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Adhesion and Running Speed of a Tropical Arboreal Ant (*Cephalotes atratus*) on Rough, Narrow, and Inclined Substrates

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Abstract

Arboreal ants must navigate variably sized and inclined linear structures across a range of substrate roughness when foraging tens of meters above the ground. To achieve this, arboreal ants use specialized adhesive pads and claws to maintain effective attachment to canopy substrates. Here, we explored the effect of substrate structure, including small and large-scale substrate roughness, substrate diameter, and substrate orientation (inclination), on adhesion and running speed of workers of one common, intermediately-sized, arboreal ant species. Normal (orthogonal) and shear (parallel) adhesive performance varied on sandpaper and natural leaf substrates, particularly at small size scales, but running speed on these substrates remained relatively constant. Running speed also varied minimally when running up and down inclined substrates, except when the substrate was positioned completely vertical. On vertical surfaces, ants ran significantly faster down than up. Ant running speed was slower on relatively narrow substrates. The results of this study show that variation in the physical properties of tree surfaces differentially affects arboreal ant adhesive and locomotor performance. Specifically, locomotor performance was much more robust to surface roughness than was adhesive performance. The results provide a basis for understanding how performance correlates of functional morphology contribute to determining local ant distributions and foraging decisions in the tropical rainforest canopy.

Introduction

An animal's ability to locomote over the range of conditions that occur within its local environment ultimately affects its survival (Arnold 1983; Vogel 1988; Bennett and Huey 1990; Garland Jr and Losos 1994; Ricklefs and Miles 1994; Alexander 2003; Irschick and Higham 2016; Biewener and Patek 2018). For cursorial animals, substrate characteristics are strong selective pressures; camels and mountain goats have limb and biomechanical modifications that promote efficient locomotion on sand and rock faces (respectively), but not vice versa (Dagg 1974; Lewinson and Stefanyshyn 2016). Such traits facilitate foraging and escape from predation, but are only effective across a range of substrate characteristics. Thus, selection should favor biased use of pathways that minimize costs relative to benefits (e.g., Schoener 1979; Stephens and Krebs 1986; Ydenberg et al. 1994; Yunger et al. 2002). Potentially important costs include metabolic energy expenditure, frequency of injury, and duration of exposure to hazards (Stephens and Krebs 1986).

The biased use of pathways to minimize costs is particularly relevant for small organisms like ants, where local habitat complexity rapidly increases as body size decreases (Kaspari and Weiser 1999; Yanoviak and Kaspari 2000; Grevé et al. 2019). The duration of foraging trips and the frequency of worker loss are likely to increase in more complex habitats (Fewell et al. 1996). Longer foraging trips result in reduced food intake rate (Carroll and Janzen 1973; Fewell 1988) and lost workers are costly to ant colonies as lost biomass (Wilson 1968). Thus, ants and other small foragers commonly choose paths of least resistance (i.e., shorter, less complex routes; Fewell 1988; Torres-Contreras and Vasquez 2004; Devigne and Detrain 2006; Farji-Brener et al. 2007; Clay et al. 2010).

Worker ants that forage primarily in tree crowns must maintain contact with three-dimensional substrates to avoid falling. Indeed, unique behaviors among small wingless arboreal organisms such as gliding and swimming suggest that falling is costly and climbing should be optimized (Cartmill 1985; Yanoviak et al. 2005; Yanoviak et al. 2009; Yanoviak and Frederick 2014; Yanoviak et al. 2015). Arboreal ants generally use compliant tarsal pads coated with an adhesion-mediating fluid and curved claws to adhere to, and mechanically interlock with canopy substrates, respectively (Federle et al. 2002; Endlein and Federle 2008). Although adhesion and locomotion are functionally linked (Federle and Endlein 2004; Endlein and Federle 2008), direct comparisons between adhesive performance and locomotor performance are few, particularly under variable environmental conditions (but see Federle et al. 2000; Stark and Yanoviak 2018).

Given that the energetic costs of locomotion in ants are relatively low (e.g., Fewell 1988), and endurance constraints (e.g., oxygen debt during bursts of activity; Bennett and Huey 1990) are trivial at this small scale, the maintenance of secure attachment and consistent motion during foraging bouts in a variety of environmental conditions presumably are key limiting factors. Locomotor speed likely is constrained by the robustness and versatility of the adhesive system in complex environments, especially for broad-ranging foragers. Thus, when attachment is compromised, we expect a compensatory reduction in locomotor performance (measured as running speed); however, this expectation is not always supported, suggesting that the functional morphology of locomotion in ants is very robust to environmental variation (Federle et al. 2000; Clay et al. 2010; Yanoviak et al. 2012; Stark and Yanoviak 2018).

Although the forest canopy is a linear network of stems from the perspective of small cursorial organisms like ants, rough, narrow, and steeply inclined surfaces are difficult to avoid in this setting (Schoener 1968; Mattingly and Jayne 2004). Stem roughness is likely to be especially problematic for ants because adhesive performance is dependent on the relationship between surface asperity size and adhesive unit size (e.g., tarsal pad, claw tip). In instances where the asperity size and adhesive unit size match, adhesion and mechanical interlocking can fail due to a decrease in contact area (Figure 1; Scholz et al. 2010; Zhou et al. 2014; Song et al. 2016). Conversely, as surface asperity size increases, adhesive performance should increase, although this is dependent on body size (Figure 1; Yanoviak et al. 2017). In addition to surface roughness, natural vegetative substrates have diverse features that potentially impact ant performance (Federle et al. 2000; Koch et al. 2008; Barthlott et al. 2017). Finally, arboreal ants commonly locomote on the stems of climbing plants (e.g., vines) that vary in inclination and diameter, and these factors potentially influence ant foraging behavior (Clay et al. 2010; Yanoviak et al. 2012).

