Kinematic study of six mangrove ant species (Hymenoptera: Formicidae) reveals different swimming styles and abilities

Patrick Schultheiss & Benoit Guénard

Abstract

Most insects are morphologically and behaviourally adapted to a terrestrial lifestyle, and many species struggle if they fall onto the water surface. Yet, some terrestrial species exhibit an efficient aquatic locomotion ability that enables them to escape such perilous environments. Here, we perform a comparative study that investigates swimming behaviour in six taxonomically diverse arboreal species of ants from a mangrove habitat and describe the leg kinematics in detail. Across species, we find large differences in the speed and directedness of their swimming locomotion, and correspondingly large differences in swimming styles, that is, leg kinematics and synchronisation patterns. Our results demonstrate that some species do in fact display behavioural adaptations for efficient and directed swimming, and that their locomotion patterns are not analogous to those observed during walking. Ultimately, we suggest that the study of swimming behaviour in ants may provide an interesting system for investigating adaptive locomotion kinematics in contrasting environments.

Key words: Aquatic, behaviour, gait analysis, locomotion, orientation, skototaxis.

Patrick Schultheiss (contact author) & Benoit Guénard, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China. E-mail: ppschultheiss@gmail.com

Introduction

Terrestrial lifestyles have shaped the morphology and behaviour of millions of organisms, among which insects have been the most successful. They are efficient at walking and running, with legs that are adapted for gripping rigid surfaces. However, even terrestrial insects face the risk of falling into water. Lacking secondary adaptations for aquatic environments, for example the paddle-shaped hind legs seen in some aquatic beetles (Taylor & Chapman 2013), they are highly vulnerable to predation or drowning. Small insects are invariably trapped by the surface tension and fail to escape. Larger ones often struggle to propel themselves forward with their slender legs, although some species are able to achieve directed motion on the water surface through coordinated leg movements; examples include mantids (Miller 1972), grasshoppers (Franklin & al. 1977), and cockroaches (Lawson 1965). This behaviour allows the insects to escape from entrapment on the water surface or small islands and may therefore be of adaptive significance.

Perhaps unexpectedly, swimming behaviour has also been reported in several species of ants. *Colobopsis schmitzi* (Stärcke, 1933) ants inhabit the hollow tendrils of *Nepenthes* pitcher plants and readily enter the pitcher fluid to capture invertebrates (Clarke & Kitching 1995). *Polyrhachis sokolova* Forel, 1902 builds ground nests in Australian mangroves that regularly become inundated, and worker ants willingly enter water puddles to swim across (Nielsen 2011). While these two species encounter water on a daily basis during their foraging excursions, many other species of ants come into contact with water only by accident. Among 35 species of tropical canopy ants, 20 exhibited directional swimming behaviour with differing efficiency, and phylogenetic mapping points to parallel evolution of this ability (Yanoviak & Frederick 2014). It is likely that such behaviour has evolved as a means to escape danger as most swimming ants will direct their movements towards dark visual cues (skototaxis) that could signal the safety of dry land (DuBois & Jander 1985, Yanoviak & Frederick 2014, Gora & al. 2016, Gripshover & al. 2018).

Without morphological adaptations for swimming, ants need to achieve propulsion on the water by creating a discrepancy in water resistance during backward and forward leg motion. The legs can be fully extended for backward power strokes (increasing resistance) and drawn closer to the body or lifted out of the water for

Tab. 1: Summary of published kinematic parameters of ants during water surface locomotion. Studies did not always report all parameters. The study of Yanoviak & Frederick (2014) reports absolute swimming speeds of several other species but no further details on kinematics. All values in this table are averages. Speed is expressed as body lengths (BL) per second; gait is described as "tripod" when alternating tripod coordination was evident in at least some leg pairs; directionality is provided as a straightness index (see main text); leg function describes the presumed main function of the first, second, and third leg pairs in sequence, with P = propulsion and S = stabilisation. ^) front legs only; #) study reports absolute speed.

Ant species	Speed (BL/s)	BL (mm)	Gait	Stroke freq. (Hz)	Direc-tionality	Leg function	Reference
Formicinae							
Camponotus americanus		large	tripod	1.32^	high	PSS	(DuBois & Jander 1985)
Camponotus pennsylvanicus	5.7#	~8.8	tripod	-	0.9	PPS	(Gripshover & al. 2018)
Camponotus vitiosus	2.6	~4.4	tripod	2.26	0.87	PPS	this study
Colobopsis nipponica	0.6	~4.4	none	2.58	0.57	PPP	this study
Colobopsis schmitzi	4.5	~5.1	tripod	9.35	high	PPS	(Bohn & al. 2012)
Formica subsericea	2.5#	~6.7	tripod	-	0.9	PPS	(Gripshover & al. 2018)
Myrmicinae							
Crematogaster rogenhoferi	0.2	~4.6	none	1.2	0.24	PPS	this study
Dilobocondyla fouqueti	0.2	~5.9	tripod	2.01	0.48	PSS	this study
Dolichoderinae							
Dolichoderus sibiricus	0.7	~3.9	tripod	1.69	0.61	PPS	this study
Pseudomyrmecinae							
Tetraponera nitida	0.3	~4.4	none	2.09	0.39	PPP	this study
Ponerinae							
Neoponera foetida	7.2#	large	tripod	5.8	high	PPS	(Yanoviak & Frederick 2014)
Neoponera villosa	5.6#	large	tripod	6.4	high	PPS	(Yanoviak & Frederick 2014)
Odontomachus bauri	10.2#	large	tripod	4.8	high	PPS	(Yanoviak & Frederick 2014)

