Level locomotion in wood ants: evidence for grounded running

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SUMMARY
In order to better understand the strategies of locomotion in small insects, we have studied continuous level locomotion of the wood ant species *Formica polyctena*. We determined the three-dimensional centre of mass kinematics during the gait cycle and recorded the ground reaction forces of single legs utilizing a self-developed test site. Our findings show that the animals used the same gait dynamics across a wide speed range without dissolving the tripodal stride pattern. To achieve higher velocities the ants proportionally increased stride length and stepping frequency. The centre of mass energetics indicated a bouncing gait, in which horizontal kinetic and gravitational potential energy fluctuated in close phase. We determined a high degree of compliance especially in the front legs, as the effective leg length was nearly halved during the contact phase. This leads to only small vertical oscillations of the body, which are important in maintaining ground contact. Bouncing gaits without aerial phases seem to be a common strategy in small runners and can be sufficiently described by the bipedal spring-loaded inverted pendulum model. Thus, with our results, we provide evidence that wood ants perform "grounded running".

KEY WORDS
*Formica polyctena*, arthropod, ground reaction force, insect biomechanics, ant locomotion, grounded running
INTRODUCTION

Among insects, extremely fast and agile species exist that can easily adapt their locomotor performance to a variety of substrates and inclines (Graham and Cruse, 1981; Full and Tu, 1991; Duch and Pflüger, 1995; Larsen et al., 1995; Pelletier and Caissie, 2001; Goldman et al., 2006; Gladun and Gorb, 2007; Seidl and Wehner, 2008; Sponberg and Full, 2008; Weihmann and Blickhan, 2009). For example, experiments on the desert ant *Cataglyphis fortis* revealed running speeds of up to 50 body lengths per second on level ground (Seidl and Wehner, 2008; Weihmann and Blickhan, 2009). Similar relative velocities were also measured in the cockroach species *Periplaneta americana* (Full and Tu, 1991).

From a biomechanical point of view, questions regarding principles of locomotion or commonly used type of gaits arise. It is well known that insects maintain the alternating tripod gait pattern without aerial phases over wide ranges of speeds and slopes (Hughes, 1952; Delcomyn, 1971; Full et al., 1991; Full and Tu, 1991; Zollikofer, 1994; Goldman et al., 2006; Seidl and Wehner, 2008; Weihmann and Blickhan, 2009). Only at their highest speeds and in individual cases *Periplaneta americana* changed to quadrupedal and bipedal running (Full and Tu, 1991).

The faster locomotion of cockroaches was characterized as a bouncing gait, in which horizontal kinetic and gravitational potential energy of the centre of mass (CoM) change in phase (Full and Tu, 1990). This pattern of energy fluctuations are typical for running, trotting or hopping in various animals and can be described by simple spring-loaded inverted pendulum (SLIP) models (Blickhan and Full, 1993). In addition, SLIP-conform ground reaction force patterns were determined in cockroaches. While the SLIP model is valid for the sagittal plane, an analogue model for the horizontal plane - the lateral leg-spring (LLS) model - has already been developed and validated with cockroaches (Schmitt et al., 2002). The SLIP model consists of a point mass on top of a compliant spring, which is compressed during the first half and decompressed in the second half of stance. Hence, the CoM reaches its minimum height during midstance and at the same time has the lowest speed. This is different from the situation in the inverted pendulum (IP) model, which is used as a standard model for a walking gait (Cavagna et al., 1976). In this model, through vaulting over a stiff supporting leg, a cyclic exchange from kinetic to potential energy and vice versa is achieved and the CoM describes a circular path with the highest point and the lowest velocity at midstance. In reality stiff legs rarely occur and CoM-dynamics is better described by a bipedal spring-loaded inverted pendulum (BSLIP, Geyer et al., 2006) both for walking as well as bouncing gaits with double support phases. This model has been adapted to experimental data of different gaits in humans, horses, quails and cockroaches (Geyer et al., 2006; Srinivasan and Ruina, 2006; Srinivasan and Holmes, 2008; Lippert et al., 2012; Andrade et al., 2013b; Andrade et al., 2013a). Despite leg compliance the lift of the CoM at midstance remains to be the criterion for a walking gait whereas lowering the CoM indicates a bouncing gait. In both gaits, inertia contributes to energy conversion and recovery. Mechanical energy can be stored and released as elastic energy through spring elements or kinetic and potential energy can be transformed into each other by a pendular exchange (Cavagna et al., 1964; Cavagna et al., 1976; Heglund et al., 1982).

