

RESEARCH ARTICLE

Adhesive performance of tropical arboreal ants varies with substrate temperature

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ABSTRACT

The surface temperature of tree branches in the tropical rainforest canopy can reach up to 55°C. Ants and other small cursorial organisms must maintain adequate attachment in this extreme microenvironment to forage effectively and avoid falling. Ant adhesion depends on liquid secretions that should become less viscous at high temperatures, causing ants to slip. However, tropical arboreal ants have high thermal tolerance and actively forage on hot canopy surfaces, suggesting that these ants can maintain adhesion on hot substrates. We measured tarsal pad shear adhesion of 580 workers (representing 11 species and four subfamilies) of tropical arboreal ants at temperatures spanning the range observed in the field (23–55°C). Adhesive performance among species showed three general trends: (1) a linear decrease with increasing temperature, (2) a non-linear relationship with peak adhesive performance at ca. 30–40°C, and (3) no relationship with temperature. The mechanism responsible for these large interspecific differences remains to be determined, but likely reflects variation in the composition of the secreted adhesive fluid. Understanding such differences will reveal the diverse ways that ants cope with highly variable, and often unpredictable, thermal conditions in the forest canopy.

KEY WORDS: Viscosity, Panama, Canopy, Formicidae, Tarsi

INTRODUCTION

Temperature is perhaps the most influential environmental factor contributing to the survival of ectotherms. Specifically, deviations from an ectotherm's thermal tolerance range (i.e. maximum and minimum critical thermal tolerances, CT_{max} and CT_{min} , respectively) can have significant physiological consequences, ultimately affecting performance and survival (Cowles and Bogert, 1944; Huey and Stevenson, 1979; Huey, 1991; Huey et al., 1992). To overcome physiological limitations, many ectotherms have adopted specific behaviors to help regulate their internal body temperature and stay within their physiological boundaries (e.g. basking, seeking shelter from the sun; Huey, 1991; Martin and Huey, 2008; Kearney et al., 2009; Sunday et al., 2014). Although the physiological dependence of ectotherm performance and behavior has been studied extensively, investigation of other

relationships associated with performance and the thermal environment has received less attention.

The independent evolution of biological adhesive systems across many taxa (e.g. plants, insects, mollusks, reptiles) has allowed organisms to take advantage of new niches, and escape predators or competitors (Gorb, 2008; Peattie, 2009). Although adhesive performance, or how strongly an organism clings to a substrate, is influenced by physiology and behavior, fundamental physical and chemical processes are directly responsible for the adhesive mechanism. For example, 'wet' biological adhesive systems, like those used by insects, take advantage of glue-like secretions that form temporary bonds or bridges between the tarsi and the substrates to which they attach (Federle et al., 2002; Gorb et al., 2002; Dirks and Federle, 2011a; Dirks, 2014). These gluey secretions depend primarily on surface tension and viscosity to resist perpendicular loads (i.e. normal loads; Fig. 1A; Federle et al., 2002, 2004; Dirks, 2014), and fluid viscosity to resist parallel loads (i.e. shear loads; Fig. 1B; Federle et al., 2004; Dirks, 2014). Thus, the 'wet' adhesive systems of ectothermic insects are driven by the physical and chemical properties of the system (e.g. surface tension, viscosity, biochemistry), in addition to physiological and behavioral mechanisms (e.g. CT_{max} , CT_{min} , activity period, foraging behavior).


