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Research Article

A Functional Comparison of Swimming Behavior in Two Temperate Forest Ants (*Camponotus pennsylvanicus* and *Formica subsericea*) (Hymenoptera: Formicidae)

Noah D. Gripshover,[1] Stephen P. Yanoviak,[1,2] and Evan M. Gora[1,3]

[1]Department of Biology, University of Louisville, 139 Life Sciences Building, Louisville, KY, 40292, USA, [2]Smithsonian Tropical Research Institute, Balboa, Republic of Panama, and [3]Corresponding author, e-mail: evan.gora@louisville.edu

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Abstract

Water is a dangerous and often lethal obstacle for small terrestrial animals like insects. However, some ants survive this hazard by efficiently traversing the water surface. Swimming performance (velocity, acceleration, and efficiency) differs dramatically among ant species, yet the factors that affect performance remain unclear. Here, we investigate the relative importance of behavior and morphology to swimming performance using a comparative study of two temperate forest ant species having superficially similar morphology: *Camponotus pennsylvanicus* (De Geer, 1773) (Hymenoptera: Formicidae) and *Formica subsericea* (Say, 1836) (Hymenoptera: Formicidae). We also investigated how water surface tension and ant morphology enable some *F. subsericea* workers to walk across the water surface, whereas others swim partially submerged. Leg ablation experiments demonstrated that both species use their forelegs for propulsion and hind legs as stabilizers, whereas their midlegs effect both propulsion and stabilization during swimming. *C. pennsylvanicus* workers swam faster than *F. subsericea* workers, probably reflecting the larger body size and longer relative foreleg length of *C. pennsylvanicus* workers. *F. subsericea* workers that walked on water were somewhat smaller than swimming conspecifics, and no workers were able to walk on water when surface tension was reduced with ethanol. Collectively, these results and those of related studies suggest that, within a clade of ants, differences in swimming performance arise mainly from subtle morphological differences. The importance of ant ecology and cuticular chemistry in this context remains to be explored.

Key words: aquatic, water walking, performance, ablation, surface tension

Locomotion at the air-water interface presents multiple challenges for taxa that are fundamentally terrestrial (Denny 1993, Vogel 1994). In addition to the risk of predation from both above and below, treading across the water surface, or 'swimming' partly immersed in water, is hazardous for terrestrial animals. Legs adapted for moving on solid substrates often are biomechanically inefficient in fluids, and directed locomotion quickly becomes energetically costly. This is particularly problematic for smaller organisms, like insects, that can be overpowered by the surface tension of water and subsequently drown (Denny 1993, Vogel 1994).

Many riparian insects and spiders have traits that facilitate locomotion in or on the water surface (Andersen 1976, Shultz 1987, Hu et al. 2003, Bush and Hu 2006, Suter 2013). However, various other insects are able to make directed movements across water despite lacking specific morphological adaptations to aid their progress. Key examples include 'swimming' mantids (Miller 1972), grasshoppers and cockroaches (Franklin et al. 1977), and some ants (Adis 1982, DuBois and Jander 1985, Yanoviak and Frederick 2014, Gora et al. 2016). Effective aquatic locomotion in these cases is the result of specific behaviors that biomechanically coopt anatomical features otherwise adapted for locomotion on soil, leaf litter, or vegetation.

Ants in particular are a terrestrial taxon in which efficient swimming behavior is unexpected. Indeed, the best-known examples of aquatic locomotion in ants occur in species directly associated with water, including the mangrove-inhabiting *Polyrhachis sokolova* (Forel, 1902) (Hymenoptera: Formicidae) (Nielsen 1997, Robson 2010) and the *Nepenthes* pitcher plant associate *Camponotus schmitzi* (Stärcke, 1933) (Hymenoptera: Formicidae) (Clarke and Kitching 1995, Merbach et al. 2007, Bohn et al. 2012). Nonetheless, various species of arboreal and ground-dwelling ants in tropical and temperate forests are effective swimmers (Yanoviak and Frederick 2014). These nonobligate swimmers regularly encounter potentially lethal bodies of water (i.e., ground pools), while foraging or after falls from vegetation. Even a small pool on the florest floor poses a significant hazard due to the diminutive stature of these ants (<30 mm).

