



Original investigation

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



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ABSTRACT

Vertical stratification of the arboreal habitat allows the coexistence of several species in a given area, because the complex arboreal strata can be used in different ways by arboreal and scansorial mammals. The present report experimentally investigated the gait metrics on different arboreal substrates, of three sympatric rodents living in a deciduous forest in Poznań, Poland. Arboreal locomotion was compared between the burrowing striped field mouse, *Apodemus agrarius*, the scansorial bank vole, *Myodes glareolus*, and the more arboreal yellow-necked mouse, *Apodemus flavicollis*. We filmed two wild-caught individuals from each species walking on four different substrate diameters (2 mm, 5 mm, 10 mm, 25 mm) and three different inclinations (45° descending, horizontal, 45° ascending) at 240 fps and collected a set of gait parameters from a total of 273 complete cycles. Our results did not demonstrate clear relationships between arboreal locomotion and the ecology of the three species. Only *A. flavicollis* exhibited locomotor features partly associated with arboreal competence, including lower velocity and diagonality on narrow substrates and asymmetrical gaits on wider ones. On the other hand, the two *Apodemus* species, despite their different ecologies, shared a few locomotor similarities, such as velocity regulation primarily by stride frequency, and similar effects of substrate size and inclination on diagonality, duty factor, and duty factor index indicating the possibility of a phylogenetic signal. Because the selected gait parameters provided limited insight into the ability of small mammals to move competently through an arboreal habitat, these findings indicate that the relationship between behaviour and ecology is complex.

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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 (Cartmill, 1974; Grand, 1972).

In terms of locomotion, arboreal gaits function as a behavioural mechanism to enhance stability on arboreal substrates of different orientations (Lammers and Zurcher, 2011a; Schmidt and 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 (Cartmill et al., 2007; Hildebrand, 1967) 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

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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., 2007, 2002).

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 and 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., 2007, 2002). DS gaits are prevalent among primates (Cartmill et al., 2007; Hildebrand, 1967), some metatherians (Karananis et al., 2015; Pridmore, 1994; Schmitt and Lemelin, 2002; White, 1990), and the carnivorous *Potos flavus* (Lemelin and Cartmill, 2010). On the other hand, the coordinated movements of ipsilateral limbs of LS gaits, which have been functionally related to static stability (Lammers and Zurcher, 2011a), dominate in most terrestrial mammals. Nevertheless, they are used by some arboreal specialists, such as sugar gliders *Petaurus breviceps* (Shapiro and Young, 2010), callitrichid primates (Nyakatura et al., 2008; Nyakatura and Heymann, 2010) and scansorial rodents (Schmidt and 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 (Farley and Taylor, 1991; Hoyt and Taylor, 1981). In small mammals, asymmetrical gaits induce large oscillations of the spine in the sagittal plane (Schilling and 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 et al., 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 (Schmidt and Fischer, 2011; Young, 2009). 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 (Camargo et al., 2016; Delciellos and Vieira, 2006) substrates, suggesting that arboreal species are capable of utilizing dynamic stability (Schmidt and Fischer, 2011). There are multiple mechanisms to increase velocity: increasing stride frequency and decreasing stride length (Camargo et al., 2016; Delciellos and Vieira, 2006), reducing frequency and increasing stride length (Delciellos and Vieira, 2006; Larson et al., 2001), or increasing primarily stride frequency and, at a lesser rate, stride length (Karananis et al., 2015; Nyakatura et al., 2008). 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 (Delciellos and Vieira, 2006; Larson et al., 2001). 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 and 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 and Heymann, 2010), and the arboreal metatherian *Petaurus breviceps* (Shapiro and 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 and 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 and 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 and 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 (Karananis et al., 2015; Lammers, 2007; Nyakatura and Heymann, 2010; Shapiro et al., 2014; Shapiro and Young, 2010). 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 (Nyakatura and Heymann, 2010; Vilensky et al., 1994). 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 (Nyakatura and Heymann, 2010; Rollinson and Martin, 1981). 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 (Nyakatura and Heymann, 2010; Rollinson and Martin, 1981). 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 (Lammers et al., 2006; Vilensky et al., 1994). 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 and 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 and Holisova, 1974), and inhabit crop-fields and open habitats, especially along rivers and streams, as well as forest ecotones (Karaseva et al., 1992; Pucek, 1981; Zejda, 1967). 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 and Frynta, 2009). *Apodemus flavicollis* (Melchior, 1834) feed mainly on fruit and seeds (Holisova, 1967; Obrtel and Holisova, 1974), and are found primarily in forested areas (Marsh and Harris, 2000; Montgomery, 1978). The yellow-necked mice display significant arboreal behaviour and frequent use of tree branches (Borowski, 1962; Holisova, 1969; Juškaitis, 2002; Montgomery, 1980), in order to access food stores (Balát and Pelikán, 1959; Juškaitis, 2002), nesting holes (Czeszczewik et al., 2008), and to avoid predators (Montgomery and 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; Kuncová and Frynta, 2009; Zefferer, 2002). *Myodes glareolus* (Schreber, 1780) are more omnivorous than other arvicoline, with diets in between arvicoline and murids (Butet and Delettre, 2011; Watts, 1968). 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 and Olson, 2015), mainly to avoid predators (Jędrzejewska and Jędrzejewski, 1990; Jędrzejewski et al., 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 (Nations and Olson, 2015; Zefferer, 2002). 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 and Wrocławek, 1961; Chełkowska et al., 1985; Gliwicz, 1981; Heroldova, 1994; Holisova, 1969; Olszewski, 1968). 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.

Material 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. 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).