Ants constitute ca. 20% of the Earth's terrestrial animal biomass (Schultz, 2000), making the 'wet' adhesive system of ants one of the most conspicuous biological adhesive systems. To attach to smooth substrates, ants use a compliant smooth pad coated with tarsal pad secretions (TPS; Federle et al., 2002). Analysis of other insects (e.g. locust, beetle) suggests that ant TPS consist of carbohydrates, proteins, and non-polar components like fatty acids, alcohols, glycerol and hydrocarbons (Ishii, 1987; Kosaki and Yamaoka, 1996; Attygalle et al., 2000; Federle et al., 2002; Vötsch et al., 2002; Geiselhardt et al., 2010b; Dirks and Federle, 2011a; Reitz et al., 2015; Betz et al., 2016). The composition of this emulsion can vary intraspecifically and interspecifically in relation to its location on the body (e.g. non-adhesive tibia versus adhesive tarsi; Geiselhardt et al., 2009, 2010a; Jarau et al., 2012; Reitz et al., 2015; Betz et al., 2016; Gerhardt et al., 2016), and preferred habitat (e.g. non-adhesive ground dwellers versus adhesive leaf-dwellers; Peisker et al., 2014; Gerhardt et al., 2016). Furthermore, it has been suggested that insects can tune this secretion to match the surface properties of the attachment substrate (Dirks and Federle, 2011b). This broad variation suggests that environmental and behavioral factors drive temporal and spatial variability in the physicochemical properties of the 'wet' adhesive systems of ants.

In addition to their high abundance in terrestrial ecosystems, wingless worker ants are among the most thermally tolerant ectotherms (Kaspari et al., 2015, 2016; Spicer et al., 2017; Wehner et al., 1992). Consequently, the TPS of foraging workers are in direct, repeated contact with heated substrates in their environment. In general, elevated temperature reduces fluid viscosity (i.e. Arrhenius equation, Williams–Landel–Ferry equation; Williams

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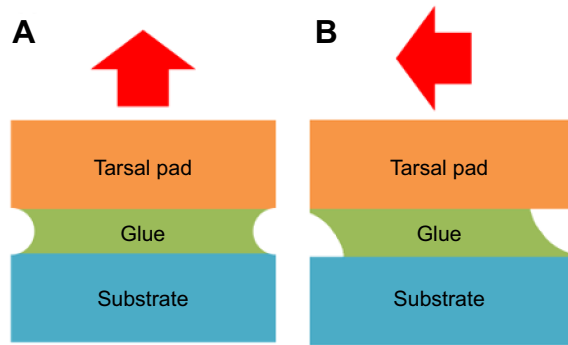


Fig. 1. The ‘wet’ adhesive system of an insect employs a gluey secretion to form a bridge between the substrate and the tarsal pad or hairs. This adhesive complex is capable of resisting normal loads (A) and shear sliding loads (B). The red arrow represents the loading direction.

et al., 1955), and low-viscosity fluids lubricate the interface between two surfaces, reducing shear adhesion (Israelachvili, 2011). Specifically, the viscosity of liquids like water and oil decreases exponentially over biologically relevant temperatures (i.e. 20–60°C; Korson et al., 1969; Roelands et al., 1963), suggesting that similar compounds in ant TPS may also experience significant declines in viscosity as a function of temperature. Indeed, the adhesive performance of the ant *Oecophylla smaragdina* decreases at high temperature (30°C versus 15°C), presumably because their TPS become more fluid at 30°C (Federle et al., 2002, 2004).

Temperature is particularly important for tropical arboreal ants that forage on tree branches, the surface temperature of which can exceed 50°C, well above the melting temperature (T_m) of many grease-like long chain *n*-alkanes (>C20) commonly found in TPS (T_m ca. >35°C; Schmidt et al., 2000; Kaspari et al., 2015; Reitz et al., 2015; Stark et al., 2017). However, physiological and behavioral studies suggest that some tropical arboreal ants can maintain adhesion in these extreme conditions. For instance, species like *Atta colombica* and *Cephalotes atratus* have high CT_{max} and forage at temperatures >30°C, supporting an alternative prediction that the physicochemical mechanisms of some tropical arboreal ant adhesive systems remain functional in these extreme conditions (Kaspari et al., 2015; Spicer et al., 2017).

The primary goal of this study was to test adhesive performance of tropical arboreal ants in ecologically relevant thermal conditions (i.e. surface temperature ranging from 23 to 55°C). We

hypothesized that the extreme thermal conditions of the tropical forest canopy affect the shear adhesive performance of arboreal ants. Given expectations based on the physicochemical properties of the ‘wet’ adhesive system (i.e. temperature-dependent viscosity and density), we expected shear adhesive performance to vary predictably with temperature. Specifically, we predicted that adhesive performance will decline exponentially as temperature increases, following the Arrhenius and Williams–Landel–Ferry equations (depending on the characteristics of the fluid; Williams et al., 1955) and non-linear trends in temperature–viscosity relationships of many compounds (Korson et al., 1969; Roelands et al., 1963). We explored this prediction among 11 common species of tropical arboreal ants.

