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RESEARCH ARTICLE

How to find home backwards? Locomotion and inter-leg coordination during rearward walking of *Cataglyphis fortis* desert ants

Sarah E. Pfeffer*, Verena L. Wahl and Matthias Wittlinger

ABSTRACT

For insects, flexibility in the performance of terrestrial locomotion is a vital part of facing the challenges of their often unpredictable environment. Arthropods such as scorpions and crustaceans can switch readily from forward to backward locomotion, but in insects this behaviour seems to be less common and, therefore, is only poorly understood. Here we present an example of spontaneous and persistent backward walking in *Cataglyphis* desert ants that allows us to investigate rearward locomotion within a natural context. When ants find a food item that is too large to be lifted up and to be carried in a normal forward-faced orientation, they will drag the load walking backwards to their home nest. A detailed examination of this behaviour reveals a surprising flexibility of the locomotor output. Compared with forward walks with regular tripod coordination, no main coordination pattern can be assigned to rearward walks. However, we often observed leg-pair-specific stepping patterns. The front legs frequently step with small stride lengths, while the middle and the hind legs are characterized by less numerous but larger strides. But still, these specializations show no rigidly fixed leg coupling, nor are they strictly embedded within a temporal context; therefore, they do not result in a repetitive coordination pattern. The individual legs act as separate units, most likely to better maintain stability during backward dragging.

KEY WORDS: Locomotion, Backward walking, *Cataglyphis* desert ant, Flexible motor control, Inter-leg coordination

INTRODUCTION

Insects, which are among the most successful groups in the animal kingdom, are mostly all capable of terrestrial locomotion. The movements of the six legs can be characterized as regularly coordinated during forward walking, which results in rhythmical stepping patterns. However, leg coordination of insects is not inflexibly predetermined; rather, it can be adapted to the behavioural context and to the environmental conditions, such as upcoming obstacles, transport of different loads or to cope with changes in walking direction (Ritzmann and Zill, 2013). Studying inter-leg coordination in different demanding situations is crucial for understanding locomotion behaviour. This might further provide an important insight into neuronal control and could be useful for the implementation of biologically inspired hexapod robots.

We know that different inter-leg coordination patterns (gaits) can be found in hexapods during forward locomotion. In wavegait

Institute of Neurobiology, University of Ulm, Ulm D-89069, Germany.

*Author for correspondence (sarah.pfeffer@alumni.uni-ulm.de)

S.E.P., 0000-0003-1470-5055

coordination, one leg is in swing phase, while the other five legs remain on the ground. The swing propagates from back to front, first on one and then on the other body side. This kind of walking behaviour is seen in some insects during very slow walking or load-carrying, observable, for example, in stick insects (*Carausius morosus*, Hughes, 1952; Wilson, 1966) and fruit flies (*Drosophila melanogaster*, Wosnitza et al., 2013). In tetrapod gait, two diagonally located legs are in swing phase at once, while the other four legs are in stance phase. This walking behaviour is seen in insects with an intermediate walking speed. Good model organisms, where the tetrapod gait pattern is well studied, are stick insects (*Carausius morosus*, Graham, 1972; *Aretaon asperrimus*, Jeck and Cruse, 2007). During tripod coordination, three legs are in swing phase, while the other three legs are in stance phase. Thereby, the front and hind legs on one body side step in unison with the middle leg of the other body side. This kind of locomotion is well known from fast walking insects, such as cockroaches (*Periplaneta americana,* Delcomyn, 1971; *Blaberus discoidalis*, Bender et al., 2011; *Blatta orientalis*, Hughes, 1952), fruit flies (*Drosophila melanogaster*, Wosnitza et al., 2013) and ants (*Cataglyphis, Formica, Lasius* and *Myrmica*, Zollikofer, 1994a).

It should be noted that this kind of classification represents idealized forms of coordination patterns, but walking insects produce also intermediate versions to avoid jerky movements (Grabowska et al., 2012). The different gait patterns described show a transition into each other, forming a continuum (Schilling et al., 2013; Wilson, 1966). Leg patterns within this continuum are interlinked by several coordination rules (Cruse, 1990; Cruse et al., 2007), which assume that legs may be coupled via mechanical and neurobiological signals. The rules were derived from behavioural experiments on different species and have been successfully implemented in hexapod robots. Rules 1–3 affect the timing of the transition between stance and swing phase. Together, they produce a back-to-front sequence of swing movements (a so-called metachronal wave). Rule 4 causes legs to be placed in locations very similar to where the anterior leg neighbour was standing. Rule 5 addresses the force distribution to spread the load efficiently. Rule 6, the treading-on-tarsus (TOT) reflex, enforces a correction step to avoid stumbling because of a leg placement error.

Arthropods with more than six legs, such as scorpions or crustaceans, can also be examined according to their walking pattern. Scorpions are known to move with two alternating tetrapods, which means that the first and third leg pairs move in synchrony as well as the second and fourth leg pairs, which could be regarded as an eight-legged extension of the insects' alternating tripod gait (Bowerman, 1981). Crustaceans show less coupling of the appendages between the contralateral body sides. The leg coupling mechanisms on the ipsilateral side, however, are much more distinctive, leading to a metachronal wave of sequential

Journal of Experimental Biology

stepping on one body side (Sleinis and Silvey, 1980; Marshall and Diebel, 1995). Scorpions or crustaceans are known to often walk backwards, demonstrating a simple reversal of the forward stepping pattern, although a slight decrease of inter-leg coupling exists (Clarac and Chasserat, 1983).

In insects, however, backward walking is less understood, simply because there are not many apparent cases. There are only a few examples mentioned in the literature, such as the escape behaviour of stick insects (Jeck and Cruse, 2007; Graham and Epstein, 1985), the manoeuvring out of blind alleys in fruit flies (Bidaye et al., 2014) or the backward rolling of dung balls in several species of dung beetles (Byrne et al., 2003; Hanski and Cambefort, 2014).

The present study provides the first quantitative analysis of backward locomotion in ants. We use the natural behaviour of rearward food dragging in *Cataglyphis fortis* (Forel 1902), where ants walk backwards, voluntarily and persistently over a long period of time. Compared with forward locomotion, we find remarkable differences in backward walking ants.

MATERIALS AND METHODS

Our experiments were conducted with *C. fortis* in its natural habitat of alluvial salt plains near Maharès, Tunisia (34.53°N, 10.54°E). All data were obtained during the summer months of 2013, 2014 and 2015.

How to convince the ants to walk backwards?

To persuade the ants to take their homebound trip backwards, we offered them a large food item, which they were not able to carry. Thus, the ants did not lift it up to carry it in a regular forward-faced body orientation (Fig. 1B,C). Instead, the load was dragged backwards. This behaviour allowed us to investigate rearward locomotion and the ants' walking performance under realistic, natural conditions.

Food load

The large food items were either made of biscuit crumbs or mealworms. To make the load sufficiently heavy we concealed a tin-solder wire in the middle of the food item. The food items

weighed 186±50 mg (mean±s.d.) and were all elongated in shape. The food loads were carefully chosen to ensure that each ant (14±4 mg) was able to drag its load backwards.

Data analysis

We made high-speed video recordings within an aluminium channel to characterize the backward walking performance (Fig. 1A). The recordings (MotionBLITZ EoSens mini1, Mikrotron, Unterschleissheim, Germany) had a sampling frequency of 250 and 500 frames s^{-1}. The backwards (*n*=20) and forwards (*n*=20) moving ants were filmed in top view. We examined at least 300 frames of backward walking or three step cycles of forward walking.

Walking speed was analyzed with Ethovision software (Noldus Information Technology, Wageningen, The Netherlands). The ants' movements were tracked automatically (sampling rate of 50 Hz). We further analyzed the lift-off and touchdown moments of the tarsal tips for each leg, which were used to determine each ant's stepping pattern. We define a leg to be in swing phase as long as the tarsal tip is lifted off the ground or is in motion, and in stance phase as long as the tarsal tip touches the ground without moving.

To visualize the sequence of swing and stance phases, we plotted podograms. To illustrate and quantify the ants' inter-leg coordination, we assigned each frame of the videos with a number and a colour that classifies each frame according to its leg combination, similar to the work of Mendes et al. (2013) and Wahl et al. (2015). We categorized each frame into one of the following groups: tripod, tetrapod, wavegait, undefined gait and hexa support phase (see Fig. S1). To visualize the variability of the inter-leg relationship according to the swing phase onsets, we used phase plots. We used the MATLAB environment (MathWorks, Natick, MA, USA) for frame-by-frame indexing, calculation of podograms and phase plots. In particular, the phase plot analysis was realized by means of the 'CircStat' toolbox (Berens, 2009), where the phase shifts of legs L1, L2 and R1–3 were calculated with respect to L3. We measured the distance between the position of the lift-off and the position of the touchdown event in every leg throughout the video and averaged the values for each leg pair to obtain a mean stride length. We defined the swing movement without the body movement as stride amplitude, which was calculated as stride length minus swing phase duration multiplied by walking speed (Wahl et al., 2015). To calculate stride frequency, we divided walking speed by stride length. To calculate swing speed, we divided stride length by the corresponding swing phase duration. We measured the x/y coordinates of the tarsal tips with respect to the petiole in the frame before and after a swing phase to quantify the ant's footfall geometry (similar to the work of Mendes et al., 2013 and Seidl and Wehner, 2008). We considered the petiole as the centre of mass (Reinhardt and Blickhan, 2014). The measurements were conducted using ImageJ (US National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