Experimental setup

For the locomotion experiments, we used a single, specially configured, filming glass terrarium (L: 90 cm × H: 40 cm × 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. 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

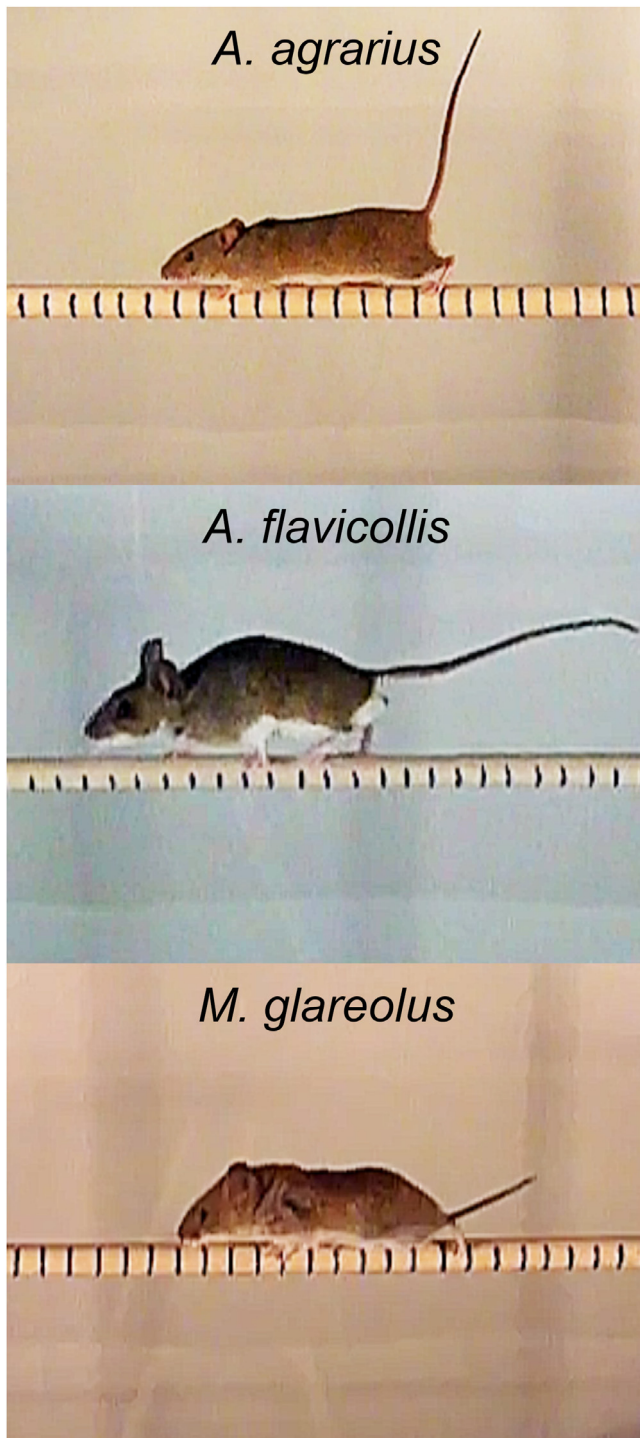


Fig. 1. Stills from the videos used for data collection. From left to right, the striped field mouse (*Apodemus agrarius*), the yellow-necked mouse (*Apodemus flavicollis*), and the bank vole (*Myodes glareolus*). The images are on the same scale. In the stills, the animals are traversing a horizontal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

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) (Cartmill et al., 2007; Hildebrand, 1967) 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 (DFh) to forelimb duty factor (DFf), calculated as $100 \cdot \text{DFh} / \text{DFf}$ (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) 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 (m s^{-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 and Jayes, 1983; Hof, 1996). The dimensionless measures were calculated as follows:

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

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

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

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

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.

Table 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			
		2 mm	5 mm	10 mm	25 mm	2 mm	5 mm	10 mm	25 mm	2 mm	5 mm	10 mm	25 mm
<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	–	–	–	–

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).

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 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), 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), 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. 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*.

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 s) 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 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 25 mm 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.

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, Figs. 2 and 4), with only a single gait being recorded over the 50.00 threshold for *A. agrarius* (Fig. 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, Figs. 2 and 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, Figs. 2 and 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,

Table 2
Means and 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 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	–	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)

$p=0.757$), 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 (Figs. 3 and 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 (Figs. 3 and 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 (Figs. 3 and 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. 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. 4 implies some interaction. Duty factor decreased with substrate size increase (Fig. 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. 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. 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. 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. 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