The primary goal of this study was to measure the effect of local substrate characteristics (roughness, diameter, and inclination) on arboreal ant adhesive and locomotor performance. We hypothesized that adhesive performance and running speed vary according to the match between substrate asperity size and attachment structure size. Specifically, we predicted lower adhesive performance on substrates with fine asperity size and higher adhesive performance on substrates with large asperities, relative to smooth surfaces (Figure 1). We expected locomotor performance, measured as running speed, to slow as a consequence of reduced adhesion on fine rough substrates and on very rough, structurally complex substrates like tree bark. Secondarily, we predicted that ant running speed would also slow with declining stem diameter and increasing angle of stem inclination, due to the biomechanical limitations of appendages and adhesive anatomy. We explored these predictions with laboratory and field experiments on artificial and natural substrates with one common tropical arboreal ant species.

Materials and Methods

Data were collected on Barro Colorado Island (BCI), Panama (9.15°N, 79.85°W) during the 2015 wet season (May - July) and in June 2019. More information about this site is available elsewhere (Croat 1978; Leigh 1996). We measured adhesive and locomotor performance of workers of a common arboreal ant species (*Cephalotes atratus* L., Hymenoptera, Formicidae, Myrmicinae). Workers were hand collected from at least two colonies daily, and housed (alive) with their nestmates in small plastic containers until needed (generally < 12 hr). Only intact individuals that could articulate all legs were used in experiments. Prior to experimental testing in the laboratory or the field, focal ants were acclimated to the appropriate conditions (laboratory: ca. 22°C and 80% RH; field: ca. 30°C and 80% RH) for at least 1 hr. All ants were weighed to the nearest 0.1 mg following experiments.

Ant Adhesive Morphology

We used tarsal pad area and tarsal claw diameter as basic measures of ant adhesive morphology. Tarsal pad area values were obtained from prior studies (Stark et al. 2018) in which digital images of tarsal pads engaged with a glass surface were measured using NIH ImageJ (version1.46r; see Stark et al. 2018 for details). Tarsal claw diameter was measured using scanning electron microscopy (SEM; Zeiss Supra 35 SEM, Jena, Germany). Ant tarsi were separated from three frozen individuals and mounted so that the claw tips were in clear view (i.e., facing up or laterally). We used NIH ImageJ to measure the diameter of the claw tips on digital SEM images. Specifically, we used the *oval measurement* tool (locking it into a circle) to outline the perimeter of the tarsal claw tip, then converted the area of the circular overlay to diameter.

Surface Characterization

We used plate glass and seven different grades of sandpaper (grit size = P2500, P2000, P1500, P320, P120, P80, and P36; Table 1) to test ant adhesion and locomotor performance on smooth and variably rough substrates. For the purposes of this project, we assumed glass roughness is effectively zero. The surface asperities of plate glass exist at the nanometer scale, thus they are at least three orders of magnitude smaller than the micrometer-scale size of the attachment structures of ants.

We quantified the asperity size of each sandpaper grit size as the average maximum length of 5 particles chosen in three different locations on a sandpaper sample (i.e., 15 particles per sandpaper type). Specifically, a digital grid was overlaid on images of each of the three sample locations on a sheet of sandpaper. Due to the broad range in asperity size, grid areas were set at 10 mm^2 for P36 grit sandpaper, 2 mm^2 for P80 grit sandpaper, 0.75 mm^2 for P120 grit sandpaper, and 0.05 mm^2 for the remaining grit sizes (P2500, P2000, P1500, P320). These grid sizes provided six complete squares per sample area; we selected the largest exposed particle within each of the 5 clearest grid squares for measurement. Digital images of the sample areas were taken with a Zeiss Axio Zoom V16 microscope fitted with an Axiocam 305 color camera (Jena, Germany). We used the *grid plug-in* and *line tool* in NIH ImageJ to draw grid lines and measure the largest dimension of selected particles, respectively.

We measured ant adhesion on leaves from five different tree species: *Anacardium excelsum, Cavanillesia platanifolia, Dipteryx oleifera, Ochroma pyramidale,* and *Pseudobombax* *septenatum*. We chose these species because they are common trees near the BCI lab clearing, and superficially appear to span a broad range of surface roughness. In each case, we harvested mature leaves from portions of the trees receiving full sun exposure. Only leaves free of conspicuous epiphylls and damage were used for experiments. We quantified the surface characteristics of each leaf species by first making sodium alginate impressions of the leaf surface, and then making plaster casts of the impressions. The roughness profiles of these casts were obtained with a Veeco contact profilometer (Plainview, NY, USA). On each cast, three linear 6.102 mm passes using 5 mg of force were made with a 12.5 μm stylus tip. These passes did not include leaf veins. Profilometry data were uploaded to TopoBank (version 0.7.5; https://contact.engineering/) to calculate root-mean-square (RMS) height deviation, following the conventions of Jacobs et al. (2017). RMS height deviation is the average of the profile height deviations from the mean height. Curvature was removed from the profile plots. The RMS height deviation of each pass was used to calculate the mean RMS height deviation for each leaf species. All leaves were measured and used in experiments within 24 h of collection. To prevent dehydration and decay between trials, leaves were stored in a plastic bag with a wet paper towel in the laboratory.

We used stems of two liana (woody vine) taxa (*Tontelea ovalifolia* and *Bauhinia* sp.) and bark from the trunks of three tree species (*Anacardium excelsum*, *Alseis blackiana* and *Dipteryx oleifera*) to characterize ant locomotor performance on non-foliar natural plant substrates. Similar to the leaf substrates, we chose these focal species because they differed conspicuously in surface roughness, which was quantified in a separate study (Yanoviak et al. 2017). Specifically, field-based laser scanning was used to measure the mean amplitude of the bark surface asperities, and a laser scanning confocal microscope was used to image surface topography of liana stem samples (Yanoviak et al. 2017). Hereafter, all natural substrates used in experiments are referred to by genus only.