We used SigmaPlot (Systat Software, San Jose, CA, USA) for statistical comparison and to generate box-and-whisker plots. For pairwise comparison of normally distributed data we applied the *t*-test, and for non-normally distributed data we applied the Mann–Whitney rank sum test (denoted as *U*-test). For a multiple comparison of normally distributed data we used one-way ANOVA; for non-normally distributed data we used ANOVA on ranks. Box-and-whisker plots show the median as the box centre, 25th and 75th percentiles as box margins, and 10th and 90th percentiles as whiskers.
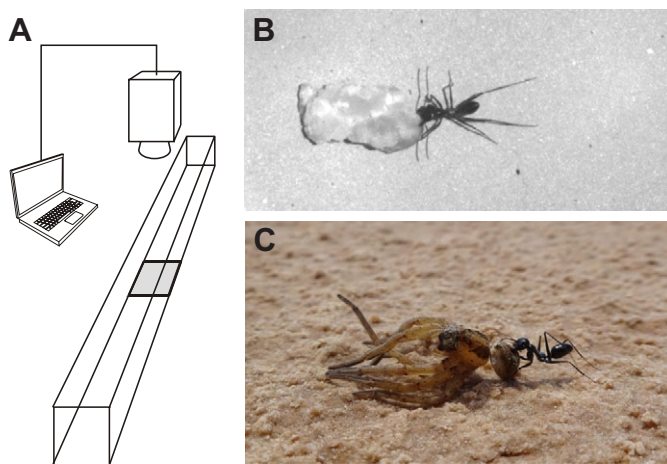

Fig. 1. Experimental setup and backward dragging ants. (A) Experimental setup. Ants were dragging a heavy food item backwards for 5 m before film recordings were performed with a resolution of 1280×1024 pixels (see grey area within the channel). (B,C) Still images showing the food-dragging behaviour (B) from a high-speed video in the channel and (C) under natural conditions.

RESULTS

We wanted to determine how ants coordinate their leg movements during backward locomotion. We made high-speed video recordings during rearward (20 animals) and forward (20 animals) walking and analyzed the stepping coordination according to several parameters that will be presented in the following sections (example videos have been uploaded to the Dryad Digital Repository; for video overview, see Fig. S4).

Stance-and-swing envelopes

To give an impression of how ants move their legs during backward and forward locomotion, we exemplarily show the spatial tarsal traces (envelopes) for one step cycle during the stance and swing phase in relation to the ant's body position (Fig. 2A). Swing and stance envelopes are a body-coordinate-based measure for the tarsal tip position during swing and stance. It is noticeable that the envelopes (from the stance phase as well as from the swing phase)
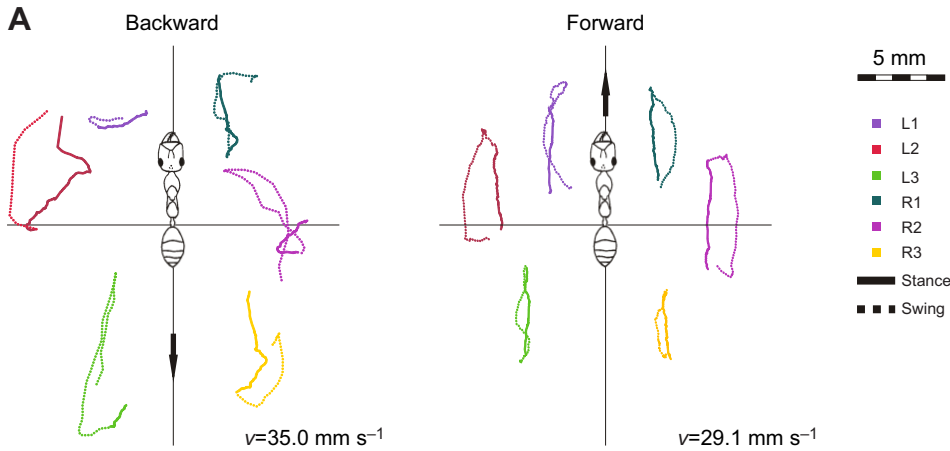

Fig. 2. Examples of stance and swing envelopes and quantitative analysis of stride amplitudes. (A) Stance and swing envelopes of the tarsal tip of each leg during one step cycle tracked relatively to a fixed position of the ant's body (2nd abdominal segment, petiole). The black arrows indicate the direction of movement. L, left, R, right body side; 1, 2 and 3, front, middle and hind leg. v, speed. (B) Quantitative comparison of the stride amplitudes. Mean stride amplitudes of each of $n=20$ backward and forward walking animals. Box plots give the 10th, 25th, 50th, 75th and 90th percentile distributions. Statistical analysis: (1) t-test, $P \leq 0.001$; (2) t-test, $P \leq 0.001$; (3) U-test, $P=0.001$; (4) ANOVA on ranks, $P=0.001$ (Tukey *post hoc* test shows difference for all pairwise comparisons, except 2nd and 3rd leg pairs), (5) one-way ANOVA, $P=0.849$. (C) Footfall geometry during backward and forward locomotion (each $n=5$). Selected videos cover the entire walking speed range. Values were normalized to body length. (Ci) Footfall position with respect to the petiole (origin of plot, marked with asterisk, considered as centre of mass). AEP, anterior extreme position; PEP, posterior extreme position. (Cii) Quantification of footprint clustering using the standard deviation. Box plots give the 10th, 25th, 50th, 75th and 90th percentile distributions. Statistical analysis: (6) U-test, $P \leq 0.001$; (7) U-test, $P \leq 0.001$; (8) t-test, $P=0.639$; (9) t-test, $P=0.876$.
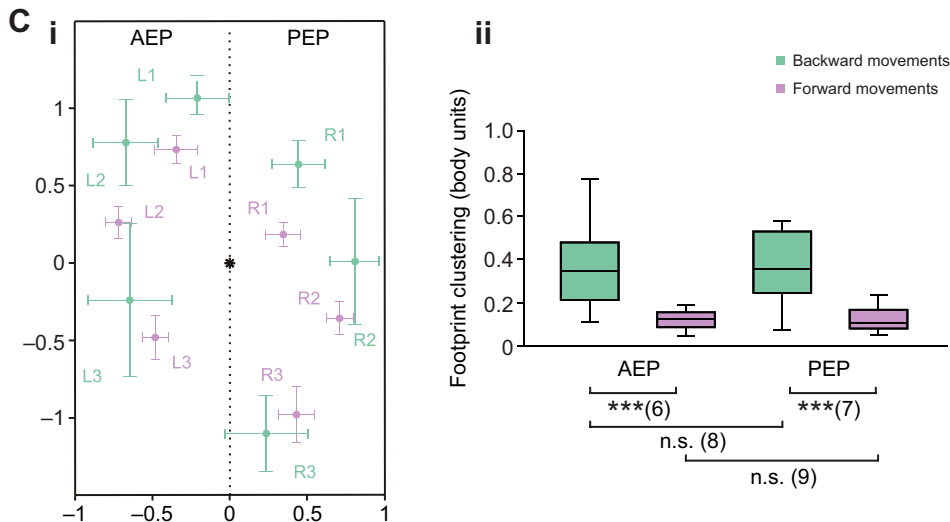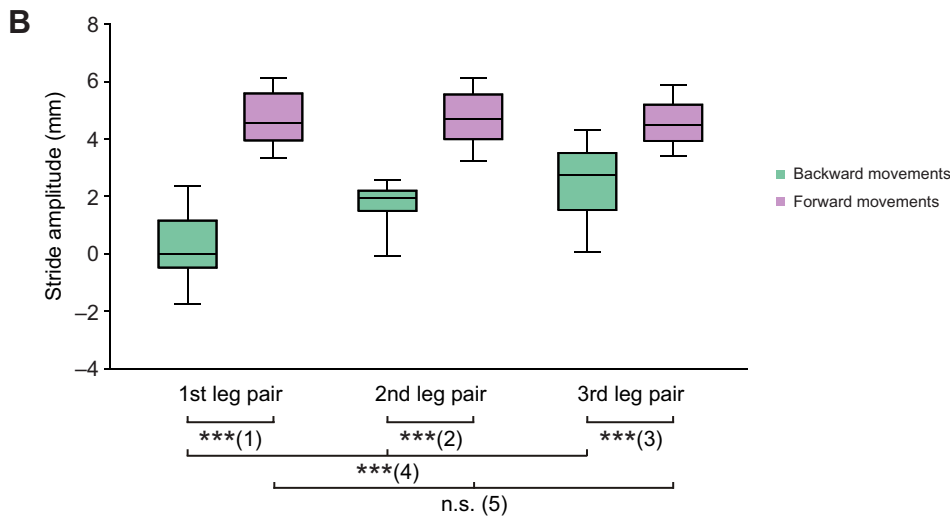
show more uneven contours and are more curved during backward locomotion compared with the envelopes from a normal forward walk.

These visually distinguishable differences can further be supported by the modified index of straightness (Batschelet, 1981). This index value sets the actual distance moved by a leg in relation to its straight line distance; values were calculated for the example envelopes shown in Fig. 2. A value of 1 describes a straight line between the stride onset and stride touchdown. The lower the value, the longer and more complex the respective swing or stance traces. The median value (for all legs) of the modified index of straightness decreases from 0.92 in the forward envelopes to 0.82 in the backward envelopes. The same is true for the swing movements, where the median index value decreases from 0.59 in the forward envelopes to 0.49 in the backward envelopes (for exact values, see Table S2).

Although we here show only one example of stance and swing envelope for each leg, one can expect quite similar envelopes for other step cycles (given the fact that we visually confirmed this in all videos). If we consider the petiole as the ant's centre of mass (in compliance with Reinhardt and Blickhan, 2014), our results suggest that rearward movements are more wobbly and it can be assumed that the ant has to balance its centre of mass more frequently.