To distinguish between bouncing and vaulting mechanics two measures can be considered: (i) the percentage of energy recovery and (ii) the percentage of congruity (Cavagna et al., 1976; Ahn et al., 2004). Congruity conveys the similarity between the curve progressions of kinetic and potential energy...
(Ahn et al., 2004). Thus, a congruity of 100% is the result when both curves have an identical trend throughout the whole gait cycle. Correspondingly, congruity compiles 0% in the case of the IP and 100% in the SLIP model. The percentage of recovery expresses the magnitude of energy exchanged between potential and kinetic energy by the pendular mechanism (Cavagna et al., 1976). Both energy forms are out of phase in the IP and consequently the percentage of energy recovery approaches 100%. This value however cannot be reached due to losses in the collision (Ruina et al., 2005). While running, there is no exchange between these energies in the SLIP, and energy recovery amounts to 0% in this conservative model. Depending on the stiffness of the legs and style, the BSLIP-model recovery can assume all values between 0% and 100% (ignoring collision loss). The recovery values measured for the cockroach varied with a mean of 15.7%, supporting a bouncing gait (Full and Tu, 1990).

Elastic structures in the legs are advantageous for bouncing gaits. Since the joint axes are aligned vertically in the hind leg of cockroaches, the exoskeleton can operate as an energy-conserving spring while running (Dudek and Full, 2006). However, this does not apply for all insect species. For instance, in the case of ants, the leg plane is orientated perpendicular to the substrate. Consequently, the joint axes are orientated parallel to the ground and the exoskeleton cannot function as a passive spring element without contribution of muscles and apodemes in the joints. Nevertheless, due to the existence of resilin, a certain degree of passive elasticity cannot be ruled out in the ant leg (Weis-Fogh, 1960). This long-chained protein is ideally suited as an energy store and has been found between the leg segments of several insects (Andersen, 1963; Andersen, 1964; Andersen and Weis-Fogh, 1964; Alexander, 1966; Anderson, 1966; Sannasi, 1969; Neff et al., 2000; Bennet-Clark, 2007; Patek et al., 2011; Michels and Gorb, 2012).

However, it is unclear to what extent resilin may support bouncing dynamics and, furthermore, the magnitude of energy recovery mechanisms in small insects has not yet been elucidated. It has even been argued that animals weighing less than 1 kg are not using energy recovery and locomotion is generated and maintained by muscle contraction alone (Reilly et al., 2007). In addition, the influence of relative friction and viscosity (damping ratio) of the limbs is notably higher in ants than in larger animals, due to their extremely low weight and size (Garcia et al., 2000). Moreover, forces like drag and those involved in securing a foothold hinder propulsion as well (Full and Koehl, 1993; Federle et al., 2000), and the energetic cost of locomotion are disproportionally high (Full, 1991).

From this point of view, it is difficult to predict which movement strategy ants are using. The concepts on global CoM-dynamics (IP and SLIP models) introduce virtual legs, which are generated by the cooperation of the legs constituting the tripod. Using compliant legs (BSLIP model), walking gaits are possible with much reduced vertical excursions of the CoM. Furthermore, the virtual leg may pivot about a virtual hip located above the animal. Nevertheless, considering the findings in other insects we expect to find a bouncing gait, if inertia plays any role for an insect of such a small size. In order to examine this issue, we analysed the three-dimensional CoM kinematics of Formica polyctena and measured its ground reaction forces (GRF).
RESULTS

Mass distribution

In Table 1 and Fig. 1 the results of the mass distribution are given. The cumulated segment masses resulted the same values as the weighing of the living animals (paired t-test, $P = 0.01$). A large proportion (58%) of the total mass ($m$) was concentrated in the gaster (abdomen). Accordingly, the total CoM has to be near this body part. The variability in the gaster mass (s.d.: 3.1 mg or 14%) was high due to its variable filling level (Josens et al., 1998). This variability determined completely the variability of the total mass of the ant. Levene’s test for homogeneity of variance revealed that the variances of both groups do not differ ($P = 0.97$). All other segments showed only minor variations of about 1% body weight. Moreover, the gaster weight strongly correlated with the total mass of the animals (Pearson correlation: $r = 0.98$). The mass of all legs added up to only 10% of body weight and all had approximately the same size. Thus, the weight of a single leg does not exceeded 2% of the ant’s total mass.