Some swimming ants modify their normal walking behaviors to effect propulsion and stability while in or on water. Specifically, *C. schmitzi* use their forelegs and midlegs for propulsion, whereas *Camponotus americanus* (Mayr, 1862) (Hymenoptera: Formicidae) use only forelegs for propulsion; both species use their hind legs as rudders (DuBois and Jander 1985, Bohn et al. 2012). By contrast, the Neotropical ants *Neoponera villosa* (Fabricius, 1804), *Neoponera foetida* (Linnaeus, 1758), and *Odontomachus bauri* (Emery, 1892) (Hymenoptera: Formicidae) use forelegs and midlegs for propulsion and hind legs for stability (Yanoviak and Frederick 2014; S. Yanoviak, personal observation). In all of these examples, the ants depend at least partly on water surface tension and hydrophobic cuticular hydrocarbons to prevent sinking (Bush and Hu 2006). However, to the best of our knowledge, the only experimental evaluation of the role of surface tension in this context was conducted for neustonic spiders (i.e., spiders foraging on the surface of water; Suter et al. 1997).

Swimming ability varies substantially among typically terrestrial ant species (Yanoviak and Frederick 2014), but the reasons for this variation are mostly unexplored. As summarized above, leg motions used during swimming differ among ant species (DuBois and Jander 1985, Bohn et al. 2012, Yanoviak and Frederick 2014) and could influence swimming ability. Similarly, ants exhibit substantial interspecific variation in morphology, and certain characteristics, such as leg length or structure, are potentially related to swimming performance. However, the relative importance of behavioral and morphological differences to swimming performance remains unknown. This knowledge gap persists, in part, because previous studies with sufficiently detailed descriptions (e.g., Dubois and Jander 1985) do not compare swimming performance among species. Our preliminary observations of two common temperate forest ants, *Camponotus pennsylvanicus* (De Geer, 1773) (Hymenoptera: Formicidae) and *Formica subsericea* (Say, 1836) (Hymenoptera: Formicidae), suggested that they exhibit a very different swimming performance despite their superficially similar morphologies and swimming motions. We used this apparent difference as the basis for a comparative study to separate the contributions of behavior and morphology to ant locomotor performance in an aquatic setting.

The primary objective of this study was to identify the morphological and behavioral characteristics that underlie differences in the swimming performance of two common ant species. We focused on three questions: 1) Are differences in swimming performance between these species related to morphological differences, particularly among their legs?; 2) Do the functional roles (i.e., behaviors) of the different pairs of legs during swimming differ between the focal species?; and 3) Do the properties of water determine whether a worker ant swims through or walks across water? Given the morphological similarities between the focal species, we expected that differences in swimming performance are caused by differences in the motions of specific leg pairs. We used a simple arena and video recordings in the laboratory to address these questions.

Materials and Methods

Ants used in this project were collected from Iroquois Park and Cherokee Park in Louisville, Kentucky, USA (38.16° N, 85.81° W) between June and August of 2016. Iroquois and Cherokee Parks are urban greenspaces dominated by oak-hickory-maple woodlands. We housed workers and partial nests (soil and woody debris) of *F. subsericea* and *C. pennsylvanicus* in clear plastic containers (15 × 15 × 30 cm) at the University of Louisville. Cotton saturated with honey and water was provided ad libitum. We collected new nest fragments weekly and used two or more colonies of each species per experiment.

Experimental Procedure

All experimental trials were conducted in a clear plastic container (15 × 15 × 30 cm) filled with water to a depth of 1 cm. The exterior sides of this arena were covered with white enamel paint to provide a uniform background. A black vertical stripe (5 × 10 cm) painted at one end of the arena served as an orientation cue for the ants (Gora et al. 2016). A small section of the arena was left unpainted for video recordings of ant profiles during swimming. Two LED lamps (800 lumens each) mounted 35 cm above the water at opposing 45° angles provided relatively uniform illumination. Air and water temperatures during trials were 25 ± 2°C.

For each experimental trial, we dropped a single worker from a 15-mL vial coated internally with Fluon (PTFE-30; BioQuip Products, Inc., Gardena, CA) into the center of the arena from a height of 30 cm. A new worker was used for each trial to maintain statistical independence. Each trial was recorded using the video function of a Canon PowerShot ELPH115 camera (Canon USA, Inc., Melville, NY) recording at 25 frames s^{-1} and mounted 25 cm above the water. We placed a 1- × 1-cm grid under the transparent base of the plastic container to provide a reference for quantification of worker performance (see below). The profiles of swimming ants were recorded using a Casio Exlim HS EX- ZR850 high-speed camera (120 frames s^{-1}; Casio Ltd., Tokyo, Japan).