In order to illustrate the leg swing independent of body movements, we calculated the stride amplitude, which is a body-coordinate-based measure for the leg in swing phase (Fig. 2B). It turns out that the stride amplitudes during backward movements are significantly shorter than those during forward movements of the respective leg pair. Further, it is noteworthy that during backward movements especially the first leg pair shows small and even negative stride amplitudes. This finding indicates that the frontal legs sometimes cover less distance than the distance of the mean body displacement. Hence, it seems that the front legs do not contribute to the same extent to the walking speed as the middle and hind legs. Contrary to backward stride amplitudes, the stride amplitudes of forward movements do not differ significantly among different leg pairs.

To further analyze the spatial properties of backward locomotion, we quantified the ants' footfall geometry. During forward locomotion, the anterior extreme position (AEP) is the most anterior footfall position of the leg after a swing phase. At the end of the stance phase, just before the leg is lifted again, a leg is in its posterior extreme position (PEP). The terms 'anterior' and 'posterior' refer to the ant's body orientation. We use these terms also for backward locomotion, but it should be noted that the anterior and posterior position of the legs are reversed within the step cycle. During rearward locomotion, the AEP is at the end of a stance phase, whereas the PEP is reached after a swing phase.

If we compare the AEPs and PEPs of backward and forward walking ants (Fig. 2Ci), we can see that during backward locomotion the tarsal contact is more spread to increase static stability. Further, the frontal legs are positioned more anteriorly. The middle legs are shifted slightly more distally and anteriorly.

The footprint clustering of AEPs and PEPs is shown in Fig. 2Cii. Here we plot the standard deviation of the mean for all AEPs and PEPs. The comparison of the clustering of the AEPs during backward and forward walking reveals a significant statistical difference. The same is true for the PEPs. However, we cannot find a difference of the AEPs and PEPs clustering within one walking direction (backwards as well as forwards).

Stepping pattern and inter-leg coordination

In the following section we quantify the locomotion pattern of backward (25.8 to 65.6 mm s^{-1}) and forward (22.6 to 64.0 mm s^{-1}) moving ants (Fig. 3) and analyze how the inter-leg movements are coordinated temporally.

Although the lower range of walking speed is overrepresented quantitatively in our data set, we are convinced that we covered a natural walking speed distribution in our analysis of backward walking ants. Fast backward walks (>50 mm s^{-1}) are less frequent, while walking speeds between 20 and 50 mm s^{-1} are much more often observed. For comparative reasons we thus adapted the analyzed walking speed range of forward walking ants (which of course can be observed in a much broader scope of walking speeds up to 600 mm s^{-1} and even greater; Wahl et al., 2015). The walking speed of backward dragging ants strongly depends on the ants' individual performance and mostly on the size and weight of the food item. If the food morsel is too lightweight, the ants would not reliably drag it backwards but try to push and carry it forwards (see Movie 3).

Fig. 3A gives an exemplary demonstration of a typical backward and forward walk within a temporal context. The podograms shown visualize the durations of stance and swing phases for each leg (Fig. 3Ai). Further, we assigned each frame of the video a colour index (Fig. 3Aii) and a number index (Fig. 3Aiii), according to the current gait pattern, as indicated in the key. In Fig. S1, a more detailed list of different leg combinations assigned to a particular gait type is shown; those that did not match with these ideal leg combinations were classified as 'undefined gait' (listed and quantified in Fig. S2). The examples show that during backward walks no regular repetition of leg combinations can be found, while in forward locomotion tripod coordination is the predominant coordination pattern.

Frame-by-frame indexing was also used for a quantitative analysis. The percentage distribution (Fig. 3Bi) of the different leg combinations is illustrated as a bar chart, where every frame of the video was taken into account. For a more specific idea of the individual's walk, we calculated the averaged number index for each video (Fig. 3Bii). During backward locomotion there is no preferred gait type; rather, wavegait (one leg in swing phase) and undefined leg combinations make up the larger part of the quantitative analysis. In contrast, forward walks show a high proportion of the tripod pattern, while the fraction of non-tripod coordination is small and can be related to the transition between one tripod group (L1, R2, L3) and the next one (R1, L2, R3). With increasing walking speed, the percentage of tripod coordination increases while the proportion of the hexa support phase (all six legs with ground contact) decreases in backward as well as in forward walks (see also Wahl et al., 2015).

During backward locomotion, a quantification of the undefined leg combinations reveals a wide range of different leg combinations, where no pattern has a clear majority. We show that 74% of the undefined leg combinations recorded during backward locomotion do not follow Cruse's rule 1 (ipsilaterally or contralaterally); this constitutes 24% of all analyzed frames. In these frames we find a simultaneous stepping of adjacent legs and therefore statically unstable leg combinations (Cruse et al., 2007). During forward walking 20% of the undefined stepping patterns also show unstable combinations in which Cruse's rule 1 is disregarded (ipsilaterally or contralaterally). However, they make up only 2% of all analyzed frames. Most undefined frames (80%) during forward locomotion can be attributed to the transition of one tripod group to the next (see Fig. S2).
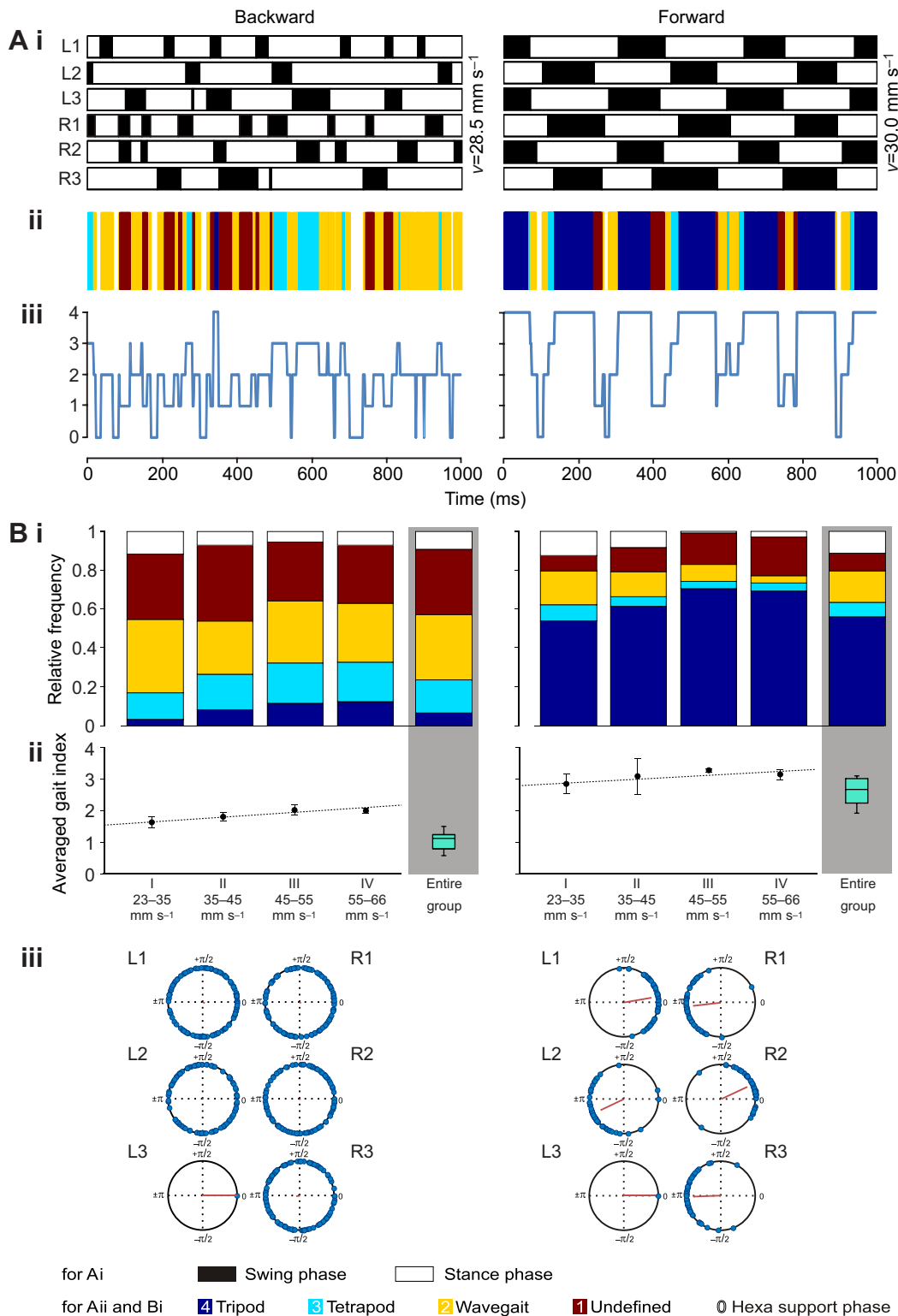
Fig. 3. Stepping pattern and inter-leg coordination. (A) Example of backward and forward locomotion. The podograms (Ai) illustrate the footfall patterns of all six legs at 28.5 mm s^{-1} for the backward and 30.0 mm s^{-1} for the forward example. White bars represent the stance phase, black bars represent the swing phase. L, left; R, right; 1, 2 and 3, front, middle and hind leg. Each frame of our examples was classified according to the prevailing gait pattern [colour (Aii) and number (Aiii) indices are indicated in the key at the bottom of the figure]. (B) Quantitative indexing analysis of each of n=20 backward and forward walking ants. Each frame was indexed with a colour and number code as in A. The summarized results for colour index analysis (Bi) were categorized according to the ants' walking speed (I–IV). For exact values, see Table S3. The averaged number index (Bii) for each video was grouped according to walking speed categories (I–IV). The data points represent the means; error bars show the s.d. Averaged number indices of the entire groups differ significantly (t-test, P≤0.001). Box plots give the 10th, 25th, 50th, 75th and 90th percentile distributions. (Biii) Phase plots as the swing phase onset of all legs in relation to L3 (reference leg). Blue circles symbolize the onset of each cycle. The mean vector is represented by the red line, with its length indicating the variance. Backward walking ants show no regular repetition of leg coordination, whereas forward walking ants show clear tripod coordination.