Adhesive Performance Experiments

The adhesive performance of ants was measured on sandpaper and tree leaves mounted to a flat surface. Adhesion to plate glass was used as a reference. In each case, tarsal adhesion was measured by clamping a thin nylon thread (Aurlfil, Milano, Italy) to a hand-held 10 or 20 g analog spring gauge (0.1g and 0.2g resolution respectively; Pesola, Schindellegi, Switzerland), and tying the free end around the ant petiole (Stark et al. 2018; Stark and Yanoviak 2018; Stark et al. 2019). We measured the adhesion of live ants clinging to experimental substrates in two orientations. First, ants were pulled approximately orthogonal to the horizontally positioned substrate surface (i.e., normal adhesion), then the substrate was mounted vertically using a stage with a clamp and ants were slid approximately parallel to the vertical substrate surface (i.e., shear adhesion; see Stark and Yanoviak 2018 for a schematic of the experimental set-up). In both orientations, each ant was induced to securely attach its adhesive pads by applying slight pressure to the gaster. Normal adhesion was defined as the maximum load (g) an ant could maintain before detaching from the experimental substrate (i.e., losing grip and springing free of the horizontal surface). Shear adhesion was defined as the maximum load (g) an ant resisted during an approximately 3 cm slide along the vertically positioned substrate. Ants were oriented head down for these experiments, so that their tarsal pads were fully engaged during vertical sliding. Each ant was tested in both orientations three times, but only the maximum of the three measurements in each orientation was used for statistical analysis. We tested normal and shear adhesion of fifteen ants per substrate.

Locomotor Performance Experiments

Locomotor performance (running speed, cm s^{-1}) was measured on 1 cm diameter wooden dowels wrapped with sandpaper, leaves attached to a flat substrate, or ca. 1 cm diameter vines. Running speed on bark was measured in the field by releasing ants on tree trunks at approximately 1.5m above the ground (Yanoviak et al. 2017). A 1 cm glass rod was used as a baseline reference for running speed on a smooth, hard substrate. We tested the effect of substrate diameter on locomotor performance by measuring ant running speed on glass rods of varying diameter (2, 5, 7, 9, 12 mm). Similarly, we tested the effects of substrate inclination on locomotor performance by measuring ant running speed on a 1 cm glass rod secured at different angles relative to horizontal (0, 30, 60, and 90°). In all experiments, running speed was measured as the time required for an ant to traverse a 10 cm section of the test substrate. Only uninterrupted, straight runs were used. Each ant was tested three times (n = 30 ants per treatment), and only the maximum speed observed for a given ant was used for statistical analysis.

Statistical Analysis

Normal and shear adhesion data did not fit the assumptions of a linear model, so we used nonparametric Kruskal-Wallis tests with post hoc Wilcoxon comparisons when significant differences occurred among treatment groups. Normal and shear adhesion where analyzed separately as a function of either sandpaper grit size or leaf species. Load resisted divided by body mass was used as the response variable to account for differences in body size. Body mass and body size measures (e.g., head width) are highly correlated in *C. atratus* and other ants (Corn 1980; Kaspari and Weiser 1999; Yanoviak et al. 2005).

Running speed data fit the assumptions of a linear model, therefore we used three separate analysis of covariance (ANCOVA) tests to investigate differences in running speed on sandpaper substrates and natural substrates (leaves, vines) in the laboratory, and tree trunks in the field. Running speed on tree trunks in the field was analyzed separately from leaves and vines because ambient temperature differences affect running speed (Hurlbert et al. 2008; Yanoviak et al. 2017). We used multiple regression to test for differences in running speed on dowels of different diameter, where mass and diameter were the independent variables. Finally, we used an ANCOVA to test for differences in running speed on 1 cm dowels secured at different angles of inclination. Ant mass was used as the covariate in all ANCOVA models. Differences among means in ANCOVA tests were determined with post hoc Tukey HSD tests. Data were transformed when necessary to meet normality and homogeneity of variance assumptions. Analyses were conducted with JMP 10.0.0 software (SAS Institute, Inc. 2012) and all means are reported ± s.e.m.

Results

Ant Morphology

The average mass of individual *C. atratus* workers used in this study was 40.3 ± 0.32 mg. The range of worker mass (12.4 - 79.0 mg) used in this study matches the range of worker sizes commonly encountered in the field (Corn 1980; Yanoviak et al. 2005). Their average tarsal pad area was 0.0140 ± 0.00078 mm^2 (Stark et al. 2018) and average tarsal claw diameter was 0.014 ± 0.0005 mm.

Surface Characterization

Sandpaper asperity sizes ranged from 0.02-1.19 mm in average maximum length (Table 1). These values are higher than reported by Federation of European Producers of Abrasives (FEPA) because we used the largest linear dimension of maximum particle size, not average particle size. The RMS height deviation of fresh leaves ranged ca. 0.01-0.02 mm (Table 2). Vine and tree bark asperity height was reported elsewhere (Yanoviak et al. 2017), and ranged ca. 0.09-2.15 mm (Table 2).

Adhesive and Locomotor Performance on Artificially Rough Substrates

Normal and shear adhesion of *C. atratus* ant workers on sandpaper substrates varied as a function of the asperity size (i.e., sandpaper grit size; Normal Adhesion: $\chi^2 = 111.32$, df = 7, $P < 0.0001$; Shear Adhesion: $\chi^2 = 98.67$, df = 7, $P < 0.0001$; Figure 2A and 2B). In general, shear adhesion was higher than normal adhesion, and substrates with larger asperity size produced higher forces. Specifically, normal adhesion was significantly different among asperity sizes except the largest sandpaper grits (P120, P80, P36). Normal adhesion to smooth glass produced the same load resistance as adhesion to sandpaper grit P320, but not smaller asperity sizes (grit > P320). Indeed, adhesion was lower (effectively zero) on these substrates than on smooth glass (0.5 ± 0.07 g load resisted). Likewise, shear adhesion differed among asperity sizes except the roughest sandpaper grits (P120, P80, P36). Adhesion consistently was higher on these rough substrates than on substrates with small asperity sizes. Shear adhesion loads did not differ for ants tested on smooth glass and on fine-grit sandpaper (P2000, P1500).

The running speed of *C. atratus* ant workers varied as a function of substrate asperity size ($F_{15,224} = 3.45$, $P < 0.0001$; Figure 2C). Specifically, running speed was consistently slower on smooth glass than on sandpaper, and tended to be marginally slower on the roughest sandpaper grits (P80 and P36).