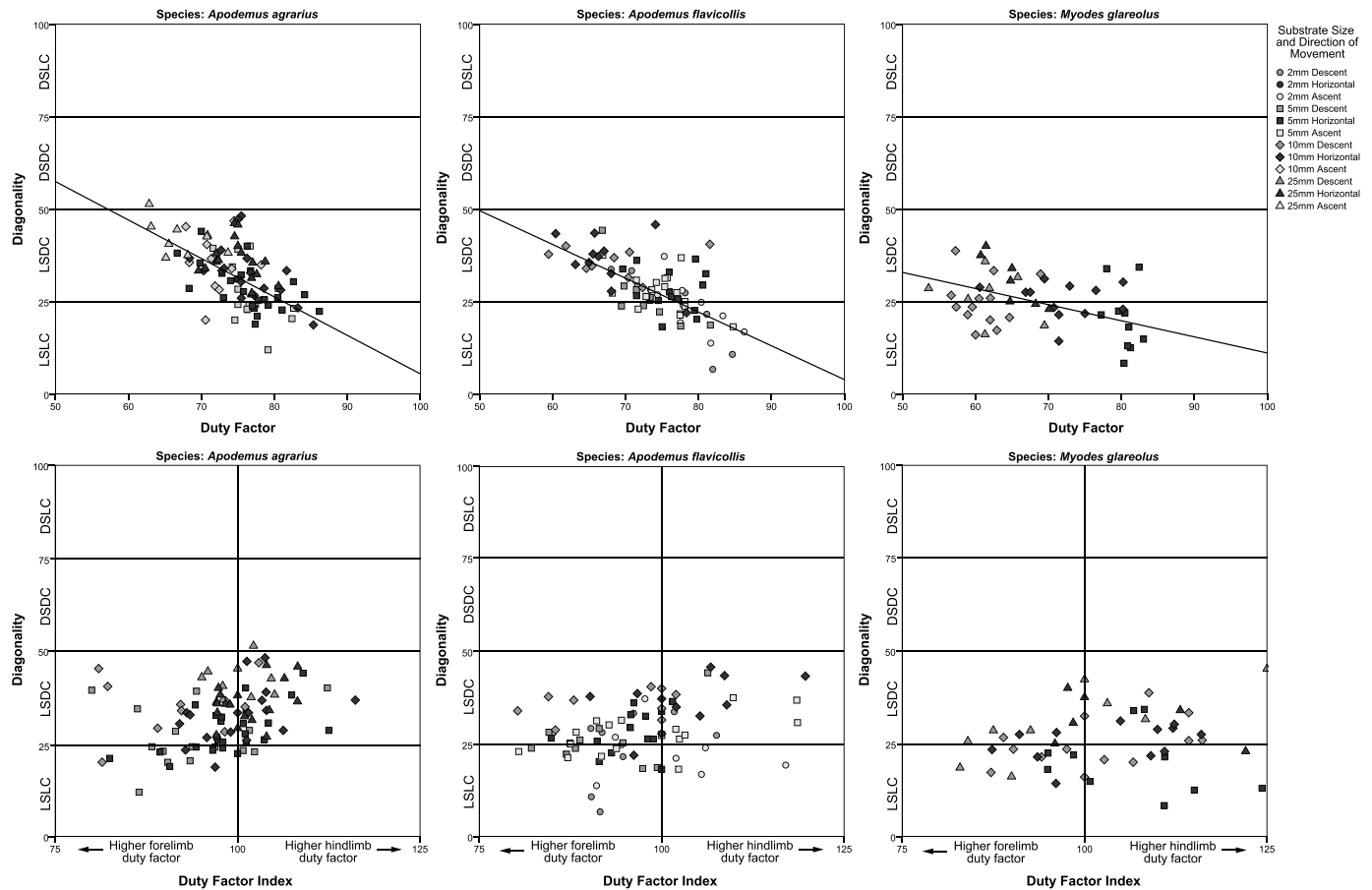


Fig. 2. Scatterplots of diagonality as a function of duty factor (top) and duty factor index (bottom) for symmetrical gaits in all species in the dataset. The horizontal lines split the sample in quadrants of decreasing diagonality (DSLC, DSDC, LSDC, LSLC, from top to bottom, acronyms explained in text). The trend line equations reported are significant at $p < 0.05$, and their functions are: *A. agrarius*, $D = 1.07E2 - 1^*DF$; *A. flavicollis*, $D = 93.51 - 0.88^*DF$; *M. glareolus*, $D = 53.83 - 0.42^*DF$. 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.

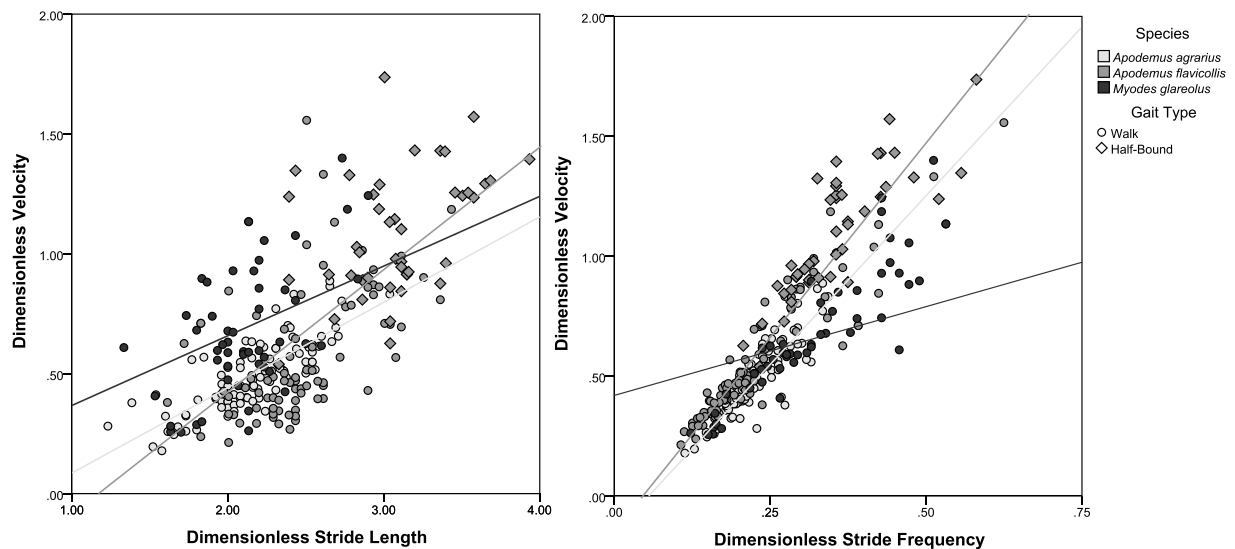


Fig. 3. Scatterplot of dimensionless velocity (V) as a function of dimensionless stride length (SL) (left) and dimensionless stride frequency (SF) (right) for all substrate sizes and directions of movement. Walks are denoted by a circle, while in asymmetrical gaits, half-bounds are represented by a diamond. The trend line equations reported are significant at $p < 0.05$. In the left graph, functions are: *A. agrarius*, $V = -0.13 + 0.36^*SL$; *A. flavicollis*, $V = -0.65 + 0.53^*SL$; *M. glareolus*, $V = 0.1 + 0.28^*SL$. In the right graph, functions are: *A. agrarius*, $V = -0.13 + 2.76^*SF$; *A. flavicollis*, $V = -0.09 + 3.07^*SF$; *M. glareolus*, $V = 0.41 + 0.75^*SF$.