the return stroke (decreasing resistance) (DuBois & Jander 1985, Bohn & al. 2012, Yanoviak & Frederick 2014). The speed of propulsion can be modulated through the length of power strokes, the stride frequency, or the duty factor – that is, the time ratio of backward to forward leg movement. However, swimming ants also need to control the direction they are headed in. Directional stability can be achieved actively through symmetric force generation by the legs on each side of the body. The highest stability is attained by coordinating leg movements, with contralateral legs moving precisely in phase or antiphase and neighbouring ipsilateral legs moving in antiphase. Indeed, leg coordination patterns of swimming ants often retain elements of the terrestrial alternating tripod gait, in which "tripods" of fore and hind legs from one side and the mid leg of the other side move in unison, alternating between sides (Cruse 1990). Directional equilibrium during forward motion is further aided passively by posteriorly elongated body features. Slight changes in yaw angle then lead to increased lateral drag on these body parts, realigning the body parallel to the flow direction like a weathervane or rudder (Dudley 2000). Most swimming ants appear to make use of this effect by stretching their posterior legs (one or two leg pairs) straight backwards, turning them into effective stabilisers.

While only a limited number of studies have so far investigated the swimming behaviour of ants, there appears to be considerable variation in their leg kinematics, as shown in our overview of key parameters in Table 1. *Colobopsis schmitzi* – evidently the only habitually aquatic ant species in this list – swims just below the surface with all legs fully submerged and moving in an alternating tripod pattern. Compared with running, the legs move with lower frequencies and are fully extended during backward strokes; the hind legs move the least and are probably used more for steering than propulsion (Bohn & al. 2012). In contrast, *Camponotus americanus* Mayr, 1862 swims supported by the water surface tension and only thrusts the front legs alternatingly downward into the water, while keeping the remaining legs immobile and stretched towards the back (DuBois & Jander 1985). The swimming motions of *Camponotus pennsylvanicus* (De Geer, 1773) and *Formica subsericea* Say, 1836 are similar but include horizontal rowing motions of the mid legs (Gripshover & al. 2018). In the ponerine ants *Neoponera foetida* (Linnaeus, 1758), *N. villosa* (Fabricius, 1804), and *Odontomachus bauri* Emery, 1892 (and sometimes in *F. subsericea*; see Gripshover & al. 2018), only the ventral mesosoma and the tarsi are in contact with the water surface, and the legs are completely withdrawn from the surface during forward motion; their swimming

style is thus akin to "walking on the water" (Yanoviak & Frederick 2014).

Overall however, the kinematics of swimming ants remain poorly investigated; the list of studied species is short, and most studies limit their measurements to swimming speed, directionality and stroke frequency (Tab. 1). While it is clear that different species have markedly different swimming abilities, it remains unclear what makes some ants better swimmers than others. Here, we perform a comparative study that investigates swimming behaviour in six taxonomically diverse species of ants and quantifies the leg kinematics and associated locomotory performance in detail. We selected arboreal ant species that inhabit tidally inundated mangroves as they occasionally fall into the water by accident, where they are exposed to water currents, salinity, and predation. Escape is only possible if they are able to reach dry surfaces (most likely tree trunks) and natural selection pressure may have favoured directional swimming ability for escape, though these species are not mangrove specialists. We aim to explore the flexibility of locomotory behaviours at the water surface and investigate behavioural adaptations for directed swimming abilities in ants. We focus on the following questions: 1) Do different species of ants from the same habitat show different locomotion kinematics when swimming? 2) Are locomotion kinematics distinct between swimming and walking? 3) Are differences in swimming kinematics related to swimming ability?

Material and methods

Colonies of arboreal ants were collected from mangrove trees in the intertidal zone of Hong Kong and kept in the laboratory at a temperature of 25 °C for a maximum of three weeks with access to food and water. Six species from six different genera and four subfamilies were collected: *Camponotus vitiosus* Smith, F., 1874 (Formicinae), *Colobopsis nipponica* (Wheeler, W.M., 1928) (Formicinae), *Dilobocondyla fouqueti* Santschi, 1910 (Myrmicinae), *Crematogaster rogenhoferi* Mayr, 1879 (Myrmicinae), *Dolichoderus sibiricus* Emery, 1889 (Dolichoderinae) and *Tetraponera nitida* (Smith, F., 1860) (Pseudomyrmecinae). After being mounted, specimens were identified by one of the authors (BG) using existing literature and the reference collection of ants at the University of Hong Kong. The swimming behaviour of ants was recorded from two or more colonies per species (except *T. nitida*, from which only one colony was collected). As the forces generated during propulsion at the water surface are scale-dependent, it was ensured that only species of a comparable size range were recorded. All workers of the species studied here (majors excluded) were of a similar body size (~3.9 - 4.6 mm in length), with *Dilobocondyla fouqueti* being slightly larger (~5.9 mm in length). To account for the remaining size variation, all measures involving distance were standardised to body size as is common practice in studies on locomotion kinematics (Zollikofer 1994, Bohn & al. 2012).