Adhesive and Locomotor Performance on Natural Substrates

Normal and shear adhesive performance of *C. atratus* workers differed among natural substrate types (i.e., leaves; Normal Adhesion: $\chi^2 = 12.84$, df = 4, P = 0.0121; Shear Adhesion: $\chi^2 = 20.74$, df = 4, P = 0.0004; Figure 3). Similar to adhesion results on sandpaper, normal adhesion was much lower than shear adhesion on leaves. Normal adhesion was higher on *Ochroma* leaves than on the *Cavanillesia* and *Dipteryx* leaves, but was similar among all other leaf comparisons. Shear adhesion was higher on *Ochroma* and *Anacardium* leaves than on *Dipteryx* and *Pseudobombax* leaves. All other paired comparisons were statistically similar.

The running speed of *C. atratus* ant workers differed as a function of leaf and vine type in the laboratory ($F_{13,196} = 2.82$, $P = 0.0010$) and bark type in the field ($F_{5,85} = 8.05$, $P < 0.0001$). Specifically, ant running speed was faster on *Dipteryx* and *Anacardium* leaves and *Bauhinia* vine stems than on *Ochroma* leaves in the laboratory (Figure 4). In the field, ant running speed was lower on *Anacardium* bark than on *Dipteryx* and *Alseis* bark (Figure 4).

Locomotion on Narrow and Inclined Substrates

The average running speed of *C. atratus* ant workers increased as substrate diameter increased ($F_{2,147} = 69.90$, $P < 0.0001$, $R^2 = 0.49$; Figure 5). Running speed also differed as a function of running orientation (running up or down an incline; $F_{15,96} = 2.35$, $P = 0.0065$; Figure

6) and substrate angle had a significant effect on running speed (interaction: F = 3.69, df = 3, P = 0.0146). The significant interaction was driven by orientation (single effect: F = 12.55, df = 1, P = 0.0006), such that ants running upward on a vertical dowel ran slower than those running downward.

Discussion

The relatively linear, reticulate substrates that characterize the tropical rainforest canopy present a variety of physical challenges for wingless arboreal ants. Reduction in worker ant adhesion and running speed on highly variable substrates may increase the likelihood of falling, extend the time needed to discover food, elevate energy requirements, and prolong exposure to dangerous abiotic and biotic factors (i.e., temperature, rain, wind, predators, competitors; Denny et al. 2001; Gissel Nielsen 2001; Sarty et al. 2006; Farji-Brener et al. 2018; Radnan et al. 2018; Stark et al. 2018). The results of this study show that the adhesive performance and locomotor performance of workers of one common arboreal ant species are resilient to variation in substrate roughness, diameter, and orientation. However, in some conditions the adhesive mechanism completely failed or running speed significantly declined. Thus, it is likely that *C. atratus* workers establish foraging routes that minimize the inclusion of substrates effecting reduced adhesion and running speed, as observed in other cursorial animals (Pounds 1988; Irschick and Losos 1999; Jones and Jayne 2012).

Surface roughness is a ubiquitous and unpredictable environmental variable that can impact the adhesive performance of many organisms, especially when asperity size matches adhesive unit size (Huber et al. 2007; Gorb and Gorb 2009; Scholz et al. 2010; Wolff and Gorb 2012; Gillies et al. 2014; Salerno et al. 2017; Pillai et al. 2020). The results of this study show that when comparing asperity size, tarsal pad area, and claw tip size of *C. atratus* workers, the finest polishing paper is similarly scaled (i.e., asperity size of P2500 is ca. 0.02 mm and tarsal pad area and claw tip diameter are ca. 0.01 mm^2 and ca. 0.01 mm wide, respectively). This similarity likely explains the low adhesive performance of *C. atratus* workers on P2500 sandpaper, even in shear sliding. Presumably this outcome is due to additional friction between the tarsal pads and claws when sliding (Dai et al. 2002; Federle et al. 2004).

By contrast, the increase in adhesive performance with asperity size is counter to theoretical predictions and other experimental results (Fuller and Tabor 1975). However, these models do not take into account mechanical interlocking and friction from claws, which only occurs at high surface asperity sizes (Dai et al. 2002). The results of this study suggest that significant mechanical interlocking and friction from claws of *C. atratus* workers begins to occur when surface asperities are ca. 0.13 mm wide (P320 sandpaper grit substrate) and dominate when surface asperities are ca. 0.4 mm wide (P120 sandpaper grit substrate). The lack of difference in adhesive and locomotor performance on grits at the larger end of our experimental range of asperity (> 0.5 mm grain size) suggests that such asperities are the equivalent of boulders for ants. Consequently, it appears that differences in size among grains averaging 0.5-1.2 mm in maximum dimension are irrelevant to locomotion in *C. atratus* workers. The results also suggest that shear sliding helps claws interlock at smaller surface asperity sizes better than when pulled orthogonally from a substrate (i.e., asperity size ≥ 0.3 mm vs. asperity size ≥ 0.4 mm, respectively). Evaluating these possibilities was beyond the scope of this study, but could be accomplished via microscopic examination of tarsal-substrate interactions on diverse surfaces (e.g., Stark et al. 2018).

Surface roughness is just one parameter that potentially influences ant performance on natural substrates; variables like wettability, trichrome density, and the presence of wax crystals are also important (Davidson et al. 1989; Federle et al. 2000; Gorb and Gorb 2011; Stark and Yanoviak 2018). The plaster casting process used in this study generates a structurally imperfect replica of leaf surface properties, which likely contributed to the lack of strong RMS height deviation differences among leaf species that were otherwise superficially distinct. Indeed, the range of RMS height deviation observed among leaf casts was small, and matched the attachment structure size of ants (tarsal pads and claws). Regardless, when qualitatively comparing adhesion to glass and rough sandpaper with leaf adhesion, adhesion of *C. atratus* workers to natural leaf substrates was much lower overall. However, the normal and shear loads ants resisted on leaves were much higher than their body mass, suggesting that the risk of falling from a leaf surface is low under most natural circumstances. While the surface roughness of sandpaper and natural substrates (leaves, bark) used in this study are not easily comparable due to differences in characterization (i.e., asperity length vs. RMS height deviation vs. peak-valley asperity height), it was clear that surface roughness is significantly smaller on leaves than sandpaper and bark surfaces. Improving surface roughness measurement and quantification of substrates in the field would be a potentially useful future direction for this work.