Because swing and stance phases are repetitive events, we can also use a circular illustration. Phase plots are a useful tool with which to analyze the variability of the inter-leg coordination. The phase plots (Fig. 3Biii) of the backward walks show a short length of the mean vector with no clear tendency towards phase coupling. There is a high variability of the blue dots, symbolizing the swing phase onsets with respect to the left hind leg (L3). This is due to the irregular leg coordination without leg synchronization. In contrast, forward runs display strong antiphase swinging of the tripods.

General walking parameters

We further analyzed the relationship between walking speed and important walking parameters (Fig. 4), where we found interesting differences between backward and forward walking.

First, during backward locomotion, walking speed is increased by longer stride lengths (Fig. 4Ai) and higher stride frequencies (Fig. 4Aii). In contrast, the forward walking ants increase only stride frequency for increasing speed (Fig. 4Bii), while the stride length (Fig. 4Bi) is kept more or less constant within the analyzed walking speed range.

Second, as opposed to forward walking ants (with almost superposed correlation lines), in backward walking ants the correlation lines tend to be distinctly separated. This is most notable in Fig. 4Ai,ii and 4Bi,ii, which might indicate a certain degree of leg specialization: during rearward locomotion, front legs tend to make small and frequent strides, whereas hind legs make large but fewer strides. At the end of the swing phase, the flexed front legs are often put close to the ant's thorax, seemingly to push the body in the direction of movement. The hind legs, however, seem to be placed far backwards and cling to the ground to pull the body (and the load) in the direction of movement.

Third, the backward swing speeds (Fig. 4Aiii) are faster than the forward swing speeds (Fig. 4Biii). The backward swing phase (Fig. 4Aiv) is short compared with the forward swing phase (Fig. 4Biv). These aspects reduce the time that the legs are in air and thus do not contribute to the stability of walking. The backward stance phases, especially those of the first leg pair, are also shortened compared with the forward stance phases.
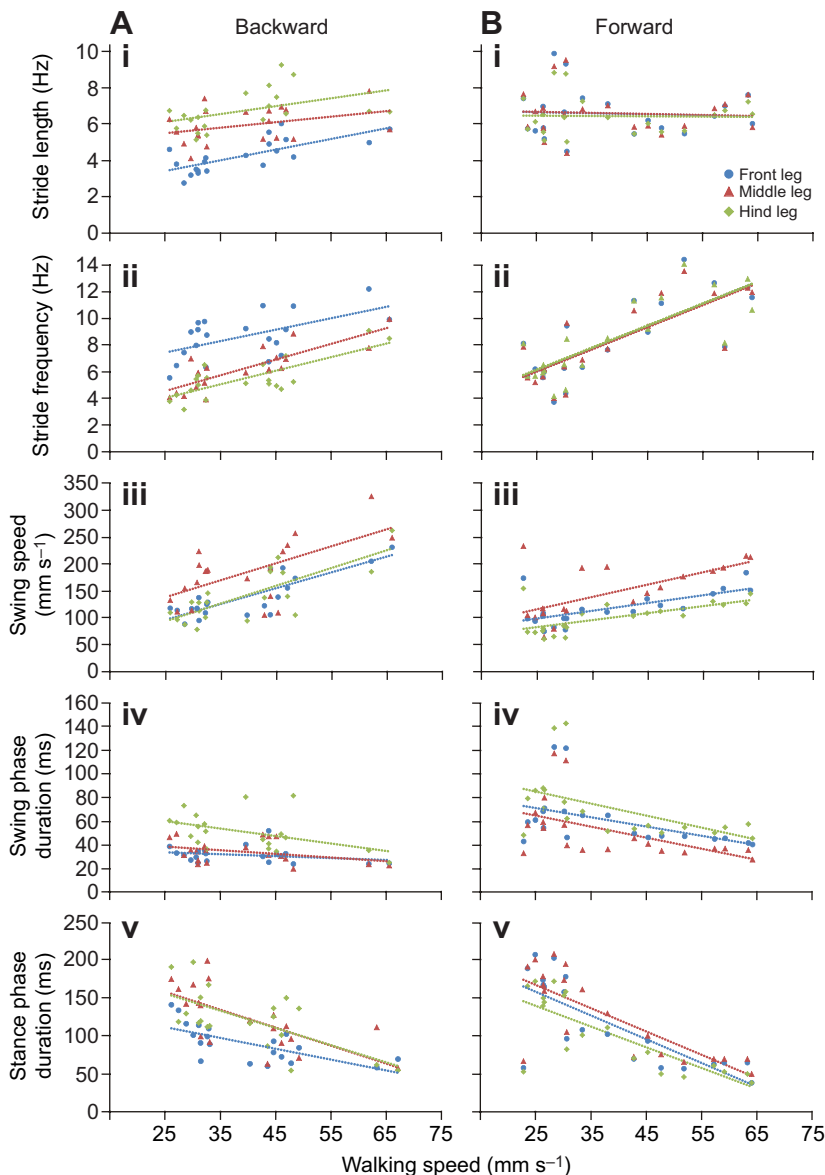

Fig. 4. General walking parameters. Front, middle and hind legs are plotted separately in each diagram. The same videos were analyzed as in Fig. 2. The linear regressions were used to analyze the general walking parameters and their relationships. For the linear regression formula and the coefficient of determination (R^2), see Table S1.

DISCUSSION

Backward walking is not an uncommon situation in an ant's life, in particular if large and heavy food items need to be transported. Interestingly, it has rarely been investigated how legs are coordinated during backward locomotion. To elucidate this fundamental question in ants, we compared the performance of backward and forward walking.

Stepping pattern of backward walking ants

Tripod, tetrapod and wavegait patterns are known to describe the inter-leg coordination in hexapods during forward locomotion. These different gait types are ideal forms of leg coordination patterns that are quite useful for classification. However, it is not possible to execute a distinct gait type in an absolute manner, which also becomes clear in our results. During forward walking, the transition from one to the next tripod group (L1, R2, L3 to R1, L2, R3 and vice versa) does not occur simultaneously for all three legs, because this would lead to an abrupt and jerky locomotion. Rather, the transitional change from one ideal leg combination to the next is discontinuous with regard to the stepping pattern and requires intermediate combinations to maintain a smooth sequence of movement.

The locomotion pattern of forward walking is well examined in ants, which show a remarkable robustness of tripod coordination over almost the entire range of walking speeds (Wahl et al., 2015), during leg loss (Wittlinger and Wolf, 2013) and even during swimming (Bohn et al., 2012). However, locomotion and inter-leg coordination during backward walking has never been studied in ants. We know that stick insects (*Carausius morosus* and *Aretaon asperrimus*) walk backwards when they try to escape. This behaviour can be elicited if the antennae are stimulated. Rearward walking lasts for 5–30 s (Graham and Epstein, 1985). In rare cases, stick insects can also walk backwards spontaneously over a few step cycles (Jeck and Cruse, 2007). These studies show that although during rearward walking the stepping pattern is irregular, the ipsilateral legs still swing in a typical sequence from front to middle to hind leg (metachronal wave). A similar picture emerges in backward walking *Drosophila* (Bidaye et al., 2014, see their supplementary material). Transgenically modified 'moonwalker flies' walk backwards at certain temperatures. They only rarely use tripod or tetrapod combinations during backward walks. Rather, their locomotion follows a non-rigid and loosely coordinated leg pattern. As in escaping stick insects, a reversed metachronal wave of leg swings on the ipsilateral side is maintained.

Contrary to the studies mentioned above, we present a naturally occurring backward locomotion that is performed voluntarily and is persistent over a long time period. Backward dragging ants do not show a periodically emerging gait pattern. Further, the metachronal wave of the ipsilateral leg swings is only visible to some extent. The wave from front to middle to hind leg is often interrupted. That means that during the backward walks we find deviations from the coordination rules of Cruse (1990). Previous experiments with *Carausius morosus* that performed straight and curved walks on a spherical treadmill already suggest that the strength and efficacy of these coordination rules are context dependent (Dürr, 2005).

The coordination rules suitably describe walking during forward locomotion, where they coherently display the coupling and alternating activity of several leg groups. In particular, rules 1–4, which presume inter-leg coordination, seem to become less important and are partly ignored during the backward walks in our study. In these rules the current state of one leg influences the motor behaviour of its neighbouring leg in several ways. It might be

that rule 5, dealing with load distribution, outcompetes the other rules during the behaviour of backward dragging. Because of the dragging of the heavy food, the ants' rearward walk becomes unstable. The maxim of coordination is now not to fall, while the performance of an ideal stepping pattern retreats into the background. That means that in our case the leg coordination might especially be determined by sensory feedback, while a regular inter-leg coordination is widely ignored. Backward dragging thus reveals the extensive flexibility of leg coordination and emphasizes the remarkable adaptability of motor programs as a result of the integration of sensory feedback. Unfortunately, in our data set we cannot assess whether rule 6, the TOT reflex, is present in *C. fortis* during backward walking.