MATERIALS AND METHODS

Field and laboratory work was conducted at the Barro Colorado Island (BCI) field station in Panama (09.15 N, 79.85 W). We collected 580 workers of 11 focal ant species (Table 1) from around the BCI laboratory. Each focal species was represented by workers from at least two colonies when possible (i.e. $N \geq 25$). These species were selected opportunistically (i.e. $N < 25$) and based on availability (i.e. collection of large sample sizes, $N \geq 25$) and phylogenetic relationship (i.e. representing as many subfamilies as possible). Collectively, these 11 species represent four of the most common arboreal subfamilies in the neotropics, and span a large range in mass (i.e. 0.1–166.0 mg).

Adhesion testing

To measure the adhesive performance of individual ants, we attached a nylon thread (Aurfil; Milano, Italy) to the petiole of each focal ant (Fig. 2). The free end of the thread was clamped to a 10 g capacity spring scale (Pesola; Schindellegi, Switzerland). Stinging and fast-moving species were anesthetized by cooling at –20°C for 10–20 min (until they stopped moving) before the thread was attached. All ants were acclimated to the laboratory temperature (ca. 23°C) for 1 h prior to testing.

Ant adhesion was tested on a piece of plate glass (20×25×0.5 cm) secured in a vertical position (Fig. 2). We used glass to ensure that the ant claws did not significantly contribute to adhesive performance measurements, i.e. the tarsal pad and TPS were responsible for adhesion. Although plate glass is not perfectly smooth, only surface asperities greater than the claw tip diameter can contribute to mechanical interlocking (Dia et al., 2002). The claw tip diameters of

Table 1. Body mass, tarsal pad area and shear adhesive performance of 11 focal ant species

Species	Body mass (mg)	Tarsal pad area (μm^2)	Maximum load (g)	Maximum load/pad area (g mm^{-2})	Best-fit prediction
<i>Atta colombica</i> (Guérin-Méneville) ($N=118$)	5.65±0.227	5.2±0.26 ($n=11$)	1.2±0.05	235.0	NL
<i>Azteca trigona</i> Emery ($N=25$)	0.91±0.056	1.7±0.21 ($n=6$)	0.3±0.03	160.8	NL
<i>Camponotus linnaei</i> Forel ($N=12$)	2.53±0.233	2.0 ($n=1$)	0.5±0.07	258.3	NR
<i>Camponotus sericeiventris</i> (Guérin-Méneville) ($N=103$)	55.71±1.850	23.0±2.00 ($n=3$)	2.1±0.09	93.0	P
<i>Cephalotes atratus</i> (Linnaeus) ($N=99$)	39.91±0.956	14.0±0.78 ($n=8$)	1.7±0.09	119.0	P
<i>Cephalotes basalis</i> (Smith) ($N=14$)	6.26±0.353	7.0±0.00 ($n=2$)	1.1±0.13	152.0	NL
<i>Crematogaster brasiliensis</i> Mayr ($N=7$)	0.48±0.114	1.0 ($n=1$)	0.3±0.04	328.6	NL
<i>Crematogaster crinosa</i> Mayr ($N=58$)	1.03±0.062	2.0±0.00 ($n=2$)	0.7±0.04	353.4	P
<i>Dolichoderus bispinosus</i> (Olivier) ($N=121$)	4.46±0.078	3.6±0.24 ($n=5$)	0.4±0.01	114.8	NL
<i>Paraponera clavata</i> (Fabricius) ($N=14$)	133.76±4.164	35.0±1.52 ($n=5$)	2.8±0.40	79.6	NR
<i>Procrystocerus belti</i> Forel ($N=9$)	1.02±0.055	1.0 ($n=1$)	0.6±0.06	588.9	NR

Study species are listed with their taxonomic authority (number of individuals used for experiments, N , is given in parentheses). Data are means±1 s.e.m. with number of individuals used to measure tarsal pad area only (n) in parentheses.