Video Analysis

We quantified ant swimming performance using NIH ImageJ following protocols established for swimming spiders and ants (Suter et al. 1997, Suter et al. 2003, Yanoviak and Frederick 2014). We converted videos to individual frames and identified a 2-s video segment that exhibited conspicuous, directed swimming for each ant. We used this short video segment to measure swimming performance as the change in location of an ant (as X and Y coordinates) in every third frame. The distance traveled was approximated by calculating the hypotenuse of the change in X and Y coordinates. We also summed the distance traveled every three frames to estimate the cumulative displacement during the 2-s segment. Total displacement (or straight-line distance) was calculated as the linear distance between the position of the ant in the first frame and the last frame of the entire 2-s video segment. Swimming efficiency was calculated as the straight-line distance divided by the cumulative displacement, and velocity equaled the straight-line distance divided by time. We also calculated maximum velocity as the greatest straight-line distance traveled between two frames analyzed in sequence (0.12 s), and maximum acceleration as the greatest change in velocity between adjacent velocity estimates.

Worker Morphometrics

Ants were individually stored in 90% ethanol after each trial. We measured body size and leg lengths of a subset of workers from each species (N = 31 *C. pennsylvanicus* and 44 *F. subsericea* workers). Specifically, we measured body length from the anterior of the clypeus to the apex of the abdomen, the total length of each leg, and the lengths of individual leg segments (femur, tibia, and tarsus). To generate proportional data for a size-independent morphological comparison between ant species, we divided the length of each leg segment by the total length of that leg and divided the total length of each leg by the total body length. Total leg and leg segment lengths were similar between individual legs of the same pair (e.g., left and right hind legs; $t < 2.01$, df = 10, $P > 0.08$). Consequently, we only measured the right legs of each worker. All workers were weighed to the nearest 0.0001 g on an electronic balance.

Leg Ablation

We ablated pairs of legs from workers of both species to determine how individual pairs of legs influence swimming performance. We used different individuals for ablations of forelegs, midlegs, and hind legs (Supplementary Table S1) and each ablation treatment had independent controls (i.e., ants that were handled similarly to treatment ants but without leg ablations). To simplify ablations, we anesthetized workers by placing them in a refrigerator (4°C) for 10 min. We then transferred each worker to a cold stage (i.e., a Petri dish filled with ice) under a dissecting scope for the ablation procedure. Pairs of legs (e.g., right and left forelegs) were removed by firmly pinching the coxa-trochanter articulation with fine-tip forceps. Workers were allowed to acclimate for >30 min following the ablation and before swimming trials. We only tested ants that appeared to behave normally after the acclimation period. All control workers were similarly anesthetized.

'Swimming' Versus 'Walking' Behaviors

All of the *C. pennsylvanicus* workers observed during this study were partially submerged as they swam (see DuBois and Jander 1985). However, workers of *F. subsericea* exhibited two distinct behaviors during preliminary swimming trials: some traversed the water partially submerged (i.e., 'swimming'), whereas others used their legs to fully support their bodies above the water surface (i.e., 'walking'). To determine whether these differences in behavior are associated with intraspecific differences in morphology, we compared body size and leg length between groups of walking and swimming *F. subsericea* using the same methods described above.

We also measured the swimming performance of *F. subsericea* workers after experimentally reducing water surface tension via addition of ethanol in 5% increments (Suter et al. 1997). Specifically, we recorded locomotion behavior (swimming vs walking) and swimming performance in solutions of 5, 10, 15, or 20% ethanol. Tap water (0% ethanol) served as the control. Each worker was observed for >30 min following swimming trials to confirm that its behavior was not conspicuously altered by exposure to ethanol. We replaced the experimental solutions every 20 min. We determined water tension, density, and viscosity of each ethanol dilution using standard values (Lide 2001; Table 1).

Statistical Analysis

Linear models and *t*-tests were performed in the R statistical environment (R Core Team 2016) and multivariate analyses were performed using Primer (version 6). For fixed effects linear models, we tested the significance of individual terms using *F*-tests and removed nonsignificant interaction terms. We tested for normality using Shapiro–Wilk tests and we examined residuals to confirm appropriate model fit, leading to the log transformation of maximum velocity and acceleration. We used *t*-tests to compare swimming performance and leg morphometrics between swimming and walking workers of *F. subsericea*. We compared the effects of ablation on swimming velocity, swimming efficiency, maximum velocity, and maximum acceleration between species with linear models including species, treatment (ablations and control), and the interaction between species and treatment as fixed effects. When the interaction between species and treatment was significant, we compared swimming performance between treatments within each species. We corrected for multiplicity using the Bonferroni adjustment and report α when different from 0.05. We assessed swimming performance among ethanol addition treatments using a fixed effects linear model and post hoc Tukey's HSD tests. Finally, we used a generalized linear model to assess the frequency of water walking and swimming behaviors exhibited by *F. subsericea*. We tested the effect of ethanol concentration on water walking frequency using likelihood ratio tests rather than *F*-tests. For simplicity, we present statistical results as inequalities when multiple tests with identical statistical structure produced the same directional results.