Although our data especially show irregular locomotion behaviour, it should be mentioned that backward tripod-like coordination is possible per se (see Movie 4). This has been observed over several consecutive step cycles; nonetheless, we never observed an ant exclusively using a tripod-like pattern during backward locomotion. Because of the fast backward leg movements, there is not much temporal overlap, and the tripod coordination pattern seems to be distorted and less exact.

Specializations of leg pairs

Although no repetitive leg coordination can be found over a longer period of time, some kind of specialization seems to occur. The front legs make frequent but small strides, putting the flexed front leg close to the thorax and pushing the body and the load into the moving direction until the front leg is extended again. In contrast, the middle and especially the hind legs make less frequent but long strides (Fig. 4). The legs are reaching far backwards in order to find some grip to cling to the ground and to pull the body and the load into the direction of movement. Note that these specializations are often present but are not mandatory. They are neither periodically repeated in a temporal context nor do they force an obligatory inter-leg coordination.

Interestingly, similar specializations of certain leg pairs have also been described in the few studied examples of backward walking insects. Rearward walking stick insects show a reversed specialization: here the front legs make larger steps at a corresponding lower frequency than the middle and hind legs (Graham and Epstein, 1985). A similar tendency can be found in the fruit flies' hind legs, which step significantly more often than the front and middle legs during backward walking (Bidaye et al., 2014). However, specialized leg pair coordination is not exclusively found during backward locomotion. In other challenging walking situations, such as forward walks on an inclined surface, a particular modification of common stepping patterns is revealed. In stick insects, the front legs become uncoupled from normal locomotion, showing multiple stepping, while the middle and hind leg coordination remains regular (Grabowska et al., 2012).

Food dragging and stability of backward walking

Our results show that the movements of ants during backward dragging become unstable, thus it can be suggested that the ant's centre of mass has to be balanced more than during forward walking. One reason for this is that backward walking ants move slowly and, hence, the support provided by dynamic stability is low (Ting et al., 1994). Further, it can be assumed that by grasping the food item with the mandibles and dragging it backwards, the ant's centre of mass is shifted to the anterior (probably even outside the tripod triangle), as is indicated in the more anterior footfall position of the front and middle legs. Therefore, the limits of a stable tripod

walk are exceeded in rearward locomotion and the ants change to a more flexible and stable leg pattern. The influence of load on locomotion was previously investigated in *C. fortis* (Zollikofer, 1994b). Compared with the present study, the load was less and the ants maintained tripod coordination. Nevertheless, carrying load led to slight deformations of the tripod triangle.

To maintain a stable walk, the tarsal positions are spatially more spread during backward than during forward locomotion. This probably increases the ants' static stability. Besides, the ants dragging the weight possess an additional support point – the mandibles clamping to the food – which might facilitate unstable gait conformations and increase static stability. This might explain why backward dragging ants are able to disregard Cruse's rule 1 to a much higher extent than during forward locomotion. Backward dragging ants could more easily compensate for unstable movements such as the simultaneous stepping of ipsilateral or contralateral legs. Further, rearward food dragging ants increased their contact with the substrate. This becomes conspicuous in our results. First, backward walks have relatively fast swing phases. This reduces the time that legs are in air and are not supporting the ant. Second, during rearward walking, leg pattern combinations are used where often more than three legs have ground contact. The proportion of tetrapod and wavegait combinations is higher in backward walks, as are 'undefined combinations', a majority of which have at least three legs on the ground simultaneously to support the ant.

Interestingly, a similar outcome was shown in experiments with *Drosophila*, where a load with twice the body mass was glued onto the flies' notum (Mendes et al., 2014). The authors report an increase in swing speed compared with unloaded flies. Further, the flies had more contact with the substrate as walking via tripod and tetrapod gaits become less frequent and wavegait and hexa support phases increased.

Final remarks

Although ants are known to be very robust tripod walkers, their leg coordination is less coupled during backward walking, demonstrating the flexibility of ant locomotion. We have to keep in mind that we examined backward dragging behaviour under idealized conditions. This means that not all food items found in an ant's natural environment have an elongated shape and can easily be grasped with the mandibles. We also tried to avoid bulky food. It might be that under natural conditions the front legs are especially hampered and therefore limited in their range of movements. Furthermore, the mass of the food load is often not as symmetrical as in our study, which might give leg and body movements a biased direction. Additionally, the natural substrate is more uneven than our sanded, plain aluminium floor (even the desert ground, see movie 1 in Pfeffer and Wittlinger, 2016). One can easily assume, therefore, that we would find even more flexibility of locomotion under natural conditions with even less coupled leg coordination and more unsteady leg and body movements. But as we know, ants actually cope well with their natural environment. That fact that the stability could be maintained during backward dragging indicates a strong influence of sensory feedback overriding the seeming rigidity of the tripod leg pattern. Here, the exciting question arises of if and how a challenging task such as odometry is accomplished with such a flexibility of the walking apparatus (Pfeffer and Wittlinger, 2016). This might also be an interesting aspect for the implementation of insect walking in robotics. Through further biomechanical measurements in backward walking ants, for example, ground reaction forces and further kinematic parameters, important information could be provided for a more precise understanding of legged locomotion and its neuronal control.

Competing interests

The authors declare no competing or financial interests.

Author contributions

The experiments were planned by S.E.P. and M.W., and carried out by S.E.P., V.L.W. and M.W. The data were analyzed by S.E.P. and V.L.W. The paper was written by S.E.P. and M.W.

Data availability

Data are available from the authors on request. Videos are available from the Dryad Digital Repository at http://dx.doi.org/10.5061/dryad.7k82t.

Supplementary information

Supplementary information available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.137778/-/DC1

References

Batschelet, E. (1981). *Circular Statistics in Biology*. London: Academic Press.

Bender, J. A., Simpson, E. M., Tietz, B. R., Daltoria, K. A., Quinn, R. D. and Ritzmann, R. E. (2011). Kinematic and behavioral evidence for a distinction between trotting and ambling gaits in the cockroach *Blaberus discoidalis*. *J. Exp. Biol.* **214**, 2057-2064.

Berens, P. (2009). CircStat: a MATLAB toolbox for circular statistics. *J. Stat. Softw.* **31**, 1-21.

Bidaye, S. S., Machacek, C., Wu, Y. and Dickson, B. J. (2014). Neuronal control of *Drosophila* walking direction. *Science* **344**, 97-101.

Bohn, H. F., Thornham, D. G. and Federle, W. (2012). Ants swimming in pitcher plants: kinematics of aquatic and terrestrial locomotion in *Camponotus schmitzi*. *J. Comp. Physiol. A* **198**, 465-476.

Bowerman, R. F. (1981). Arachnid locomotion. In *Locomotion and Energetics in Arthropods* (ed. C. F. Herreid and C. R. Fourtner), pp. 73-102. New York: Plenum.

Byrne, M., Dacke, M., Nordström, P., Scholtz, C. and Warrant, E. (2003). Visual cues used by ball-rolling dung beetles for orientation. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* **189**, 411-418.

Clarac, F. and Chasserat, C. (1983). Quantitative analysis of walking in a decapod crustacean, the rock lobster *Jasus lalandii*: I. Comparative study of free and driven walking. *J. Exp. Biol.* **107**, 189-217.

Cruse, H. (1990). What mechanisms coordinate leg movement in walking arthropods? *Trends Neurosci* **13**, 15-21.

Cruse, H., Dürr, V. and Schmitz, J. (2007). Insect walking is based on a decentralized architecture revealing a simple and robust controller. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **365**, 221-250.

Delcomyn, F. (1971). The locomotion of the cockroach *Periplaneta americana*. *J. Exp. Biol.* **54**, 443-452.

Dürr, V. (2005). Context-dependent changes in strength and efficacy of leg coordination mechanisms. *J. Exp. Biol.* **208**, 2253-2267.

Grabowska, M., Godlewska, E., Schmidt, J. and Daun-Gruhn, S. (2012). Quadrupedal gaits in hexapod animals – inter-leg coordination in free-walking adult stick insects. *J. Exp. Biol.* **215**, 4255-4266.

Graham, D. (1972). A behavioural analysis of the temporal organisation of walking movements in the 1st instar and adult stick insect (*Carausius morosus*). *J. Comp. Physiol.* **81**, 23-52.

Graham, D. and Epstein, S. (1985). Behaviour and motor output for an insect walking on a slippery surface: II. Backward walking. *J. Exp. Biol.* **118**, 287-296.

Hanski, I. and Cambefort, Y. (2014). *Dung Beetle Ecology*. Princeton, NJ: Princeton University Press.

Hughes, G. M. (1952). The co-ordination of insect movements in the walking movements of insects. *J. Exp. Biol.* **29**, 267-285.

Jeck, T. and Cruse, H. (2007). Walking in *Aretaon asperrimus*. *J. Insect Physiol.* **53**, 724-733.

Marshall, N. J. and Diebel, C. (1995). 'Deep-sea spiders' that walk through the water. *J. Exp. Biol.* **198**, 1371-1379.

Mendes, C. S., Bartos, I., Akay, T., Márka, S. and Mann, R. S. (2013). Quantification of gait parameters in freely walking wild type and sensory deprived *Drosophila melanogaster. eLife* **2**, e00231.

Mendes, C. S., Rajendren, S. V., Bartos, I., Márka, S. and Mann, R. S. (2014). Kinematic responses to changes in walking orientation and gravitational load in *Drosophila melanogaster. PLoS ONE* **9**, e109204.

Pfeffer, E. S. and Wittlinger, M. (2016). How to find home backwards? Navigation during rearward homing of *Cataglyphis fortis* desert ants. *J. Exp. Biol*. **219**, 2119-2126.