Shear adhesive performance was measured as the load an ant resisted while being pulled parallel to a vertical glass substrate (maximum load). The best-fit prediction of adhesive performance as temperature varied is listed as a negative linear relationship (NL), a second-order polynomial (P), or no relation (NR).

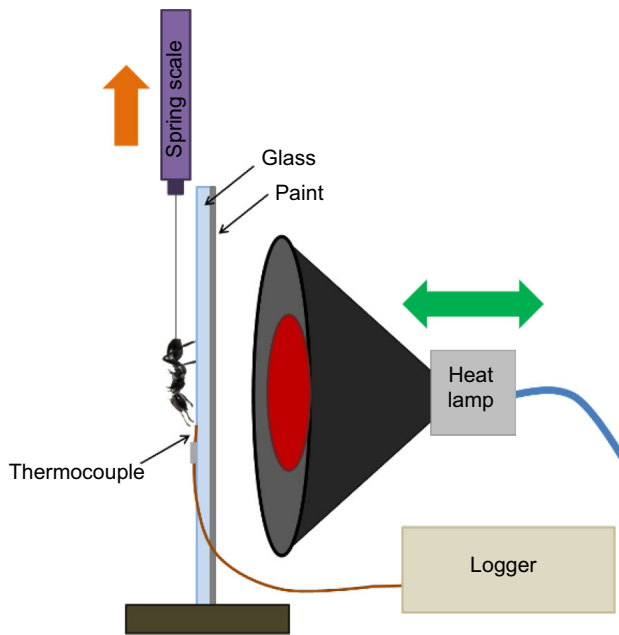


Fig. 2. Experimental set-up for measuring shear adhesive performance of tropical arboreal ants. Shear adhesive performance was measured by attaching a nylon thread to the petiole of the ant and pulling the ant in the direction of the orange arrow along a vertically positioned glass plate. A spring scale was used to measure the maximum load the ant resisted while sliding ca. 3 cm. Surface temperature of the glass substrate was manipulated by moving a heat lamp toward or away from the glass (green arrow), and measured using a wire thermocouple taped to the surface of the glass.

the 11 focal species ranged from 1 to 18 μm . Examination of the experimental surface under a light microscope revealed no surface defects in this range (i.e. all surface asperities were $<1 \mu\text{m}$ in the test region of the glass).

Preliminary observations showed that individual tarsal pads maintain varying amounts of contact with the substrate. To fully engage the tarsal pads with the glass surface, we allowed each ant to take a few steps on the glass prior to testing. Once the tarsal pads were engaged (i.e. the thread was taught) and the ant stopped moving, we gradually increased tension on the spring scale to pull the ant parallel to the glass for ca. 3 cm (Fig. 2). We recorded the highest load that the ant resisted during the 3 cm slide as the datum for each trial. This sliding load was used as a measure of the ant's viscosity-dependent shear adhesive performance. Sliding measurements were repeated three times, and only the highest load of three attempts (maximum load, in g) was used for analysis. The highest recorded load for a given ant typically occurred when all of its tarsi were in contact with the surface and its longitudinal body axis was aligned with the tether; thus, we generally aborted trials that did not meet these conditions. Before each new ant was tested, the glass was cleaned with ethanol, followed by water, to remove any adhesive residue or debris. Ants were killed by freezing (-20°C for >2 h), allowed to thaw for 30 min, and then weighed to the nearest 0.1 mg at the conclusion of all trials.

Temperature treatment

We used a 125 W UVA/UVB mercury vapor heat lamp (Solar Glo, ExoTerra; Mansfield, MA, USA) to alter the surface temperature of the vertical glass plate. To prevent light from the heat lamp from disrupting ants during experiments, heat was always applied to the back surface of the glass, which was painted grey to inhibit light transmission to the experimental surface (Fig. 2). The temperature

