

Kinematics and coordination of moth flies walking on smooth and rough surfaces

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Abstract

The moth fly, *Clogmia albipunctata*, is a common synanthropic insect with a worldwide range that lives in nearly any area with moist, decaying organic matter. These habitats comprise both smooth, slippery substrates (e.g., bathroom drains) and heterogeneous, bumpy ground (e.g., soil in plant pots). By using terrain of varying levels of roughness, we focus specifically on how substrate roughness at the approximate size scale of the organism affects kinematics and coordination in adult moth flies. Finally, we compare and contrast our characterizations of locomotion in *C. albipunctata* with previous work of insect walking in naturalistic environments.

KEYWORDS

Diptera, insect walking, limb coordination, surface roughness, walking kinematics

INTRODUCTION

Natural environments are rarely flat. As such, walking animals must navigate substrates that are rough and variable, often across multiple scales ranging from small surface variations (far smaller than the animal's characteristic length scale) to large obstacles that induce path planning.^{1,2} The effect of heterogeneity at these two extremes on kinematics and behavior has earned significant attention, with several past investigations determining the impact of small-scale roughness on footpad adhesion and friction,^{3–7} and of large-scale mechanical challenges in determining longer distance navigation and foraging strategies.^{8–10}

Variability at the intermediate scale (ground roughness regime), though ubiquitous in a wide range of natural habitats, remains relatively less studied in invertebrates, with some recent work primarily on ants.^{1,2,11} We define the ground roughness regime as comprising obstacles for which the length scale of variability ranges from approximately the size of a foothold to the size of the animal.¹ Panarthropods (a diverse clade comprising arthropods, onychophorans, and tardigrades) can adeptly navigate challenges in this regime; their ability to

do so with a simple nervous system relative to vertebrates makes them particularly compelling study organisms.¹² The natural environments of many insect species constitute a three-dimensional (3D) maze of twigs and leaf litter.^{13,14} Moss-dwelling tardigrades lumber over 3D landscapes to find food and mates.¹⁵ Ants travel over long stretches of coarse sand and gravel to forage for food.¹⁶ The geometries of these complex substrates often compromise walking stability, and efficient locomotion over such terrain necessitates the constant updating of kinematics and coordination in response to environmental variability.

The impact of interacting with terrain heterogeneity at this scale on locomotive behavior is likely dependent on the species, substrate properties, and speed of walking.¹² Few studies have considered the effects of substrates that more closely mimic natural terrain.^{2,6,14,17–19} The complex heterogeneities inherent to such substrates pose significant challenges in characterizing their impact on animal behavior and biomechanics; this likely skews studies toward the use of more simple, interpretable experimental setups that incorporate localized disruptions of steady walking. Indeed, the majority of past work within this regime has focused on the impact of localized obstacles on walking kinematics, including gap crossing^{20–22} or step climbing.^{23,24}

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Another factor constraining our generalized understanding of invertebrate walking along naturalistic terrain is the limited choice of model species. Several species have been observed to alter their coordination or foot placement when faced with variable terrain: Centipedes running along blocked surfaces reverse the direction of their wave-like, interlimb coordination pattern,²⁵ and crabs deviate from their standard alternating tetrapod coordination to a variable, random stepping pattern when walking on rough ground.²⁶ Other studies have observed stable stepping patterns in other species—cockroaches running along blocks with Gaussian-distributed heights¹⁴ and ants walking on checkerboard mostly maintain their stepping patterns.² Given the differences observed between species and the wide phylogenetic spread of such a sparse number of species, the possibility of discerning conserved coordination patterns or biomechanical strategies across panarthropods is limited.^{1,12}

Within the insects, dipterans are a diverse and speciose clade that display significant morphological variability and thrive in a wide range of habitats. However, the majority of studies into the behavior of fly species have focused on the model organism *Drosophila melanogaster*. The ease of culture and genetic manipulation of *Drosophila* has allowed for several rigorous investigations into the link between neuromechanics and behavior.^{27–29} However, as mentioned above, this narrow focus constrains the identification of any wider biomechanical or evolutionary patterns among arthropods. In an effort to expand analyses beyond the standard model dipteran, we explore walking kinematics and coordination over variable terrain in the moth fly *Clogmia albipunctata*, a species that diverged from *Drosophila* over 250 million years ago.³⁰

C. albipunctata is a dipteran with a wide geographical range, living and moving through a variety of complex environments. These environments vary widely, from slippery shower drains to coarse granular soil found in plant pots, encompassing the entirety of the ground roughness regime. Although many factors likely contribute to the ability of this species to effectively locomote through its many natural environments, we here focus on the navigation of obstacles from about 2% to 25% of body length. To characterize the walking behavior of this species along naturalistic terrain, we use sandpaper of varying grit size, which simultaneously creates structural heterogeneity in upward, lateral, and forward directions along the walking track (Figure 1). We hypothesize that larger grit sizes, corresponding to rougher terrain, will result in lower walking speed, decreased leg coordination, and increased variance in other kinematic parameters such as step length and period. Similar studies in foraging ants show decreased walking speeds on rough substrates, suggesting that uneven ground disrupts stability. The authors showed that this manifests in preferences for flat substrates, which improve foraging efficiency.² We further characterize how other kinematic parameters, including stride length, frequency of stepping, and interlimb coordination, are affected by both walking speed and substrate. These findings are presented in the context of *C. albipunctata*'s natural habitat and life history and in comparison to results from investigations of kinematic changes in other insect species in response to challenging terrain.