Reinhardt, L. and Blickhan, R. (2014). Level locomotion in wood ants: evidence for grounded running. *J. Exp. Biol.* **217**, 2358-2370.

Ritzmann, R. and Zill, S. N. (2013). Neuroethology of insect walking. *Scholarpedia* **8**, 30879.

Schilling, M., Hoinville, T., Schmitz, J. and Cruse, H. (2013). Walknet, a bio-inspired controller for hexapod walking. *Biol. Cybern.* **107**, 397-419.

Seidl, T. and Wehner, R. (2008). Walking on inclines: how do desert ants monitor slope and step length. *Front. Zool.* **5**, 8.

Sleinis, S. and Silvey, G. E. (1980). Locomotion in a forward walking crab. *J. Comp. Physiol. A* **136**, 301-312.

Ting, L. H., Blickhan, R. and Full, R. J. (1994). Dynamic and static stability in hexapedal runners. *J. Exp. Biol.* **197**, 251-269.

Wahl, V., Pfeffer, S. E. and Wittlinger, M. (2015). Walking and running in the desert ant *Cataglyphis fortis*. *J. Comp. Physiol. A* **201**, 645-656.

Wilson, D. M. (1966). Insect walking. *Annu. Rev. Entomol.* **11**, 103-122.

Wittlinger, M. and Wolf, H. (2013). Homing distance in desert ants, *Cataglyphis fortis*, remains unaffected by disturbance of walking behaviour and visual input. *J. Physiol.* **107**, 130-136.

Wosnitza, A., Bockemühl, T., Dübbert, M., Scholz, H. and Büschges, A. (2013). Inter-leg coordination in the control of walking speed in *Drosophila*. *J. Exp. Biol.* **216**, 480-491.

Zollikofer, C. (1994a). Stepping patterns in ants – influence of speed and curvature. *J. Exp. Biol.* **192**, 95-106.

Zollikofer, C. (1994b). Stepping patterns in ants – influence of load. *J. Exp. Biol.* **192**, 119-127.

Tripod gait (4)

Tetrapod gait (3)

Wavegait (2)

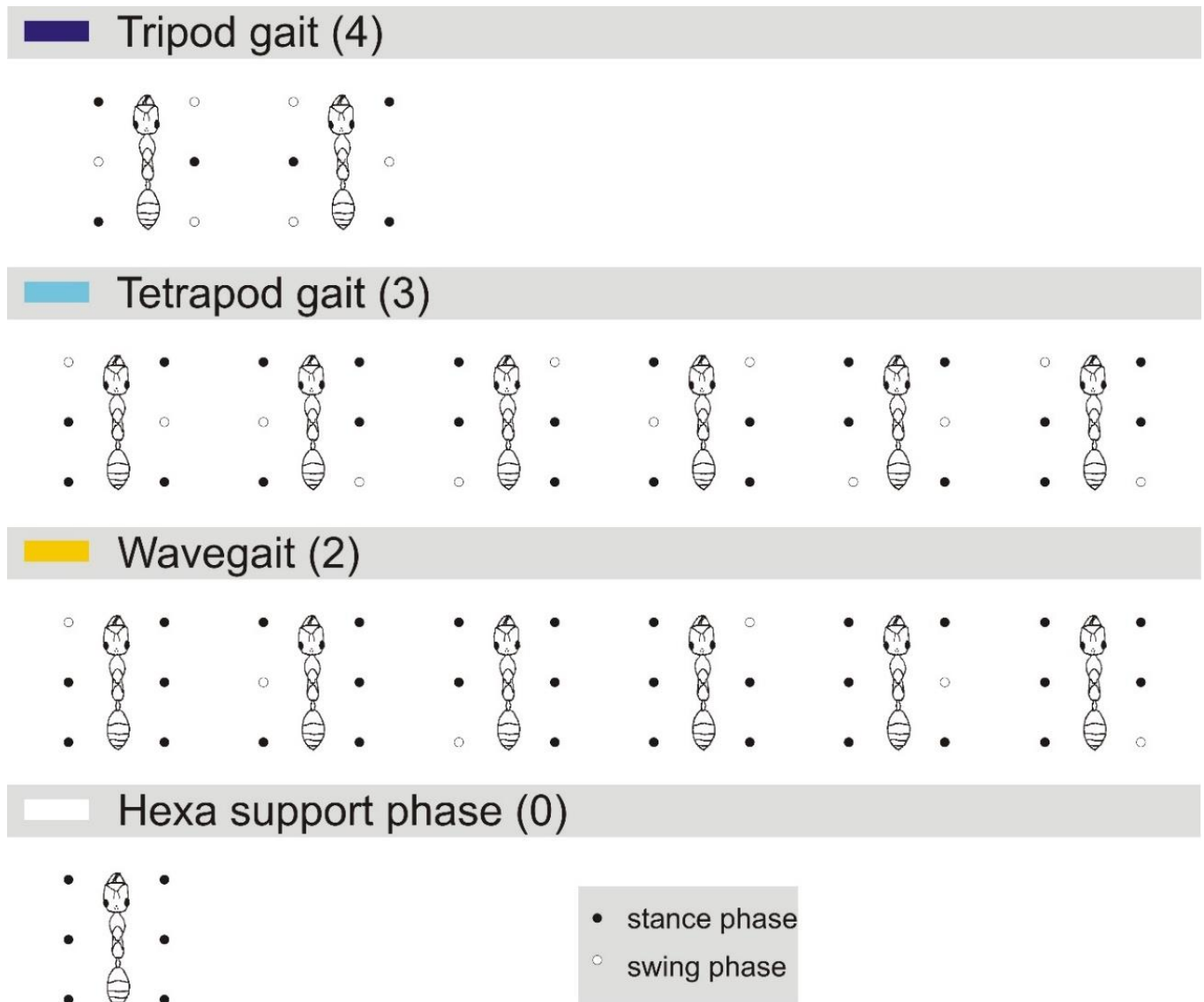## Hexa support phase (0)

- • stance phase
- ○ swing phase

**Fig. S1. Different leg combinations assigned to different gait patterns.**

In hexapods, several leg coordination patterns (gaits) are known that rhythmically emerge during locomotion. The current understanding is that the different gait patterns are part of a continuum with a continuous transition from tripod to tetrapod to wavegait coordination with decreasing walking speed (Wilson 1966, Schilling et al. 2013). Further, it is plausible that also within one gait type during the transition between one coupled leg group to the next, deviant leg combinations emerge to avoid jerky and abrupt movements. This also means that the different gaits are sometimes difficult to distinguish. Nevertheless, the classification of distinct gaits is a very helpful concept for the analysis of locomotion. In simple terms, we can define a tripod gait where three legs, a tetrapod gait where four legs, and a wavegait where five legs

engage simultaneously in stance phase, while the other legs are in swing. We analysed the stepping within a temporal context by assigning each frame a number and a colour according to the present leg combination as it is shown in this figure. If none of the listed leg combinations could be found, the frame was classified as 'undefined' and respectively the leg pattern is shown in the next figure (S2). This method is useful to illustrate leg combinations over time, but as well to quantify the different gait patterns and to further evaluate the ants' stepping behaviour.