The results of this study highlight the robustness of running speed across a variety of natural and artificial surface asperity sizes. Specifically, the running speed of *C. atratus* workers is very consistent across a range of leaf, vine, bark, and sandpaper substrates, as observed in related studies (Yanoviak et al. 2017). However, the slower running speeds on *Anacardium* bark (and our observations in the field) suggest that roughness amplitudes matching larger anatomical structures of worker ants, such as tarsi and legs, impose significant obstacles to locomotion

(Figure 1; Yanoviak et al. 2017). Very rough or unstable substrates like bark or gravel potentially reduce foraging efficiency (e.g., causing dropping of heavy loads when returning to the nest; Bernadou et al. 2011), making navigation on rough substrates even more costly. Thus, we would expect *C. atratus* workers to avoid foraging on very rough bark and very smooth substrates (at the roughness scale of glass), which also reduced running speed.

A key result of this work is the comparison of adhesive performance and running speed. In particular, while running speed across artificial and natural substrates did not show strong differences, adhesion varied significantly. This countered our prediction that adhesive performance dictates running speed (Figure 1). The mechanism responsible for this discontinuity has not been resolved (Stark et al. 2015; Stark and Yanoviak 2018). Many biological materials like ant adhesive pads are viscoelastic, and thus dependent on the rate of material extension (Federle et al. 2004; Vincent 2012). Therefore, it is possible that speed is maintained on poorly adhesive substrates by increasing shear sliding rate (i.e., strain rate; Stark et al. 2015). It is also possible that ants do not need their adhesive structures while running. For example, ants running horizontally and vertically can make contact mainly with their proximal tarsal segments (i.e., running on their "heels"), and use tarsal hairs for additional friction as needed (Reinhardt et al. 2009; Wöhrl et al. 2017).

Running up or down inclined substrates often reduces running speed (Birn-Jeffery and Higham 2014), and running on narrow substrates likely makes correct tarsal placement more difficult, thereby slowing forward progress (Lammers and Biknevicius 2004; Frantsevich and Cruse 2005). Given the prevalence of narrow and inclined substrates in arboreal habitats, (e.g., 50% of the surfaces were < 0.8 cm in diameter and angled at ca. 60° in a Bahamian forest; Mattingly and Jayne 2004), arboreal organisms likely routinely encounter physical challenges that impact running speed. The results of this work show that substrate inclination and diameter affect running speed of *C. atratus* workers, although such effects are limited to extreme circumstances (i.e., narrow or vertical substrates). Similar inclination-related results were reported in the field and laboratory for ants running up and down slopes (Seidl and Wehner 2008; Ravindra 2014), but other studies found limited effects of substrate inclination (Seidl and Wehner 2008; Weihmann and Blickhan 2009; Holt and Askew 2012; Khuong et al. 2013; Norton et al. 2014). Inclination generally slows ascent in cursorial animals (Jayne and Byrnes 2015), and different experimental outcomes likely reflect differences among species (Grevé et al. 2019) and slope (i.e., most studies were not conducted on vertical substrates). Likewise, only very narrow vine substrates seem to constrain foraging substrate choice in arboreal ants (Clay et al. 2010). This pattern supports the habitat constraint hypothesis - i.e., that foraging worker ants should avoid substrates and conditions that negatively impact performance (Irschick and Losos 1999).

Conclusion

The results of this study show that canopy substrates can affect adhesive performance of foraging worker ants, but that locomotor performance is maintained in most contexts. Given this outcome, we would expect ants like *C. atratus* (with relatively larger workers) to avoid foraging in locations of the canopy where adhesion is compromised (i.e., smooth substrates). Conversely, we do not expect foraging paths to be influenced by substrate structure except where substrates are vertical or narrow. Indeed, running down a vertical substrate (verses a lower angle of inclination) may maximize time and energy benefits. Future work should focus on field observations of foraging arboreal ants in the canopy and more detailed analyses of the kinematics of adhesion and locomotion on variable substrates.

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Data Availability Statement

The data underlying this article will be shared on request to the corresponding author.

Figure Captions

Figure 1. Predicted performance (adhesion and running speed) of adhesive animals on rough substrates. On smooth to rough substrates performance is expected to be lower on fine-scale rough substrates that match the asperity size of the attachment structures (pads, claws) due to low contact area. Within this regime the attachment structure size dominates performance. On very rough substrates, body size effects dominate and performance is lowest on very rough substrates that match the size scale of feet and legs. Under this circumstance, surface asperities become significant obstacles. As substrate roughness increases, performance increases because the asperities essentially become hills at the scale of the whole animal while being relatively smooth at the adhesive interface. Ultimately, larger and larger hills increasingly have their own surface asperities (i.e., at the micrometer scale), which interact with adhesive structures at the smooth to rough end of the scale while also resisting the gravitational forces of inclination. This latter complex interaction is not illustrated here, but was partially explored experimentally.

Figure 2. Normal adhesion (A), shear adhesion (B), and running speed (C) of *Cephalotes atratus* ant workers on sandpaper substrates that vary in asperity size (i.e., grit size) and plate glass (reference substrate). Treatment groups denoted with the same letter are not significantly different. Boxes in A and B represent the 25th and 75th percentiles, the line marks the median, bars are the 90th and 10th percentiles. Filled circles in C are means and error bars are ± s.e.m.

Figure 3. Normal adhesion (A) and shear adhesion (B) of *Cephalotes atratus* ant workers on leaves. Substrates are abbreviated by genus name (ANA: *Anacardium*; CAV: *Cavanillesia*; DIP:

Dipteryx; OCH: *Ochroma*; PSE: *Pseudobombax*). Treatment groups denoted with the same letter are not significantly different. Plotted values are described in Figure 2A and B.