of the experimental surface of the glass was regulated by adjusting the distance between the lamp and the glass. A T-type wire thermocouple (HYP-1, Omega Engineering; Stamford, CT, USA) was taped to the front (experimental surface) of the glass within a few centimeters of the ant, and a data logger (RDXL4SD, Omega Engineering) recorded the temperature of the glass surface every 2 s during each trial (Fig. 2). Ants were tested at a range of temperatures (ca. $23\text{--}55^\circ\text{C}$) that replicated the range of tree branch surface temperatures measured in the field (Kaspari et al., 2015; Stark et al., 2017). Within a species, test temperatures were as evenly distributed between these extremes as possible. For example, adhesion tests on *Atta colombica*, *Camponotus sericeiventris*, *Cephalotes atratus* and *Dolichoderus bispinosus* were conducted with a minimum of 15 individuals (from three different colonies) tested every ca. 5°C over the full temperature range. Thus, we tested ca. 100 workers for each of these species. Because of time and logistical constraints, sample sizes for *Azteca trigona*, *Camponotus linnaei*, *Cephalotes basalidis*, *Crematogaster brasiliensis*, *Crematogaster crinosa*, *Paraponera clavata* and *Proccryptocerus belti* were considerably smaller. Regardless, in all cases the total number of workers obtained was distributed approximately evenly across the range of focal temperatures.

Tarsal pad area

Images of ant tarsal pads were captured using a digital video microscope (Dino Lite 2.0, AnMo Electronic Co.; Hsinchu City, Taiwan), which was fastened below a horizontally mounted thin glass plate (1.6 mm thick), and focused on the top surface of the glass (Fig. 3A). Tethered ants were allowed to walk naturally on the glass and engage their tarsal pads. We applied tension to the thread to drag each ant across the glass, causing its tarsal pads to extend as they passed through the field of view (Federle et al., 2001). Video recordings of the engaged pads were converted to images of individual frames for analysis (Movie 1). Only clear images with retracted claws and fully extended pads were selected for measurement. We used NIH ImageJ version 1.51a to measure the area of each tarsal pad by tracing its perimeter (Fig. 3B). Tarsal pad area was measured from at least one individual per focal species (Table 1).

Statistical analysis

To compare relative shear adhesion among the 11 focal ant species, we divided average maximum load by average tarsal pad area for each species. To investigate how adhesion varies with temperature in each species, we used a partial F -test to determine the appropriateness of a simple linear model for all maximum load–temperature relationships that were significant. Specifically, we compared the R^2 value of the simple linear model with a model treating temperature as a categorical variable binned into 10°C increments (cf. Federle et al., 2004). When the increase in R^2 was significant, we used a quadratic model for the analysis, as predicted by the non-linear change in fluid viscosity over a range of temperatures in water and oil (see Introduction). Species that showed no significant linear relationship between adhesive performance and surface temperature were denoted as such. Maximum load data were log transformed before analysis to meet model assumptions. All means are presented ± 1 s.e.m.

RESULTS

Relative shear adhesion, measured as maximum load resisted by an ant, ranged from 0.1 to 5.6 g among the 11 ant species tested. This represented resistance of loads 12,000 times the body mass of some individuals. Maximum load normalized by tarsal pad area revealed that comparatively, *P. belti*, *C. crinosa* and *C. brasiliensis* adhered

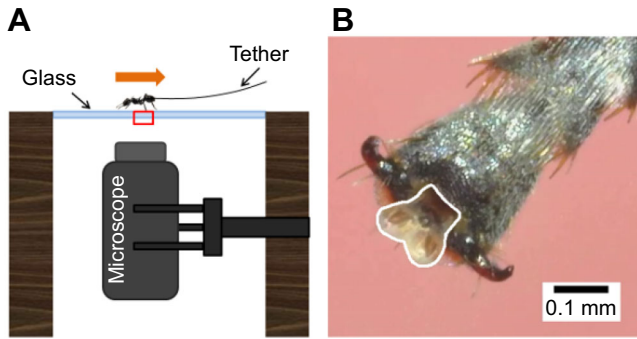


Fig. 3. Diagram of tarsal pad area video capture set-up, and example tarsal pad area of *Cephalotes atratus*. (A) Ants were slid across a glass substrate using a tether in the direction of the orange arrow. The focal area for the tarsal pad area image capture is boxed in red, and located above the vertically mounted video microscope. (B) Tarsal pad area was measured by outlining the extended tarsal pad during the slide.

to the glass substrate more strongly than all other species, regardless of temperature (Table 1). These species were also among those with the lowest mass tested.