MATERIALS AND METHODS

Animals

We used five adult wild-type individuals of *C. albipunctata* (Figure 1A), obtained from cultures kept in the Schmidt-Ott lab at the University of Chicago. Larvae were reared on Petri plates containing damp cotton and fed dried, powdered parsley weekly. Because adult flies only survive for about a week after eclosion, adults were removed from culture and used in trials less than 48 h after eclosion. At least 1 h before an experiment, each individual was anesthetized with carbon dioxide, and the wings were trimmed to about 25% of their original size with sharp microscissors. Wing removal has been used previously in walking studies with *Drosophila* to allow visualization of legs from above.³¹

Experimental setup and video acquisition

Each experimental track consisted of a ~1 cm × 2 cm rectangle of sandpaper glued to a microscope slide and bordered with foam tape (Figure 1). Sandpaper is fabricated by gluing objects (grains of sand or small pieces of metal, typically) to a paper or fabric substrate. For clarity, we refer to these objects as abrasive grains. Sandpapers are graded by grit, a metric corresponding with the size of the abrasive grains.³² We used four different sandpaper grits, with abrasive grains ranging in size from ~25% of the fly's body length (24 grit) to ~2.7% of the fly's body length (150 grit). See Table 1 for additional details of the sandpapers that were used. We also used one track consisting of a bare microscope slide with no sandpaper. Each track was covered with an additional microscope slide to prevent flies from escaping.

Animals were presented with each track in a randomized order. Animals that did not complete every treatment were excluded from the analysis. During each trial, a fly was placed on a track and filmed from above using a smartphone (Pixel 6a, Google) with a 25× macro lens attached (Shenzhen Laika Technology Co., Ltd). Videos were recorded at 240 frames per second. We recorded each fly for several minutes, ensuring to capture as many stride bouts as possible. Stride bouts were chosen to consist of at least three continuous full strides. We excluded any stride bouts with turns or those that did not include three strides for each leg. (See Table S1 for the sampling for each individual.)

Gait tracking and analysis

Videos were evaluated using the MTrackJ plugin³³ in ImageJ.³⁴ Exact time and location of liftoff (swing initiation) and touchdown (stance initiation) events for the tarsus of each foot, as well as the frame-by-frame position of anterior, midpoint, and posterior points of the body, were visually determined and tracked manually. All measurements were made in ground-fixed coordinates. Data obtained were processed using in-house Python scripts; all analysis code is available at <http://github.com/jnirody/clogmia>.

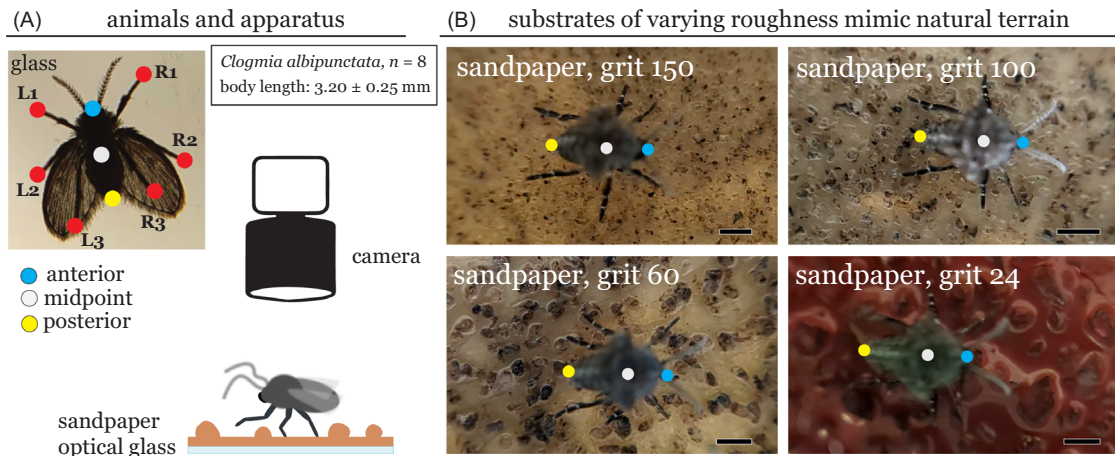


FIGURE 1 Animals and experimental setup. (A) Setup used for measuring kinematics on different substrates. Flies were filmed walking on glass from above, as well as over substrates of varying roughness glued onto a microscope slide. An image taken from a video of *C. albipunctata* walking on glass is shown with right (R1–R3, from anterior to posterior) and left (L1–L3) legs highlighted in red. (B) Cuts of sandpaper with different grit sizes (150, 100, 60, and 24) were used to explore walking kinematics over substrates that imitate naturalistic terrain, with varying obstacle sizes along vertical and horizontal axes. Images show screenshots from trials on each sandpaper grit. The flies look slightly different than in (A) due to wing removal. Anterior, midpoint, and posterior points are marked as in (A). Black bars in each image correspond to 1 mm.