Figure 4. Average running speed of *Cephalotes atratus* ant workers on natural substrates (A, leaves and vine stems; B, tree bark). Substrates in A are abbreviated by genus name (ANA: *Anacardium*; BAU: *Bauhinia* (vine); CAV: *Cavanillesia*; DIP: *Dipteryx*; OCH: *Ochroma*; PSE: *Pseudobombax*; TON: *Tontelea* (vine)). Treatment groups denoted with the same letter are not significantly different. Error bars are ± s.e.m.

Figure 5. Running speed of *Cephalotes atratus* ant workers on horizontal glass rods that vary in diameter. Error bars are ± s.e.m.

Figure 6. Running speed of *Cephalotes atratus* ant workers on a glass rod (1 cm) positioned at various angles relative to horizontal (0, 30, 60, and 90°). Ants were positioned on the rod and induced to either run up or down. Means that differ are indicated with an asterisk. Error bars are ± s.e.m.

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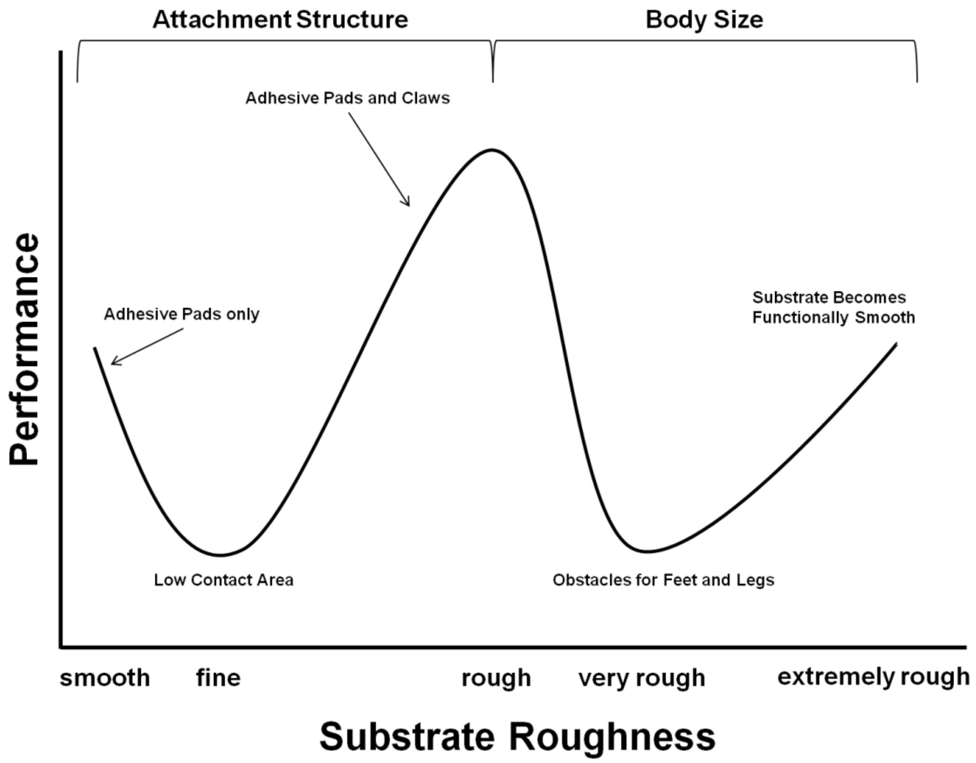

Figure 1. Predicted performance (adhesion and running speed) of adhesive animals on rough substrates. On smooth to rough substrates performance is expected to be lower on fine-scale rough substrates that match the asperity size of the attachment structures (pads, claws) due to low contact area. Within this regime the attachment structure size dominates performance. On very rough substrates, body size effects dominate and performance is lowest on very rough substrates that match the size scale of feet and legs. Under this circumstance, surface asperities become significant obstacles. As substrate roughness increases, performance increases because the asperities essentially become hills at the scale of the whole animal while being relatively smooth at the adhesive interface. Ultimately, larger and larger hills increasingly have their own surface asperities (i.e., at the micrometer scale), which interact with adhesive structures at the smooth to rough end of the scale while also resisting the gravitational forces of inclination. This latter complex interaction is not illustrated here, but was partially explored experimentally.

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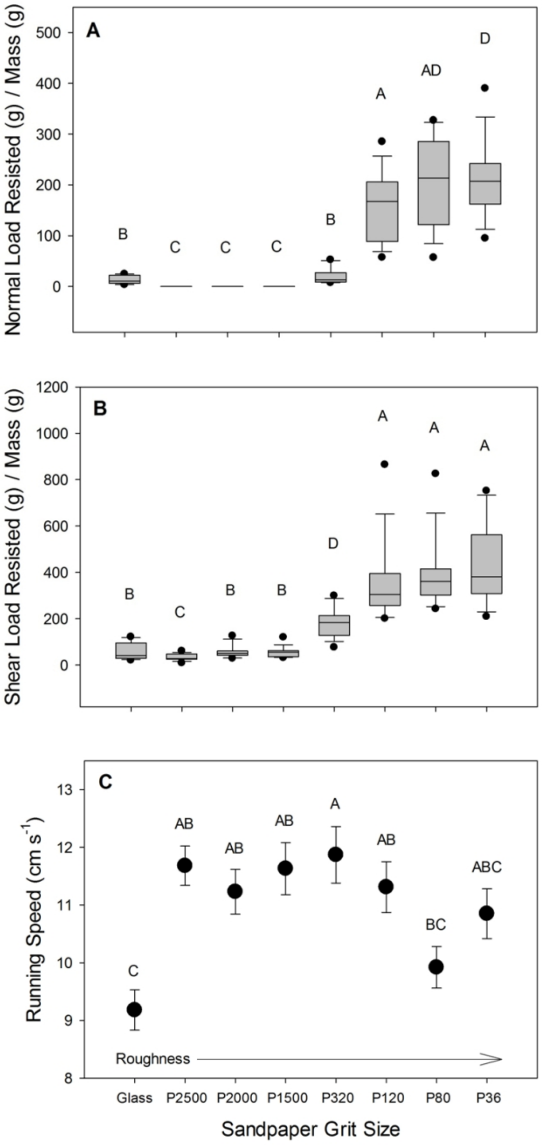

Figure 2. Normal adhesion (A), shear adhesion (B), and running speed (C) of *Cephalotes atratus* ant workers on sandpaper substrates that vary in asperity size (i.e., grit size) and plate glass (reference substrate). Treatment groups denoted with the same letter are not significantly different. Boxes in A and B represent the 25th and 75th percentiles, the line marks the median, bars are the 90th and 10th percentiles. Filled circles in C are means and error bars are ± s.e.m.