Maximum load declined monotonically with increasing temperature in *A. colombica* ($F_{1,116}=117.75$, $R^2=0.50$, $P<0.0001$), *A. trigona* ($F_{1,23}=7.18$, $R^2=0.24$, $P=0.0134$), *C. basalis* ($F_{1,12}=25.90$, $R^2=0.68$, $P=0.0003$), *C. brasiliensis* ($F_{1,5}=9.43$, $R^2=0.65$, $P=0.0278$) and *D. bispinosus* ($F_{1,119}=44.24$, $R^2=0.27$, $P<0.0001$; Fig. 4). The adhesive performance of *C. sericeiventris*, *C. atratus* and *C. crinosa* was non-linear (based on partial F -test results). For all of these species, adhesive loads followed a hump-shaped (quadratic) relationship with temperature (Fig. 4). Peak adhesive performance in *C. sericeiventris* occurred between 30 and 40°C, whereas peak adhesive performance in *C. atratus* and *C. crinosa* occurred more abruptly between 35 and 40°C (Fig. 4). All other species showed no clear relationship between temperature and adhesive performance (i.e. linear regression $P>0.05$; Table 1).

Some species exhibited distinctive temperature-related behaviors during the experimental trials. Specifically, workers of *C. sericeiventris* began to move erratically at temperatures $>50^\circ\text{C}$. Likewise, *D. bispinosus* tended to curl up at these elevated temperatures. No conspicuously erratic behaviors were observed in other species (*A. colombica*, *C. atratus*, *A. trigona*, *C. crinosa*) during the experiments.