A BACKWARD - undefined gaits

B FORWARD - undefined gaits

○ stance phase

● swing phase
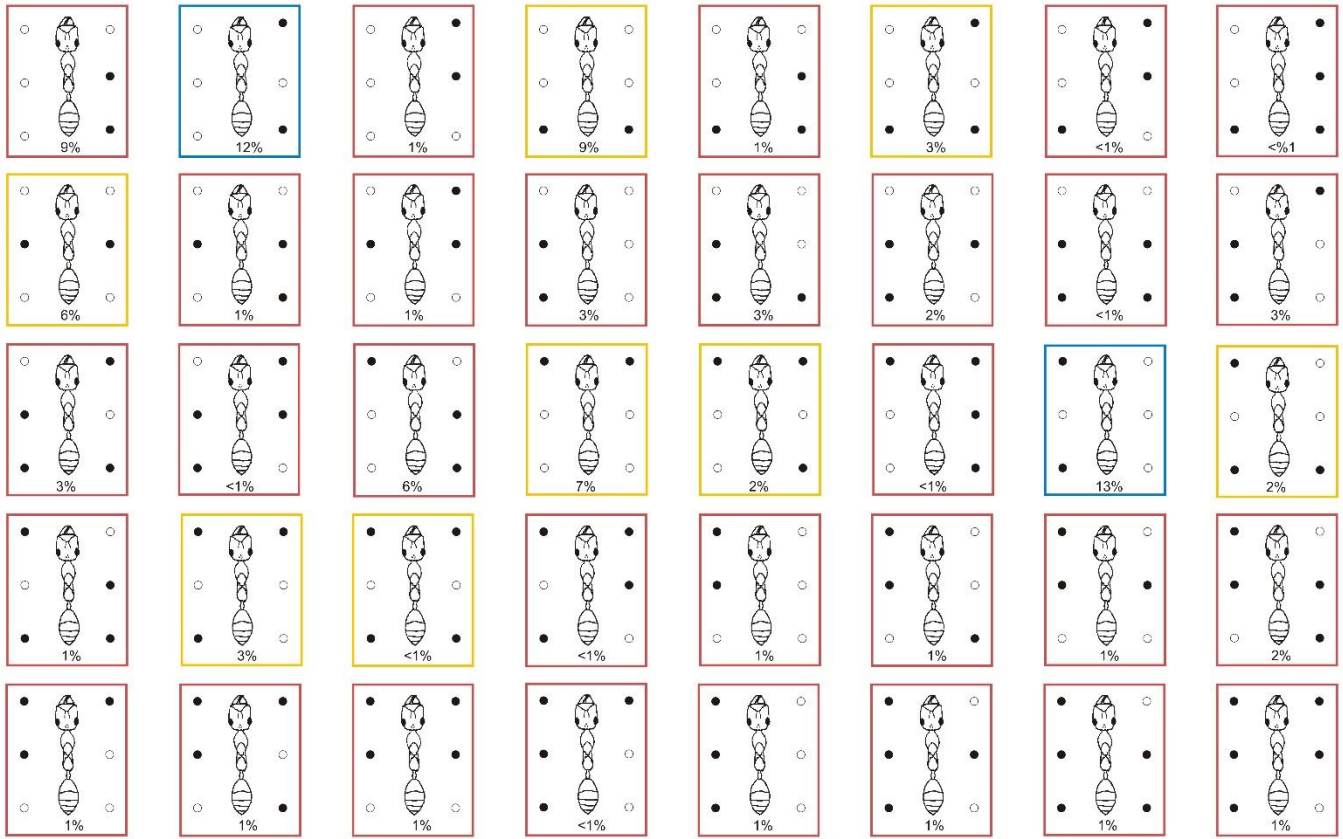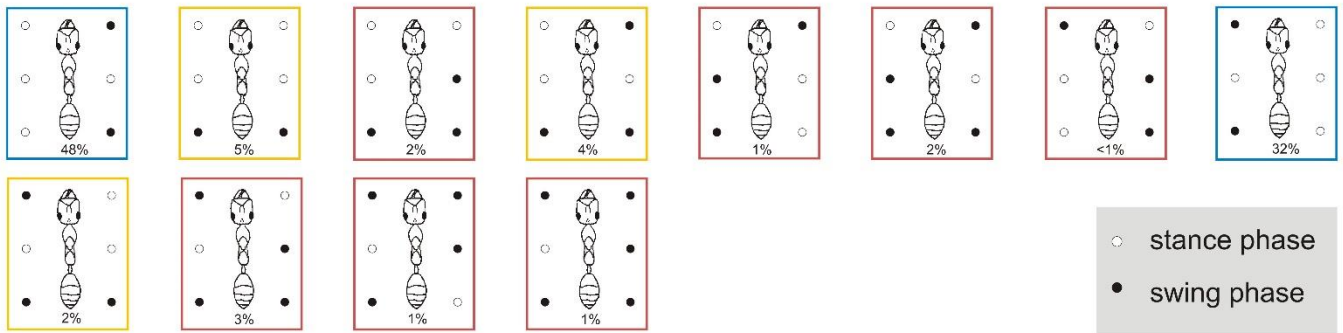
C VALIDITY OF RULE 1

		Invalidity of rule 1 *ipsilateral*	Invalidity of rule 1 *contralateral*	Validity of rule 1 *ipsi- and contralateral*
BACKWARD	Proportion of *undefined* frames	44 %	30 %	26 %
	Proportion of *all* frames	14 %	10 %	8 %
FORWARD	Proportion of *undefined* frames	9 %	11 %	80 %
	Proportion of *all* frames	1 %	1 %	6 %

Fig. S2. Undefined leg combinations.

Leg combinations that did not match with one of the ideal gait patterns as shown in figure S1 were classified as 'undefined' and are illustrated in this graph. The different leg combinations that occur during backward (Fig S2 A) and forward (Fig S2 B) locomotion are listed and quantified. The corresponding relative occurrence of each leg combination is indicated as percentage information below each ant.

We further analysed theses undefined leg combinations whether they are in conformance with Cruse's rule 1. This rule states that as long as a leg is in swing phase, it has an inhibitory influence on the anterior leg of the ipsilateral side. If one of the listed undefined leg combinations deviates from this rule we marked it with a *red frame*. Further studies suggests that Cruse's rule 1 might also be applicable to the contralateral leg (Dean and Wendler 1983). If an inhibitory effect to the contralateral leg was disregarded, we marked the respective leg combination with *a yellow frame*. A disregard of these rules (ipsilaterally or contralaterally) means an overlapping of swing movements of two adjacent legs and therefore a statically unstable leg combination. If rule 1 is fulfilled (ipsilaterally as well as contralaterally) the respective leg combination is indicated with a *blue frame*.

Most leg combinations that have been classified as 'undefined' deviate from either one of these rules (red and yellow frames), but their quantities differ noteworthy depending on the walking direction (Fig S2 C). Rearward walking can be characterized by a wide range of different types of leg combinations, where none is occurring in a clear majority. The undefined combinations that contradicts rule 1 either ipsilaterally or contralaterally (red and yellow frames) make up a percentage of 24% of all analysed frames, while during forward locomotion they account for only 2%. Nevertheless, the two leg combinations that do not contradict rule 1 (ipsilaterally and contralaterally, blue frames) make up 26% of the undefined combinations during backward locomotion. These two leg combinations are also found during forward locomotion, with a large proportion of 80% of all undefined leg combinations. These two combinations are representing an inchoate tripod coordination, missing the respective middle leg. They emerge during the transition between one tripod group (L1, R2, L3) to the next one (R1, L2, R3), where the middle legs are the last legs touching the ground (see phase plot analysis, Fig 3 B iii).

It should be noted that we applied the rule for this analysis in a deterministic manner. This means, for example, if one leg is in swing and its anterior or contralateral neighbour is also in

swing phase we marked the respective leg combination as violation to Cruse's rule 1. The rules generally should not be considered as deterministic but rather stochastic (Dürr 2005). Hence, rule 1 does not strictly prevent, the lift-off of the anterior and contralateral neighbour. Rather it affects the likelihood of a stance-swing transition.
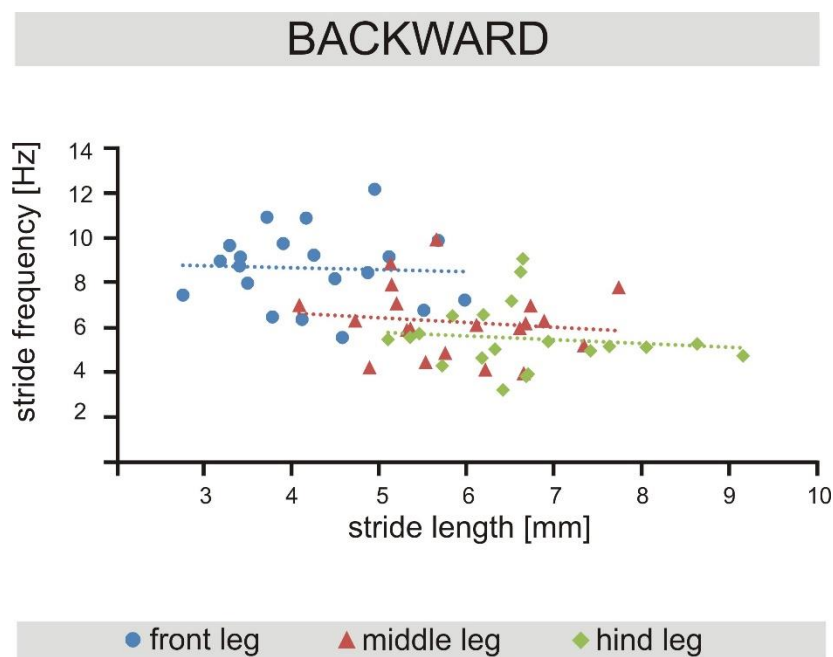
**Fig. S3. Stride length versus stride frequency during backward and forward walking.**

During backward walking stride length and stride frequency show no correlation. Note that the regression lines of the three leg pairs are separated in backward walks. This indicates a leg pair specific stepping specialisation, as already shown and discussed in our work.

