)	40.45 (4.5)	103.16 (11.83)	0.122 (0.095)	6.93 (0.55)	0.75 (0.30)	10.59 (3.92)	0.21 (0.16)	2.13 (0.20)	0.13 (0.05)	6.09 (2.23)
	Horizontal	21	52.43 (11.59)	48.93 (7.48)	91.2 (17.52)	0.115 (0.96)	7.6 (0.96)	0.85 (0.29)	11.2 (3.45)	0.20 (0.17)	2.41 (0.30)	0.15 (0.05)	6.34 (1.95)
	Ascent	8	51.65 (3.05)	42.08 (3.84)	106.72 (13.51)	0.091 (0.014)	6.85 (0.49)	0.77 (0.12)	11.18 (1.61)	0.16 (0.02)	2.08 (0.15)	0.14 (0.02)	6.49 (0.93)
10mm	Descent	9	49.32 (9.38)	28.23 (6.11)	83.44 (12.91)	0.066 (0.007)	7.64 (0.43)	1.17 (0.15)	15.28 (1.78)	0.12 (0.01)	2.36 (0.15)	0.21 (0.03)	8.77 (1.08)
	Horizontal	42	55.95 (8.64)	31.64 (6.29)	105.46 (26.05)	0.07 (0.007)	8.64 (1.55)	1.24 (0.23)	14.37 (1.43)	0.12 (0.01)	2.72 (0.48)	0.22 (0.04)	8.18 (0.84)
	Ascent	16	52.73 (8.4)	33.22 (4.11)	102.44 (12.44)	0.073 (0.010)	7.31 (1.11)	1.02 (0.22)	13.81 (1.65)	0.13 (0.02)	2.24 (0.34)	0.18 (0.04)	7.96 (0.96)
25mm	Descent	8	39.24 (9.98)	31.44 (5.48)	82.1 (21.45)	0.071 (0.008)	8.57 (1.24)	1.23 (0.13)	14.3 (1.68)	0.13 (0.01)	2.71 (0.30)	0.22 (0.02)	8.02 (0.76)
	Horizontal	23	53.00 (5.39)	35.11 (9.21)	99.08 (15.1)	0.14 (0.117)	8.36 (1.54)	0.86 (0.39)	10.37 (4.22)	0.25 (0.21)	2.62 (0.49)	0.15 (0.07)	5.94 (2.44)
	Ascent	5	50.3 (10.15)	35.51 (3.87)	96.8 (15.03)	0.083 (0.009)	7.55 (1.15)	0.91 (0.14)	12.08 (1.35)	0.14 (0.02)	2.29 (0.33)	0.16 (0.02)	7.01 (0.86)
Total Mean		181	52.5 (9.61)	39.11 (10.5)	99.34 (19.6)	0.102 (0.76)	7.69 (1.39)	0.93 (0.34)	11.96 (3.47)	0.18 (0.14)	2.41 (0.44)	0.17 (0.06)	6.83 (1.99)

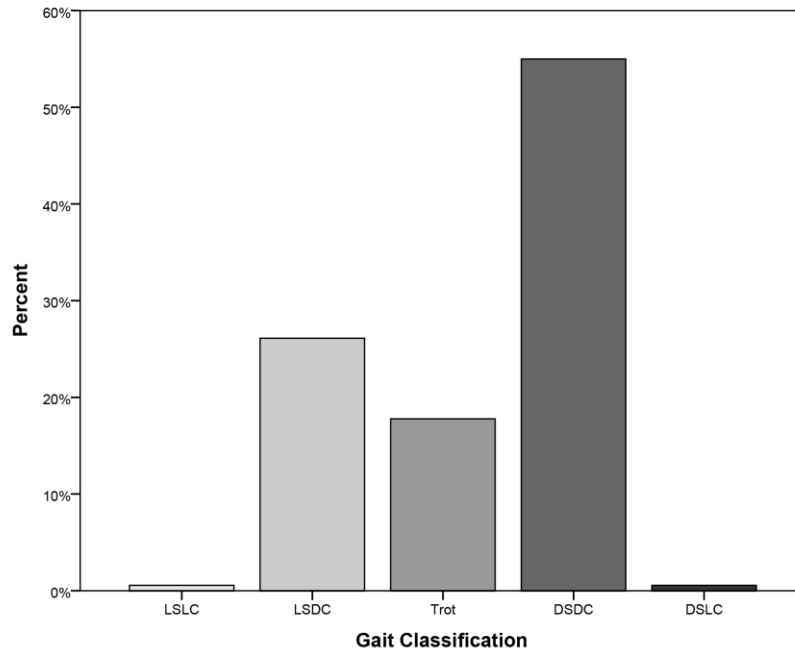


Figure 7.2. Overall percentages of the different gait types of *Acrobates pygmaeus*.

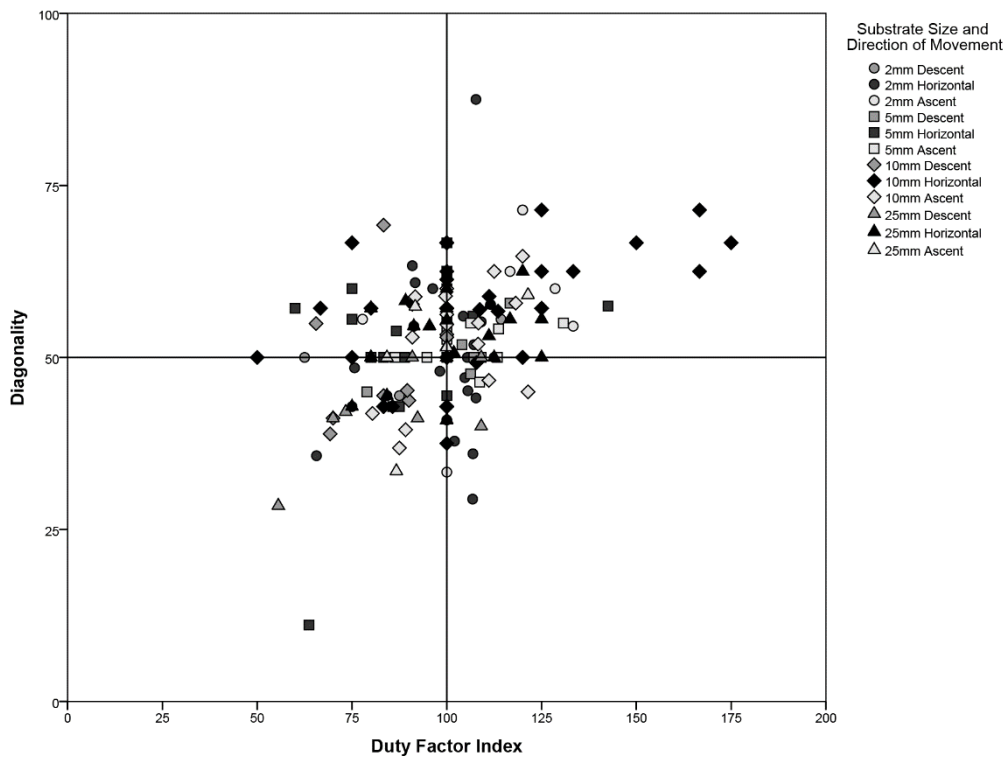


Figure 7.3. Scatterplot of diagonality as a function of duty factor. Horizontal line corresponds to diagonality 50 (trot) and vertical line corresponds to duty factor index 100 (equal duty factors of forelimbs and hind limbs).

Velocity and stride parameters

In order to estimate locomotor efficiency in *A. pygmaeus*, we analyzed velocity, stride frequency, stride length, and stride duration as dimensionless parameters (sensu Hof, 1996). Velocity correlated to substrate size and increased with increased size ($F=49.55$, $p<0.001$; Fig. 7.5a). Moreover, stride frequency (controlling for the effect of stride length, $F=28.55$, $p<0.001$; Fig. 7.5b) and stride length (controlling for the effect of stride frequency, $F=13.35$, $p<0.001$, Fig. 7.5c) also increased from smaller to larger substrate sizes. The construction of a stepwise regression model, on the effects of stride frequency and stride length on velocity, showed that stride frequency was the main factor for velocity regulation ($R^2=62.88\%$, $F=549.874$, $p<0.001$; Fig. 7.6a). Stride length also played a significant role, but explained a much smaller amount of variation in velocity ($R^2=22.85\%$, $F=2468.52$, $p<0.001$; Fig. 7.6b). Additionally, there was no correlation between stride length and stride frequency ($R=-0.137$, $p=0.067$). Both parameters seem to significantly act as regulating mechanisms on velocity, though independently of one another and at different rates.

Substrate Size	Direction of Movement	Classification of Strides				
		LSLC	LSDC	Trot	DSDC	DSLCL
2mm	Descent	0 (0.0%)	2 (25.0%)	2 (25.0%)	4 (50.0%)	0 (0.0%)
	Horizontal	0 (0.0%)	11 (50.0%)	2 (9.1%)	8 (36.4%)	1 (4.5%)
	Ascent	0 (0.0%)	1 (8.3%)	3 (25.0%)	8 (66.7%)	0 (0.0%)
5mm	Descent	0 (0.0%)	2 (28.6%)	2 (28.6%)	3 (42.9%)	0 (0.0%)
	Horizontal	1 (4.8%)	3 (14.3%)	5 (23.8%)	12 (57.1%)	0 (0.0%)
	Ascent	0 (0.0%)	1 (12.5%)	3 (37.5%)	4 (50.0%)	0 (0.0%)
10mm	Descent	0 (0.0%)	5 (55.6%)	0 (0.0%)	4 (44.4%)	0 (0.0%)
	Horizontal	0 (0.0%)	8 (19.0%)	7 (16.7%)	27 (64.3%)	0 (0.0%)
	Ascent	0 (0.0%)	5 (31.3%)	0 (0.0%)	11 (68.8%)	0 (0.0%)
25mm	Descent	1 (12.5%)	1 (12.5%)	5 (62.5%)	2 (25.0%)	0 (0.0%)
	Horizontal	0 (0.0%)	3 (13.0%)	5 (21.7%)	15 (65.2%)	0 (0.0%)
	Ascent	0 (0.0%)	1 (20.0%)	1 (20.0%)	3 (60.0%)	0 (0.0%)
All sizes	Descent	1 (3.1%)	14 (43.8%)	6 (18.8%)	11 (34.4%)	0 (0.0%)
	Horizontal	1 (0.9%)	25 (23.1%)	19 (17.6%)	62 (57.4%)	1 (0.9%)
	Ascent	0 (0.0%)	8 (19.5%)	7 (17.1%)	26 (63.4%)	0 (0.0%)
Total		2 (1.1%)	47 (26.0%)	32 (17.7%)	99 (54.7%)	1 (0.6%)

Table 7.2. Summarized statistics of gait diagonality classifications for *Acrobates pygmaeus*.