Stride pattern

In our experiments, Formica polyctena showed a tripodal stride pattern with no more than three legs in the air at any time. There were overlapping phases of both tripods (double support) in which all legs were on the ground partially. The middle leg initiated the contact phase and touched the ground a bit earlier than the other two legs of the same tripod (R2, Fig. 2). An average step cycle in the investigated velocity range (9.5 cm/s to 12.5 cm/s) lasted 85.8±3.2 ms (mean ± s.d.) which corresponds to a stepping frequency ($f$) of 11.7±0.4 Hz. For all legs, the contact phase was longer than the swing phase. This applied in particular to the middle legs, where the duty factor (du) was 0.62 (cp. Table 2). At the front and hind legs the contact phase was clearly shorter and the duty factor accordingly smaller. Most step parameters were speed-dependent (Fig. 3). With increasing speed ($v$), the contact time ($t_{co}$) linearly decreased while swing time ($t_{sw}$) remained constant at about 35 ms (Fig. 3A). The intersection point of both regression lines was at 18.8 cm/s. Thus, at speeds above this value, the swing phase was longer than the ground contact and the duty factor fell below 50% (Fig. 3C). As expected the contact ($s_{co}$) and swing width ($s_{sw}$) increased with speed. Again, there was a linear relationship between both variables (Fig. 3B). Stride length ($s_{cy}$) and stepping frequency were also linearly dependent on speed (Fig. 3E-F).

Three-dimensional motion of the CoM

In a single step cycle, the CoM covered a distance of 9.4±0.7 mm in antero-posterior direction ($x$, Fig. 4). The forward speed ($v_x$) rapidly decreased in the double support phase and steadily increased during the remainder of the gait cycle. Correspondingly, negative accelerations in the x-direction ($a_x$) were found during the double support. In the lateral direction ($y$), the CoM oscillated around zero. Around midstance the maximum lateral displacement of about 0.1 mm was reached at the side where two legs were on the ground, and subsequently the CoM swung back to the other side. On average the CoM was 2.5±0.4 mm above the ground ($z$) and fluctuated around this value sinusoidal with double step frequency. In the initial stance phase until the end of double support, the CoM was lowered by about 0.1 mm. Thereafter it was raised again to its highest point at midstance. In accordance with this, the vertical velocity was positive until midstance. In the phases where all legs were on the ground, accelerations of up to 5 m/s² were registered.
CoM mechanics in the contact phase

For evaluating the gait dynamics, we calculated the CoM energetics during the contact phase of one tripod in all investigated steps. Besides linear regression analysis (Fig. 3G-I), the influence of running speed on CoM mechanics was determined by classifying the trials into two velocity groups (G) using a median split. Thus, we obtained one sample with slower (G1, \( v < 11.5 \) cm/s, \( N = 43 \)) and one with faster runs (G2, \( v > 11.5 \) cm/s, \( N = 42 \)). Both in G1 and in G2 potential (\( E_{\text{pot}} \)) and kinetic (\( E_{\text{kin}} \)) energy proceeded nearly in phase in a sinusoidal pattern (Fig. 5A). Around 20-30% of contact a first minimum occurred in \( E_{\text{pot}} \) and \( E_{\text{kin}} \). Kinetic energy reached its maximum value between 65 and 75% at all speeds. The maximum of the potential energy, i.e. the highest CoM position, was found between 60 and 80%, whereby the maximum was reached later at G2. The CoM was raised at higher speeds (Fig. 5B and Table 3). Although the regression analysis revealed only a weak correlation (\( r^2 = 0.1 \)) between \( v \) and \( h_{\text{CoM}} \) (Fig. 3G), we found significantly higher values for \( h_{\text{CoM}} \) (two-tailed \( t \)-test, \( P = 0.02 \)) in G2. Consequently, the potential energy oscillated around a higher value at G2 (0.56 µJ) compared to G1 (0.48 µJ, cp. Fig. 5A). Due to the greater velocities (cp. Table 3), \( E_{\text{kin}} \) was higher in G2, as well. For the percentage of mechanical energy recovery (\%Rec) we calculated values around 16-18% for the two speed ranges, which are not significantly different from each other (Fig. 5C and Table 3). In accordance to this, we found high values (60-70%, Fig. 5D and Table 3) for the congruity of the curve progressions of kinetic and potential energy (\%Con). However, the comparison of means revealed significantly lower congruity values in G2 than in G1 (two-tailed \( t \)-test, \( P = 0.02 \)). Nevertheless, \%Con was on average higher than 50% in both groups what indicates a running gait.