The linear regression lines of front, middle and hind leg do not differ significantly in a multiple comparison regarding the slope and y-intercept (t-test for regression coefficient, $p<0.05$). Linear regressions for backward walking: front legs (y=-0.09x +9.05; R^2=0.0023), middle legs (y=-0.21x + 7.48; R^2=0.0157), hind legs (y=-0.17 + 6.63; R^2=0.0148).
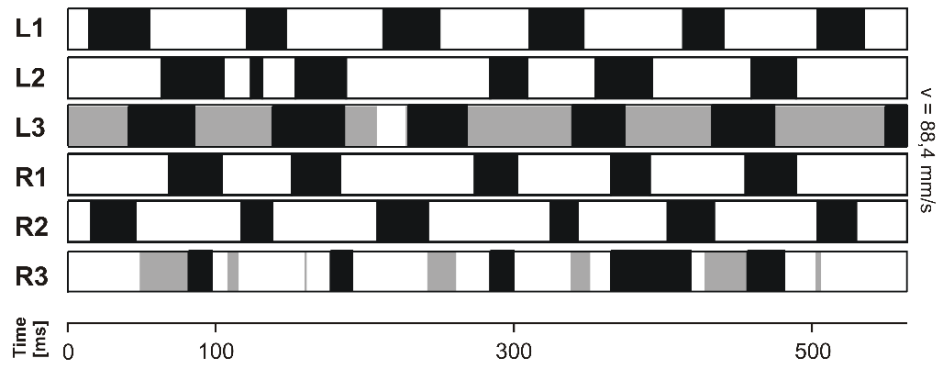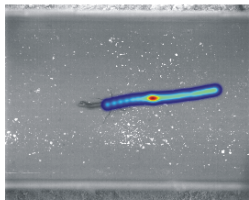
Video 1: Backward walking
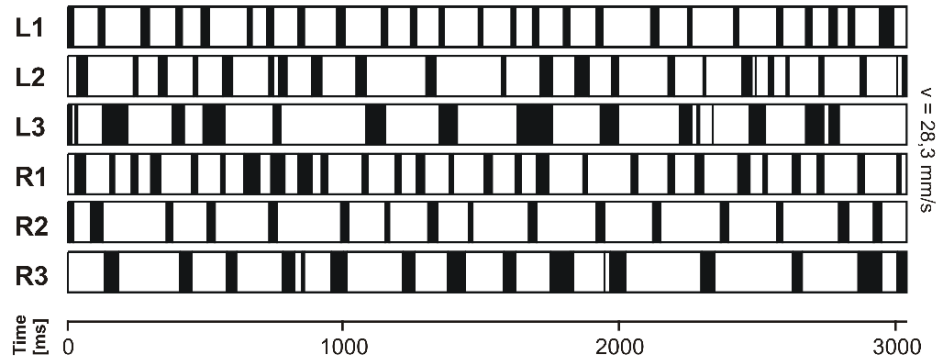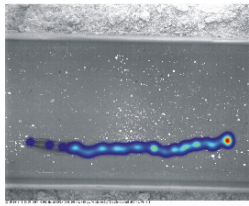
Video 2: Forward walking
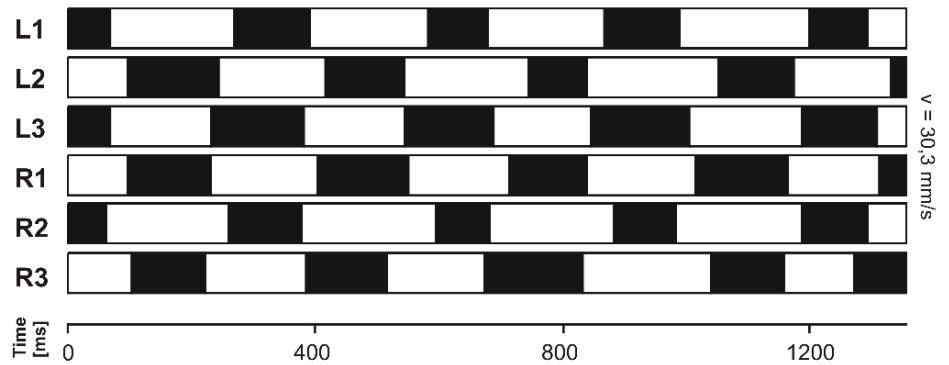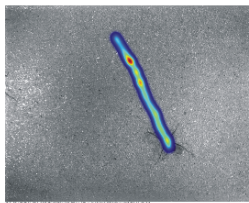
Video 3: Forward pulling

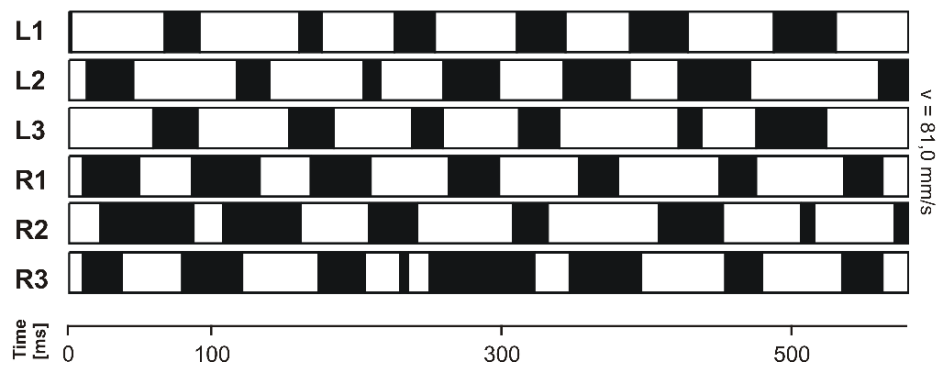## Video 4: Backward tripod-like coordination
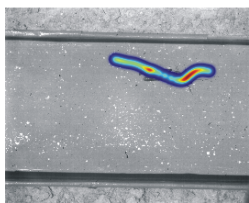
Fig. S4. Overview of supplementary movies.

Heatmaps are shown on the left side, while the corresponding podograms are shown on the right side for each video. Heatmaps give an impression about the walking speed of the ant. The warmer the colour (from dark blue to dark red), the slower the motion. Dark blue shows highest walking speeds. Podograms give an overview of inter-leg coordination. Black bars represent swing phases, white bars stance phases and grey bars leg dragging (tarsus moving along the floor without lifting off). L, left; R, right; 1, 2, 3, front leg, middle and hind leg. The videos are shown with 16.7 times slow motion.

Supplementary table 1. Linear regression functions and coefficients of determination (compare Fig. 4).

			Backward locomotion		Forward locomotion	
			linear regression formula	coefficient of determination	linear regression formula	coefficient of determination
A	stride length vs. walking speed	front	y = 0.0583x + 1.9617	R^2 = 0.5173	y = -0.0059x + 6.7386	R^2 = 0.0041
		middle	y = 0.0301x + 4.7375	R^2 = 0.1236	y = -0.0057x + 6.7401	R^2 = 0.0042
		hind	y = 0.0433x + 5.0196	R^2 = 0.2035	y = -0.0016x + 6.4333	R^2 = 0.0005
B	frequency vs. walking speed	front	y = 0.0855x + 5.3113	R^2 = 0.3127	y = 0.1217x + 1.331	R^2 = 0.6016
		middle	y = 0.1175x + 1.6271	R^2 = 0.6743	y = 0.1181x + 1.4614	R^2 = 0.617
		hind	y = 0.1015x + 1.5161	R^2 = 0.5877	y = 0.12x + 1.4469;	R^2 = 0.6314
C	swing speed vs. walking speed	front	y = 3.042x + 20.724	R^2 = 0.689	y = 1.4316x + 60.717	R^2 = 0.4376
		middle	y = 3.2678x + 58.231	R^2 = 0.3783	y = 2.2782x + 56.744	R^2 = 0.4003
		hind	y = 3.409x + 8.8227	R^2 = 0.5865	y = 1.289x + 48.329	R^2 = 0.4148
D	swing phase duration vs. walking speed	front	y = -0.0002x + 0.0378	R^2 = 0.0701	y = -0.0008x + 0.0903	R^2 = 0.2275
		middle	y = -0.0003x + 0.0465	R^2 = 0.1302	y = -0.0009x + 0.0877	R^2 = 0.2762
		hind	y = -0.0006x + 0.0765	R^2 = 0.0119	y = -0.001x + 0.1099	R^2 = 0.2753
E	stance phase duration vs. walking speed	front	y = -0.0015x + 0.1472	R^2 = 0.2075	y = -0.0032x + 0.2384	R^2 = 0.5892
		middle	y = -0.0024x + 0.2171	R^2 = 0.4691	y = -0.0031x + 0.2462	R^2 = 0.6011
		hind	y = -0.0023x + 0.2123	R^2 = 0.4438	y = -0.0028x + 0.2098	R^2 = 0.6098
F	cycle period duration vs. walking speed	front	y = -0.0017x + 0.187	R^2 = 0.55	y = -0.0039x + 0.3287	R^2 = 0.5342
		middle	y = -0.0025x + 0.2435	R^2 = 0.3178	y = -0.004x + 0.3339	R^2 = 0.5433
		hind	y = -0.0031x + 0.2919	R^2 = 0.5561	y = -0.0038x + 0.3197	R^2 = 0.5321

Supplementary table 2. Modified index of straightness for stance- and swing envelopes shown in Fig. 2 A.

The table shows the values of the modified index of straightness (Batschelet, 1981) describing the tortuousness of each stance- and swing envelope illustrated in figure 2. The median at the end of the table includes all legs of the respective column.

	Backward locomotion		Forward locomotion	
	stance phase	swing phase	stance phase	swing phase
L1	0.85	0.49	0.90	0.75
L2	0.65	0.80	0.93	0.60
L3	0.87	0.14	0.88	0.58
L1	0.86	0.57	0.94	0.48
L2	0.48	0.11	0.89	0.46
L3	0.80	0.48	0.94	0.79
median	**0.82**	**0.49**	**0.92**	**0.59**

Supplementary table 3. Exact percentage values of the walking speed groups (I-V) shown in Fig. 3.

The summarized results for the colour index analysis (Fig 3 Bi) were categorized according to the ants' walking speed I. 23-35 mm/s; II. 35-45 mm/s; III. 45-55 mm/s; IV. 55-66 mm/s; E, Entire group. The values are rounded percentage information.

	Backward locomotion					Forward locomotion				
	I	II	III	IV	E	I	II	III	IV	E
Number of videos	10	4	4	2	20	11	2	3	4	20
Tripod	3	8	12	12	7	54	61	70	69	56
Tetrapod	14	19	21	21	18	10	6	4	5	9
Wavegait	38	27	32	30	34	17	13	9	3	16
Undefined gait	33	39	30	30	32	7	12	16	20	8
Hexa support phase	12	7	5	7	9	12	8	1	3	11

References

Batschelet, E. (1981). Circular Statistics in Biology. London: Academic Press.

Dean, J. and Wendler, G. (1983). Stick insect locomotion on a walking wheel: interleg coordination of leg position. *J. Exp. Biol.* **103**, 75-94.

Dürr, V. (2005). Context-dependent changes in strength and efficacy of leg coordination mechanisms. *J. Exp. Biol.* **208**, 2253-2267.

Schilling, M., Hoinville, T., Schmitz, J. and Cruse, H. (2013). Walknet, a bioinspired controller for hexapod walking. *Biol. Cybern.* **107**, 397-419.

Wilson, D. M. (1966). Insect walking. *Annu. Rev. Entomol.* **11**, 103-122.