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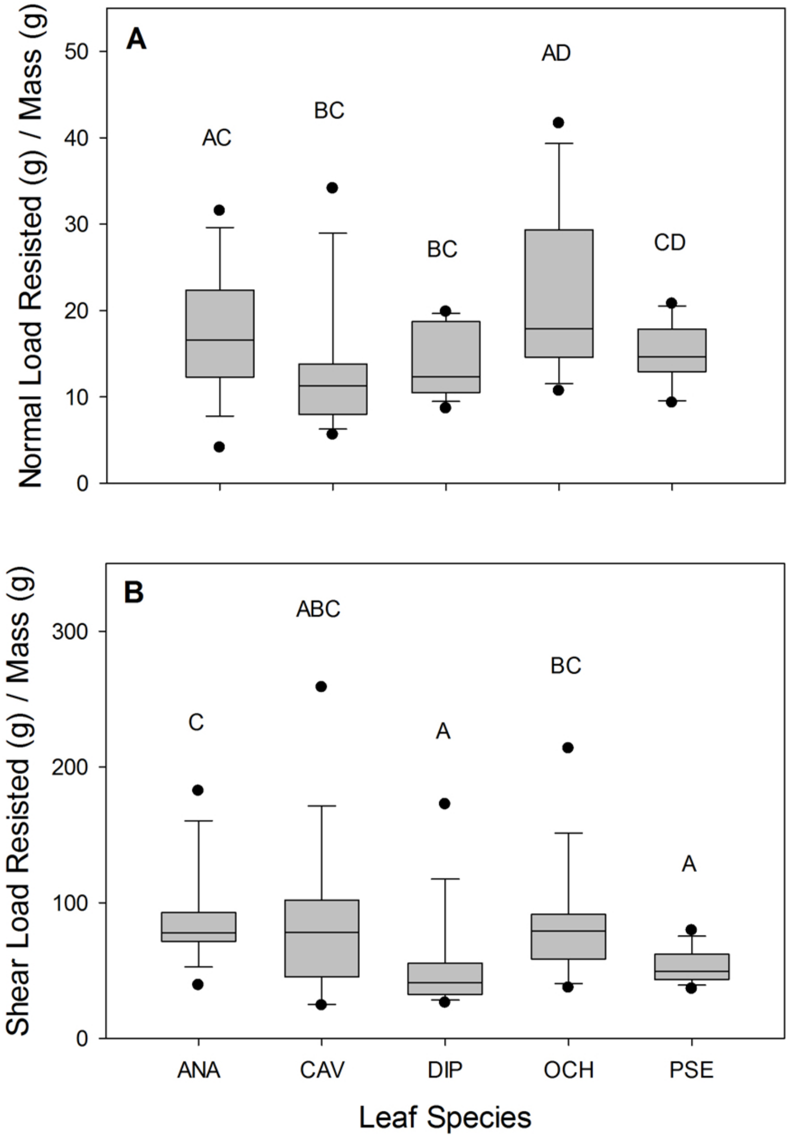

Figure 3. Normal adhesion (A) and shear adhesion (B) of *Cephalotes atratus* ant workers on leaves. Substrates in are abbreviated by genus name (ANA: *Anacardium*; CAV: *Cavanillesia*; DIP: *Dipteryx*; OCH: *Ochroma*; PSE: *Pseudobombax*). Treatment groups denoted with the same letter are not significantly different. Plotted values are described in Figure 2A and B.

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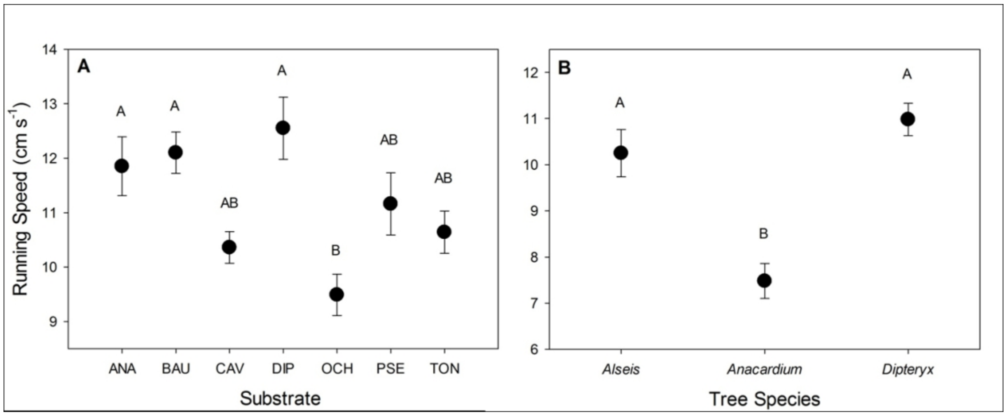

Figure 4. Average running speed of *Cephalotes atratus* ant workers on natural substrates (A, leaves and vine stems; B, tree bark). Substrates in A are abbreviated by genus name (ANA: *Anacardium*; BAU: *Bauhinia* (vine); CAV: *Cavanillesia*; DIP: *Dipteryx*; OCH: *Ochroma*; PSE: *Pseudobombax*; TON: *Tontelea* (vine)). Treatment groups denoted with the same letter are not significantly different. Error bars are ± s.e.m.