Three-dimensional body kinematics

In Fig. 6 the trajectories of the body markers of all investigated steps as well as the tarsi positions of all legs are shown in the sagittal and horizontal plane in a body-fixed coordinate system. Tarsi positions are slightly curved (Fig. 6B). Consequently, during one contact phase the CoM swung to the side where two legs were on the ground and back into the initial \( y \)-position. The Marker B3 was located on average less than 0.3 mm away from the CoM. The points digitized more cranially (B1/B2) seemed to move along an arcuate path around a pivot point located close to the CoM (Fig. 6A). However, the gaster tip (B4) remained nearly at the same spot and kept its orientation (see below). Moreover, during the entire individual step cycle, the angular position of the three main segments to each other varied little. The peak-to-peak amplitudes of all rotational movements amounted to less than 5° (Fig. 7A). In particular, the angle between caput and thorax (\( \beta \)) almost did not change but remained at 139.4±1.9° (Table 4). The same applied to the gaster-substrate-angle (\( \epsilon \)), which on average remained at 41.4±2.3° with an angular velocity around zero (Fig. 7B). The most distinct rotational motion was the pitch movement of the thorax-caput complex around the petiolus and is reflected in \( \alpha \) and \( \delta \). Due to the steady gaster position both showed an almost identical cosinusoidal time course with two oscillations per gait cycle. On average \( \alpha \) oscillated around 22.7±2.3° while \( \delta \) amounted to 161.3±2.3°. This rotation was performed at angular velocities of up to ±500 °/s (Fig. 7B). We calculated the rotational energy of pitch and found maximum values of 0.4 nJ. These are about 50 times smaller than the fluctuations of the kinetic and potential energy of the CoM (Fig. 7C). On average these two energy forms oscillated in a range of ±20 nJ with the same amplitudes. The yaw (\( \zeta \)) angle had a sinusoidal profile around 0° with one oscillation per stepping cycle.
Three-dimensional ground reaction forces

The front and middle leg pair generated only minor propulsive forces over the entire contact phase \((F_x > 0, \text{Fig. 8})\), and braked especially during the first half of stance. Contrary to this, the hind legs produced relatively high positive forces in running direction before slightly braking towards the end. In the lateral \((F_y)\) direction, the middle as well as the hind leg produced consistently negative forces (i.e. pressing them outward). However, we registered no significant forces in the lateral direction in the front legs. All legs generated positive, substrate normal forces (pressing on the substrate), whereby the time courses of the middle and hind leg were very similar. Up until 30% of stance both time courses steeply increased up to 0.5 body weight and subsequently fell off continuously. At midstance, the three legs all had the same force values (one-third body weight). The front legs generated the same vertical force over a major part of the contact. Thus, the body weight was shifted more and more towards the cranial legs. With knowledge of the individual leg forces and the stride pattern, we calculated the total leg-generated force acting on the CoM (Fig. 9A). While the vertical \((F_z, \text{red})\) and fore-aft force \((F_x, \text{blue})\) went through two cycles per step, only one period was to be seen in the lateral direction \((F_y, \text{green})\). \(F_z\) on average corresponded to body weight and oscillated around this value with an amplitude of 0.4 body weight. After the overlap phase until midstance this force was higher than the weight force. The maximum force was generated at about 20% and 70% of the gait cycle and then continuously decreased until the next double support to its minimum. \(F_x\) oscillated around zero and was negative during double support. At around midstance this force component reached its maximum of 0.3. \(F_y\) fluctuated around zero in a sinusoidal pattern with amplitudes below 0.1.