The swimming ability of workers (majors excluded) from all six species was quantified in a glass aquarium (60 × 40 × 39 cm) filled with 2 cm of clean seawater. The ants had no prior experience of the setup. All four walls were covered in white paper, with a single vertical black bar (6 cm wide) presented on one short wall acting as a visual aid for orientation. The side of this visual cue was balanced between the two short walls of the aquarium. Each ant was gently placed onto the water surface at the centre of the aquarium and filmed from above at 40 fps (iPhone 5S, Apple, Cupertino, USA) for up to three minutes or until it reached within 1 cm of an aquarium wall. Ants that managed to break free from the water surface and enter the water column were removed from further analysis (1 *Dolichoderus sibiricus* and 2 *Crematogaster rogenhoferi* workers). The videos were converted to image sequences (OpenShot software, version 2.5.1, <https://www.openshot.org>), and the ant path trajectories were digitised at 4 fps (Fiji ImageJ software, version Madison; Schindelin & al. 2012) and smoothed using a three-point moving average. Directional heading was measured when the ant first reached a distance of 5 cm from the release point (ants that did not achieve a displacement of 5 cm were removed from this analysis), speed was calculated as total path length over time, and a straightness index was calculated by dividing the total displacement by total path length.

Additional videos at close range and high frame rate were recorded for analysing the leg kinematics. The camera (iPhone 6S) was placed so that it covered a visual field of about 6 × 10 cm and filmed from above at 240 fps. A formal biomechanical analysis of the drag and thrust forces involved was not attempted as all six species intermittently positioned their legs at the water-air boundary, in the water column, or on occasion even raised them above the water surface; in such a complex physical environment, any quantification of mechanical forces would remain imprecise and unreliable. Sequences from all six species were recorded while swimming and, for comparison, while walking on a white paper surface, with different individuals being used in all conditions. One sequence per individual (n = 5 per species) was selected in which the ant moved straight ahead without turning for three full strides of the right front leg. In walking ants, a stride is defined as lasting from the touchdown of a tarsus on the substrate to the subsequent touchdown of that tarsus, and thus encompasses one full backward and forward stroke of a leg. During the backward stroke (also called power stroke), the tarsus is in contact with the substrate, and during the return stroke it is lifted up as the leg moves forward. As there is no substrate contact in swimming ants, the start of the power stroke was used to define the beginning and endpoint of a stride instead. As preliminary investigations showed that power strokes are often interrupted by short periods of no motion, the beginning of a new power stroke had to be preceded by a return stroke, and not by lack of motion alone. These three-stride sequences were used to visualise gait patterns and calculate kinematic parameters
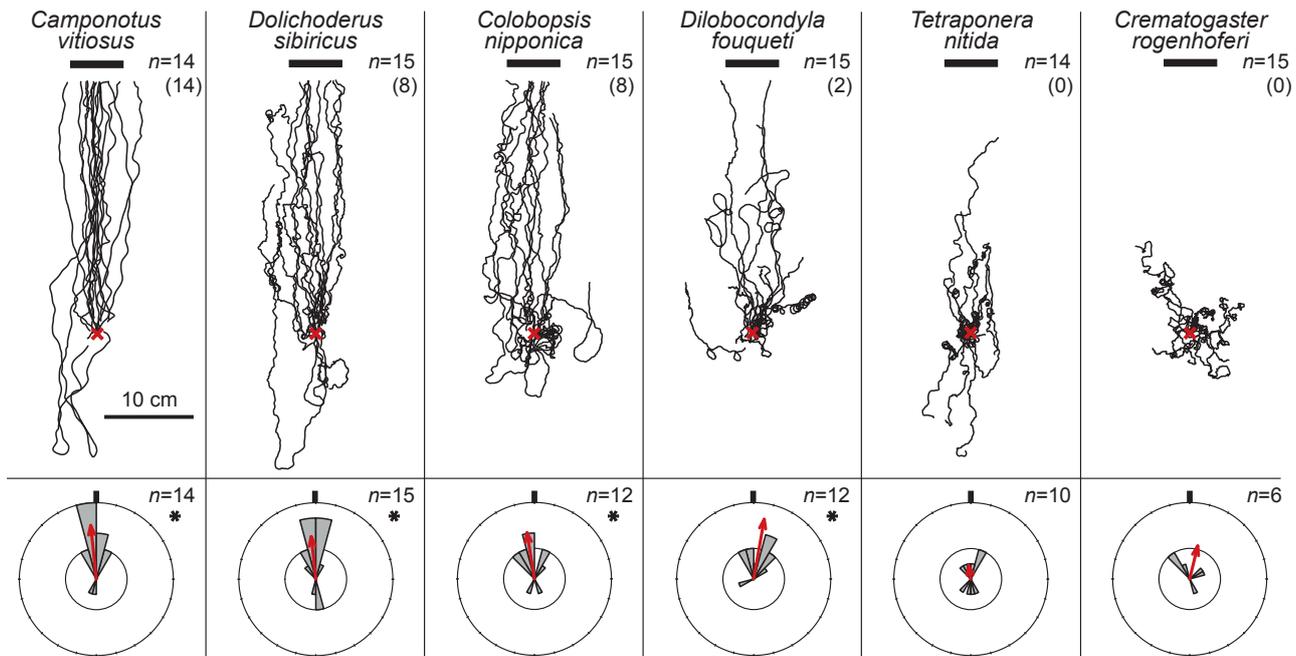
Fig. 1: Swimming behaviour of six arboreal ant species. **Top:** Movement trajectories of ants at the water surface. The red cross marks the release point at the centre of the aquarium, and the black bar at the top shows the location of the visual cue. The position of the visual cue was balanced between the two short sides of the aquarium, but all paths are aligned here with the cue at the top for easier comparison. The six species display large differences in their ability for directed movement. Ants with directed movements show a strong attraction to the visual cue. Sample size is shown near the top of each panel, with the number of ants that reached the cue given in brackets. **Bottom:** Circular histograms of directional heading at a distance of 5 cm from the release point. Note that not all ants reached the required distance (compare n-values between top and bottom panels); the proportion of unsuccessful ants increases from left to right in this figure. Black rectangles mark the direction of the visual cue, red arrows show the mean vectors, and asterisks show significant orientation (Rayleigh and V-tests for cue direction. Test results from left to right: Rayleigh: $p < 0.001$, $p < 0.01$, $p < 0.01$, $p < 0.001$, $p = 0.73$, $p = 0.32$; V-tests: $p < 0.001$; $p < 0.001$; $p < 0.01$; $p < 0.001$). The order of the species here and elsewhere reflects their decreasing swimming ability from left to right, based on path metrics.