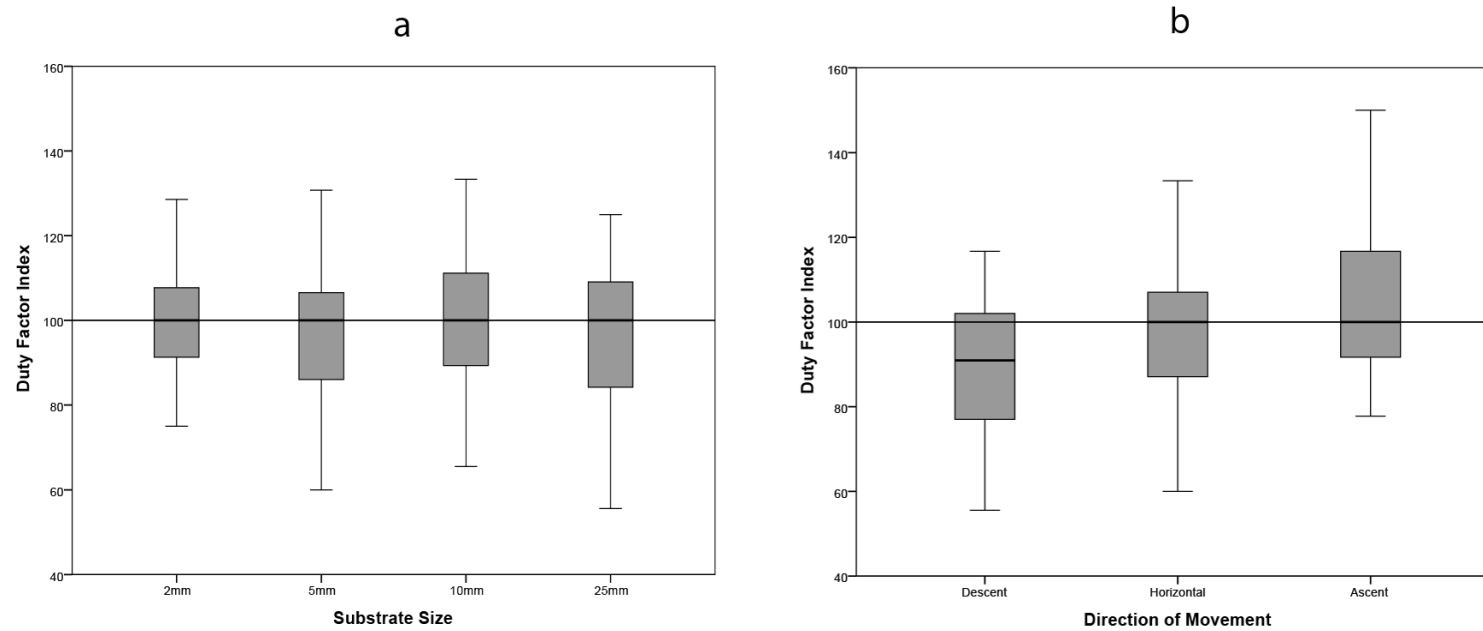


Figure 7.4. Boxplot of duty factor index for each substrate size (a) and direction of movement (b) category (middle point represents median duty factor index, box represents duty factor index from the 1st to the 3rd quartile, and whiskers min-max range).

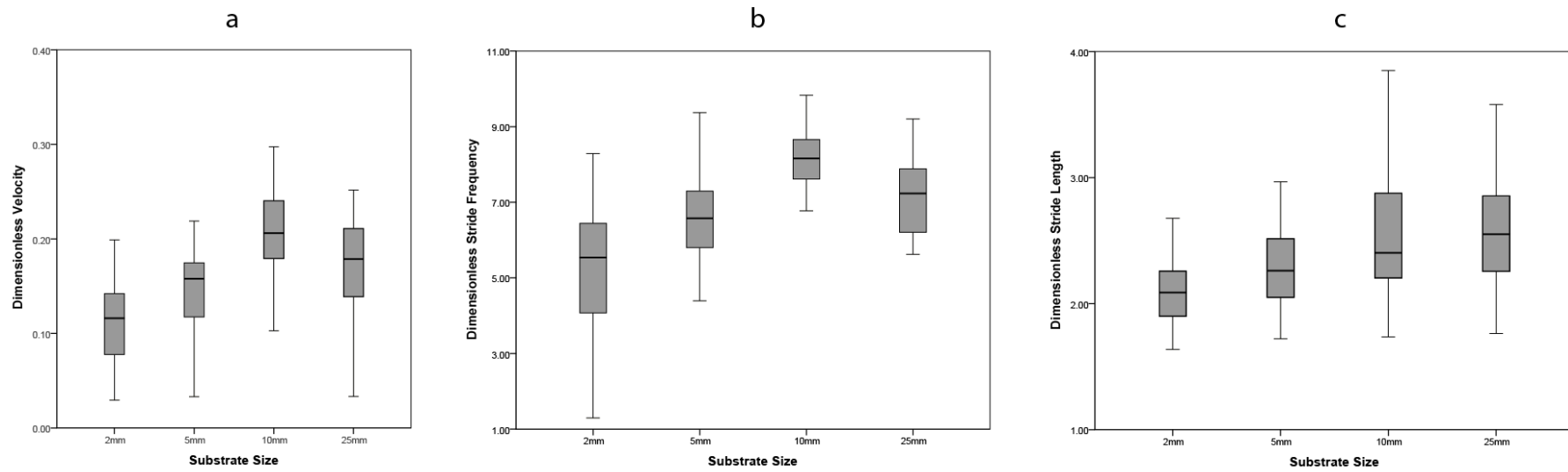


Figure 7.5. Boxplot of dimensionless velocity (a), stride frequency (b), and stride length (c) for each substrate size (middle points represent median, boxes represent values from the 1st to the 3rd quartile, and whiskers min-max range).

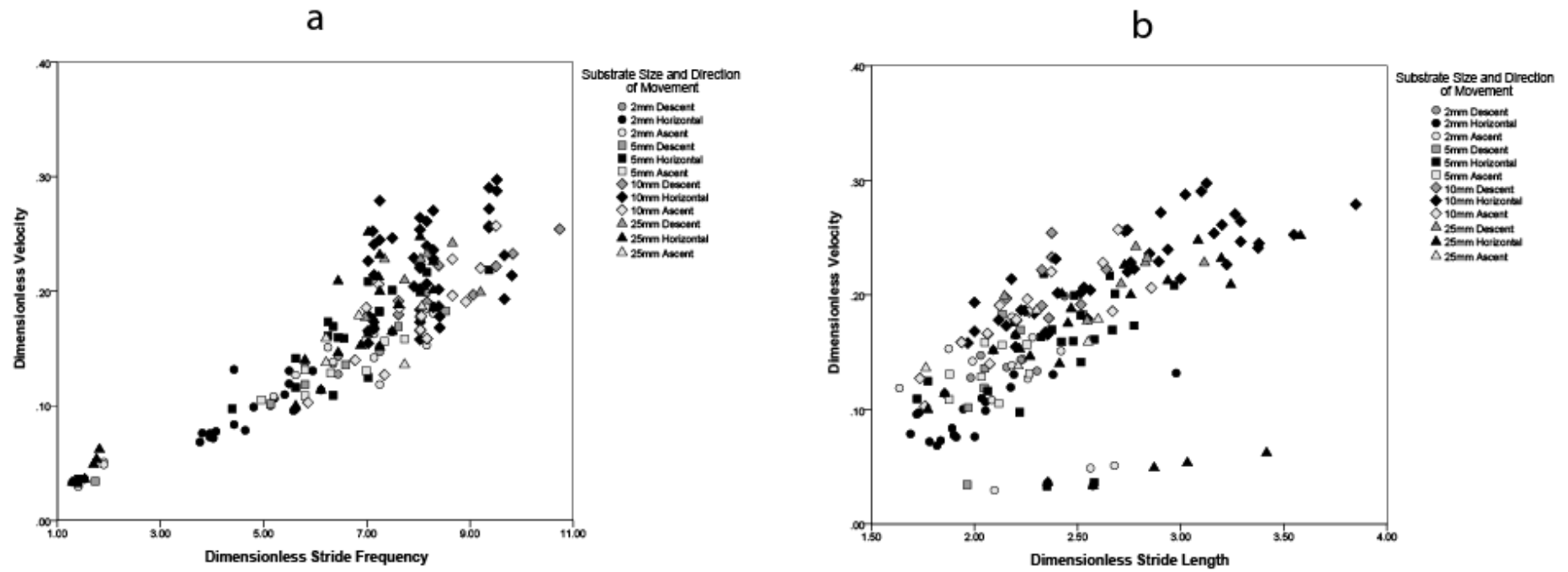


Figure 7.6. Scatterplots of dimensionless velocity as a function of stride frequency (a) and stride length (b).