TABLE 1 Relevant statistics of sandpapers used in the experiment.

| | 24 grit | 60 grit | 100 grit | 150 grit |
|---------------------------------------|-----------------|------------------|----------------|------------------|
| Grit size range (μm) | 710–850 | 250–300 | 125–150 | 75–106 |
| Grit size/fly body length (%) | 24.0–24.6 | 8.4–8.6 | 4.2–4.3 | 2.5–3.0 |
| Grit size/fly femur length (%) | 130–150 | 44–53 | 22–27 | 13–19 |
| Grit density (grits/cm ²) | 125.2 \pm 7.9 | 268.4 \pm 21.4 | 511 \pm 43.3 | 880.2 \pm 34.0 |

Note: Femur lengths were calculated from animals not included in this experiment.

Calculation of kinematic parameters

Walking speed was calculated as the change in the measured position of the midpoint of the body divided by the time interval among frames. A stride period for an individual leg was defined as the time difference between two consecutive liftoff events. Each stride comprised a stance and a swing. The durations of swing and stance were calculated as the time difference between a liftoff event and the subsequent touchdown (swing) or the time difference between a touchdown and the subsequent liftoff (stance).

Temporal coordination of leg pairs was determined using the relative timings of swing onsets. To describe the walking patterns observed, we measured pairwise inter-leg coordination between ipsilateral and contralateral neighboring legs. Phase differences between ipsilateral leg pairs (neighboring legs on the same side of the body; e.g., R1 and R2, see Figure 3A illustration) were denoted as ϕ_I and phase differences between contralateral leg pairs (legs directly opposite each other; e.g., L2 and R2) were denoted as ϕ_C . The leg within a pair that swings first within a full cycle (comprising swing events of all six legs) is considered the reference leg; the phase offset is normalized with respect to the stride period. For example, let us consider the contralateral leg pair-

ing (R1, L1); assume R1 lifts off before L1. R1, therefore, serves as the reference leg in the pair, and consecutive swing initiations of R1 demarcate the boundaries of the period (t_0, t_1). Then, if R1 swings at time t_s , the phase difference $\phi_C^{(R1-L1)}$ is given by $\phi_C^{(R1-L1)} = (t_s - t_0)/(t_1 - t_0)$. Phase differences between ipsilateral leg pairs were calculated in the same way.

Spatial coordination of legs was determined using the (x, y) positions during transitions between consecutive swing and stance phases. Step length for an individual leg was determined as the two-dimensional (x, y) distance between the point of liftoff (posterior extreme position [PEP]) and the location of the subsequent touchdown (anterior extreme position [AEP]). We do not consider stride length (calculated as the distance between two consecutive AEPs). This is because factors unrelated to active choices by the animal (e.g., slipping on low-friction substrates like glass) have a greater effect on stride length than on step length.¹⁴ The clustering of step positions was quantified as the distance (in mm) between the position of liftoff (PEP) of a reference leg and the position of subsequent touchdown (AEP) of the ipsilateral leg directly posterior; for instance, the distance between the PEP of the middle right limb (R2) and the AEP of the hind right limb (R3).