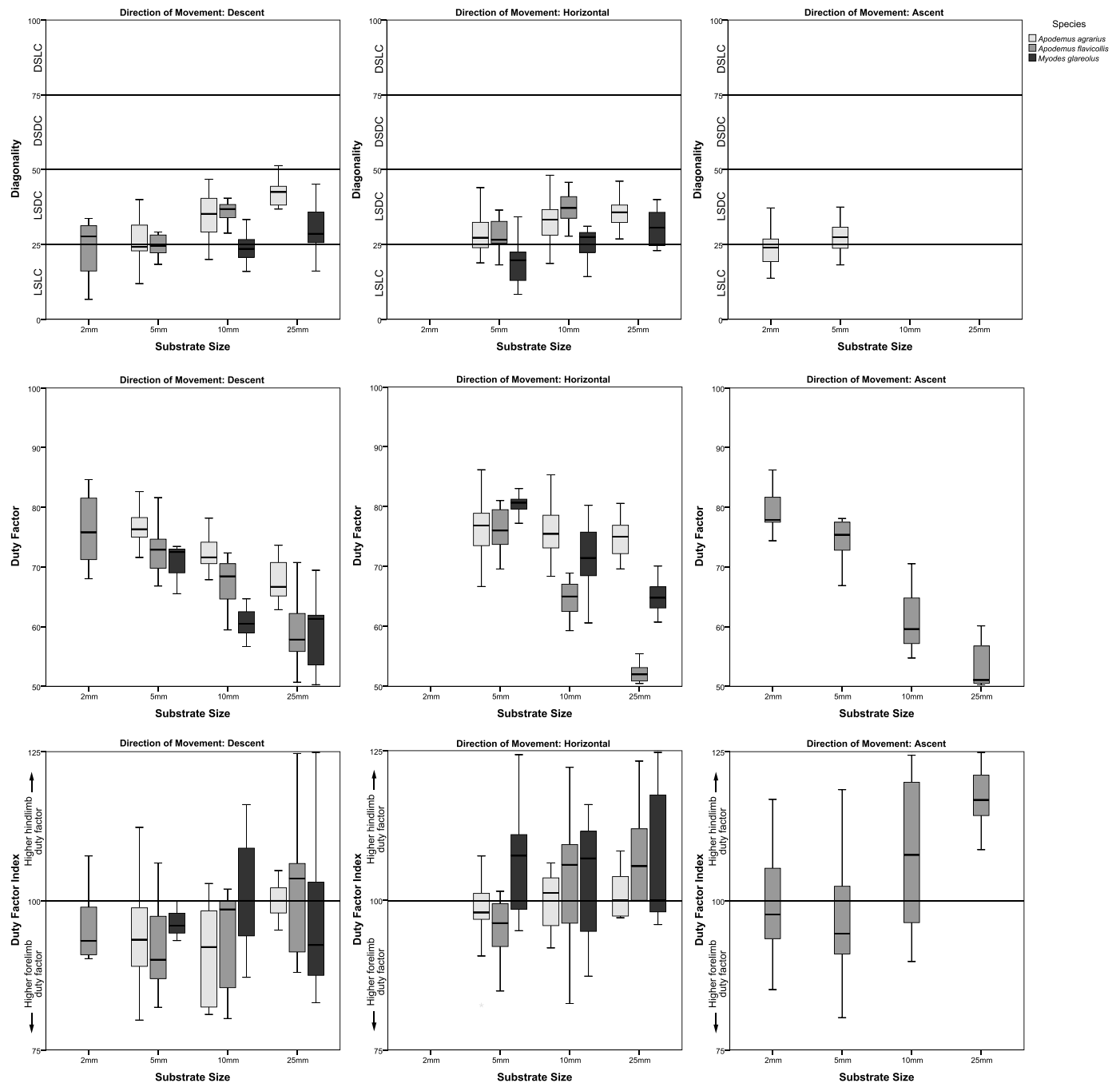


Fig. 4. Boxplots of the distribution of gait diagonality (top), duty factors (middle) and duty factor indices (bottom) in the substrate size categories examined, split into three graphs for each direction of movement. In *Apodemus flavicollis* both symmetrical and asymmetrical gaits have been included. 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 (DSIC, DSDC, LSIC, LSDC, 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.

in *A. flavicollis* ($N = 123$, $F_{(2,122)} = 1.61$, $p = 0.211$). Despite its great variability (Table 2, Fig. 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. 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. 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 $N = 54$,