of swimming and walking ants. The positions of the anterior tip of the pronotum were digitised at 240 fps (Fiji ImageJ software, version Madison; Schindelin & al. 2012), as were the posterior tip of the propodeum and the tarsal tips of all six legs. From these, stride frequency was calculated as the average number of stride initiations per leg and second, duty factor as the time ratio of backward to forward / no motion, and stroke length as the linear distance between the anterior and posterior points of power strokes (relative to the ant's pronotum). Two representative three-stride sequences (one for swimming, one for walking locomotion) were chosen per species to visualise in detail the trajectories of leg movements relative to the mesosoma. For all recorded ants, mesosoma length (Weber's length, WL) was measured as a proxy for body size (Weber 1938). As the ant mesosoma is a rigid structure, this measure is preferred over total body length, which can change with body posture and levels of physogastry and includes softer tissue.

Analysis: Statistical analyses were conducted in R v3.6.0 (R Core Development Team 2019). Directional headings of ants were analysed using circular statistics (Batschelet 1981). Rayleigh tests were used to test for non-uniformity, and V-tests to inspect if ants were headed

in the direction of the visual cue. All comparisons of kinematic parameters between species were performed with generalised linear mixed modelling (GLMM) in the package MASS (Venables & Ripley 2002). The function '*glmmPQL*' with a Gaussian or quasi-Poisson distribution for non-normal data was used, to fit mixed-effect models for fixed (= species) and random effects (= individual identity). Within species, the same function structure as above was used to compare between swimming and walking modalities (= fixed effect). Alpha was set at 0.05 for all statistical tests. For ease of visual comparison, in all figures the ant species were sorted in order of descending swimming ability, based on the calculated straightness indices of trajectories.

Results

When placed on the water, the floating bodies (head, mesosoma, and gaster) of all ants remained dorsally dewetted and in ventral contact with the water surface at all times (*Crematogaster rogenhoferi* workers sometimes lifted the gaster above the surface, as they do when walking). Swimming ants used their legs for generating forward propulsion and kept them at the surface or immersed in the water most of the time, only occasionally lifting them
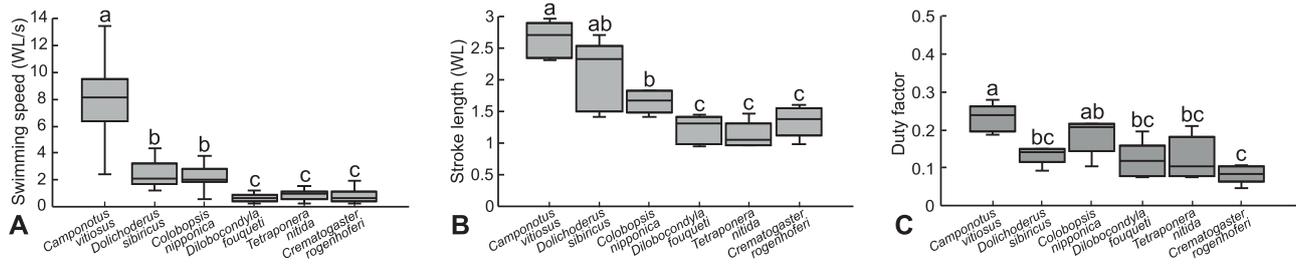
Fig. 2: Kinematic parameters of swimming ants. Distance measures are normalised to mesosoma length (Weber's length, WL), as body size varies between species and individuals. **(A)** Swimming speed of the ants in Figure 1. *Camponotus vitiosus* is by far the fastest swimmer, *Dolichoderus sibiricus* and *Colobopsis nipponica* show intermediate speeds, and the remaining three species are very slow swimmers (overall effect: Wald χ^2 = 543.06, p < 0.001; degrees of freedom 5,88). **(B)** Inter-species differences in power stroke length match the pattern of swimming speed seen in panel (A) (overall effect: Wald χ^2 = 112.77, p < 0.001; df 5,30). **(C)** Duty factor is slightly higher in *Camponotus vitiosus* and *Colobopsis nipponica* than in the other species (overall effect: Wald χ^2 = 55.69, p < 0.001; df 5,30). Panels (B) and (C) are based on the movements of all six legs over three full strides (five individuals per species). Full statistical results are compiled in Table S1.