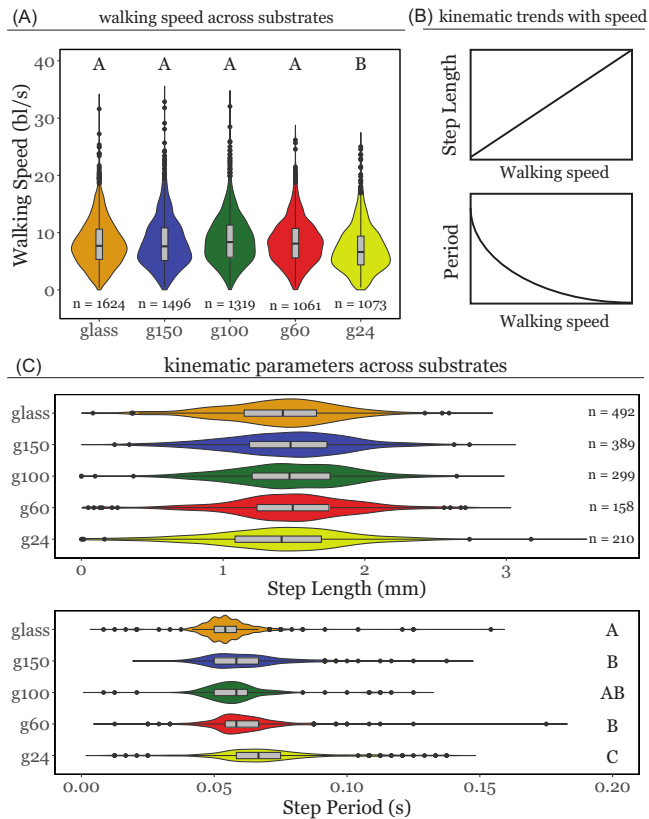


FIGURE 2 Effects of substrate roughness on walking kinematics. (A) *C. albipunctata* walking speed does not change across the considered substrates of varying roughness, except in the case of the most extreme roughness (grit 24). (B) Graphs showing generalized relationships between walking speed and step length (top) and stride frequency (bottom). Both stride length and frequency increase with walking speed, with stride frequency plateauing at high speeds. (C) *C. albipunctata* do not increase step length (top), although stride period (bottom) does generally increase as substrate roughness increases. Step period (C) therefore seems to be the predominant factor in changes in speed (A). Groups sharing a letter are not significantly different from one another at $p < 0.05$; see the Supporting Information for exact p values and degrees of freedom. Box plots show median (center line) and first and third quartiles (box borders). Panel B is adapted from Nirody et al.¹⁵

Statistical analysis

Linear mixed effects models implemented by the R package *lme4*³⁵ were developed to model the effects of treatments on each kinematic variable (speed, step length, and step period) (Figure 2) plus spatial coordination (Figure 5). First, response variables were visually inspected with histograms and quantile–quantile plots to assess normality. Period, speed, and spatial coordination were square root transformed to improve normality. Step length was not transformed. Next, we built models for each response variable, incorporating random effects, and selected the best models using AIC as the selection criterion. For each model, treatment was the fixed effect. Random effects were individual ID for spatial coordination; individual ID, date and time of measurement, and treatment order for speed; and indi-

vidual ID and date and time measured for step period and speed. Treatment groups were directly compared by inputting the models into the package *emmeans*,³⁶ which uses estimated marginal means while correcting for multiple comparisons. Data is presented as pooled across walking legs. Joint distributions of phase angles were computed using a kernel density estimate using Gaussian kernels in the Python package *scipy.stats*.³⁷ Plots in Figures 2 and 5 were made using *ggplot2*³⁸ in R.

Note that for step length and step period, a data point consisted of a single step for a single leg; therefore, each stride bout contributed ~18 data points (6 legs \times 3 steps each). Data points for speed consisted of instantaneous speed for each frame interval; therefore, each stride bout contributed approximately 60 data points.

RESULTS

Walking speed and step period change across treatment, but not step amplitude

C. albipunctata walking speeds were about the same for all treatments (about 7.8 ± 0.4 body lengths/s) except at the most extreme grit size (24 grit) (7.4 ± 0.4 body lengths/s) (Figure 2A). In this treatment, speed significantly decreased compared to all other treatments (for all p values and degrees of freedom, see Tables S2–S4).

Invertebrates tune both the length of their steps and the amount of time devoted to each step to modulate their speed of locomotion; these trends are consistent with our observations in walking *C. albipunctata* (Figure 2B). Step length generally shows a linear relationship with speed across walking speeds.^{2,29,39,40} To increase stride frequency, organisms can reduce the *period* of each step cycle by either shortening the swing or stance phase of the cycle. In general, walking speeds across panarthropod species are modulated by stance duration. In contrast, swing duration generally decreases only slightly with speed at low-to-medium speeds and is constant at high speeds.^{28,40,41} This observed trend lends support to the idea that mechanically mediated load-based coordination is a widespread control strategy.²⁹

We find that walking speeds only decrease in the roughest treatment (24 grit) compared to all other treatments. Step length did not significantly change across treatments (Figure 2C). However, stride period increased as substrate roughness increased from 0.55 ± 0.013 s on glass to 0.63 ± 0.02 s on 150 grit sandpaper, 0.61 ± 0.02 s on 100 grit sandpaper, 0.60 ± 0.01 s on 60 grit sandpaper, and 0.71 ± 0.02 s on 24 grit sandpaper. Taken together, these results suggest that step phasing is interrupted at much smaller grit sizes, but speed is only impacted once the obstacles become much larger.