We used principal components analysis (PCA) to compare the proportional and absolute morphometrics between species and between workers of *F. subsericea* with distinct swimming behaviors, respectively. PCA was performed with correlation matrices and we confirmed that axes were informative by comparing them with random configurations (Monte Carlo randomization). Axis loadings > 0.3 were considered to contribute significantly to the variation explained by an axis. We compared groups (species or swimming/walking workers) using PERMANOVA of Euclidean distance (9999 permutations).

Results

Interspecific Comparison of Morphology and Performance

Despite their superficially similar morphologies, swimming performance and overall size differed between workers of *C. pennsylvanicus* and *F. subsericea*. Swimming velocity, maximum velocity, and maximum acceleration of *C. pennsylvanicus* were approximately double that of the smaller *F. subsericea*, but swimming efficiency was similar for both species (Table 2). Workers of *C. pennsylvanicus* were longer and heavier than *F. subsericea*, but the magnitude of these differences was proportionally less than the differences in performance (Table 2). Although body length was strongly correlated with leg morphometrics for both ant species (*C. pennsylvanicus:* $r \geq 0.44$, $P < 0.01$; *F. subsericea*: $r \geq 0.39$, $P \leq 0.014$), it was not a good predictor of swimming performance ($F < 2.32$, df = 1, 70, $P > 0.13$). Similarly, velocity, efficiency, maximum velocity, and maximum acceleration were not correlated with body length ($r < 0.31$, df ≥ 28, $P > 0.10$) or mass for either species ($r < 0.34$, df ≥ 28, $P > 0.06$). Body mass was strongly correlated with leg lengths for *C. pennsylvanicus* workers ($r > 0.77$, df = 41, $P < 0.001$), but not for *F. subsericea* workers ($r < 0.37$, df = 28, $P > 0.02$).

The proportional morphometrics of workers differed between the two focal species (pseudo-F = 20.56, df = 1, 71, $P < 0.001$; Fig. 1). Loadings for PCA axis 1 indicated that midleg tarsi and hind leg tarsi were relatively larger in *F. subsericea* than in *C. pennsylvanicus* (Table 3). By contrast, the total length of forelegs, and the tibia length

Table 1. Physical properties of experimental ethanol solutions and their effects on the swimming behavior and performance of *F. subsericea* workers

Ethanol concentration (%)	Density (Kg m^{-3})	Viscosity (Pa s)	Surface Tension (N m^{-1})	Sample Size (N)	Frequency of walking (%)	Velocity (±SE) (cm s^{-1})	Efficiency (±SE)
0	0.999	1.00	72.88	17	23.5	1.9 (0.12)	0.9 (0.01)
5	0.979	1.46	48.57	19	10.5	1.8 (0.14)	0.9 (0.01)
10	0.963	2.32	37.16	20	0	1.4 (0.09)	0.9 (0.01)
15	0.947	2.47	36.12	10	0	N/A	N/A
20	0.939	2.85	37.97	10	0	N/A	N/A

Table 2. The performance and overall size of worker ants tested during the morphological comparisons along with statistical results

Characteristics	*Camponotus pennsylvanicus*	*Formica subsericea*	*F*
Velocity (cm s^{-1})	5.0 (0.21)	1.7 (0.08)	250.0*
Efficiency	0.9 (0.01)	0.9 (0.01)	3.2
Length (mm)	8.8 (0.14)	6.7 (0.06)	218.6*
Mass (mg)	17.3 (0.90)	8.3 (0.03)	119.2*

df = 1, 71 for all tests.
**P* < 0.001.