7.4. Discussion

One of the keystone apomorphies of the order Primates is the way they move quadrupedally along single, small, arboreal substrates. This quadrupedal progression is characterized by the frequent use of diagonal sequence diagonal couplets compliant gaits, especially on smaller substrates, reduced peak reaction forces and increased forelimb protraction and overall contribution to body progression (Cartmill *et al.*, 2002). Given that these morpho-behavioural novelties likely evolved during early primate evolution (Gebo, 2004; Sargis, 2007), their adaptive significance can only be assessed by the use of extant analogues. In effect, a finer look at other arboreal mammals, especially marsupials, indicates that some of these behavioural features, such as the frequent use of diagonal gaits (DS), are not exclusive to primates (White, 1990; Pridmore, 1994; Cartmill *et al.*, 2002; Schmitt & Lemelin, 2002; Lemelin & Cartmill, 2010). However, a major drawback of these extant analogues is their size. Small body size has been considered integral to early primate evolution (Cartmill, 1974a; Larson *et al.*, 2000; Gebo, 2004), but the best candidate model so far, the woolly opossum (*Caluromys philander*), weighs around 300 g, contrasting with predictions and fossil evidence of early primate body mass [e.g., Micromomyidae: 30 - 40 g (Bloch & Boyer, 2007), early euprimates: *Altanius orlovi*: 30 g (Gingerich, Dashzeveg & Russell, 1991); *Altiatlasius koulchii*: 30 - 60 g (Bajpai *et al.*, 2008), *Donrussellia provincialis*: 40 g (Gilbert, 2004); *Teilhardina belgica*: 40 - 80 g (Gebo *et al.*, 2000), and possibly even smaller ancestors (Gebo, 2004)]. In contrast, feathertail gliders, *A. pygmaeus*, with a body mass of 12 g, a clawless opposable hallux (Rosenberg & Rose, 1999) and terminal branch feeding habits (Ward & Woodside, 2008), appear more suited to modeling behavioural adaptations in the small branch milieu.

The current study of quadrupedal locomotion of feathertail gliders showed that these tiny-sized marsupials mainly used diagonal sequence (DS) gaits, fast velocities and low duty factors. Diagonality did not correlate to duty factor, but increased as substrate size decreased, and from descending to ascending locomotion. Furthermore, the duty factor index increased in more diagonal gaits and ascending locomotion. Finally, velocities were lower on smaller substrates, and were mainly regulated by stride frequency and, to a lesser degree, stride length.

During quadrupedal locomotion, *A. pygmaeus* used high velocities extensively and consequently had low duty factors. This is likely a common behaviour of small arboreal marsupials, related to their small size and elevated metabolism (Delciellos & Vieira, 2007). These relatively high speeds may account for the observed high rates of DS gaits (Tables 1, 2), a common trend for some arboreal marsupials, which usually shift to LS gaits at lower speeds (Pridmore, 1994; Shapiro & Young, 2010; Biknevicius *et al.*, 2013). Unfortunately, our sample of high duty factor gaits was restricted ($n=30$, across all categories) and therefore it was difficult to obtain statistically robust conclusions on the use of DS gaits at lower speeds. Nevertheless, our analyses showed no significant covariance between diagonality and duty factor, while controlling for the effect of substrate size and direction of movement. This indicates that the feathertail gliders may not shift to LS gaits at lower speeds, as would be expected, and may likely retain their DS gaits during walking gaits as well. However, the current data may represent a sample at the lower tail end of the duty factor distribution. Thus, it is not certain that the higher tail end of duty factors would be governed by the same statistical relationship. Consequently, similar conclusions should be very cautiously considered, but would suggest that some small arboreal mammals with prehensile extremities may predominantly utilize DS gaits, regardless of the velocity in which they move. If this holds true, it may bear important implications for the evolution of such behaviour in early, supposedly small-bodied, primate ancestors (Sargis *et al.*, 2007).

The importance of DS gaits for *A. pygmaeus* was further substantiated by their overall preferential use over LS gaits, regardless of any observed variation on different substrates and orientations. Thus, diagonality increased on smaller substrates, as well as from descents to horizontal locomotion and ascents (Table 7.3a,b). This adaptive plasticity is comparable to that observed in most primates, which adjust their gaits in accordance to substrate characteristics (Stevens, 2006), and raises several interesting questions. First, Vilensky and Larson (1989) proposed that selecting between DS and LS gaits does not impact locomotor stability, but the dominance of DS gaits in primates is a side effect of primate-specific brain reorganization. However, the discovery that several marsupials, such as *Caluromys philander* (Schmitt & Lemelin, 2002; Lemelin *et al.*, 2003), *Trichosurus vulpecula* (White, 1990) and *A. pygmaeus* (this study), frequently employ DS gaits on arboreal substrates does not seem to support this explanation. Even though stability might not be the advantage procured by DS gaits, Cartmill *et al.* (2002) proposed

instead that diagonal sequence diagonal couplets (DSDC) gaits allow an animal to bear its mass on a hind limb, while its diagonal forelimb tests a new substrate during a crossing. If the new substrate is unstable, the animal can fall back swiftly and safely to the previous substrate, still anchored by the hind limb. Thus, diagonality should be negatively correlated to substrate size. Our results seem to support this claim. We found that, while controlling for the effect of the direction of movement, diagonality decreased on larger substrates (Table 7.3a), reaffirming predictions by Cartmill et al. (2002). In effect, *A. pygmaeus* seemed to move more diagonally (Table 7.3a) and at slower paces (Table 7.1) on the smaller of substrates. This indicates that, despite their small size, which might impose different biomechanical constraints in relation to slender substrates (Preuschoft, 2002), feathertail gliders very likely require behavioural adjustments related to DS gaits in order to safely negotiate the smallest of branches.

Regarding direction of movement, diagonality was lower in descents than in horizontal locomotion and ascents. This indicates that feathertail gliders tended to use more lateral gaits while descending, and more diagonal gaits while moving horizontally and during ascents. Declining substrates can also be the outcome of slender peripheral branches bent under an animal's weight. In this case, they may consist of rather unstable substrates that would require safe anchoring of the hind limbs and the use of DS gaits, according to Cartmill et al. (2002). However, this was not substantiated by our observations on feathertail gliders, as is also the case for other arboreal primates and non-primate mammals (Prost & Sussman, 1969; Lammers, 2007; Nyakatura *et al.*, 2007; Nyakatura & Heymann, 2010; Shapiro & Young, 2010; Shapiro *et al.*, 2014). In fact, in descents, arboreal mammals face a cranial/anterior shift of the centre of mass, which is associated with increasing contact time and a higher fraction of vertical impulse on the forelimbs, enhancing their regulative and supportive role (Rollinson & Martin, 1981; Lee *et al.*, 2004; Nyakatura *et al.*, 2008; Young, 2012). Additionally, during descending locomotion, lateral gaits allow the forelimbs to provide retardation through a “stop-jolt” before the hind limbs contact the substrate (Rollinson & Martin, 1981). In feathertail gliders, this retardation of the forelimbs may be further related to the lower duty factor indices observed during descents (Table 7.1, Fig. 7.4b). A lower duty factor index indicates an increased forelimb duty factor compared to that of the hind limb, emphasizing an enhanced anchoring role of the forelimbs (Lee *et al.*, 2004; Nyakatura *et al.*, 2008). On the other hand, compared to descents, both duty factor index and

diagonality increased on inclined substrates. This indicates that diagonal sequence gaits and hind limb duty factor, compared to that of the forelimb, increase in ascents. In effect, increased diagonality on inclined substrates is associated with a caudal shift of the centre of mass and the reduction of the retarding role of the forelimbs in the first part of the stance phase (Nyakatura *et al.*, 2007). This enhances the load-bearing role and mediates the propulsive role of the hind limbs (Lee *et al.*, 2004; Young, 2012). These mechanisms very likely promote safe ascending progression and enable a farther forelimb reach, in a manner comparable to vertical climbing and clinging (Hirasaki, Kumakura & Matano, 2000; Johnson, 2012).

In *A. pygmaeus*, the functional interplay between diagonality and duty factor index in different substrate categories was also corroborated by their correlation, although with moderate strength. This is also the case for most primates (Cartmill *et al.*, 2002), with the exception of small-bodied callitrichids which employ LS gaits very often (Schmitt, 2003a; Nyakatura *et al.*, 2007; Nyakatura & Heymann, 2010). In primates, this correlation has been observed mainly for walking gaits, whereas duty factor index and diagonality can also be correlated at higher speed in non-primate mammals as well (Cartmill *et al.*, 2002). As the majority of the feathertail gliders' gaits were ambles and runs, a similar conclusion should be treated with caution, as it may be a simple result of the higher speeds encountered in this tiny marsupial.