Alternating tripod coordination is maintained across conditions

In addition to the kinematic parameters discussed above, many species also shift the temporal coordination among legs. Inter-leg

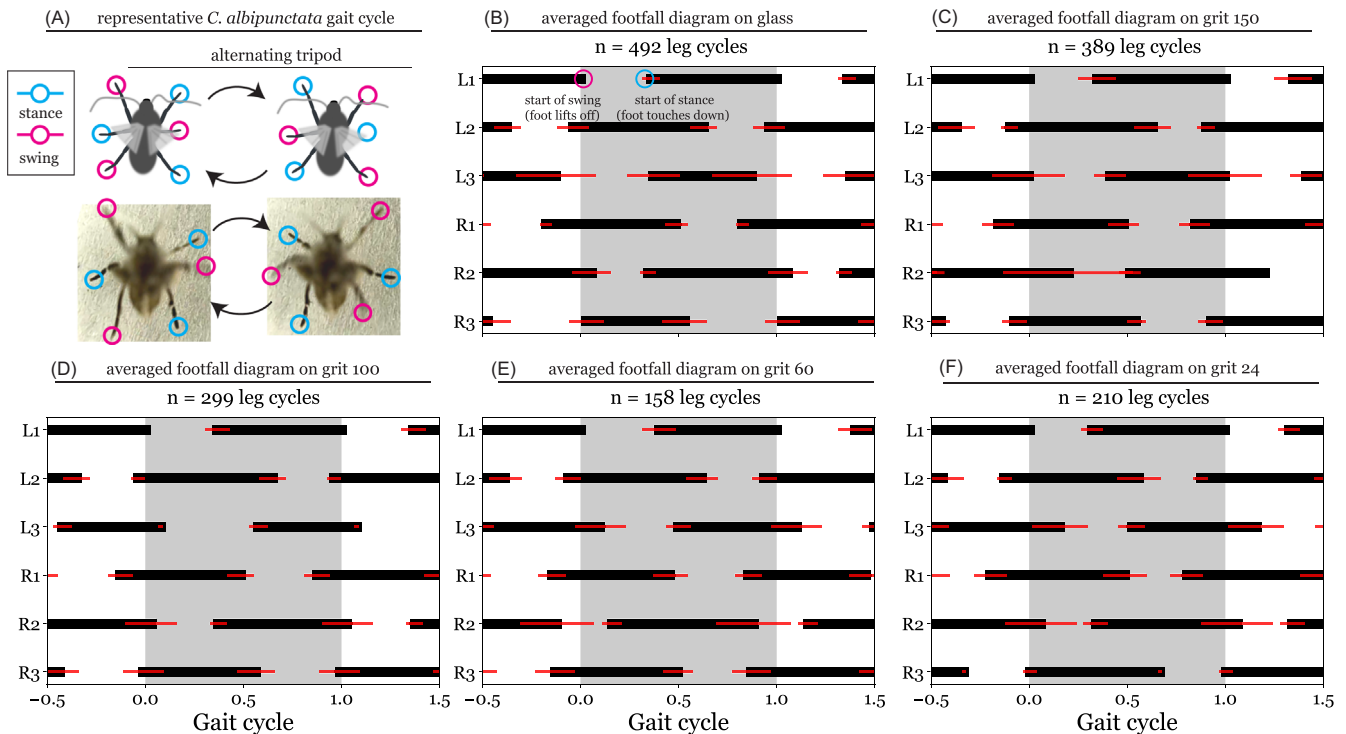


FIGURE 3 Overview of moth fly inter-leg coordination patterns. (A) Schematic of the canonical tripod stepping pattern in the hexapod *C. albipunctata*. In tripod coordination, three limbs swing simultaneously. The transitions among configurations are shown to reflect the posterior-to-anterior propagation of ipsilateral swing events. (B–F) Podograms show the average temporal sequence of ground contacts for legs L1–L3 (left, anterior to posterior) and R1–R3 (right, anterior to posterior) for each treatment. Values are normalized to the cycle period of the left front leg L1, shown within the gray shaded region (0.0–1.0). Extrapolated sequences for previous [–0.5, 0) and subsequent (1, 1.5) periods are shown outside the shaded area. Here, we define “cycle” as a sequence containing one full stride from each leg. Mean \pm SD is depicted; SD is indicated by red lines.

coordination parameters are sometimes thought to be of secondary importance for modulating walking speed but are essential for static and dynamic stability.^{15,29}

Previous studies have shown that coordination patterns in several insects, including dipterans like *Drosophila*, exist along a speed-dependent continuum.^{15,27,28,40} At their slowest speeds, fruit flies display pentapodal wave coordination, in which a single leg swings (lifts off) at a time; this transitions into a tetrapodal stepping pattern in which two legs are in swing simultaneously. However, at the majority of walking speeds, *Drosophila* prefers a tripod coordination in which two pairs of three legs swing in alternating sequence (Figure 3A). This spectrum of coordination patterns can be characterized by measuring the phase offset in the initiation of swing phase (lift off) between ipsilateral (Φ_I) and contralateral leg pairs (Φ_C): In hexapods, wave coordination shows $\Phi_I = \frac{1}{6}$, tetrapod coordination shows $\Phi_I = \frac{1}{3}$, and tripod $\Phi_I = \frac{1}{2}$. All characterized patterns show antiphase contralateral leg coordination $\Phi_C = \frac{1}{2}$.¹⁵

In our experiments, *C. albipunctata* displays a strong preference for tripod coordination across walking speeds on glass, with alternating simultaneous or near-simultaneous liftoffs between triplets of legs L1–R2–L3 and R1–L2–R3 (Figure 3A,B). This corresponds to an ipsilateral and contralateral phase offset pairing

(Φ_I, Φ_C) = ($\frac{1}{2}, \frac{1}{2}$) (Figure 3C). When challenged to walk along rougher terrain, moth flies generally maintain this coordination pattern across all sandpaper substrates considered; however, variability in the timing of stepping patterns increases with roughness (Figure 4).