above the water surface during the return stroke (more frequently in *Colobopsis nipponica* workers). There were pronounced differences in swimming ability between the six species of ants. *Camponotus vitiosus* workers swam in a very directed manner, as did the majority of *Dolichoderus sibiricus* and *Colobopsis nipponica* workers. Only a few individuals of *Dilobocondyla fouqueti* and *Tetraponera nitida* showed directionality, and the trajectories of *Crematogaster rogenhoferi* resembled random diffusion (Fig. 1, top). Directional clustering was evident in the trajectories of *Camponotus vitiosus*, *Dolichoderus sibiricus*, *Colobopsis nipponica*, and *Dilobocondyla fouqueti*, but not in *T. nitida* or *Crematogaster rogenhoferi* (Fig. 1, bottom). The four directionally oriented species were all oriented towards the dark visual cue on the aquarium wall, revealing an innate attraction (skototaxis).

The six species swam at different speeds, with *Camponotus vitiosus* swimming the fastest, *Dolichoderus sibiricus* and *Colobopsis nipponica* at intermediate speeds, and the remaining three species moving very slowly (Fig. 2A, Tab. S1 in the Appendix, as digital supplementary material to this article, at the journal's web pages). A similar pattern was apparent in the straightness indices of paths (Fig. S1, Tab. S1). In addition, the length of the backward stroke differed significantly between species. *Camponotus vitiosus* displayed the longest strokes, followed by *Dolichoderus sibiricus* and *Colobopsis nipponica* (Fig. 2b, Tab. S1). Duty factors were generally quite low in swimming ants (well below 0.5) and species-specific; they were highest in *Camponotus vitiosus* and *Colobopsis nipponica* (Fig. 2C, Tab. S1). All species except *Crematogaster rogenhoferi* swam with similar stride frequencies (Tab. S1).

Figure 3 (upper panels) provides overviews of spatial and temporal leg kinematics during swimming and shows that the six species used their legs very differently for forward propulsion. *Camponotus vitiosus* and *Dolichoderus sibiricus* swung their fully extended front and mid leg pairs backwards in wide, sweeping motions; the sweep

of the front legs often ended in a fast, downward motion into the water column. On the return stroke, these legs were drawn closer to the body. Their motions had a high degree of coordination, with left and right legs moving either synchronously or alternately, and front and mid legs of the same side moving in antiphase. However, leg synchrony patterns were not sufficiently stereotypical for a numerical phase analysis. The hind legs were outstretched towards the back and kept almost motionless. *Colobopsis nipponica* showed no such coordination; they moved all six legs rather erratically and only occasionally swung them backwards in wide sweeps. *Dilobocondyla fouqueti* moved their front legs in short strokes (often directed downwards), made little use of their mid legs, and kept the hind legs motionless at the back. *Tetraponera nitida* used all six legs for propulsion, with little rhythmicity or temporal coordination. The leg movements of *Crematogaster rogenhoferi* showed almost no discernible pattern, except that the hind legs were kept immobile; no distinct strides could be discerned for the remaining legs.