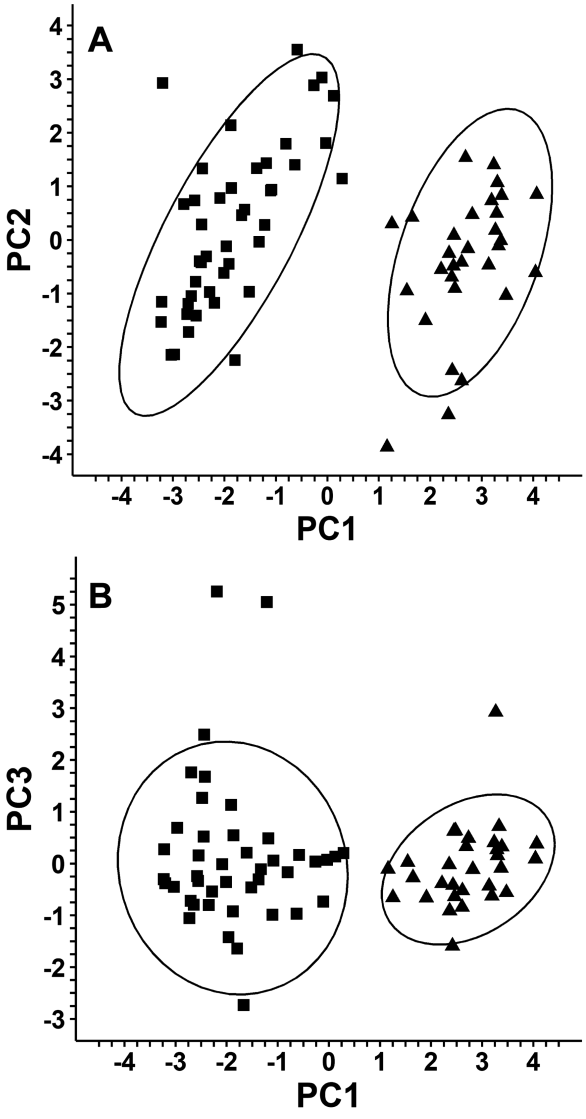

Fig. 1. PCA of the proportional morphometrics of *C. pennsylvanicus* (squares) and *F. subsericea* workers (triangles). Panel A depicts PC axis 1 (48.5% of variation) versus PC axis 2 (17.9%), whereas panel B depicts PC axis 1 versus PC axis 3 (14.3%). The ellipses depict the 95% confidence interval for the location of each group centroid.

of all legs, was larger in *C. pennsylvanicus* than in *F. subsericea*. Workers from both species completely overlapped across PCA axes 2 and 3, indicating that leg measurements associated with these axes do not differ between species (Table 3).

Table 3. Axis loadings for the PCA contrasting the relative morphometrics of *C. pennsylvanicus* and *F. subsericea* workers

Leg or leg segment	PC1	PC2	PC3
Right front femur	0.101	–0.018	–0.639
Right front tibia	0.378	–0.155	0.163
Right front tarsus	–0.131	0.087	–0.619
Right front leg	0.301	0.424	0.080
Right middle femur	0.266	–0.114	0.246
Right middle tibia	0.379	–0.144	–0.158
Right middle tarsus	–0.317	0.152	0.211
Right middle leg	0.223	0.554	–0.048
Right hind femur	0.282	–0.116	0.142
Right hind tibia	0.379	–0.147	–0.113
Right hind tarsus	–0.343	0.197	0.092
Right hind leg	0.186	0.595	0.028

Bold text indicates loading values > 0.3.

Ablation Experiments

Ablations decreased two or more measures of swimming performance in each species. Overall swimming velocity decreased with each leg ablation for both species (*C. pennsylvanicus*: $F = 19.7$, df = 3, 81, $P < 0.001$, $\alpha = 0.025$; *F. subsericea*: $F = 19.6$, df = 3, 79, $P < 0.001$, $\alpha = 0.025$; $t > 4.8$, $P < 0.001$), but the change in velocity associated with each leg pair was inconsistent between species (Fig. 2A; species x ablation interaction: $F = 38.7$, df = 1, 160, $P < 0.001$). Maximum velocity also responded differently between species (Fig. 2B; species x ablation interaction: $F = 5.1$, df = 3, 160, $P = 0.002$). Maximum velocity of *F. subsericea* decreased with midleg and foreleg ablations ($F = 14.1$, df = 3, 79, $P < 0.001$, $\alpha = 0.025$; $t > 4.9$, $P < 0.001$) but not with hind leg ablation ($t = 0.64$, $P = 0.92$), whereas maximum velocity of *C. pennsylvanicus* only decreased in response to midleg ablations ($F = 3.6$, df = 3, 81, $P = 0.016$, $\alpha = 0.025$; $t > 3.0$, $P = 0.019$). By contrast, none of the ablations affected maximum acceleration (Fig. 2C, $F = 0.92$, df = 3, 160, $P = 0.43$).

Swimming efficiency decreased with midleg and hind leg ablations for both species (Fig. 2D; $F = 10.3$, df = 3, 163, $P < 0.001$; $t > 4.6$, $P < 0.001$). However, swimming efficiency of workers with ablated forelegs did not differ from controls ($t = 2.1$, $P = 0.17$) and was greater than those with ablated midlegs ($t = 2.7$, $P = 0.037$). Regardless of treatment, swimming efficiency of *F. subsericea* was less than that of *C. pennsylvanicus* ($F = 8.7$, df = 1, 163, $P = 0.004$). Despite differences in their performance, the behavioral (i.e., biomechanical) process of swimming was similar between the two species (Fig. 3; Supplementary Videos S1 and S2). Specifically, the experimental ablations demonstrate that midlegs were important to both propulsion and stability, whereas forelegs were used exclusively for propulsion and hind legs functioned as stabilizers.