We find that across all treatments, contralateral legs maintain an antiphase relationship, whereas there is more variation in ipsilateral legs, including a hotspot of frequency where Φ_I is close to 1. This is in accordance with results found in *Drosophila* by Szczecinski et al.⁴² Interestingly, previous work noted the opposite pattern in response to sensed changes in loading—contralateral legs tend to shift to in-phase coordination to counteract slipping on low-friction substrates.¹² We hypothesize that animals walking along terrain exhibiting roughness at the intermediate scale prioritize gait or COM stability over forward processivity (as they might on slippery substrates), resulting in a relaxed constraint on Φ_I and a more strictly-maintained value for Φ_C . We also anecdotally observed that the third legs seem to drag behind the animal more frequently, suggesting that they are perhaps less important in generating forward momentum during the stride cycle. Therefore, these legs may move in-phase with one another more often without having a strong effect on kinematic parameters such as speed.

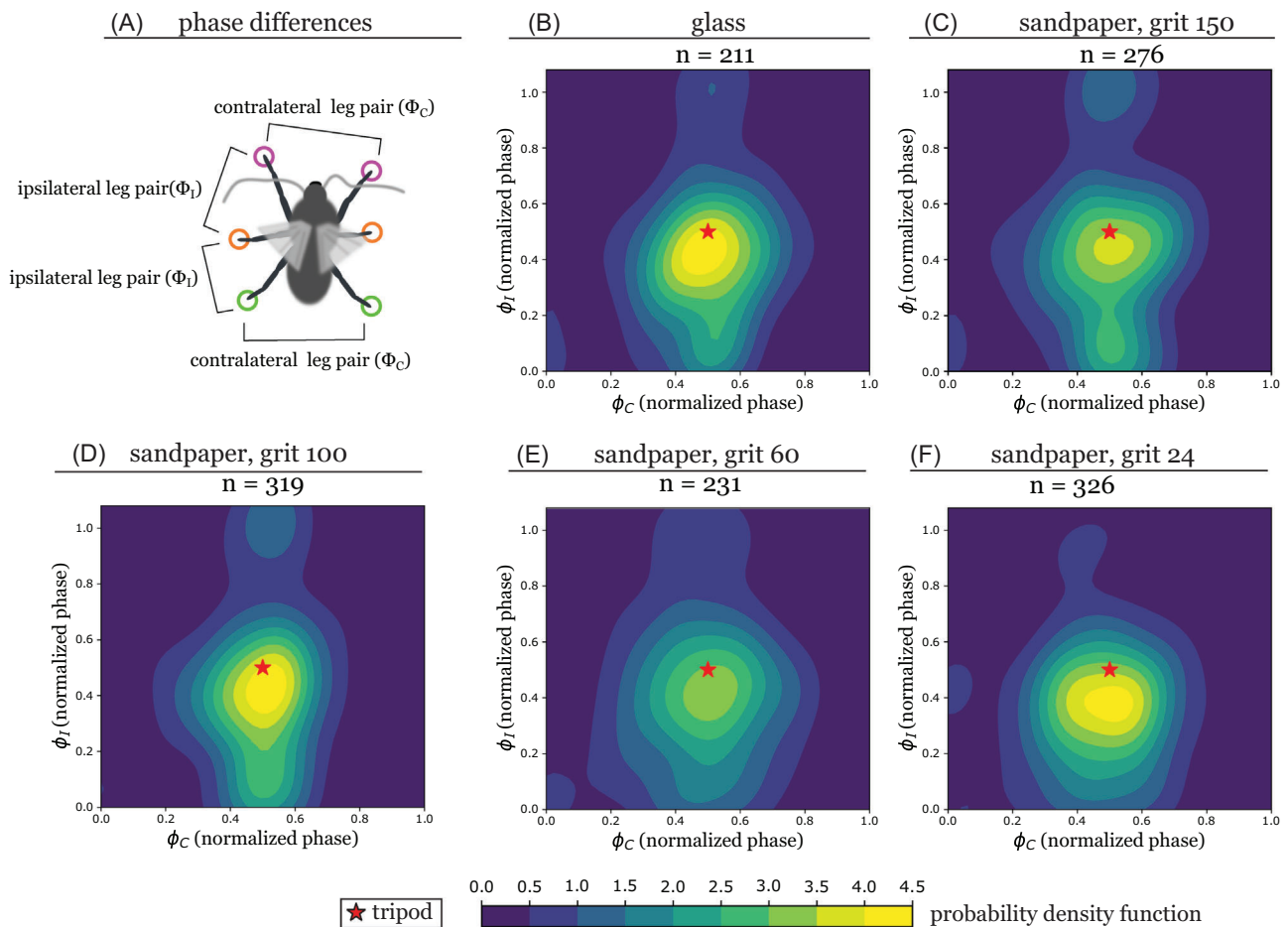


FIGURE 4 Joint distribution of the phase difference between contralateral (Φ_C) and ipsilateral (Φ_I) leg pairs in *C. albipunctata* when walking on terrain of varying roughness. (A) Illustration of contralateral and ipsilateral leg pairs. Legs sharing a color circle on opposite sides of the body are contralateral, whereas those immediately adjacent to one another on the same side of the body are ipsilateral. Phase differences on (B) glass; (C) sandpaper of 150 grit; (D) sandpaper of 100 grit; (E) sandpaper of 60 grit; (F) sandpaper of 24 grit. Phase differences corresponding to a classic tripod stepping pattern (Φ_I, Φ_C) = (0.5, 0.5) are shown as red stars. Flies show a preference for tripod across terrains but display higher variability in coordination pattern on rougher substrates.

Spatial coordination increases with substrate roughness

The spatial placement of limbs can be affected when moving along uneven or heterogeneous substrates due to the limited number of stable footholds. Several studies have demonstrated an increase in targeted footholds on rough terrain; in this way, animals are able to take advantage of successfully determined landing points by the steps made by previous legs.²⁸ For instance, in the alternating tripod pair of legs, L1–R2–L3 and R1–L2–R3, the foothold used by R2 can be used by R3 after R2 lifts off (Figure 5).

We characterize spatial coordination during stepping by quantifying the distance between the lift-off point of a reference leg (e.g., the PEP of R2) and the subsequent touch-down point of the posterior ipsilateral leg (e.g., the AEP of R3). We find that the separation between ipsilateral footholds on glass (0.69 ± 0.15 mm on glass) is further spaced than on rougher substrates (Figure 5); separation between footholds is