Subsumption

In the following paragraph, we use a representative image sequence (Fig. 10) to describe the stepping pattern, the kinematics, and dynamic contributions of the individual legs during level locomotion in *Formica polyctena*. This comprehensive description provides the background for the considerations in the discussion. All three legs can be considered as two-segmented in a simplified manner. Viewed in the sagittal plane, the distal segment (tibia, metatarsus and tarsus) of the front leg reaches the ground at an angle of about 50° at the moment of touchdown, while the femur is orientated approximately 10° to the horizontal. Consequently, the angle between both segments is around 120°. In the course of stance, this angle decreases up to 55° while the distal segment tilts over the contact point and the angle of attack is around 110° at lift-off. Meanwhile, the hind leg extends during the stance phase. As a result, the angle in the femur-tibia joint increases from around 55° to 140° while the approach angle of the distal segment decreases from 65° to 30°. A different situation exists in the middle leg, since it operates sideways in an extended position. The almost constant angle in the femur-tibia joint causes only small changes in the effective leg length, i.e. the distance between coxa and tarsus tip. Due to the same coxa height, these changes can be determined in the top view and amounts to less than 7%. The angle of attack of the effective leg ranges between 25° and 30°. This happens while the leg is loaded with a large portion of the body weight, especially in the vertical and lateral direction and particularly during the first half of stance \((F_y \text{ and } F_z, \text{Fig. 8})\). Thus, a relatively high leg stiffness is indicated, which is in any case sufficient to compensate the lateral forces of the contralateral legs. This causes the CoM to be slightly deflected to the side were two legs are on the ground (\(y, \text{Fig. 4 and Fig. 6B}\). However, when considering the sagittal projection, the leg is clearly compressed during midstance. Compared with the length at the
moment of touchdown or lift-off (2.8 mm), effective leg length is reduced by 18% at midstance. An even
greater shortening can be found in the front leg. The sagittal coxa-tarsus-distance decreases from about
4.5 mm at touchdown to 3 mm at midstance and finally to 2.5 mm at lift-off. Thus, leg length is reduced
by 33% already in the first half, and by another 11% in the second half of the contact phase. This
happens mainly through flexion in the femur-tibia joint, which causes an erection of the femur and
forward pitching of the thorax (decreasing \( \alpha \), Fig. 7A). The gaster position (i.e. its height and its angle to
the substrate; \( \epsilon \), Fig. 7A), remains unaffected by pitch. Instead, its fluctuations are erratic, uncoordinated
and clearly decoupled from the thorax oscillations (Fig. 7B). In this process, the front leg is braking
\( (F_x < 0, \text{Fig. 8}) \) before it contributes slightly to propulsion in the second part of contact \( (F_x > 0, \text{Fig. 8}) \).
Since the hind leg is pushing forward as well, running speed \( (v_x, \text{Fig. 4}) \) and kinetic energy \( (E_{kin}, \text{Fig. 5A}
and Fig. 7C) \) continue to increase until the legs of the other tripod hits the ground.

**DISCUSSION**

**Tripod conservation**

Similar to other fast moving insects, the walking legs of wood ants are organised in stereotyped
alternating tripods. The front and rear leg of one side and the middle leg of the other side move
simultaneously during a step, while each moves out of phase with its contralateral pair (Hughes, 1952;
Delcomyn, 1971; Zollikofer, 1994). Throughout the entire contact period a statically stable situation was
ensured, since the CoM (red dot, Fig. 10) was always within the support area of the tripod (Ting et al.,
1994).