Swimming Versus Walking Workers

Some *F. subsericea* workers walked across the water surface (i.e., with the body elevated above the surface and legs supported by surface tension), whereas others 'swam' (i.e., with the ventral surface of the body in contact with the water surface and legs penetrating the surface film). Despite this conspicuous behavioral difference (walking vs swimming), ants in the two groups did not differ conspicuously in morphology or swimming performance. The average body length and hind leg tarsus length of walking workers were somewhat shorter than those of swimming workers (Table 4). However, no other individual legs or leg parts differed between them ($t < 1.83$, df ≥ 27, $P = 0.08$). Mass also was similar between the groups, and

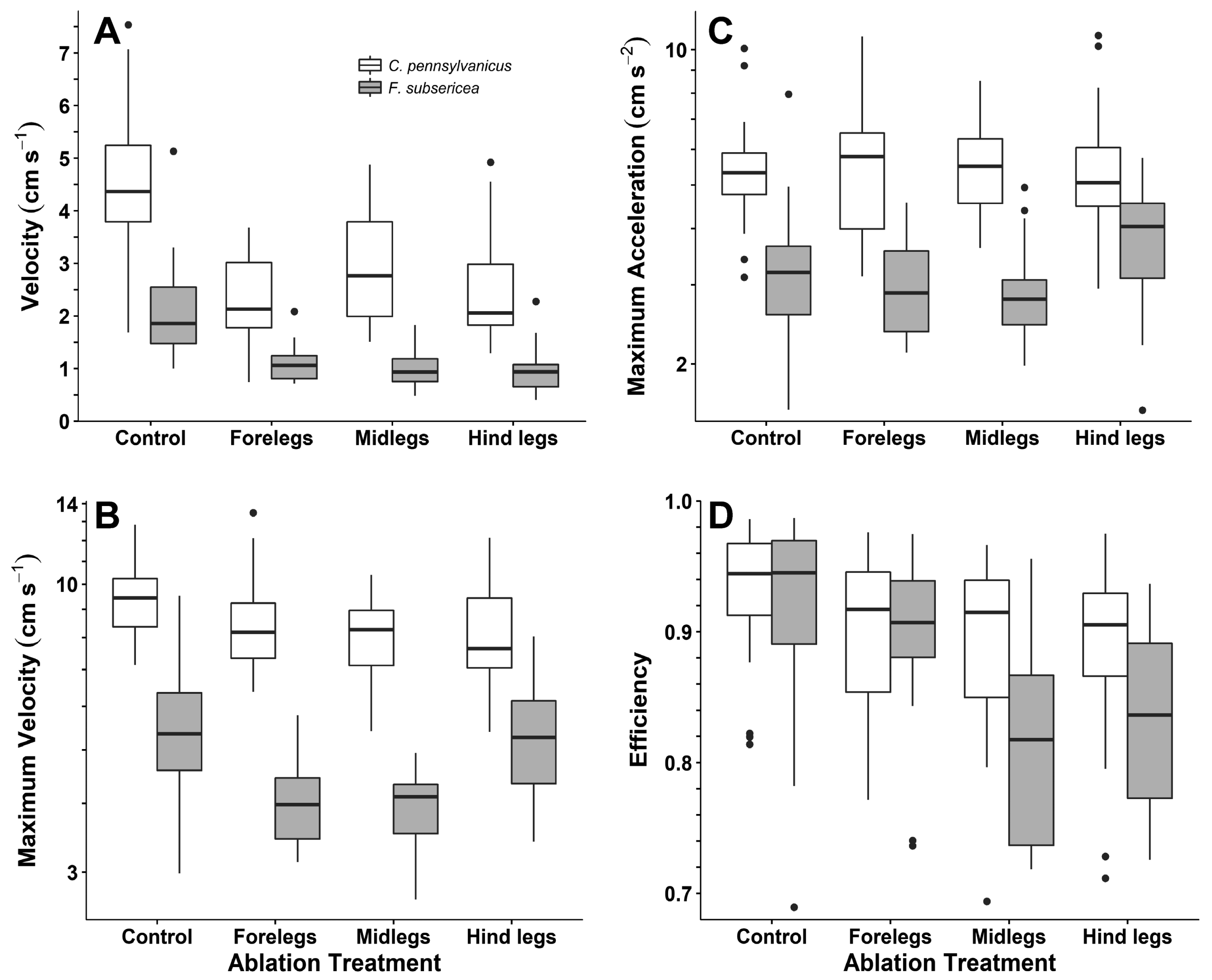

Fig. 2. Boxplots of the effects of foreleg, midleg, and hind leg ablations on overall velocity (A), maximum velocity (B), maximum acceleration (C), and swimming efficiency (D).