0.65 ± 0.15 mm on 150 grit sandpaper; 0.61 ± 0.16 mm on 100 grit sandpaper; 0.66 ± 0.16 mm on 60 grit sandpaper; 0.70 ± 0.17 mm on 24 grit sandpaper.

DISCUSSION

Terrestrial legged animals must navigate obstacles across different length scales when moving across natural terrain. Here, we focus on exploring how the moth fly *C. albipunctata* adapts its kinematics and coordination patterns while walking on substrates showing variability in the ground roughness regime (i.e., substrates with obstacles roughly the order of the animal's body size). At this scale, roughness can disrupt foot–ground contact, affecting the timing and placement of steps as well as body stability⁴; to accommodate these challenges, animals often reduce walking speeds on uneven substrates.^{2,16,43} We overall find that our hypothesis of walking locomotion being disrupted by

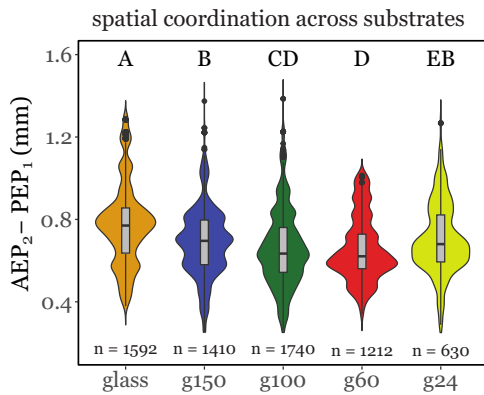


FIGURE 5 Quantification of spatial limb coordination across flat and uneven terrain. Clustering between consecutive footfalls is characterized as the distance between the lift-off location (x_1, y_1) of a reference leg (e.g., the posterior extreme position, or PEP, of R2) and the subsequent touch-down point (x_2, y_2) of the ipsilateral posterior leg (e.g., the anterior extreme position, or AEP, of R3). In general, spatial coordination decreases with increasing grit size. Groups sharing a letter are not significantly different from one another at $p < 0.05$; see the Supporting Information for exact p values and degrees of freedom. Box plots show median (center line) and first and third quartiles (box borders).

larger obstacle sizes to be partially supported. Although speed, step period, and spatial coordination did change at these more extreme conditions, the results for step amplitude and inter-leg coordination were more equivocal. We find that walking speed in *C. albipunctata* only decreases in the treatment with the largest obstacles, on the order of ~25% of their body length. These findings are in line with previous studies in other species accustomed to living in heterogeneous, cluttered habitats like cockroaches¹⁴ and tropical canopy ants,¹¹ and are likely due in part to the stability of the alternating tripod coordination pattern observed across walking speeds in these insects.^{29,44} This result also agrees with previous work that determined that this stability begins to falter in several ant species when the amplitude of substrate roughness approaches approximately 33% of the animal's body length.^{1,11} This tolerance is likely significantly higher in cockroaches.¹⁴ Future work with engineered substrates with higher roughness amplitude will serve to hone this limit for *Clogmia*.