The swimming speed of ants is much slower than their walking speed (4-fold to 30-fold; Figs. 2A, S2A and Tab. S3). The leg kinematics are also very different between swimming and walking modalities, which is most noticeable in the placement and timing of leg movements (Fig. 3). All species walked at very similar speeds (Tab. S2), using a highly regular and stereotypical tripod gait, in which the tripod of legs L1 / R2 / L3 is moved simultaneously and alternately with the tripod R1 / L2 / R3 while all tarsi are kept in a narrow lateral range at the side of the body because lateral leg movements are not possible during the power stroke of walking (Fig. 3). Swimming gaits are distinctly altered in all species as not all legs are used for propulsion and the hind legs tend to be used for stabilisation instead (Figs. 3 and S3). Leg coordination patterns vary considerably between species and are highly flexible (Fig. S4). Similarities to the walking tripod coordination pattern are noticeable in the front two leg pairs of
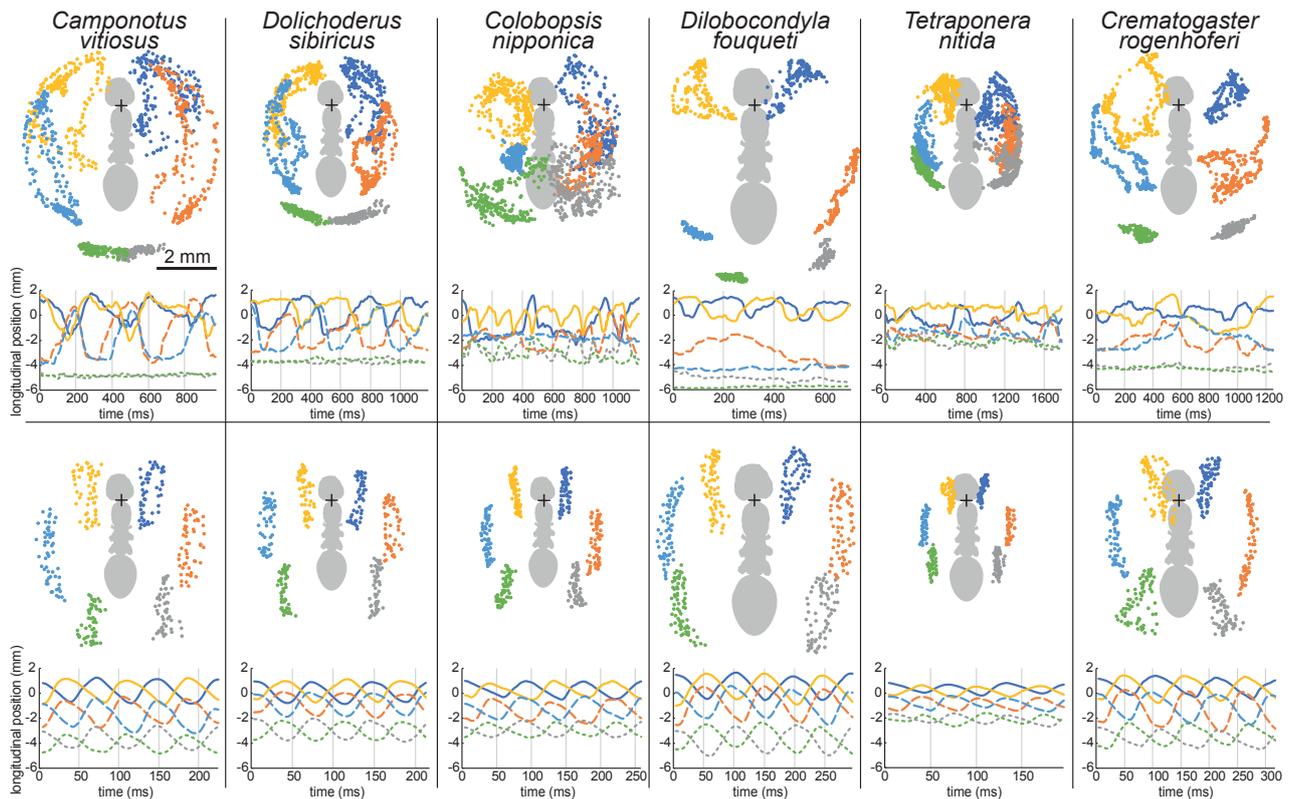
Fig. 3: Foot placement patterns in top view of swimming **(top)** and walking **(bottom)** ants over three complete strides of the right front leg. The dots show the positions of all six tarsi in every video frame (240 fps) in reference to the tip of the pronotum (small cross). Dots are coloured according to leg identity, and ant body outlines are shown for body size reference only and do not reflect actual body shape. Swimming ants show large variation, with some species displaying wide, sweeping motions. All walking ants place their tarsi in narrow patches at the side of the body. Below each foot placement plot, a graph shows the oscillations of all six legs along the longitudinal body axis over time (note differences in scale on the time axis). When swimming, only some ants show consistent inter-leg coordination, while all species walk with a highly coordinated tripod gait.

Camponotus vitiosus and *Dolichoderus sibiricus* and the front legs of *Dilobocondyla fouqueti* (Fig. 3). Swimming locomotion also involves a significant reduction of duty factors (Figs. 2C, S2C and Tab. S3) and stride frequencies (Fig. S3 and Tab. S3). Stroke length differs overall between swimming and walking (Figs. 2B, S2B and Tab. S3), but the differences are species-specific: *Camponotus vitiosus*, *Dolichoderus sibiricus*, and *Colobopsis nipponica* use longer strokes while swimming, but no differences are seen in *Dilobocondyla fouqueti*, *Tetraponera nitida*, and *Crematogaster rogenhoferi*.

Discussion

Ants are typically terrestrial insects under natural circumstances and do not encounter open water very often. Thus, one should not expect workers to have evolved an ability for directed swimming. Our results, however, confirm that some species do in fact display behavioural adaptations for efficient and directed swimming, and that their locomotion patterns differ significantly from those observed during walking. Even though all six species live in the same habitat, their leg kinematics during swimming varied greatly, as did the resulting trajectories in terms of straightness, orientation, and speed. *Camponotus vitiosus*

was the strongest swimmer, while *Dolichoderus sibiricus* and *Colobopsis nipponica* swam at slower speeds but showed a strong ability to direct their movements. The remaining three species swam at so slow speeds that under natural conditions their propulsive abilities would be immediately counteracted by even a slight wind or water current. We do not consider these to have any meaningful swimming ability. Skototaxis toward the black visual cue on the aquarium wall was strong among all ants with swimming ability (Fig. 1). This behavioural response is also seen in other swimming ants (Yanoviak & Frederick 2014, Gora & al. 2016) and indeed in falling canopy ants when using directed aerial descent to reach the closest tree trunk (Yanoviak & al. 2005). For mangrove ants that fall into the water, skototaxis will lead them to close-by tree trunks (which will appear as dark, vertical objects) and the safety of dry land. Familiarity with the visual surroundings might further aid in identifying the correct tree trunk to approach, and the intertidal ant *Polyrhachis sokolova* is indeed known to form visual memories for navigation (Narendra & al. 2013), as are many other walking and flying insects (Schultheiss & al. 2020). So far, however, it is not known whether such navigational mechanisms could be used while swimming.