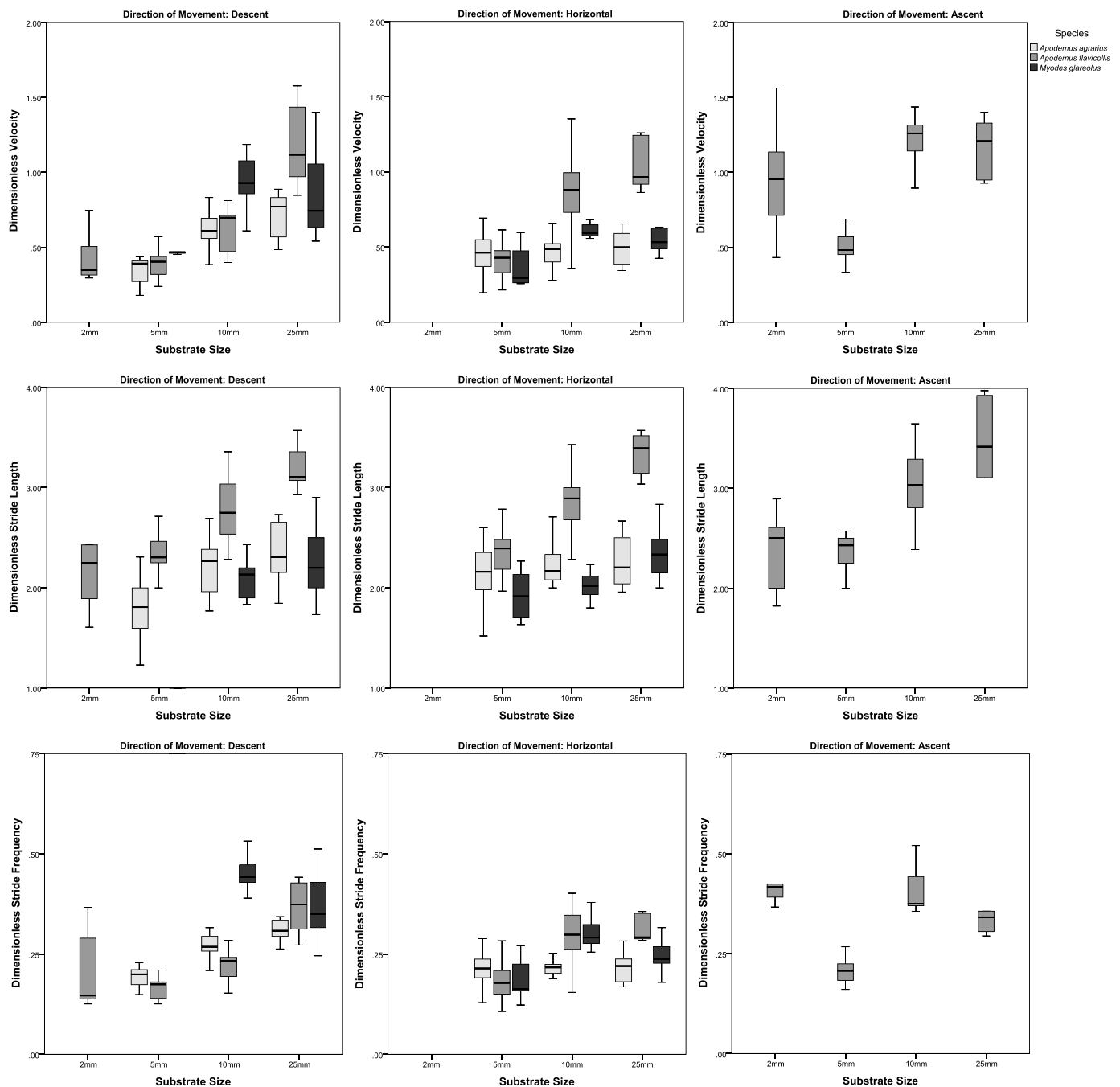


Fig. 5. Boxplots of the distribution of dimensionless velocity (top), dimensionless stride length (middle) and dimensionless stride frequency (bottom) in the substrate size categories examined, split into three graphs for each direction of movement. The top and bottom sides of the box denote the upper and lower quartiles, the line within the box is the median, while the top and bottom whiskers represent the maximum and minimum values obtained.

$F_{(1,53)} = 54.67$, $p < 0.001$, respectively). In contrast, in *Apodemus flavicollis*, stride frequency increased from descents to horizontal locomotion to ascents ($N = 123$, $F_{(2,122)} = 13.64$, $p < 0.001$).

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

(Buesching et al., 2008; Holisova, 1969; Olszewski, 1968; Tattersall and Whitbread, 1994), 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 (Camargo et al., 2016; Delciellos and Vieira, 2006; Schmidt and Fischer, 2011). 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 (Lammers, 2007; Nyakatura et al., 2008; Schmidt and Fischer, 2010; Shapiro and Young, 2012; Stevens, 2006). Upon arboreal substrates, the coordinated movements of ipsilateral limbs of LS gaits appear to provide more static locomotor stability than DS gaits (Lammers and 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 and 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 and Wrocławek, 1961; Gliwicz, 1981; Heroldova, 1994). It is very likely that the morphology of bank voles, and arvicolines in general, characterised by a short compact body, short limbs and lack of prehensile extremities (Nations and Olson, 2015; Zefferer, 2002) 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 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 (Karananis 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 and 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 (Karananis et al., 2015; Lammers et al., 2006; Nyakatura et al., 2008). 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 (Nyakatura et al., 2008; Rollinson and Martin, 1981). 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 and Fischer, 2011), and is usually linked to arboreal adaptations (Camargo et al., 2016; Delciellos and Vieira, 2006). 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 and 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 and 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 and 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 and Zurcher, 2011a). This profile better suits the more arboreal yellow-necked mice, which engage in intense arboreal activities, especially under sympatric competition (Andrzejewski and Wrocławek, 1961; Gliwicz, 1981; Heroldova, 1994; Holisova, 1969; Tattersall and 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 scanorial arvicoline *M. glareolus*, velocity depended almost equally on stride length and stride frequency, a pattern common in most small mammals (Strang and 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 and Vieira, 2006; Karantanis et al., 2015). In contrast, primates (Larson et al., 2001, 2000) and medium and large-bodied marsupials (Delciellos and 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 and 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 and 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 and Fischer, 2011; Shapiro et al., 2016). In mammals, asymmetrical gaits assist in increase of velocity, enhance manoeuvrability, and minimize the energetic cost of locomotion (Farley and Taylor, 1991; Gasc, 2001; Hoyt and Taylor, 1981). They also confer advantages in stability by reducing peak vertical forces, limiting centre of mass movements, and using shorter and more frequent strides (Schmidt and Fischer, 2011; Shapiro et al., 2016; Young, 2009). 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 (Chełkowska et al., 1985; Montgomery, 1980; Zejda, 1967), 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 (Nations and Olson, 2015; Zeffere, 2002). 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.