In our experiments, we found no significant adoptions in gait dynamics to running speed over the
investigated range from 7.8 to 20.8 cm/s (10-26 body lengths/s). In none of the recorded video
sequences a flight phase was observed and the tripodal gait pattern with double support phases was
instead maintained. As shown in Fig. 3C the duty factor decreased linearly with increasing speed. At
speeds of 8 cm/s, this parameter was around 0.65. Through linear regression, we estimated that the
duty factor should fall below 0.5 at a velocity of approximately 19 cm/s. Consequently, the double support
phase should disappear and flight phases occur. Thus, taking the flight phase as a criterion a gait change
would be the result. In contrast, this could not be proven, although we determined values below 0.5 in a
few trials. This is most likely due to measurement inaccuracies. Since the sample rate of kinematics was
500 Hz phase durations could only be determined with an error of ±4 ms. Based on linear regression
analysis we expect a gait cycle time around 70 ms at running speeds near 20 cm/s (Fig. 3A). With an
assumed contact duration of 35 ms (duty factor of 0.5), the estimated duty factor can range between
0.42 and 0.59 within the specified range of error. Since we could not find runs above 21 cm/s and we
did not observe aerial phases, we conclude that *Formica polyctena* does not perform a gait change.
Therefore, we conclude that this is the maximum possible velocity of this species.

This assumption is confirmed by other studies on the closely related species *Formica pratensis*. In more
than hundred trials, the authors did not find velocities above 21.8 cm/s (Seidl and Wehner, 2008;
Weihmann and Blickhan, 2009). The preferred speed range of *F. pratensis* was between 11 and 14 cm/s.
Furthermore, a mean CoM height of 1.9 mm with typical vertical oscillations of 0.1-0.2 mm was
determined (Weihmann and Blickhan, 2009), which is congruent with our results. In addition, Seidl and
Wehner found a linear relationship between speed and step frequency as well, with maximum values of
20 Hz in their fastest trials (Seidl and Wehner, 2008). In our fastest runs the durations of the contact and swing phase were measured around 30 ms (Table 2 and Fig. 3A). This is associated with a stepping frequency of approximately 16 Hz (Fig. 3F). Across the investigated speed range, the swing phase duration remained nearly constant at 35.4±4.4 ms. Hence we conclude that the ants performed the swing phase always as quickly as possible, most likely in order to quickly reach ground contact again. Since the step length is limited by leg length, higher velocities could only be achieved by higher step frequencies. This in turn means that both the contact and the swing time must be reduced if the duty factor should stay above 0.5. Thus, the maximum speed seems to be determined by the duration of the swing phase or rather the ability of the animals to perform this movement.

**Vaulting or bouncing**

At first glance, the absence of aerial phases and the related duty factors above 0.5 suggest a walking gait. This type of gait is typically described by the inverted pendulum model, whereby the CoM is vaulting over a stiff supporting leg (Cavagna et al., 1976). Following this model, it is to be expected that the CoM reaches its highest point at midstance. Furthermore, kinetic and potential energy should be out of phase, which would also be expressed in a high percentage of energy recovery and a small percentage of congruity (Cavagna et al., 1976; Ahn et al., 2004). Our findings do not support major energy exchange, since kinetic and potential energy are in phase for long periods of the contact phase (Fig. 5A and Fig. 7C). Accordingly, the congruity between the curve progressions of both energy forms is relatively high. We determined congruity values of 60-70% and a recovery of 16-18% (Fig. 3H-I, Fig. 5C-D and Table 3). Furthermore, the CoM did not reach its highest point before 60% of stance time (Fig. 4 and Fig. 5A). These results appear to be consistent with those of the cockroach *Blaberus discoidalis*. Over a speed range from 0.08 to 0.66 m/s (2-16 body lengths/s), these fast-moving insects use the alternating tripod gait without aerial phases as well (Full and Tu, 1990). Furthermore, the maximum stride frequency of 13 Hz was very close to that of *Formica polyctena*. For the cockroaches, recovery values varied around a mean of 15.7% and did not vary as a function of speed. This is equivalent to our calculations and leads the authors to also reject the inverted pendulum model.

It should be noted that in the transition region between walking and bouncing (i.e. especially within the transition region to grounded running) the measures of recovery and congruity are ill-defined. This transition region is characterised by small excursions of the CoM. With diminishing vertical excursions of the CoM and constant variance, recovery approaches zero and the phase of the potential energy cannot be determined reliably. In our case the vertical displacements of the CoM of about 10% body height is within the magnitude of the standard deviation.