Feathertail gliders seemed to regulate velocity primarily by stride frequency and to a lesser extent by stride length (Fig. 7.6a,b). A similar behavioural mechanism of speed regulation has been observed in another small-bodied marsupial *Gracilinanus microtarsus* (mass < 35g; Delciellos & Vieira, 2007). This is dissimilar to primates and the marsupial *Caluromys*, which increase velocities by increasing stride length instead of frequency (Larson *et al.*, 2000, 2001). This way of increasing speed of progression is considered safer within an arboreal setting, as it enables a farther reaching of the forelimb in combination with secure grasping, and thus reduces involuntary branch swaying (Demes *et al.*, 1994). In contrast, regulation by stride frequency, although energetically costly (Strang & Steudel, 1990), appears to be better suited for small-bodied arboreal mammals. Due to their small size and size-to-substrate ratio, branch swaying during arboreal quadrupedal bouts may be negligible, but body oscillations (Delciellos & Vieira, 2007), which produce moments which disrupt continuous progression and may cause toppling over, are not. This may be particularly important when negotiating small,

slender substrates in tree peripheries. Regulation by stride frequency does not immediately implicate elongated contact times of the limbs on the branches, but reduces involuntary oscillations and could thus be safer (Delciellos & Vieira, 2007). However, feathertail gliders also involved, to a lesser extent, stride length as a regulative mechanism of velocity. This mechanism may be related to the increased grasping capacities of these marsupials contributing to reduced branch oscillations (Demes *et al.*, 1994; Delciellos & Vieira, 2007). But how are these mechanisms related to substrate size? Our analyses demonstrated that velocity, stride frequency and stride length increased with increased substrate diameter. This implies that during the negotiation of slender substrates, *A. pygmaeus* reduces speed, stride frequency and, to a lesser extent, stride length (Fig. 7.5a,b,c). This behavioural adaptation contrasts with the strategies adopted by most other small-bodied marsupials, but is similar to that of *C. philander* (Schmitt & Lemelin, 2002; Delciellos & Vieira, 2007), and some prosimians (though not in a significant way; see Stevens, 2008). *Acrobates pygmaeus* does not possess particularly long limbs and mainly relies on its very prehensile extremities to establish firm contact with the substrate (Rosenberg & Rose, 1999). Cautious locomotion upon fragile substrates, by reducing the basic gait parameters, probably minimizes any unwanted substrate sways and oscillations, facilitating a controlled and secure body displacement within the fine branch milieu. This strategy may be suited for small-bodied arborealists, which can be quite flexible in the way they navigate and negotiate unstable, slender substrates (Stevens, 2006, 2008).

The current study showed that the feathertail glider, a regular rover of fine branches of tree peripheries and bushes (Ward, 1990; Ward & Woodside, 2008), possessing grasping extremities and a divergent clawless hallux (Rosenberg & Rose, 1999), exhibited some of the behavioural features that characterize primate quadrupedalism. Compared to *C. philander* and *P. breviceps*, *A. pygmaeus* is very small. The small body mass and reduced size-to-substrate ratio of feathertail gliders, which approximates more the estimated size of early primates, impose different mechanical constraints and generate different behavioural responses to those demonstrated by previous marsupial models (Delciellos & Vieira, 2006, 2007). Nevertheless, *A. pygmaeus* showed interesting convergence in some quadrupedal features considered characteristic of primates, and more particularly the common use of DS gaits, especially on smaller and on horizontal and inclined substrates, an increased duty factor index with more DS gaits,

and the lower velocities on smaller substrates, as well as the involvement of stride length in the contribution of stride frequency in regulating speed. Some of these behavioural convergences may be slightly equivocal and these results should be treated with some caution. In our study, diagonal sequence gaits may have been the by-product of high velocities, while the contribution of stride frequency to velocity regulation, that of small size-to-substrate ratio. Both of these differences are likely related to the small body mass of our model mammal. However, the existing fossil evidence supports the suggestion that early primate ancestors were smaller than 100 g (Gebo, 2004). In this case, *A. pygmaeus* could represent a lightweight, agile, arboreal ancestral primate stage that would have started to commonly employ DS gaits on most arboreal substrates, with increased duty factor indices, and be regulating its velocity primarily by stride frequency, but with a lesser, albeit significant, effect of stride length. All these features appeared to be more dominant during the use of smaller and horizontal and inclining substrates. This could eventually fit well in stage (c) of the Sargis *et al.* (2007) scenario, and could be preceded by a stage characterized by equally small size, a divergent clawed hallux, agile arboreal locomotion, and habitual use of a fine-branch milieu, as represented by Eurasian harvest mice *Micromys minutus* (Urbani & Youlatos, 2013). Subsequently, as body size increased, the already present alternative method of modifying velocity by stride length would be favored, as it would produce relatively less branch swaying, speed was lowered and DS was established as the main gait. We believe that *A. pygmaeus* possesses the set of morphological and behavioural features that make it a highly promising model for early stages of primate evolution. We hope that this study will serve as a good starting point towards research to this end, in order to elucidate discussions on primate origins and early primate evolution.

Chapter 8: Conclusions

The results obtained from the case studies show that gaits relate to arboreality in various ways, which are often not uniform. Arboreal species display behaviours which maximize their abilities to efficiently utilize arboreal substrates. More terrestrial species may not be as able to negotiate slender substrates, however, they modify their behaviour appropriately to cope with the challenges of arboreality. In this last section, the results from the previous chapters will be comparatively discussed to uncover any trends related to substrate size and direction of movement (i.e. ascent, descent, or horizontal locomotion). Moreover, I proceeded to a meta-analysis of selected trends in an attempt to link gait parameters to an animal's degree of arboreality, which would provide insights on common patterns for an arboreal lifestyle among mammals.

8.1. The Effect of Substrates on Gait Metrics

Substrate size

Overall, the effect of substrate size on gaits, across the small mammals examined, was rather uniform (Table 8.1), except for diagonality (D). Duty factor (DF) was reduced on narrower substrates whereas duty factor index (DFI) was not affected by substrate size across the tested species. Diagonality (D) was the only parameter subject to variability across species. Only *Acrobates pygmaeus* increased D on narrow substrates in contrast to *Apodemus agrarius*, *A. flavicollis* and *Micromys minutus* in which D decreased. *Thallomys paedulus* and *Myodes glareolus* showed no effect of substrate size on D, and among the two glirids, *Glis glis* made exclusive use of asymmetrical gaits, while *Muscardinus avellanarius* used LS symmetrical gaits only on the narrowest substrates, switching to asymmetrical gaits on all other. Similar results for most gait parameters, excluding D, reported for other mammals of various taxa and body sizes (Lammers & Biknevicius, 2004; Stevens, 2006; Nyakatura *et al.*, 2008; Shapiro *et al.*, 2016), indicate that the effect of substrate size on these gait parameters may be conserved across mammalian groups.

Table 8.1. Effect of substrate size on various gait metrics for each species examined, as determined by ANCOVA tests (See respective chapters for exact results). *Positive* indicates that the metric increases on larger substrates. *Negative* indicate a decrease in the metric on larger substrates. Positive and negative results are significant at $p < 0.05$. *None* indicates no significant relationship ($p > 0.05$), and *Unknown* means that there is not enough data to compute relationships.

Species	Diagonality	Duty Factor	Duty Factor Index	Velocity	Stride Length	Stride Frequency
<i>Acrobates pygmaeus</i>	Negative	Negative	None	Positive	Positive	Positive
<i>Muscardinus avellanarius</i>	Unknown	Negative	None	Positive	Positive	Positive
<i>Glis glis</i>	Unknown	None	None	None	None	None
<i>Myodes glareolus</i>	None	Negative	None	Positive	Positive	None
<i>Apodemus agrarius</i>	Positive	Negative	None	Positive	Positive	Positive
<i>Apodemus flavicollis</i>	Positive	Negative	None	Positive	Positive	Positive
<i>Micromys minutus</i>	Positive	Negative	None	Positive	Positive	Positive
<i>Thallomys paedulcus</i>	None	Negative	None	Positive	Positive	Positive

These observations have immediate implications for theories regarding gait adaptations during arboreal locomotion. It has been hypothesised that the diagonal-sequence diagonal couplet (DSDC) footfall pattern (i.e. increased D) is favourable to arboreal mammals. It allows them to explore new, potentially unstable substrates with their forelimbs, while their safely anchored contralateral grasping hind limbs permit them to withdraw in case of instability, keeping their centre of mass within the support polygon of the limbs (Cartmill *et al.*, 2007). Consequently, the frequency of DS gaits should increase on fine branches, which are more prone to bending or breakage. On one hand, D was indeed found to increase on narrow substrates in the arboreal specialist *A. pygmaeus*, characterized by grasping hind and forefeet (Rosenberg & Rose, 1999), overall faster locomotion, and a predominant use of DS gaits, contrary to the LS gaits of the other species examined here. Although testing new substrates through DS gaits seems improbable during high speed locomotion (Nyakatura *et al.*, 2008), the diagonally paired

limbs of DS gaits may confer better dynamic stability (i.e. during fast locomotion), allowing possibly a better control and transfer of moments and torques across the body axes (Lammers & Zurcher, 2011a). Increased D on narrow branches, as exemplified by *A. pygmaeus*, with DSDC gaits, produces medially-oriented forces (Schmitt, 2003b; Lammers & Biknevicius, 2004; Schmidt & Fischer, 2010), and allow animals to seize the branch and increase the force applied, and consequently friction, which makes slipping less likely (Lammers & Zurcher, 2011a). Moreover, opposing mediolateral and angular impulses generated by the contralateral limbs may reduce mediolateral deviations from the long axis of the branch (Shapiro & Raichlen, 2007). Although the previous explanation for DS gaits focuses on grasping hind feet (Cartmill *et al.*, 2007), this highlights that powerful or at least strong grasping forelimb capacity may be integral for effective DS gaits, as the combined function of fore- and hind limbs would contribute to increasing stability. *Muscardinus avellanarius*, for instance, moves even faster than *A. pygmaeus* at the narrowest substrates, hence requiring good dynamic stability utilised LSDC gaits, however. Although both animals possess grasping hind feet (Stafford, Rosenberger & Beck, 1994; Haffner, 1998), *A. pygmaeus* is further differentiated by its increased forelimb grasping ability (Rosenberg & Rose, 1999). However, further research is required regarding the contribution of grasping forelimbs in DS gaits to test this hypothesis. Lastly, the gaits of *A. pygmaeus* are also often ambles, in which D is over 50, and DF below 50, and at least one limb is on the substrate (Schmitt *et al.*, 2006). The lack of an aerial phase in ambling gaits limits the dorsal and ventral accelerations of the centre of mass, thus reducing peak vertical forces, which in turn reduce the vertical oscillations of narrow branches (Young, 2009) on which *A. pygmaeus* specialises.