Conflict of interest

None.

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References

- Alexander, R.M., Jayes, A.S., 1983. A dynamic similarity hypothesis for the gaits of quadrupedal mammals. *J. Zool.* 201, 135–152.
- Alexander, R.M., Dimery, N.J., Ker, R.F., 1985. Elastic structures in the back and their role in galloping in some mammals. *J. Zool.* 207, 467–482.
- Alexander, R.M., 1977. Terrestrial locomotion. In: Alexander, R.M., Goldspink, G. (Eds.), *Mechanics and Energetics of Animal Locomotion*. Chapman & Hall, London, pp. 168–203.
- Andrzejewski, R., Wroclawek, H., 1961. Mass occurrence of *Apodemus agrarius* (Pallas, 1771) and variations in the number of associated Muridae. *Acta Theriol.* 5, 173–184.
- ASAB/ABS, 2012. Guidelines for the treatment of animals in behavioural research and teaching. *Anim. Behav.* 83, 301–309.
- Balát, F., Pelikán, J., 1959. On *Apodemus flavicollis* (Melch.) occupying nest boxes for birds. *Zool. List* 3, 257–287.
- Biewener, A.A., 2005. *Animal Locomotion*. Oxford University Press, Oxford.
- Borowski, S., 1962. *Apodemus flavicollis* (Melchior, 1834) in the tops of tall trees. *Acta Theriol.* 6, 314–314.
- Brown, D., 2009. Tracker Video Analysis and Modeling Tool.
- Buesching, C.D., Newman, C., Twell, R., Macdonald, D.W., 2008. Reasons for arboreality in wood mice *Apodemus sylvaticus* and bank voles *Myodes glareolus*. *Mamm. Biol. – Zeitschrift Säugetierkd* 73, 318–324.
- Butet, A., Deletré, Y.R., 2011. Diet differentiation between European arvicoline and murine rodents. *Acta Theriol.* 56, 297–304.
- Camargo, N.F., Sano, N.Y., Ribeiro, J.F., Vieira, E.M., 2016. Contrasting the realized and fundamental niche of the arboreal walking performance of neotropical rodents. *J. Mammal.* 97, 155–166.