Among our six species, the swimming performance of *Camponotus vitiosus* was in fact quite remarkable, with highly directed paths and speeds averaging a quarter of walking speed. Several other species of *Camponotus* – as well as other members of the Camponotini tribe – from different habitats and biogeographic regions are now known to be capable swimmers: *Camponotus americanus* (see DuBois & Jander 1985), *Camponotus pennsylvanicus* (see Gripshover & al. 2018), *Camponotus senex* (Smith, F., 1858) (Yanoviak & Frederick 2014), *Camponotus sericeiventris* (Guérin-Méneville, 1838) (Yanoviak & Frederick 2014), *Colobopsis schmitzi* (see Bohn & al. 2012), *Polyrhachis sokolova* (see Robson 2009, Nielsen 2011), and *Colobopsis nipponica* (this study). It is conceivable that their shared evolutionary history makes them particularly well-disposed for efficient swimming behaviours. Both morphological (Gripshover & al. 2018) and behavioural traits (this study) seem to be advantageous in this regard.

Our analysis of leg kinematics has highlighted which behavioural traits convey such swimming ability in ants. All species studied here, with the exception of *Crematogaster rogenhoferi*, extended the front legs during power strokes and drew them closer to the body for return strokes, producing a differential in water resistance and resulting in forward propulsion (to varying degrees). This pattern was most pronounced and consistent in *Camponotus vitiosus* and *Dolichoderus sibiricus*, where it is also clearly seen in the rowing motions of the mid legs (Fig. 3, top). *Colobopsis nipponica* additionally raised the mid and hind legs above the water frequently for reduced resistance during the return stroke. Our analysis further showed that ants with swimming ability do indeed employ longer strokes than poor swimmers and to a lesser degree have higher duty factors. Similar measurements have only been published for very few other ant species: *Colobopsis schmitzi*, which habitually enters and swims in the fluids of tropical pitcher plants (Bohn & al. 2012), and the tropical canopy ants *Odontomachus bauri*, *Neoponera foetida*, and *Neoponera villosa* (see Yanoviak & Frederick 2014). *Colobopsis schmitzi* is roughly the same size as the species considered here, while the other three are much larger; all can be considered strong swimmers. While no numerical comparisons can be made because of methodological differences, *Colobopsis schmitzi* swims with shorter strokes but higher duty factors and stride frequencies than our best swimmer, *Camponotus vitiosus*. *Odontomachus bauri*, *N. foetida*, and *N. villosa* all swim with similar stride frequencies to *Camponotus vitiosus* (Tab. 1).

Directional stability was achieved in two ways – the coordination of leg movements and the use of hind legs as stabilising rudders. *Camponotus vitiosus* (good swimmers) showed very strong coordination in the movements of their front and mid leg pairs, while always using their hind legs as rudders (Fig. 3). The same coordination pattern is obvious in one slower swimmer (*Dolichoderus sibiricus*) but not in the other (*Colobopsis nipponica*). Both do not appear able to sustain a stable and consistent heading for

long durations but need to re-orient repeatedly, as shown by the small-scale meander in their swimming paths (Fig. 1). The tendency to use the hind legs for directional stabilisation was seen in all our six species (Fig. S3) and may be a general feature in ants swimming on the water surface as it is seen in all species studied so far (DuBois & Jander 1985, Robson 2009, Bohn & al. 2012, Yanoviak & Frederick 2014, Gripshover & al. 2018; Tab. 1).

Leg kinematics of swimming ants are different from walking movements in several ways, and different species do not always modulate the same parameters or do not modulate them to the same degree (Figs. 2 and S2). Walking ants move their legs in a highly regular tripod gait (Fig. 1, bottom), and previous studies have suggested that swimming ants use a modification of this pattern. Swimming *Colobopsis schmitzi* ants indeed show a clear tripod gait (Bohn & al. 2012), while other species display alternating coordination patterns in at least some leg pairs (DuBois & Jander 1985, Yanoviak & Frederick 2014, Gripshover & al. 2018). The present study shows that swimming *Camponotus vitiosus* and *Dolichoderus sibiricus* use gaits similar to *Camponotus pennsylvanicus* and *Formica subsericea* (with mid legs used in synchrony or in alternation) and that *Dilobocondyla fouqueti* has a gait similar to *Camponotus americanus* (with mid legs mostly immobile). All these gait patterns still display some elements of the walking tripod gait – from which they are presumably derived – as contralateral legs are mostly moved in alternation, although some leg pairs acquire a new motor pattern as they are moved in contralateral synchrony or kept entirely immobile for stabilisation. However, we also found that three ant species show poorly coordinated leg movements on the water surface. Of these, *Tetraponera nitida* and *Crematogaster rogenhoferi* are indeed poor swimmers, yet *Colobopsis nipponica* ants are able to propel themselves forward at moderate speeds and with strong directedness. Hence, our study highlights that the swimming styles of ants on the water surface are more diverse than previously recognised.

The leg kinematics of swimming ants are influenced both by active movements on the part of the ants and passive responses to forces at the water surface. However, as all ants were exposed to similar conditions and were of relatively similar size, the observed variation in motor patterns can reasonably be assigned to differences in the active control of movement. The considerable inter- and intra-individual flexibility make it seem unlikely that neural central pattern generators are strongly involved, or at least not to the same degree in all species. Rather, the movements of individual legs might rely more heavily upon sensory feedback at the proprioceptive level and on intersegmental neural connections through which the motion of one leg influences that of its neighbours (Taylor & Chapman 2013). There is evidence that this kind of motion control is in effect during walking as well (Cruse 1990, Cruse & al. 2007). In contrast to walking on firm substrates, however, a swimming ant's body weight is fully supported by the surface tension of the water,

eliminating the need to acquire a physically stable posture and allowing the legs to move more freely. Similar conditions appear to be met when ants slowly drag large food items backwards, resulting in highly irregular stepping patterns (Pfeffer & al. 2016). In summary, we show that locomotion behaviour in ants is surprisingly flexible, and we suggest that aquatic locomotion in ants may provide an interesting study system for investigating adaptive locomotion kinematics in contrasting environments.