Conversely, most of the sampled species displayed lower D on narrower substrates, regardless of the presence of grasping hind feet [in *T. paedulus* (De Graaff, 1978), *M. minutus* (Haffner, 1998), and *A. flavicollis* (Krattli, 2001)], or lack of hind foot grasping ability [in *A. agrarius* and *M. glareolus* (Krattli, 2001; Zefferer, 2002)]. This prevalent use of LS gaits by most scansorial and arboreal small mammals examined, as well as the decrease of D on narrow substrates, implies behavioural adaptations for increased static stability (slow locomotion) on arboreal substrates. This is due to the fact that the centre of gravity always passes through the support polygon during LS gaits, provided that three or four limbs are in contact with the substrate, hence at high DFs (Lammers & Zurcher, 2011a), as was observed in most of the examined scansorial

species. For an animal that habitually uses LS gaits, it is therefore possible that lower D combined with a high DF is preferable, and optimises arboreal stability compared to gaits of higher D. Moreover, this strategy may be best suited for mammals that do not possess strong grasping abilities of the hind or forefeet and possibly cannot apply very strong contralateral forces during arboreal locomotion.

Duty factor (DF) increased progressively on narrower substrates for most of the examined species. The shortening of swing phases relative to stance phases was in all cases accompanied by a predicted decrease in velocity, suggesting cautiousness in locomotion on narrow substrates. These results are in line with observations in arboreal metatherians and primates (Delciellos & Vieira, 2006; Nyakatura & Heymann, 2010). On the other hand, some arboreal neotropical rodents seem to increase velocity on narrow substrates (Camargo *et al.*, 2016), possibly enhancing their dynamic stability (Schmidt & Fischer, 2011) by producing non-zero angular momentum through small distortions of their body such as the rotation of the limbs around the hip joint or the centre of rotation of the scapula (Lammers & Zurcher, 2011a). Nevertheless, the reduction of velocity seems to provide different advantages. Narrow substrates impose a more mid-sagittal placement of the limbs than wider substrates, producing narrower support polygons (Cartmill *et al.*, 2007; Lammers & Zurcher, 2011a). As a result, there is an increased probability of the centre of mass being positioned outside the support polygon due to the laterally-directed destabilising torques at touchdown (Lammers & Zurcher, 2011b). The observed higher DFs and lower velocities increase the number of simultaneous contact points and the relative contact time with the substrate, assisting in counteracting the destabilising torques (Lammers & Gauntner, 2008; Lammers & Zurcher, 2011b). Moreover, the inverse correlation between D and DF, observed in most species examined here and in other arboreal mammals which habitually employ LSDC gaits, has been shown to optimise the support polygons produced during a stride cycle (Cartmill *et al.*, 2002, 2007). This further adds to the argument that LSDC gaits may be preferable in terms of static stability (i.e. slower locomotion), reducing the danger of toppling over and maximizing safe navigation at lower speeds (Lammers & Zurcher, 2011a).

The duty factor index (DFI) is unaffected by changes in substrate diameter in all species examined (Table 8.2). Even though DFI indicates some interspecific variation, implying elevated dominance of the forelimbs or hind limbs in different species, it seems that the roles of the two sets of limbs remain constant regardless of the narrowness of

substrates. This is similar to findings in primates (Nyakatura *et al.*, 2008) and rats (Schmidt & Fischer, 2010), and this was partly expected, as any anterior or posterior weight distribution does not depend on substrate diameter, but on substrate inclination and ascending or descending locomotion (Preuschoft *et al.*, 1995; Preuschoft, 2002). Moreover, it highlights that despite the grasping capabilities of the hind limbs of many of the species studied, the hind limbs may not be biomechanically involved in increasing stability on narrower substrates by an increased stance to swing phase ratio.

In contrast, when small mammals are on larger substrates, they maximise their locomotor competence by increasing velocity, and swing to stance ratios of the limbs (lower DF). As a result, small mammals, which are often exposed to high predation pressures, are able to move swiftly between stops, minimising exposure and maximising vigilance for predators (McAdam & Kramer, 1998). Often, this increase in velocity is achieved by switching to asymmetrical gaits (Hildebrand, 1977; Gasc, 2001; Young, 2009; Shapiro *et al.*, 2016), as displayed by *A. flavicollis*, *T. paedulus*, and *M. avellanarius*. Overall, asymmetrical gaits may be of high importance for improving arboreal locomotion (Flaherty *et al.*, 2010). Their role in an arboreal context has been overlooked, but they have been gaining attention recently (i.e. Young, 2009; Shapiro *et al.*, 2016). Asymmetrical gaits may confer a few advantages compared to symmetrical gaits at higher velocities. For instance, the transition from symmetrical trots to asymmetrical gallops in horses has been shown to contribute to decreasing peak substrate reaction forces, which in turn significantly reduces the considerable strain born by the musculoskeletal system during high-speed locomotion (Farley & Taylor, 1991). The same transition, from trot to gallop, may also reduce metabolic costs, making asymmetrical gaits more economical at a higher velocity (Hoyt & Taylor, 1981). Moreover, when asymmetrical gaits are used in an arboreal context, they have several characteristics that increase stability. Marmosets, squirrel monkeys, and red tree squirrels, all avoid full-body suspension phases, increase the duration during which limbs are in contact with the substrate, and also increase the time interval between the touchdown of trailing and leading limbs during asymmetrical gaits (Young, 2009; Schmidt & Fischer, 2011; Shapiro *et al.*, 2016). These special characteristics of arboreal asymmetrical gaits contribute to arboreal stability of small mammals at higher velocities.

To sum up, small mammals tend to respond to substrate diameter in various ways, all of which tend to increase their stability and safety. The mechanisms which are

common among most species are the use of LS gaits concurrently with an increased DF and a reduction in velocity, all of which increase the static stability of locomotion. On the other hand, DFI is not affected, implying maintenance of the roles of fore- and hind limbs in locomotion regardless of substrate diameter. Last, the use of DS gaits displayed by *A. pygmaeus* may relate to dynamic stability and an increased degree of arboreality, especially when accompanied by enhanced grasping capacities in both fore- and hind limbs.

Direction of movement

Results regarding the relationship of gait metrics to ascending, descending, and horizontal locomotion were not as clear cut as was observed for substrate diameter (Table 8.2). In most species for which symmetrical gaits were recorded direction of movement had no effect on D, except for *A. pygmaeus* and *M. minutus*. In *A. pygmaeus* D was higher during horizontal locomotion than during descents or ascents, which scored similarly. In *M. minutus*, D was lower in horizontal locomotion than during descents or ascents, which also scored similarly. DFI was in most cases higher in ascents, followed by horizontal locomotion, and descents. No effect of the direction of movement on DFI was observed in *M. glareolus* and *T. paedulcus*. The duty factor (DF) was, in most cases, higher in horizontal compared to descending locomotion. However, *A. flavicollis* and *T. paedulcus* did not adjust DF according to their direction of movement.

During ascents, DS gaits along with a higher DFI are thought to assist in generating additional propulsion (Nyakatura *et al.*, 2008). In these gaits, the hind limbs touch down shortly before their contralateral forelimbs. As a result, a considerable proportion of the hind limbs' stance phase has elapsed before the touchdown of the diagonal forelimbs. This temporal spacing brings the hind foot spatially closer to the pivot of the extremity which is producing propulsion, or may have already started contributing to propulsion itself. As a result, an increased D during ascents may have an adaptive advantage, by reducing the retarding role of the forelimbs in the first part of their stance phase, while the autopodium is still anterior to the pivot of the extremities (Nyakatura *et al.*, 2008). However, none of the species studied increased D in ascents, except for *M. minutus*. On the other hand, the higher DFI observed in most species during ascents, i.e. a higher DF of the hind limbs in relation to the DF of the forelimbs, confirms that during ascents there

is a higher reliance on hind limbs for propulsion due to a posterior weight shift (Preuschoft *et al.*, 1995; Preuschoft, 2002). In an opposite manner, downward locomotion causes an anterior weight shift, which in some species such as *A. pygmaeus*, *A. flavicollis*, and *M. minutus*, caused a reduction in DFI, suggesting a higher reliance on the forelimbs. This is due to a higher portion of vertical impulse transferred to the forelimbs which enhances their regulative and supportive role (Rollinson & Martin, 1981; Nyakatura *et al.*, 2008). However, in some species (e.g. *M. glareolus* and *T. paedulcus*), DFI remained constant, regardless of the direction of locomotion. The lack of changes in the DFI implies that the roles of the two sets of limbs in locomotion may not be affected by the inclination of the substrate, as has been proposed based on similar results in rats and European red squirrels (Schmidt & Fischer, 2011).

In most studied species, velocity increased during ascents compared to horizontal locomotion or descents. This observation contrasts previous results in a number of mammalian and non-mammalian vertebrates moving on inclines (Birn-Jeffery & Higham, 2014). Our observations, however, may relate to the specific challenges of arboreal locomotion. Velocity increase on inclines has been also observed in European red squirrels (*Sciurus vulgaris*), and has been functionally related to the maintenance of dynamic stability (Schmidt & Fischer, 2011) by non-zero angular momentums (Lammers & Zurcher, 2011a). On the other hand, *T. paedulcus* was slower in ascents, which may promote static stability during arboreal locomotion (Schmidt & Fischer, 2011) through more controlled, cautious locomotion. Moreover, for those species for which descents were recorded, velocity was higher compared to that of horizontal locomotion. This may possibly indicate that animals take advantage of gravity when descending, in order to reduce their energy expenditure (Birn-Jeffery & Higham, 2014).

Table 8.2. Effect of the direction of movement on various gait metrics for each species examined, as determined by ANCOVA tests (See respective chapters for exact results). Comparisons between descents (Des), horizontal locomotion (Hor) and ascents (Asc) at the $p < 0.05$ level of significance. (“=” stands for similar; “>” stands for larger; “≥” stands for larger but insignificant; “unknown” stands for lack of data).