- Cartmill, M., Lemelin, P., Schmitt, D., 2002. Support polygons and symmetrical gaits in mammals. *Zool. J. Linn. Soc.* 136, 401–420.
- Cartmill, M., Lemelin, P., Schmitt, D., 2007. Primate gaits and primate origins. In: Ravosa, M., Dagosto, M. (Eds.), *Primate Origins: Adaptations and Evolution*. Springer, New York, pp. 403–435.
- Cartmill, M., 1974. Pads and claws in arboreal locomotion. In: Jenkins, F.A. (Ed.), *Primate Locomotion*. Academic Press, New York, pp. 45–83.
- Chełkowska, H., Walkowa, W., Adamczyk, K., 1985. Spatial relationships in sympatric populations of the rodents: *Clethrionomys glareolus*, *Microtus agrestis* and *Apodemus agrarius*. *Acta Theriol.* 30, 51–78.
- Czesczewik, D.C., Walankiewicz, W.W., Stańska, M.S., 2008. Small mammals in nests of cavity-nesting birds: why should ornithologists study rodents? *Can. J. Zool.* 86, 286–293.
- Delciellos, A.C., Vieira, M.V., 2006. Arboreal walking performance in seven didelphid marsupials as an aspect of their fundamental niche. *Austral Ecol.* 31, 449–457.
- Demes, B., Larson, S.G., Stern Jr, J.T., Jungers, W.L., Biknevicius, A.R., Schmitt, D., 1994. The kinetics of primate quadrupedalism: hindlimb drive reconsidered. *J. Hum. Evol.* 26, 353–374.
- Farley, C.T., Taylor, C.R., 1991. A mechanical trigger for the trot-gallop transition in horses. *Science* 253, 306–308.
- Galetti, M., Rodarte, R.R., Neves, C.L., Moreira, M., Costa-Pereira, R., 2016. Trophic niche differentiation in rodents and marsupials revealed by stable isotopes. *PLoS One* 11, e0152494.
- Gasc, J.-P., 2001. Comparative aspects of gait, scaling and mechanics in mammals. *Comp. Biochem. Physiol. – Part A Mol. Integr. Physiol.* 131, 121–133.
- Gliwicz, J., 1981. Competitive interactions within a forest rodent community in central Poland. *Oikos* 37, 353–362.
- Grand, T.I., 1972. A mechanical interpretation of terminal branch feeding. *J. Mammal.* 53, 198–201.
- Gurnell, J., 1985. Woodland rodent communities. *Symp. Zool. Soc. Lond.* 55, 337–411.
- Harrell, F.E., 2001. Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis. Springer-Verlag, New York.
- Heroldova, M., 1994. Diet of four rodent species from *Robinia pseudo-acacia* stands in South Moravia. *Acta Theriol.* 39, 333–337.
- Hildebrand, M., 1967. Symmetrical gaits of primates. *Am. J. Phys. Anthropol.* 26, 119–130.
- Hildebrand, M., 1976. Analysis of tetrapod gaits: general considerations and symmetrical gaits. In: Herman, R.M., Grillner, S., Stein, P.S.G., Stuart, D.G. (Eds.), *Neural Control of Locomotion*. Plenum Press, New York, pp. 203–236.
- Hildebrand, M., 1977. Analysis of asymmetrical gaits. *J. Mammal.* 58, 131–156.
- Hildebrand, M., 1995. Analysis of Vertebrate Structure, 4th ed. John Wiley & Sons, Inc., New York.
- Hof, A.L., 1996. Scaling gait data to body size. *Gait Posture* 4, 222–223.
- Holisova, V., 1967. The food of *Apodemus agrarius* (Pall.). *Zool. List* 16, 1–14.
- Holisova, V., 1969. Vertical movements of some small mammals in a forest. *Zool. List* 18, 121–141.
- Hoyt, D.F., Taylor, C.R., 1981. Gait and the energetics of locomotion in horses. *Nature* 292, 239–240.
- Jędrzejewska, B., Jędrzejewski, W., 1990. Antipredatory behaviour of bank voles and prey choice of weasels – enclosure experiments. *Ann. Zool. Fennici*.
- Jędrzejewski, W., Rychlik, L., Jędrzejewska, B., 1993. Responses of bank voles to odours of seven species of predators: experimental data and their relevance to natural predator-vole relationships. *Oikos* 68, 251.
- Jenkins, F.A. (Ed.), 1974. *Primate Locomotion*. Academic, New York, pp. 85–116.
- Juškaitis, R., 2002. Spatial distribution of the yellow-necked mouse (*Apodemus flavicollis*) in large forest areas and its relation with seed crop of forest trees. *Mamm. Biol. – Zeitschrift Säugetierkd* 67, 206–211.
- Karantanis, N.-E., Youlatos, D., Rychlik, L., 2015. Diagonal gaits in the feather-tail glider *Acrobates pygmaeus* (Acrobatidae, Diprotodontia): insights for the evolution of primate quadrupedalism. *J. Hum. Evol.* 86, 43–54.
- Karaseva, E.V., Tichonova, G.N., Bogomilov, P.L., 1992. Distribution of the Striped field mouse (*Apodemus agrarius*) and peculiarities of its ecology in different parts of its range. *Zool. Z.* 71, 106–115.
- Kratthli, H., 2001. Struktur und funktion des extremitäteninteguments bei einheimischen mäuseartigen (Muridae, Rodentia). PhD Dissertation. University of Zurich.
- Kuncová, P., Frynta, D., 2009. Interspecific morphometric variation in the postcranial skeleton in the genus *Apodemus*. *Belgian J. Zool.* 139, 133–146.
- Lammers, A.R., Zurcher, U., 2011a. Stability during arboreal locomotion. In: Klika, V. (Ed.), *Theoretical Biomechanics*. Available from: <http://www.intechopen.com/books/theoretical-biomechanics/stability-during-arboreal-locomotion>.
- Lammers, A.R., Zurcher, U., 2011b. Torque around the center of mass: dynamic stability during quadrupedal arboreal locomotion in the Siberian chipmunk (*Tamias sibiricus*). *Zoology* 114, 95–103.
- Lammers, A.R., Earls, K.D., Biknevicius, A.R., 2006. Locomotor kinetics and kinematics on inclines and declines in the gray short-tailed opossum *Monodelphis domestica*. *J. Exp. Biol.* 209, 4154–4166.
- Lammers, A.R., 2007. Locomotor kinetics on sloped arboreal and terrestrial substrates in a small quadrupedal mammal. *Zoology* 110, 93–103.
- Larson, S.G., Schmitt, D., Lemelin, P., Hamrick, M., 2000. Uniqueness of primate forelimb posture during quadrupedal locomotion. *Am. J. Phys. Anthropol.* 112, 87–101.
- Larson, S.G., Schmitt, D., Lemelin, P., Hamrick, M., 2001. Limb excursion during quadrupedal walking: how do primates compare to other mammals? *J. Zool.* 255, 353–365.
- Lemelin, P., Cartmill, M., 2010. The effect of substrate size on the locomotion and gait patterns of the kinkajou (*Potos flavus*). *J. Exp. Zool. Part A Comp. Exp. Biol.* 313A, 157–168.
- Marsh, A.C.W., Harris, S., 2000. Partitioning of woodland habitat resources by two sympatric species of *Apodemus*: lessons for the conservation of the yellow-necked mouse (*A. flavicollis*) in Britain. *Biol. Conserv.* 92, 275–283.
- Mazurkiewicz, M., 1994. Factors influencing the distribution of the bank vole in forest habitats. *Acta Theriol.* 39, 113–126.
- Montgomery, W.I., Gurnell, J., 1985. The behaviour of *Apodemus*. *Symp. Zool. Soc. London* 55, 89–115.
- Montgomery, W.I., 1978. Studies on the distributions of *Apodemus sylvaticus* (L.) and *A. flavicollis* (Melchior) in Britain. *Mamm. Rev.* 8, 177–184.
- Montgomery, W.I., 1980. The use of arboreal runways by the woodland rodents, *Apodemus sylvaticus* (L.), *A. flavicollis* (Melchior) and *Clethrionomys glareolus* (Schreber). *Mamm. Rev.* 10, 189–195.
- Nations, J.A., Olson, L.E., 2015. Climbing behavior of northern red-backed voles (*Myodes rutilus*) and scansoriality in *Myodes* (Rodentia, Cricetidae). *J. Mammal.* 96, 957–963.
- Nyakatura, J.A., Heymann, E.W., 2010. Effects of support size and orientation on symmetric gaits in free-ranging tamarins of Amazonian Peru: implications for the functional significance of primate gait sequence patterns. *J. Hum. Evol.* 58, 242–251.
- Nyakatura, J.A., Fischer, M.S., Schmidt, M., 2008. Gait parameter adjustments of cotton-top tamarins (*Saguinus oedipus*, Callitrichidae) to locomotion on inclined arboreal substrates. *Am. J. Phys. Anthropol.* 135, 13–26.
- Obtrel, R., Holisova, V., 1974. Trophic niches of *Apodemus flavicollis* and *Clethrionomys glareolus* in low-land forest. *Acta Sci. Nat. Acad. Sci. Bohemicae Brno* 8, 1–37.
- Olszewski, J.L., 1968. Role of uprooted trees in the movements of rodents in forests. *Oikos* 19, 99–104.
- Pontzer, H., 2005. A new model predicting locomotor cost from limb length via force production. *J. Exp. Biol.* 208, 1513–1524.
- Pontzer, H., 2007a. Effective limb length and the scaling of locomotor cost in terrestrial animals. *J. Exp. Biol.* 210, 1752–1761.
- Pontzer, H., 2007b. Predicting the energy cost of terrestrial locomotion: a test of the LiMb model in humans and quadrupeds. *J. Exp. Biol.* 210, 484–494.
- Pridmore, P.A., 1994. Locomotion in *Dromiciops australis* (Marsupialia, Microbiotheriidae). *Aust. J. Zool.* 42, 679–699.
- Pucek, Z., 1981. Keys to Vertebrates of Poland, Mammals. PWN – Polish Scientific Publishers, Warszawa.
- Reilly, S.M., McElroy, E.J., Biknevicius, A.R., 2007. Posture, gait and the ecological relevance of locomotor costs and energy-saving mechanisms in tetrapods. *Zoology* 110, 271–289.
- Rollinson, J.M., Martin, R.D., 1981. Comparative aspects of primate locomotion, with special reference to arboreal cercopithecines. *Symp. Zool. Soc. London* 48, 377–427.
- Schilling, N., Hackert, R., 2006. Sagittal spine movements of small therian mammals during asymmetrical gaits. *J. Exp. Biol.* 209, 3925–3939.
- Schmidt, A., Fischer, M.S., 2010. Arboreal locomotion in rats—the challenge of maintaining stability. *J. Exp. Biol.* 213, 3615–3624.
- Schmidt, A., Fischer, M.S., 2011. The kinematic consequences of locomotion on sloped arboreal substrates in a generalized (*Rattus norvegicus*) and a specialized (*Sciurus vulgaris*) rodent. *J. Exp. Biol.* 214, 2544–2559.
- Schmitt, D., Lemelin, P., 2002. Origins of primate locomotion: gait mechanics of the woolly opossum. *Am. J. Phys. Anthropol.* 118, 231–238.
- Shapiro, L.J., Young, J.W., 2010. Is primate-like quadrupedalism necessary for fine-branch locomotion? A test using sugar gliders (*Petaurus breviceps*). *J. Hum. Evol.* 58, 309–319.
- Shapiro, L.J., Young, J.W., 2012. Kinematics of quadrupedal locomotion in sugar gliders (*Petaurus breviceps*): effects of age and substrate size. *J. Exp. Biol.* 215, 4049–4049.
- Shapiro, L.J., Young, J.W., VandeBerg, J.L., 2014. Body size and the small branch niche: using marsupial ontogeny to model primate locomotor evolution. *J. Hum. Evol.* 68, 14–31.
- Shapiro, L.J., Kemp, A.D., Young, J.W., 2016. Effects of substrate size and orientation on quadrupedal gait kinematics in mouse lemurs (*Microcebus murinus*). *J. Exp. Zool. Part A Ecol. Genet. Physiol.* 325, 329–343.
- Stevens, N.J., 2006. Stability, limb coordination and substrate type: the ecorelevance of gait sequence pattern in primates. *J. Exp. Zool. Part A Comp. Exp. Biol.* 305A, 953–963.
- Stevens, N.J., 2008. The effect of branch diameter on primate gait sequence pattern. *Am. J. Primatol.* 70, 356–362.
- Strang, K.T., Steudel, K., 1990. Explaining the scaling of transport costs: the role of stride frequency and stride length. *J. Zool.* 221, 343–358.
- Tattersall, F., Whitbread, S., 1994. A trap-based comparison of the use of arboreal vegetation by populations of bank vole (*Clethrionomys glareolus*), woodmouse (*Apodemus sylvaticus*) and common dormouse (*Muscardinus avellanarius*). *J. Zool.* 233, 309–314.
- Vilensky, J.A., Moore, A.M., Libii, J.N., 1994. Squirrel monkey locomotion on an inclined treadmill: implications for the evolution of gaits. *J. Hum. Evol.* 26, 375–386.