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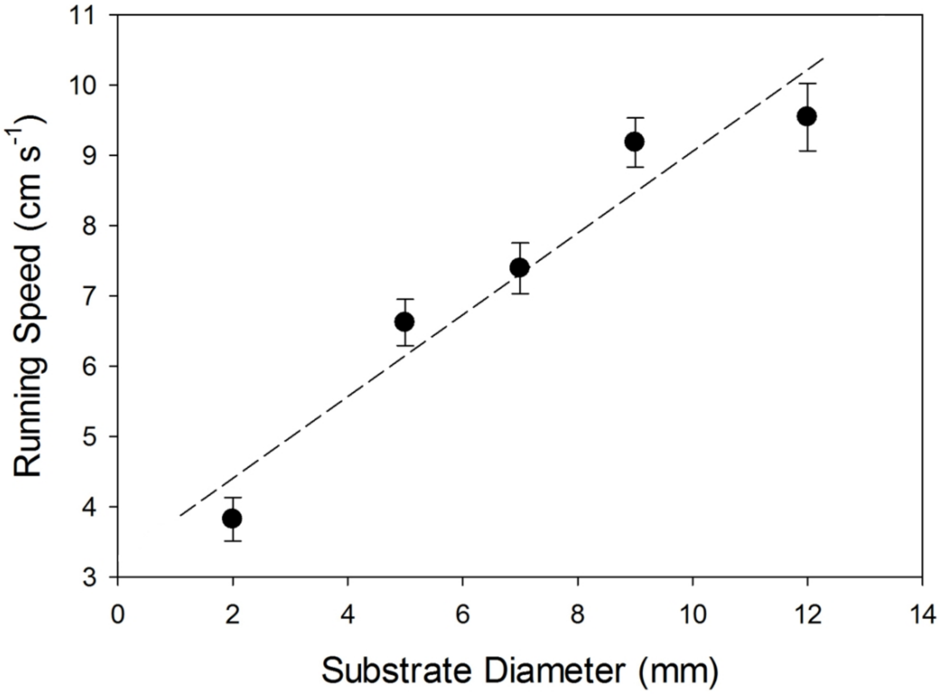

Figure 5. Running speed of *Cephalotes atratus* ant workers on horizontal glass rods that vary in diameter. Error bars are ± s.e.m.

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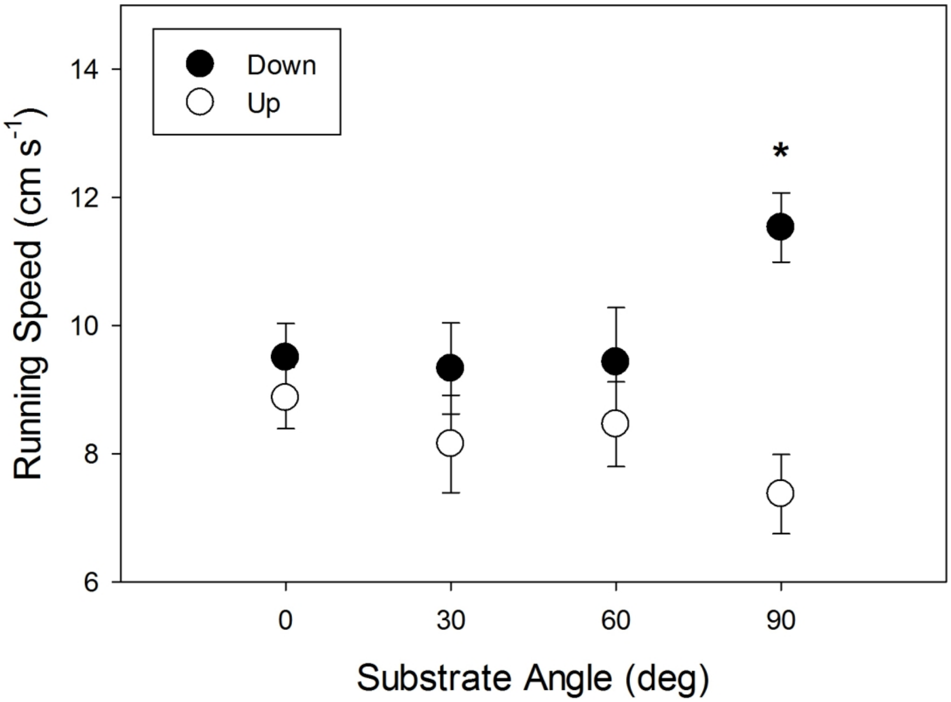

Figure 6. Running speed of *Cephalotes atratus* ant workers on a glass rod (1 cm) positioned at various angles relative to horizontal (0, 30, 60, and 90°). Ants were positioned on the rod and induced to either run up or down. Means that differ are indicated with an asterisk. Error bars are ± s.e.m.

76x60mm (600 x 600 DPI)

Table 1. Average maximum asperity size (measured as particle length) of sandpaper substrates characterized by commercially available grit size. Federation of European Producers of Abrasives (FEPA) asperity size values for the same grit size sandpaper substrates are provided as reference. Means are reported ± s.e.m.

Sandpaper Grit Size (FEPA)	**Average Maximum Asperity Size (mm)**	**FEPA Asperity Size Reference Values (mm)**
P36	1.186 ± 0.0891	0.538
P80	0.521 ± 0.0232	0.201
P120	0.354 ± 0.0190	0.125
P320	0.130 ± 0.0066	0.0462
P1500	0.035 ± 0.0008	0.0126
P2000	0.031 ± 0.0012	0.0103
P2500	0.022 ± 0.0008	0.0084

Table 2. Average asperity size of natural leaf substrates. Vine and bark substrate asperity sizes are reported elsewhere (Yanoviak et al. 2017). Surface asperity size is measured as root mean square (RMS) height deviation. Means are reported ± s.e.m.

Species	**Surface Asperity Size (mm)**
Anacardium excelsum	0.016 ± 0.0023
Cavanillesia platanifolia	0.018 ± 0.0009
Dipteryx oleifera	0.010 ± 0.0009
Ochroma pyramidale	0.012 ± 0.0018
Pseudobombax septenatum	0.016 ± 0.0031