Species	Diagonality	Duty Factor	Duty Factor Index	Velocity	Stride Length	Stride Frequency
<i>Acrobates pygmaeus</i>	Hor > Des = Asc	Hor > Des = Asc	Asc > Hor > Des	Asc = Des > Hor	Des = Hor = Asc	Des = Asc > Hor
<i>Muscardinus avellanarius</i>	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
<i>Glis glis</i>	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
<i>Myodes glareolus</i>	Des = Hor	Hor > Des	Des = Hor	Des > Hor	Des = Hor	Des > Hor
<i>Apodemus agrarius</i>	Des = Hor	Hor > Des	Hor > Des	Des ≥ Hor	Des = Hor	Des > Hor
<i>Apodemus flavicollis</i>	Des = Hor = Asc	Des = Hor = Asc	Asc > Hor > Des	Asc > Hor > Des	Des = Hor = Asc	Asc > Hor > Des
<i>Micromys minutus</i>	Des = Asc > Hor	Hor = Asc > Des	Asc (=Des) > Hor (=Des)	Des = Asc > Hor	Des = Asc > Hor	Des (=Asc) > Hor (=Asc)
<i>Thallomys paedulus</i>	Hor = Asc	Hor = Asc	Hor = Asc	Hor > Asc	Hor > Asc	Hor = Asc

8.2. Adaptive Patterns and Processes in Mammalian Arboreality

In order to diagnose whether the degree of arboreality and gait metrics relate, the eight species examined were initially ranked by degree of arboreality based on existing ecological data. The metatherian *A. pygmaeus* is exclusively arboreal (Goldingay & Kavanagh, 1995), and although it is possible, I have not found any reports of them descending to the ground. The two glirids, *M. avellanarius* and *G. glis*, are extensively arboreal, staying primarily on trees and bushes, but also occasionally descent to the ground to burrow (Juškaitis, 2008; Kryštufek, 2010). *Thallomys paedulus* relies heavily on arboreal locomotion on acacia trees, however also digs burrows and finds shelter among the roots of trees (Earl & Nel, 1976; De Graaff, 1978). *Micromys minutus* is primarily arboreal during the breeding season, but also spends extensive periods on the ground (Nordvig *et al.*, 2001). Among the remaining rodents, *A. flavicollis* often climbs on trees (Borowski, 1962; Montgomery, 1980), while *M. glareolus* is an occasional climber (Montgomery, 1980), though both are primarily terrestrial. Lastly, *A. agrarius* is possibly exclusively terrestrial (Böhme, 1978), as there are no reports of it being captured or observed on on trees or bushes.

An ANCOVA was carried out for the gait metrics (diagonality, duty factor, duty factor index, dimensionless velocity, dimensionless stride length, and dimensionless stride frequency) to explore if significant differences exist between species. As ascending and descending locomotion was missing for some species, only data on horizontal locomotion were used. Furthermore, in order to compensate for differences in body size, substrate diameters were divided by the mean head-body length of each species, to obtain a measure of adjusted dimensionless substrate size (Alexander, 1977). The adjusted substrate size was inserted as covariate into the ANCOVA model for all tests. All obtained ANCOVA results were significant at $p < 0.001$ (Fig. 8.1, Table 8.3). Moreover, a Principal Component Analysis (PCA) was performed using mean gait parameters (diagonality, duty factor, duty factor index, dimensionless velocity, dimensionless stride length, and dimensionless stride frequency) for each species on each substrate used. *Glis glis* was excluded from the analysis (exclusively asymmetrical gaits, therefore lack of data on diagonality). In the PCA, the first two axes explained 86.16% of the observed variance (axis 1: 60.86%; axis 2: 23.50%) and all observations were subsequently plotted and examined in the space described by these two axes (Fig. 8.2; Table 8.4).

Among the metrics examined, duty factor was lower ($N=7$, $F_{(7,434)}=143.084$, $p<0.001$) and stride frequency higher ($N=7$, $F_{(7,434)}=100.482$) in more arboreal species, and both seemed to be most consistent in predicting the degree of arboreality (Fig. 8.1). Duty factor and dimensionless stride frequency were also the primary contributors to Factor 1 of the PCA which separated arboreal species from terrestrial ones (Table 8.4; Fig. 8.2). Diagonality ($N=6$, $F_{(6,323)}=8.166$) was an adequate predictor of arboreality in the most arboreal species, and was overall positively linked with levels of arboreality. However, the pattern of higher diagonality for increased arboreality did not hold in the most terrestrial species (Fig. 8.1). All remaining metrics, although significantly different between species, did not seem to relate to the degree of arboreality. Moreover, dimensionless velocity, although overall higher in more arboreal than in more terrestrial species (Delciellos & Vieira, 2006; Camargo *et al.*, 2016), was not a reliable predictor of their degree of arboreality (Fig. 8.1; Table 8.3).

Table 8.3. Estimates of gait metric means (and SD given in brackets) based on ANCOVA for all study species ordered by degree of arboreality. ANCOVA F values and significance levels are also reported for each metric. “Dim.” means dimensionless values of the respective gait metrics.

Arboreality Rank	Species	N	Diagonality	Duty Factor	Duty Factor Index	Dim. Velocity	Dim. Stride Length	Dim. Stride Frequency
1	<i>Acrobates pygmaeus</i>	6	52.7 (0.80)	42.12 (0.84)	99.80 (1.52)	1.637 (0.049)	2.452 (0.047)	0.654 (0.014)
2	<i>Muscardinus avellanarius</i>	1	39.0 (2.62)	44.00 (1.39)	109.51 (2.52)	2.109 (0.081)	3.417 (0.078)	0.599 (0.023)
3	<i>Glis glis</i>	1	No Symmetrical Gaits	55.26 (1.60)	111.06 (2.90)	0.887 (0.094)	1.946 (0.090)	0.453 (0.026)
4	<i>Thallomys paedulus</i>	12		64.28 (1.25)	100.06 (2.28)	1.249 (0.074)	2.698 (0.070)	0.423 (0.020)
5	<i>Micromys minutus</i>	6	32.1 (1.01)	70.64 (1.06)	92.57 (1.92)	0.536 (0.062)	2.073 (0.059)	0.251 (0.017)
6	<i>Apodemus flavicollis</i>	2	32.9 (1.63)	69.65 (1.70)	99.12 (3.08)	0.771 (0.065)	2.852 (0.063)	0.265 (0.018)
7	<i>Myodes glareolus</i>	2	25.0 (1.52)	72.89 (1.59)	105.90 (2.88)	0.527 (0.093)	2.084 (0.089)	0.249 (0.026)
8	<i>Apodemus agrarius</i>	2	32.3 (1.07)	75.43 (1.12)	100.01 (2.02)	0.491 (0.065)	2.213 (0.063)	0.217 (0.018)
ANCOVA			$F_{(6,323)}=8.166$ $p<0.001$	$F_{(7,434)}=143.084$ $p<0.001$	$F_{(7,434)}=6.636$ $p<0.001$	$F_{(7,434)}=73.939$ $p<0.001$	$F_{(7,434)}=44.449$ $p<0.001$	$F_{(7,434)}=100.482$ $p<0.001$