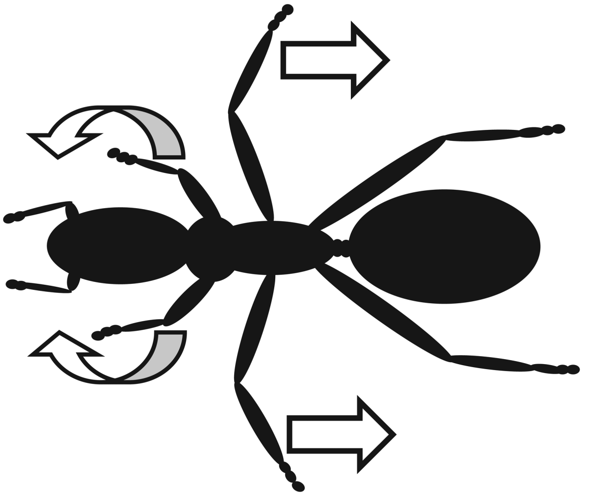

Fig. 3. A diagram of leg motions during swimming for both ant species. Forelegs extended downwards into the water in a propulsive motion, whereas midlegs performed a bilaterally synchronous rowing motion. Hind legs were generally inactive as they appeared to function as rudders.

Table 4. A comparison of performance and size characteristics (±SE) between swimming and walking *F. subsericea* workers

Characteristics	Swimming	Walking	*t*-Statistic
Velocity (cm s^{-1})	1.7 (0.10)	2.0 (0.13)	1.84
Efficiency	0.9 (0.01)	0.9 (0.01)	1.45
Maximum velocity (cm s^{-1})	3.3 (0.17)	4.2 (0.37)	1.13
Maximum acceleration (cm s^{-2})	2.2 (0.12)	2.5 (0.23)	2.24*
Length (mm)	6.8 (0.07)	6.5 (0.09)	2.10*
Mass (mg)	8.0 (0.27)	8.6 (0.47)	1.00
Hindleg tarsus (mm)	1.7 (0.08)	1.4 (0.11)	2.11*

*$P < 0.05$.
df = 44 for all tests.

overall morphometrics were only marginally different between them (Fig. 4; pseudo-$F = 2.66$, df = 1, 52, $P = 0.054$). In terms of swimming performance, walking workers exhibited higher maximum velocity, but walking and swimming individuals had similar overall velocity, maximum acceleration, and efficiency (Table 4).

The frequency of walking behavior declined with increasing ethanol concentration ($X^2 = 5.79$, df = 1, $P = 0.016$; Table 1). A few workers

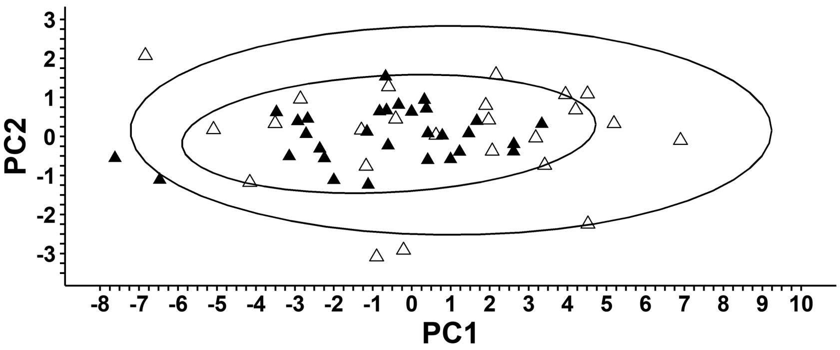

Fig. 4. PCA of the absolute morphometrics of *F. subsericea* workers that exhibit swimming (filled triangles) or walking (hollow triangles) behavior during aquatic locomotion. The ordination depicts PC axis 1 (73% of variation) versus PC axis 2 (7%). The ellipses depict the 95% confidence interval for the location of each group centroid.

fell through the water surface with 10 and 15% ethanol concentrations, and none of the workers was able to stay on the water surface with 20% ethanol concentration ($N = 10$; Table 1). The performance of swimming *F. subsericea* workers also declined with increasing ethanol concentration (Table 1). Swimming velocity in water with 10% ethanol addition was decreased relative to both the 0 and the 5% ethanol solutions ($F = 6.50$, df = 2, 53, $P = 0.003$; $t > 2.58$, $P < 0.034$), but worker performance was similar in 0 and 5% ethanol solutions ($t = 0.93$, $P = 0.63$). Swimming efficiency was reduced in the 10% ethanol solution relative to the 0% control treatment ($F = 3.27$, df = 1, 53, $P = 0.046$; $t = 2.56$, $P = 0.036$), but did not differ between the 5% ethanol treatment and the 0 or 10% treatments ($t < 1.45$, $P > 0.32$; Table 1).