- Watts, C.H.S., 1968. The foods eaten by wood mice (*Apodemus sylvaticus*) and bank voles (*Clethrionomys glareolus*) in Wytham Woods, Berkshire. *J. Anim. Ecol.* 37, 25–41.
- White, T.D., 1990. Gait selection in the brush-tail possum (*Trichosurus vulpecula*), the northern quoll (*Dasyurus hallucatus*), and the virginia opossum (*Didelphis virginiana*). *J. Mammal.* 71, 79–84.
- Witte, H., Preuschoft, H., Fischer, M.S., 2002. The importance of the evolutionary heritage of locomotion on flat ground in small mammals for the development of arboreality. *Z. Morphol. Anthropol.* 83, 221–233.
- Young, J.W., 2009. Substrate determines asymmetrical gait dynamics in marmosets (*Callithrix jacchus*) and squirrel monkeys (*Saimiri boliviensis*). *Am. J. Phys. Anthropol.* 138, 403–420.
- Zefferer, M., 2002. Adaptation des terminalen zehengliedes an die fortbewegung bei kleinsaugern (Mammalia). Bachelor Thesis. Zoologisches Museum der Universität Zurich.
- Zejda, J., 1967. Habitat selection in *Apodemus agrarius* (Pallas, 1771) (Mammalia: Muridae) on the border of the area of its distribution. *Zool. List* 16, 15–30.