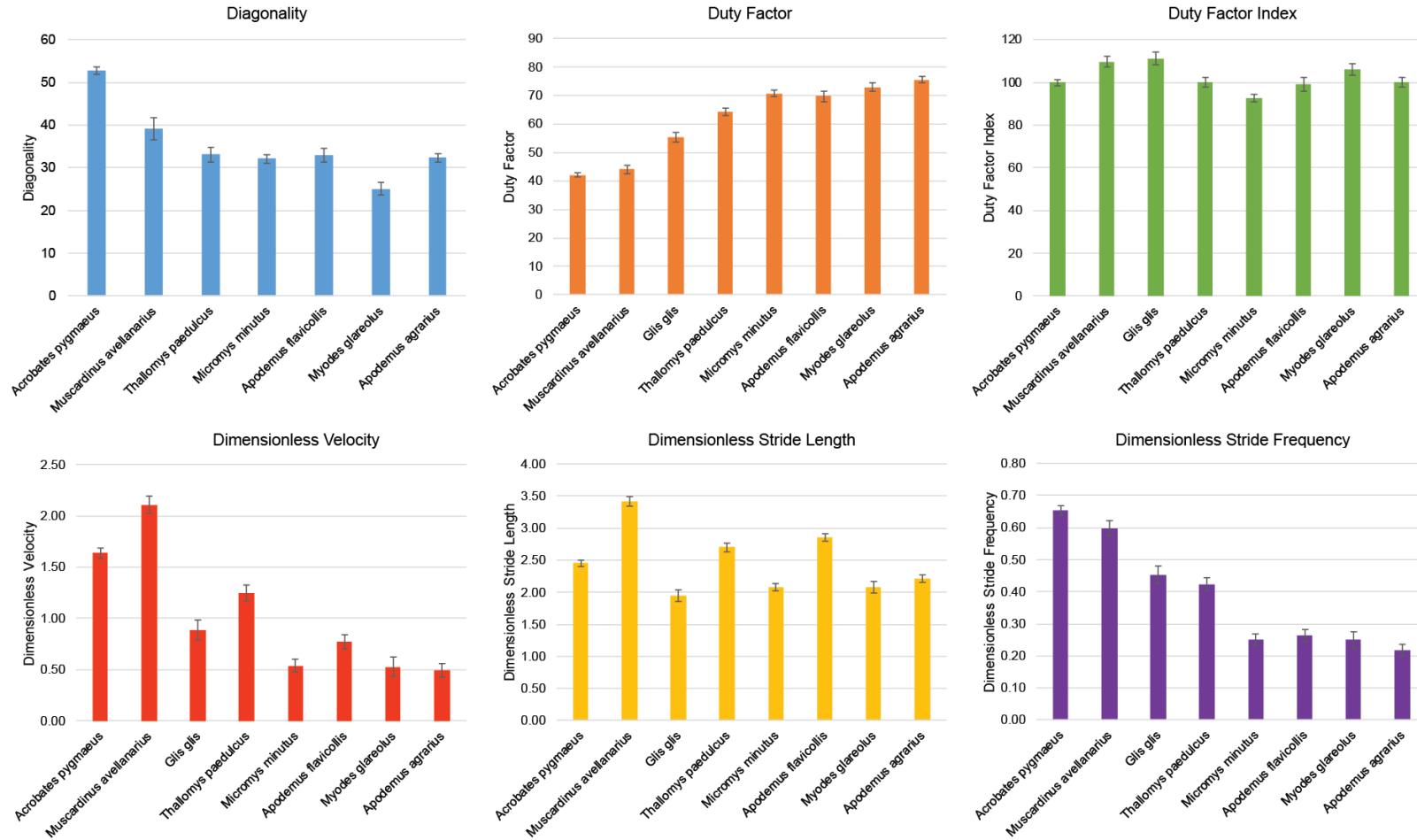


Figure 8.1. Gait metric means for all studied species as estimated through ANCOVAs. Estimates were predicted using dimensionless conversions of substrate diameters and inserting them as covariates in the ANCOVA model. In the diagonality graph, *Glis glis* is absent, due to lack of symmetrical gaits. Jackknives on each bar represent estimates of standard deviation. Species are ordered in decreasing arboreality from left to right.

Table 8.4. Principal components obtained from the covariance matrix of gait parameters. Cases used for the principal component analysis were mean gait parameters (diagonality, duty factor, duty factor index, dimensionless velocity, dimensionless stride length, and dimensionless stride frequency) for each species on each substrate diameter used.

	Factor 1	Factor 2
Diagonality	-0.375	-0.396
Duty Factor	0.503	-0.007
Duty Factor Index	0.0734	0.738
Dimensionless Velocity	-0.496	0.175
Dimensionless Stride Length	-0.330	0.505
Dimensionless Stride Frequency	-0.496	-0.110
Eigenvalue	3.652	1.410
% of variance explained	60.86	23.50

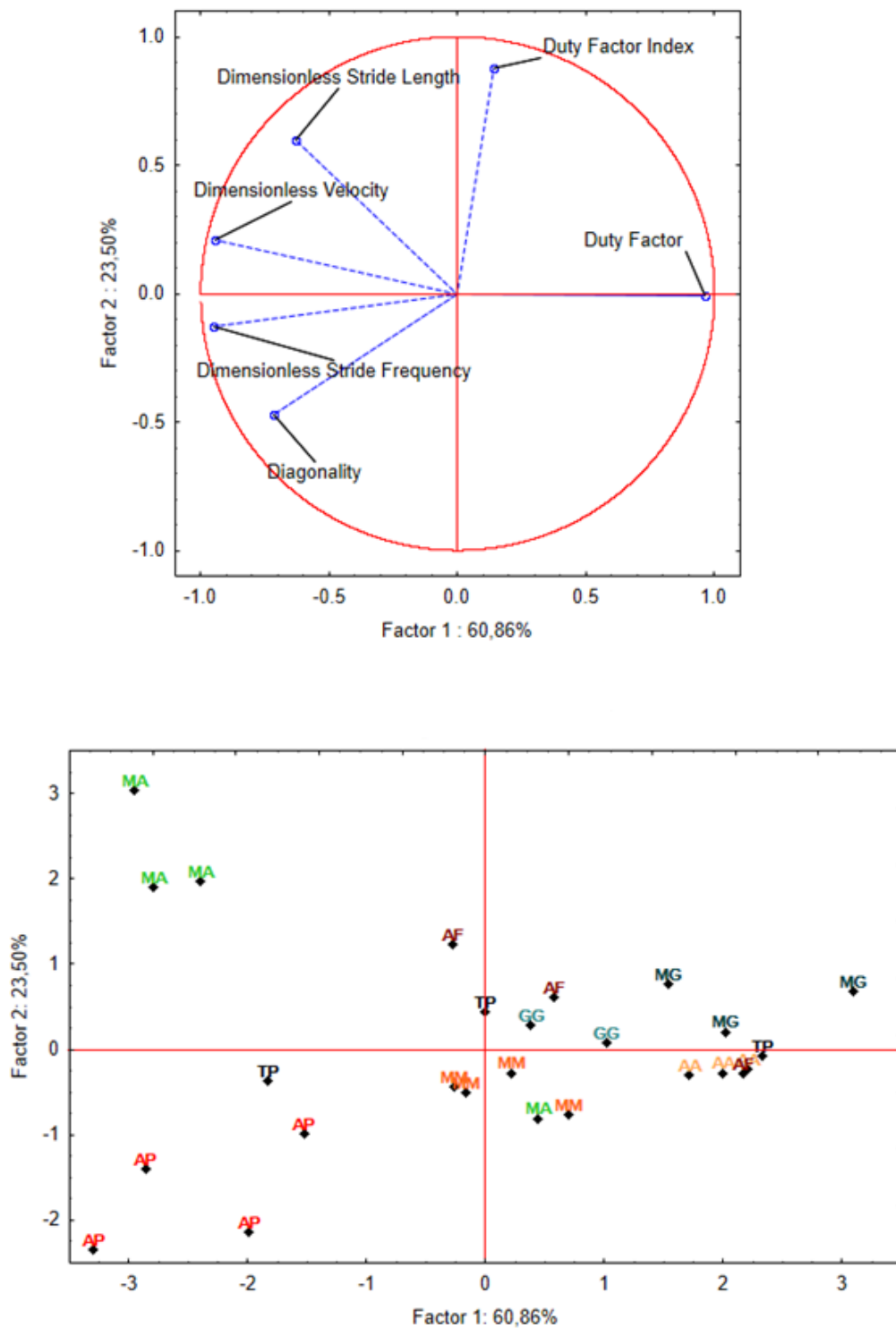


Figure 8.2. Projection of variables used in the the PCA (top) and of species (bottom) on the plane described by the first two factors. Species are represented using initials (AP: *Acrobates pygmaeus*; AA: *Apodemus agrarius*; AF: *Apodemus flavicollis*; GG: *Glis glis*; MM: *Micromys minutus*; MA: *Muscardinus avellanarius*; MG: *Myodes glareolus*; TP: *Thallomys paedulus*). Each point corresponds to each substrate diameter category with observations for given species.

A higher duty factor represents larger percentages of a gait cycle in which the limbs are on the substrate. It accommodates arboreal stability by reducing touchdown torques, increasing simultaneous contact points and contact periods with the substrate (Lammers & Zurcher, 2011b). The increased duty factor in more terrestrial species implies that this may be a behavioural response to increase stability on narrow substrates for which they are not well-adapted. On the other hand, arboreal species, adapted to the challenges of the arboreal milieu, maintained longer swing phases relative to stance phases. Towards increasing stability and safety, less arboreal mammals are also characterised by lower stride frequency, which along with the higher DF, and an overall lower velocity than more arboreal mammals, indicate a more cautious locomotion. Cautiousness may be essential for arboreal locomotion because of the lack of morphological or behavioural mechanisms that specifically facilitate arboreal stability in less arboreal mammals. This would then also translate to longer reaction times to avoid obstacles, and a consequent reduced likelihood for potentially fatal errors. On the other hand, mammals that are more inclined to use the arboreal milieu may have ultimately developed morphological adaptations that allow them to move more rapidly and decrease their DF. Moreover, hip and scapular mobility, often encountered among more arboreal mammals (Cartmill, 1985), combined with a higher velocity may assist in increasing stability by counteracting the torques generated at limb touchdown (Lammers & Gauntner, 2008; Lammers & Zurcher, 2011b). The counteraction of torques is achieved by generating non-zero angular momentums through small movements in their body, such as rotations of the limbs around the hip joint or the scapula, ultimately increasing dynamic stability on arboreal substrates (Lammers & Gauntner, 2008; Lammers & Zurcher, 2011a).

Moreover, more arboreal mammals display a higher stride frequency which essentially implies more frequent contact with the substrate. This enables a greater control of movement, allowing easier halts and swifter changes of direction when obstacles are encountered (Kram & Taylor, 1990; Hoyt, Wickler & Cogger, 2000; Hanna, 2006; Hanna & Schmitt, 2011b). The facilitation of obstacle avoidance may be of adaptive importance on arboreal substrates which are characterised by irregularities on their surface such as protrusions of branches, branch stubs, and crevices in the bark. Moreover, branches are mobile, both around and perpendicularly to their longitudinal axis, and leaves or other branches may block an animals' path. During fast locomotion, these distractions require swift action, coupled with minimising the possibility of correcting

errors and may induce a lack of stability. Furthermore, stride frequency is a more significant factor to velocity increase than stride length in all the small mammals examined here, regardless of their degree of arboreality. Regulation of velocity by stride frequency decreases body oscillations, which may disrupt continuous progression and may thus be more advantageous for smaller arboreal and scansorial mammals (Strang & Steudel, 1990; Delciellos & Vieira, 2007). Lastly, in some scansorial and arboreal mammals, the increase in velocity is further facilitated by the use of asymmetrical gaits on larger substrates. Fast asymmetrical gaits may be favourable over fast symmetrical gaits for arboreal locomotion (Young, 2009; Flaherty *et al.*, 2010; Schmidt & Fischer, 2011; Shapiro *et al.*, 2016) as they may cause less strain on the muscles and bones and result in a lower energetic expenditure (Farley & Taylor, 1991; Wickler *et al.*, 2003). Nevertheless, more research on the detailed kinematics of asymmetrical gaits is required in order to understand how small mammals specifically use and adapt these gaits on arboreal substrates where a loss of balance is dangerous.