We also find that *C. albipunctata* adjust their underlying walking kinematics on substrates at roughness amplitudes far below the 33% body length threshold (Figure 2C). In terrestrial legged locomotion, walking speed is influenced by both stride period, the length of time required to complete a stride, and the step length, the distance traversed in a single step¹⁵; previous work in Argentine ants determined that stride frequency, rather than step length, modulated walking speeds on uneven substrates.² Similarly, we find that the stride period in *C. albipunctata* increases with the amplitude of substrate irregularities (Figure 2B). This observed trend may arise from the need for more carefully planned strides due to disrupted foot-ground interactions that can occur even at lower roughness amplitudes. Furthermore,

increasing stride period allows for more time to integrate sensory feedback from terrain heterogeneities and accordingly adjust leg coordination and foot placement.^{1,12} Larger obstacles may constrain stable foot placement, limiting further increases in step length and defining the observed roughness threshold above which walking speed declines.^{2,11}

In accordance with these observations, we find that footprint clustering increases when walking on rough substrates as compared with walking on glass (Figure 5). This *follow-the-leader* pattern occurs when the landing positioning of a posterior leg is targeted to land on the foothold that ipsilateral leg immediately anterior to it successfully utilized in a previous step.^{14,45} This increase in precision during stepping can serve to ensure that legs are placed on safe ground when walking along rough terrain and is largely dependent on sensory feedback,^{24,28} the effect of which may be enhanced as stride period is increased. Targeting mechanisms and spatial limb coordination have been observed in several panarthropod species^{14,15,24,28}; the larger variation observed around tripod coordination on rougher substrates (Figure 3) may arise in part from divergent strides caused by increased planning or searching movements on uneven terrain. Further analysis of 3D limb trajectories during both swing and stance on rough terrain will be crucial in elucidating the generality of such movements across species.

Finally, it should be noted that obstacle size is not the only factor that could contribute to the results presented here and represent areas for future inquiry. For instance, the different sandpapers may be fabricated using different materials that might vary in terms of micro-asperities in the abrasive grains that could provide footholds. These differing materials might also vary in terms of their material properties, which could interact with the attachment mechanisms of the feet in different ways.^{46–52} These factors are likely important contributing factors to variation in locomotion across treatments but are beyond the scope of the present investigation. However, we note that we most often observed fly feet sliding off abrasive grains in each treatment rather than engaging with them.

CONCLUSION

Terrestrial legged animals must often navigate rough, uneven terrain. Panarthropods can control multiple appendages and navigate a wide range of challenging environments with relatively small numbers of neurons, making them compelling model systems for biomechanical and bioinspiration studies. Natural environments comprise substrates that display variability across multiple scales, from microscopic roughness to obstacles that surpass the animal's body length. We focus here on ground heterogeneity based on the order of the body length of the animal (ground roughness regime). Variability at this scale can disrupt stable foot-ground contacts and alter both temporal and spatial aspects of coordination and kinematics.^{1,12}

Previous explorations at this scale have largely focused on the effects of discrete obstacles like gaps^{20,22} or steps.²⁴ Such studies

have been largely limited to a few species, sparsely spread across the breadth of Panarthropoda.^{2,14,24–26} With this study, we explore how ground variation at the intermediate scale affects coordination and kinematics in the moth fly *C. albipunctata*, a synanthropic dipteran that can be found on many different surfaces with varying obstacle sizes. We find that walking speed in *C. albipunctata* is resistant to surface roughness below a certain threshold, consistent with results in other species.^{11,14} We note that below this threshold, step phase increases while speed is maintained, suggesting that speed in this species is robust to minor changes in kinematics up until more extreme roughness conditions. Understanding the nature of this roughness threshold at which performance metrics such as speed are diminished across species may be an interesting direction for future study. We also report that flies maintain a preference for tripod coordination across conditions considered, a trend observed in some¹⁴ but not all^{25,26} panarthropod species. We hope that this study serves to emphasize the importance of making deeper connections about conserved patterns in panarthropod locomotion in natural environments, as well as to encourage both (1) increasing the number of invertebrate species that are studied and (2) biomechanical analyses performed with naturalistic behaviors in mind.

AUTHOR CONTRIBUTIONS

J.A.N. and E.E.B. conceptualized the work. E.E.B. performed experiments. E.E.B., M.R.M., and J.A.N. analyzed data and reviewed and edited the final version of the manuscript. J.A.N. prepared the initial draft of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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