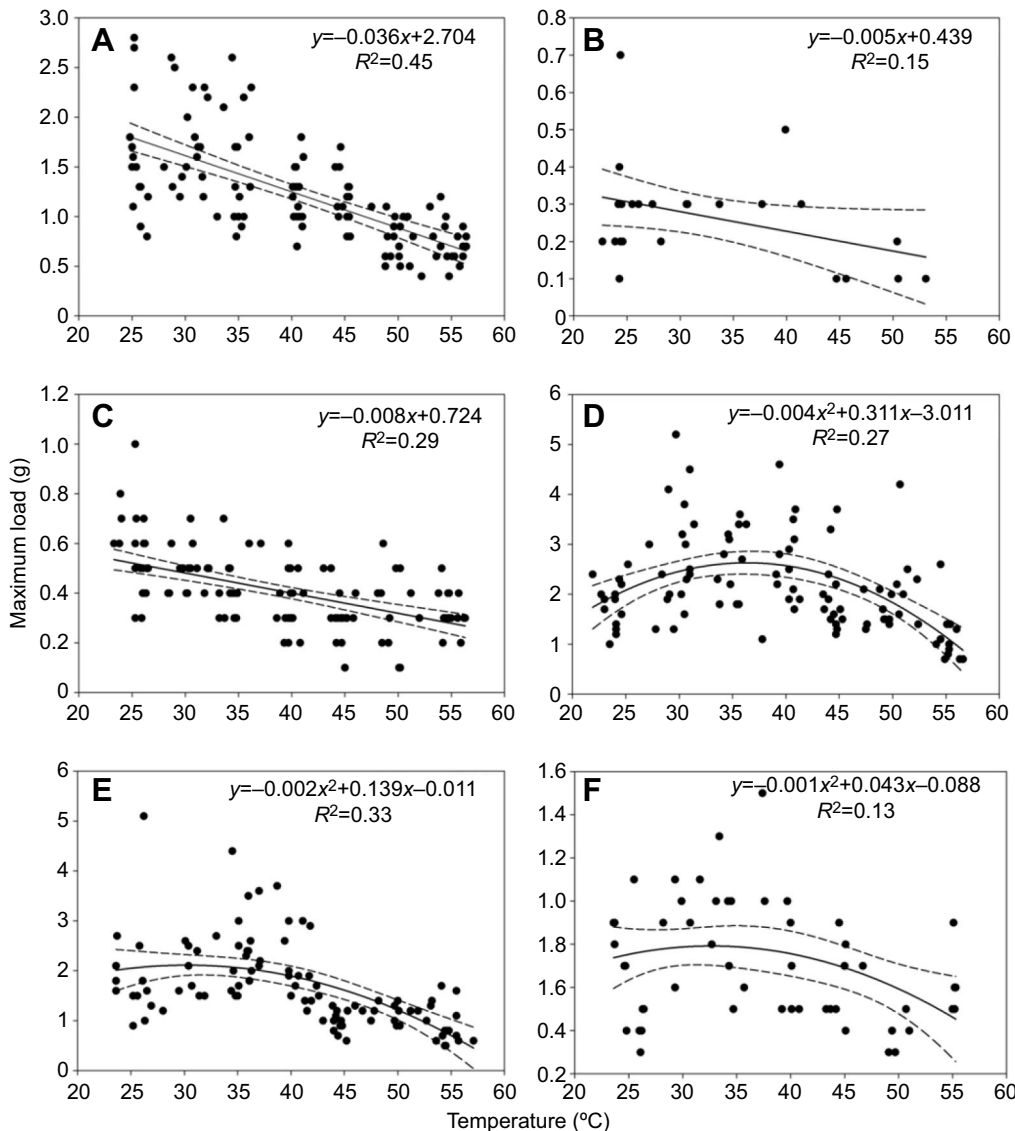


Fig. 4. Shear adhesion varies with surface temperature in six species of tropical arboreal ants. Specifically, maximum load decreased linearly in *Atta colombica* (A), *Azteca trigona* (B) and *Dolichoderus bispinosus* (C), whereas a second-order polynomial better fitted the data for *Camponotus sericeiventris* (D), *Cephalotes atratus* (E) and *Crematogaster crinosa* (F). The dashed lines represent 95% confidence intervals. Sample size $N \geq 25$.

DISCUSSION

The results of this study show that shear adhesion of 11 tropical arboreal ants varies as a function of ecologically relevant surface temperatures (i.e. 23–55°C). While the mechanism responsible for this variation is beyond the scope of this study, the linear and curvilinear relationship between adhesion and surface temperature suggests that physical, and particularly chemical, components of the adhesive system affect adhesive performance in thermally variable environments. Specifically, the hump-shaped temperature–adhesion relationship we observed for some species follows expectations of the Arrhenius and Williams–Landel–Ferry equations (Williams et al., 1955) and the exponential change in fluid viscosity of polar and non-polar compounds with temperature (Korson et al., 1969; Roelands et al., 1963). The significant linear decline in adhesion as temperature increased was not anticipated, and suggests that the relevant factors driving adhesive performance in tropical arboreal ants vary across species in ways we do not yet understand.

Species exhibiting hump-shaped relationships had peak adhesive loads within the range 30–40°C. This temperature range coincides with two relevant temperatures for the canopy ant adhesive system: (1) the T_m of semi-solid components of the tarsal pad (i.e. long chain *n*-alkanes; T_m ca. >35°C; Schmidt et al., 2000; Reitz et al., 2015); and (2) the average median temperature of a canopy tree branch on sunny days (ca. 35°C; Stark et al., 2017). Although maximum branch temperatures can be much higher in full sun (>50°C; Kaspari et al., 2015), the results of this study show that many tropical arboreal ant adhesive systems are tuned to perform optimally under the most commonly encountered surface temperatures in the canopy, and this tuning may be related to the semi-solid components of the tarsal pad secretions.

There is no obvious unifying factor that is common to all species that showed a linear decrease in performance with increasing temperature. For instance, two of these species differ in CT_{max} (*A. colombica* ca. 50°C; *D. bispinosus* ca. 46°C), adhesive performance (i.e. *A. colombica* is about twice as adhesive as *D. bispinosus*), nest site location (i.e. *A. colombica* – ground nesting; *D. bispinosus* – canopy nesting), and behavioral response to high temperatures (i.e. *D. bispinosus* is less likely to return to heated patches than *A. colombica*; Kaspari et al., 2015; Spicer et al., 2017). Similar results in canopy-nesting Asian weaver ants (*O. smaragdina*) were attributed to a reduction in TPS viscosity (Federle et al., 2004). Variation in physiological, ecological, behavioral and physical properties in these species suggest that either: (1) there is no selective pressure to retain high adhesive performance at elevated temperatures; or (2) a series of complex adhesion-related trade-offs result in reduced adhesion at high temperature. Measuring such factors will help clarify interspecific variation in shear adhesion among tropical arboreal ants clinging to hot substrates.