However, even without aerial phases, the gait of cockroaches was classified as a bouncing gait (Full and Tu, 1990; Full et al., 1991; Blickhan and Full, 1993; Srinivasan and Holmes, 2008). This is not an isolated case as several species, such as birds, crabs, primates, horses, marsupials and elephants show bouncing mechanics with duty factors above 0.5 (e.g. Alexander and Jayes, 1978; Blickhan and Full, 1987; Gatesy and Biewener, 1991; Kimura, 1996; Muir et al., 1996; Gatesy, 1999; Schmitt, 1999; Hutchinson et al., 2003; Schmitt, 2003; Srinivasan and Holmes, 2008; Biknevicius et al., 2013). These gaits are known under terms as “compliant walking” (Alexander and Jayes, 1978), “grounded running” (Rubenson et al., 2004) or “Groucho running” (McMahon et al., 1987). So far, the most suitable model to describe all these types of locomotion is the bipedal spring-mass model (BSLIP, Geyer et al., 2006).
In this model, during compliant walking, the CoM is at its apex at midstance similar to the inverted pendulum. Nevertheless, the BSLIP is purely conservative and entails energy storage. Correspondingly, energy recovery in the model is diminished to values less than 50%. Small birds for example only reached values around 30% during walking (Nyakatura et al., 2012). The BSLIP model can also be used to describe grounded running (Andrada et al., 2013a). In this gait, the CoM is not raised above its height at touch down, but double support is maintained. This gait seems to play a very prominent role in small animals with compliant legs (i.e. small birds). In these animals the recovery values during grounded running are about 7% (Nyakatura et al., 2012). Our findings give rise to the assumption that wood ants also use this type of locomotion. More specifically the mechanical compliance, demonstrated in the front and middle leg of *Formica polyctena*, leads to only small vertical oscillations of the CoM and supports nevertheless the maintenance of ground contact. We conclude that this is therefore very likely a main objective during locomotion in these animals.

**Elastic elements in the insect leg**

On the assumption that the investigated ants conducted grounded running, we hypothesise that elastic elements could be of advantage. As previously mentioned a rubber-like protein called resilin is known as a component of the insect cuticle and can provide elasticity to the legs (Weis-Fogh, 1960). In cockroaches resilin was studied at the tibio-tarsal joint and the articulation between the fourth and fifth tarsal segments of the hind leg (Neff et al., 2000). This spring-like elasticity in the cockroaches' hind leg was provided by resilin in connection with the exoskeleton, as demonstrated by Dudek and Full (2006). According to the authors, as much as 75% of the mechanical energy of the CoM may be returned each step. Moreover, 40% of the external mechanical work done to the CoM can be stored and returned due to the elasticity of the leg. Nevertheless, this is considered as an overestimate of energy storage and return in this system and it is assumed that in reality these springs only contribute marginally in improving energetic efficiency in cockroaches (Patek et al., 2011). More likely, it is supposed that the leg springs function as a kind of shock absorber, which responds to perturbations more quickly than a neural signal could (Koditschek et al., 2004; Dudek and Full, 2006, 2007). Thus, locomotion can be stabilised by elastic legs. As resilin pads can be found within the joint apparatus they are predestined to act as elastic antagonists to the leg muscles (Neff et al., 2000). In this way, it is possible to produce restoring forces without muscle activity. Additionally, it is also conceivable that this effect is used as a drive for the leg extension in the swing phase. As previously mentioned, leg compliance in the front leg of *Formica polyctena* is mainly reached by flexion in the femur-tibia joint. This leads to an eccentric load of the leg extensor muscles during the contact phase. Therefore, we assume that there is a considerable contribution to leg compliance by muscle activity. However, in future studies, skeletal anatomy and muscle architecture of walking legs of fast running ant species should be examined in more detail to allow for a discrimination of the mechanisms.

**Mechanical cost of transport**

The investigated ants moved with a preferred speed around 11 cm/s, a step length of approximately 9 mm and a step frequency of 12 Hz. Consequently, they pass 111 gait cycles in 9 s to cover a distance of 1 m. On their daily foraging trips wood ants travel distances of up to 200 m (Kirchner, 2001). This calculates to 22,200 steps on level surfaces. However, the habitat of wood ants is far away from being...
flat and they permanently have to face obstacles along their path when moving over stick and stone. Consequently, it can be assumed that they take many more steps to cover such distances in the wild. Furthermore, the locomotion is acyclic and intermittent, and therefore conditions for energy-saving mechanisms through pendular or spring mechanics are rather disadvantageous.