Another common pattern observed here is that small clawed scansorial and terrestrial mammals primarily opted for lateral-sequence (LS) gaits during arboreal locomotion. Moreover, even on the narrowest substrates utilised they reduced D rather than increasing it (i.e. using DS gaits). Most mammalian species examined, terrestrial or scansorial, as well as most reptiles, display LS footfall patterns (Hildebrand, 1976; Cartmill *et al.*, 2002; Witte *et al.*, 2002; McElroy, Hickey & Reilly, 2008), contrary to the DS footfall pattern most often observed among arboreal mammals (Cartmill *et al.*, 2002, 2007). The most parsimonious hypothesis, therefore, is that LS gaits likely represent an ancestral state, whereas DS gaits are derived. The retention of the ancestral LS footfall pattern, along with a reduction in the relative swing phases of the limbs, can confer competent arboreal stability by keeping the centre of mass always within the support polygon (Lammers & Zurcher, 2011a). On the other hand, the most arboreal *A. pygmaeus* was isolated from other species on the negative side of the PC axes (Fig. 8.2). This placement was related to high dimensionless stride length, stride frequency, velocity, and diagonality (Fig. 8.2, Table 8.3). Feathertail gliders were the only species in this study that habitually employed diagonal-sequence diagonal couplets (DSDC) gaits. However, contrary to *A. pygmaeus*, the similarly sized and highly arboreal *M. avellanarius* used equally narrow substrates, moved at higher velocities than *A. pygmaeus*, and utilized LSDC gaits. The highly arboreal *M. avellanarius* was isolated on the positive side of axis 2 and

the negative side of axis 1, due to their high stride length, stride frequency, velocity, and DFI (Fig. 8.2, Table 8.3). Among other things, *M. avellanarius* differs from *A. pygmaeus* in the lack of increased grasping abilities of the forelimb (Haffner, 1998). As argued earlier, it is possible that grasping by both the manus and the pes is required to make DSDC more useful than LSDC gaits in an arboreal setting. If grasping forefeet are present, and arboreality is a primary component in an animal's ecology, the evolution of DS gaits may be favourable, as they produce medially-oriented forces (Schmitt, 2003b; Lammers & Biknevicius, 2004; Schmidt & Fischer, 2010) and allow animals to apply contralateral pressure on substrates and consequently increase friction, making slipping less likely (Lammers & Zurcher, 2011a). However, the results from this research cannot support this hypothesis. Future research into the forces applied by both clawed and non-clawed mammals which utilise both DS and LS gaits and especially in relation to substrate use may be more informative in relation to this question. Nevertheless, as D is not the most reliable predictor of arboreality in the meta-analysis carried out it seems that DSDC gaits may not be essential for successful arboreal locomotion as its occurrence does not increase with increased arboreality. Scansorial mammals can be also successful in achieving arboreal stability by using LS gaits, and utilise other mechanisms alongside this footfall pattern, such as increased DF and lower stride frequency.

Levels of arboreality have no overall effect on the duty factor index. Most of the studied scansorial/arboreal mammals displayed an increased hind limb to forelimb duty factor ratio (DFI) when ascending, with the opposite trend observed while descending. The higher DFI observed during ascents indicates a higher reliance on the hind limbs for propulsion due to a posterior weight shift (Preuschoft *et al.*, 1995; Preuschoft, 2002). On the other hand, the anterior shift in weight during descents causes more vertical impulse on the forelimbs which then assume a more pronounced regulatory and support role (Rollinson & Martin, 1981; Nyakatura *et al.*, 2008). Moreover, as exhibited by *M. minutus*, this compartmentalisation in fore- and hind limb use may also be of great importance during vertical climbing. The separation in the roles of the fore- and hind limbs may be especially significant for both scansorial and arboreal mammals as perfectly horizontal substrates are rarely encountered. Similarly, the high duty factor index observed during above-branch locomotion in both glirids, *M. avellanarius* and *Glis glis*, implies hind limb dominance in locomotion. Both glirid species examined here are primarily arboreal animals which often move vertically between the canopy strata, yet also descend to the

ground for burrowing (Juškaitis, 2008; Kryštufek, 2010). Thus, they engage in frequent climbing, hanging and clambering, facilitated by the possession of long digits, curved claws, a tendon-locking mechanism, and pedal grasping (Haffner, 1996, 1998; Krattli, 2001; Zefferer, 2002). Their ecology and morphological adaptations highlight a possible hind limb dominance which has been functionally linked to habitual vertical climbing (Preuschoft, 2002). A high duty factor index was also observed in the highly terrestrial *M. glareolus*. Even though their gross morphology is linked with digging and terrestrial locomotion, some *Myodes* species, including bank voles, occasionally vertically climb arboreal substrates (Buesching *et al.*, 2008; Nations & Olson, 2015) and would thus benefit from an elevated duty factor index. Nevertheless, the observed hind limb dominance may relate to other aspects of their ecology, as they are much more terrestrial in their locomotor habits (Buesching *et al.*, 2008). However, since the present study lacks adequate data, especially vertical locomotion observations, this is speculative and requires further research. Most other species displayed duty factor indices close to 100 which may be an indication of different but equal contributions by the forelimbs and the hind limbs to locomotion (Schmidt & Fischer, 2011). The data on DFI that I have collected here produce more questions than answers and more observations, as well as more individuals tested, in arboreal small mammals are essential to determine how and why forelimbs and hind limbs participate in arboreal locomotion.

Interestingly, the four studied murid rodents (*A. agrarius*, *A. flavicollis*, *M. minutus*, *T. paedulcus*), as well as the cricetid *M. glareolus*, were more or less clustered closely together in the PCA (Fig. 8.1). They appear to be characterised by higher duty factor, but lower velocity, stride length and stride frequency than more arboreal species (Table 8.3, Fig. 8.1). The more terrestrial *A. agrarius*, along with *M. glareolus*, were placed at the positive end of this cluster, on the opposite side to the arboreal *A. pygmaeus* and *M. avellanarius*, probably in relation to their higher duty factors (Fig. 8.1). On the other hand, the more arboreal *T. paedulcus*, *M. minutus*, and *A. flavicollis* were located at the centre of the axes, between the most arboreal and the most terrestrial species, possibly implying an intermediate degree of arboreality. However, besides the lower duty factor and stride higher frequency of the more arboreal murids, *T. paedulcus*, *M. minutus*, and *A. flavicollis* did not differ greatly from the terrestrial representatives. Although arboreal and terrestrial murids differ substantially in appendicular morphology (Krattli, 2001; Zefferer, 2002; Samuels & Van Valkenburgh, 2008; Kuncová & Frynta, 2009), our results

suggest that gaits may be more or less conserved within the family. However, since both terrestrial and arboreal locomotion are implicated in most of the examined species it may also be that the terrestrial component is what shapes their gaits most strongly. For instance, all rodents use LS gaits which may be more stable in terrestrial locomotion than DS gaits (Lammers & Zurcher, 2011a).

These common patterns also bear some implications for mammalian locomotor evolution. The oldest known eutherians (Ji *et al.*, 2002; Luo *et al.*, 2011) and metatherian (Luo *et al.*, 2003) were small and scansorial suggesting that both characteristics may have also been featured in the common ancestor of the two lineages. As argued earlier, the LS footfall pattern may represent an ancestral state, being the most common pattern among mammals and reptiles (Hildebrand, 1976; Cartmill *et al.*, 2002; Witte *et al.*, 2002; McElroy *et al.*, 2008). Moreover, most small mammals examined in this research, which are similarly sized to *Eomaia* (20-25 g), *Juramaia* (15-17 g), and *Sinodelphys* (~30 g), used LS gaits during arboreal locomotion as well. Thus, it is highly probable that the LS footfall pattern characterised early mammals as well. These ancestors were most probably able to climb on shrubs and trees and would also have engaged in above-branch locomotion. In order to increase their stability, we would expect them to shift to gaits of lower D, higher DF, and lower velocity, as substrates became narrower, as well as increase velocity by stride frequency. Ascents, whether inclined or vertical, would be facilitated by increased input of propulsion by their hind limbs rather than the forelimbs. On the other hand, descents would place an emphasis on regulation and control of locomotion by their forelimbs. Thus, they would be able to ascend from the ground up to trees and bushes as fast as possible and descend head-first, being able to safely control their motion and scan the ground for dangers or food.

To conclude, small clawed mammals are integral in understanding how arboreality has evolved throughout mammalian history. Despite limitations, they can be used as living models to understand morpho-behavioural adaptations in extinct and ancestral mammalian species. Moreover, they represent different adaptive scenarios compared to larger mammals which tend to possess special morphological adaptations in order to navigate the arboreal milieu. Moreover these large mammals are much more derived than ancestral mammalian morphotypes, such as *Eomaia* and *Juramaia*. This research, though by far non-exhaustive, shows that several small mammals display similar patterns in their gait which tend to optimize their ability to utilise arboreal substrates. Nevertheless, there

is still much to be done. One important future addition would be more extended studies on the vertical locomotion of scansorial mammals, as climbing is a very demanding and important aspect of their ecology. Furthermore, the parameters studied herein effectively demonstrate some overall patterns and assist in constructing a gross locomotor profile of these species in relation to substrate properties. More detailed kinematic analyses of how small mammals adapt their gait and limb movements for arboreal locomotion is essential, however. Especially, higher resolution data on the spatial placement of the limbs on different substrates, as well as adjustments in the motion of joints coupled to measurements of locomotor forces and pressures would be insightful. Lastly, due to the small differences in size this study is not informative on the differences between smaller and larger mammals. Further comparative research on gait differences in animals of different body sizes can inform us on how size affects quadrupedal arboreal locomotion, an issue that has not been addressed adequately thus far.

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Appendix

Dataset available at:

<https://figshare.com/s/4d496e3c0bc8eaac98f9>