Acknowledgements

References

Batschelet, E. 1981: Circular statistics in biology. – Academic Press, London, UK, 371 pp.

Bohn, H.F., Thornham, D.G. & Federle, W. 2012: Ants swimming in pitcher plants: kinematics of aquatic and terrestrial locomotion in *Camponotus schmitzi*. – Journal of Comparative Physiology A 198: 465-476.

Clarke, C.M. & Kitching, R.L. 1995: Swimming ants and pitcher plants: a unique ant-plant interaction from Borneo. – Journal of Tropical Ecology 11: 589-602.

Cruse, H. 1990: What mechanisms coordinate leg movement in walking arthropods? – Trends in Neurosciences 13: 15-21.

Cruse, H., Dürr, V. & Schmitz, J. 2007: Insect walking is based on a decentralized architecture revealing a simple and robust controller. – Philosophical Transactions of the Royal Society A-Mathematical, Physical and Engineering Sciences 365: 221-250.

DuBois, M.B. & Jander, R. 1985: Leg coordination and swimming in an ant, *Camponotus americanus*. – Physiological Entomology 10: 267-270.

Dudley, R. 2000: The biomechanics of insect flight. – Princeton University Press, Princeton, NJ, 496 pp.

Franklin, R., Jander, R. & Ele, K. 1977: The coordination, mechanics and evolution of swimming by a grasshopper, *Melanoplus differentialis* (Orthoptera: Acrididae). – Journal of the Kansas Entomological Society 50: 189-199.

Gora, E.M., Gripshover, N. & Yanoviak, S.P. 2016: Orientation at the water surface by the carpenter ant *Camponotus pennsylvanicus* (De Geer, 1773) (Hymenoptera: Formicidae). – Myrmecological News 23: 33-39.

Gripshover, N.D., Yanoviak, S.P. & Gora, E.M. 2018: A functional comparison of swimming behavior in two temperate forest ants (*Camponotus pennsylvanicus* and *Formica subsericea*) (Hymenoptera: Formicidae). – Annals of the Entomological Society of America 111: 319-325.

Lawson, J.W.H. 1965: The behaviour of *Periplaneta americana* in a critical situation and the variation with age. – Behaviour 24: 210-228.

Miller, P.L. 1972: Swimming in mantids. – Journal of Entomology Series A, General Entomology 46: 91-97.

Narendra, A., Raderschall, C. & Robson, S. 2013: Homing abilities of the Australian intertidal ant, *Polyrhachis sokolova*. – Journal of Experimental Biology 216: 3674-3681.

Nielsen, M.G. 2011: Ants (Hymenoptera: Formicidae) of mangrove and other regularly inundated habitats: life in physiological extreme. – Myrmecological News 14: 113-121.

Pfeffer, S.E., Wahl, V.L. & Wittlinger, M. 2016: How to find home backwards? Locomotion and inter-leg coordination during rearward walking of *Cataglyphis fortis* desert ants. – Journal of Experimental Biology 219: 2110-2118.

R Core Development Team 2019: R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Version 3.6.0. – <https://www.r-project.org/>, retrieved on 30 April 2019.

Robson, S. 2009 [2010]: Ants in the intertidal zone: colony and behavioural adaptations for survival. In: Lach, L., Parr, C.L. & Abbott, K.L. (Eds.): Ant ecology. – Oxford University Press, New York, NY, pp. 185-186.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P. & Cardona, A. 2012: Fiji: an open-source platform for biological-image analysis. – Nature Methods 9: 676-682.

Schultheiss, P., Wystrach, A., Lihoreau, M. & Schwarz, S. 2020: Insect navigation. In: Vonk, J. & Shackelford, T. (Eds.): Encyclopedia of animal cognition and behavior. – Springer, Cham; doi: 10.1007/978-3-319-47829-6_1120-1.

Taylor, G. & Chapman, R. 2013: Legs and locomotion. In: Chapman, R., Simpson, S. & Douglas, A. (Eds.): The insects. – Cambridge University Press, Cambridge, UK, pp. 157-192.

Venables, W.N. & Ripley, B.D. 2002: Modern applied statistics with S. 4th edition. – Springer, New York, NY, 498 pp.

Weber, N.A. 1938: The biology of the fungus-growing ants. Part 4. Additional new forms. Part 5. The Attini of Bolivia. – Revista de Entomologia 9: 154-206.

Yanoviak, S.P., Dudley, R. & Kaspari, M. 2005: Directed aerial descent in canopy ants. – Nature 433: 624-626.

Yanoviak, S.P. & Frederick, D.N. 2014: Water surface locomotion in tropical canopy ants. – Journal of Experimental Biology 217: 2163-2170.

Zollikofer, C. 1994: Stepping patterns in ants. II. Influence of body morphology. – Journal of Experimental Biology 192: 107-118.