The results of this study show that size-related morphological traits (i.e. mass and tarsal pad area) did not influence shear adhesive performance on hot surfaces. While we noticed behaviors that likely reduced adhesive performance in some species, these behaviors only occurred in species with temperature-dependent adhesion (i.e. $P \leq 0.05$). Although we cannot rule out Type II errors due to low sample size, it is possible that some tropical arboreal species are adapted to adhere well to a broad range of canopy surface temperatures (i.e. the null hypothesis is supported; Stark et al., 2017). In support of this conclusion, two species with low sample sizes had significant negative relationships between temperature and adhesive performance [i.e. *C. brasiliensis* ($N=7$) and *C. basalis* ($N=14$)], suggesting that the lack of a strong temperature dependence in other species with similarly small sample sizes is real (i.e. *C. linmaei*, *P. clavata* and *P. belti*).

Several factors can explain the changes in adhesive performance with temperature observed in this study. First, viscosity may have increased, rather than decreased, with temperature via a change in the chemical composition of the TPS. This is possible given that ants have a variety of chemicals at their disposal (e.g. cuticular hydrocarbons, pheromones; Hölldobler and Wilson, 1990; Geiselhardt et al., 2010a). Second, variation in the volume of TPS during shear sliding could cause adhesion to be maintained over a larger temperature range than expected. For instance, most pheromones evaporate rapidly as temperature increases (Van Oudenhove et al., 2012), and TPS production declines with repeated or sustained use (Dirks and Federle, 2011b). Such a reduction in TPS would increase shear adhesion via an increase in friction between the pad and glass substrate (Drechsler and Federle, 2006), making the viscoelastic properties of the tarsal pad material (i.e. chitin) also relevant (Gorb et al., 2000; Dirks, 2014). Regardless, the results of this study show that arboreal ant shear adhesion is both temperature dependent and species specific.

Tropical arboreal ants have remarkable adhesive capabilities, even on hot surfaces. The results of this study suggest that adhesive performance does not limit the ability of most tropical arboreal ants to exploit their thermally dynamic canopy environment (i.e. shear adhesion is still able to support their body weight across a range in surface temperature). We suggest three key areas for future research on this topic: (1) the physicochemical mechanism of the TPS (i.e. variation in viscosity, amount and chemistry); (2) ecological and behavioral relationships between temperature and adhesion (i.e. activity periods, nesting location, habitat use, applied loads on the adhesive system); and (3) the contribution of morphology and biomechanics to temperature-dependent adhesive performance (i.e. variation in tarsal pad shape, stiffness, load application). This study fills an important gap in our understanding of biological adhesive systems and their function in relevant environmental conditions, and provides a foundation for understanding the potential effects of adhesive performance on ant distribution and behavior in the tropical canopy.

Acknowledgements

We thank B. J. Adams, Evan Gora, Roberta Ethington and Caryn Walker for help with sample collection and processing, and Oris Acevedo, Melissa Cano and the staff of the Smithsonian Tropical Research Institute for logistical support in Panama.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: A.Y.S., S.Y.; Methodology: A.Y.S., K.A., S.Y.; Formal analysis: A.Y.S., S.Y.; Investigation: A.Y.S., K.A., S.Y.; Resources: S.Y.; Data curation: K.A.; Writing - original draft: A.Y.S., K.A., S.Y.; Writing - review & editing: A.Y.S., S.Y.; Visualization: K.A.; Supervision: A.Y.S., S.Y.; Funding acquisition: S.Y.

Funding

This research was supported by National Science Foundation grant DEB-1252614 to S.P.Y.

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.171843.supplemental>

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