Discussion

Upon falling into water, many terrestrial arthropods avoid death by coopting structures adapted for terrestrial locomotion to traverse across the water surface. Here, we explored how morphology and behavior influenced the swimming performance of two common temperate forest ant species. The results of this study suggest that differences in swimming performance between the focal ant species are largely influenced by their morphology, rather than by differences in leg motions as we predicted. Specifically, one morphological difference that is particularly important to swimming—greater relative foreleg length—resulted in faster swimming by *C. pennsylvanicus* workers (DuBois and Jander 1985, Bohn et al. 2012, Yanoviak and Frederick 2014). Additionally, the greater mass of *C. pennsylvanicus* workers (100% heavier than *F. subsericea*) relative to their length (only 20% longer than *F. subsericea*) could be associated with greater muscle mass and thus faster swimming. Greater musculature presumably increases the propulsive force applied during swimming, thereby increasing velocity and acceleration as observed here. Although both species adopted a distinct gait for locomotion on water (vs solid substrates), the similarities in their swimming gait, specifically the motions of the mid and hindlegs, suggest that their behavioral and biomechanical approaches to aquatic locomotion are similar.

Differences in ecology between these species likely affect their encounters with water. The greater tendency for *C. pennsylvanicus* workers to forage on aboveground vegetation (relative to *F. subsericea* workers) increases the probability that they will inadvertently and uncontrollably encounter a ground pool or larger body of water (i.e., by falling from a branch overhanging a stream or pond). By contrast, workers of ants like *F. subsericea* that forage mainly on the ground are more likely to encounter water under circumstances that allow decision-making (i.e., a change in course, or a voluntary attempt to swim or walk across the surface). Although neither of these species habitually associate with water, their workers exhibit directed swimming behaviors distinct from their normal walking gait and this enables them to overcome an otherwise lethal hazard.

Both species in this study exhibited similar swimming behaviors and shared some elements with the swimming motions described for various Neotropical and Nearctic ant species (DuBois and Jander 1985, Bohn et al. 2012, Yanoviak and Frederick 2014). The propulsive foreleg and rudder-like hind leg motions were particularly similar to *C. americanus* (DuBois and Jander 1985), whereas the midleg motions resembled bilaterally synchronized versions of the *N. villosa* rowing motion (Supplementary Videos S1 and S2, Yanoviak and Frederick 2014). Despite these similarities, the combination of leg motions described for *C. pennsylvanicus* and *F. subsericea* represents a novel swimming behavior. The various swimming motions exhibited by ants suggest that these typically terrestrial taxa employ diverse mechanisms for traversing the water surface.

The dual swimming behaviors exhibited by *F. subsericea* provide insight into the mechanisms that enable organisms to walk on water (Hu et al. 2003, Bush and Hu 2006). Although the addition of ethanol has multiple effects on the characteristics of the fluid substrate, our observations suggest that reductions in surface tension caused the lower frequency of walking behavior. The difference in size between walking and swimming workers also suggests that swimming workers exceed the threshold ratio between worker size and tarsal surface area that breaks the local surface tension of water (Hu et al. 2003). This could explain why *C. pennsylvanicus* workers do not exhibit water walking behavior, as *C. pennsylvanicus* workers are generally larger than the walking *F. subsericea* tested in this study. Apart from overall size, the only structures of *F. subsericea* that were proportionally larger than those of *C. pennsylvanicus* were the midleg tarsi and hind leg tarsi, which coincidentally are the stabilizing leg segments that directly contact the water surface. The hydrophobic properties of body parts that directly interact with the water surface are important to water walking behavior (Suter 2013), and thus, the hydrophobic properties of *F. subsericea* tarsi warrant further exploration.

Ultimately, the results of this study indicate that differences in morphology underlie the differences in swimming performance and behaviors observed in the two focal species. Forelegs and tibiae could enhance swimming performance via multiple mechanisms (overall size, hair density, or hydrophobicity), and thus, further exploration is necessary to identify the specific mechanism of their action. Additionally, the dual nature of *F. subsericea* swimming behaviors presents a model system for studying the traits (e.g., body size, tarsal morphology, and hydrophobicity) that help an organism transition from swimming through water to walking across its surface. Expanding this work to a broader range of taxa would enable comparative tests of these proposed mechanisms and provide insight into the evolutionary history of terrestrial invertebrate swimming.

Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

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