It has been unclear whether these strategies play a role in small animals, with a body weight below 1 kg (Reilly et al., 2007). Although the relative amount of external mechanical energy used to move the CoM is independent of body size, the metabolic costs of transport are much higher in smaller animals (Full, 1991; Reilly et al., 2007). Reilly et al. (2007) came to the conclusion that locomotion is generated and maintained by muscle contraction alone and that these animals benefit little from passive energy-saving mechanisms. For lizards (mass = 21 g) and squirrels (mass = 238 g) they calculated that the metabolic energy saved by pendular exchange and elastic energy recovery is less than 2%. This holds especially true for the crouched posture, which is typical for small animals, and is associated with more abducted and flexed limbs and causes higher metabolic cost due to a higher muscular effort (Biewener, 1990; Reilly et al., 2007; Biknevicius et al., 2013). Furthermore, it was shown that metabolic costs are mainly reduced by an adjustment of the stepping frequency (Reilly et al., 2007). Experiments on stick insects, cockroaches and mice showed that passive forces of leg muscles and joint structures are relatively large in animals with low-weight limbs compared with gravitational forces (Garcia et al., 2000; Hooper et al., 2009). This is particularly reflected in a higher muscular activity during the swing phase in order to overcome these forces and results in a further increase of metabolic costs. Under these circumstances, running (i.e. bouncing dynamics) is preferred over walking in small animals (Garcia et al., 2000). The same most likely also applies to ants and confirms the hypothesis that ants perform grounded running. Since the mass of the abdomen, which amounts to nearly 60% of the body mass (Table 1 and Fig. 1), swings independent from the rest of the body, these fluctuations do not contribute to the rhythmic changes of the body’s rotational energy. The decoupling indicates a compliant connection and significantly reduces the amount of rotational energy of the animal’s body.

Ground reaction forces

Due to the modified experimental procedure (cp. materials and methods) we caused a behaviour modification in comparison to our previous studies (Reinhardt et al., 2009). In this way, exploratory behaviour was strongly reduced and the ants were trained on steady fast locomotion. Naturally, these changes were associated with changes in the ground reaction forces. This is particularly evident in the substrate-parallel forces ($F_x, F_y$) of the front and middle leg. In the previous experiments, the lateral force of these legs showed a sinusoidal curve progression, which is equivalent to an initial outward pushing and a later inward pulling during the second part of stance. The situation was similar in the fore-aft force of the front leg, which firstly braked and later accelerated the body forwards. Our recent investigations determined a clearly smaller forward impulse of the front leg in the second part of the contact phase and no sign change in the lateral force of the middle leg. The front leg produced nearly no force in this direction. However, the middle leg hardly contributes to propulsion. Almost identical curve progressions were identified for the hind leg, providing evidence again for its role as the primary propulsion unit. The force patterns of the first test series revealed similarities to vertical climbing cockroaches, which were particularly apparent in the lateral force component of the two front legs (Goldman et al., 2006; Reinhardt et al., 2009). The ants probably ensured ground contact through permanently clinging to the
substrate by laterally pulling towards the midline during the second half of stance. Thus, the behaviour of the animals was primarily aimed at exploring the unfamiliar surroundings and not at overcoming a known path as fast as possible. This is also confirmed by the detected ground contact of the gaster tip, which is necessary to set up a pheromone trace (Horstmann, 1976). Since the gaster touched the ground in none of the analysed trials of the current test series, we conclude that an ant trail was already established through the modified experimental procedure. As our results indicated, the ants ran significantly faster on this familiar path. Hence, it is assumed that the recent results reflect rather the fast, straight locomotion in *Formica polyctena* than the previous ones.

When evaluating the ground reaction forces more similarities emerge to those of fast running than to climbing cockroaches (cp. Full et al., 1991; Goldman et al., 2006). The vertical force component regularly predominates and the sagittal force vectors are therefore standing steep over a wide duration of the contact phase in all legs (Fig. 9B). Furthermore, no inward directed lateral forces were found in any leg. The sum of the leg-generated forces (Fig. 9A), which could be considered to be the total force acting on the CoM, was oscillated in a similar manner (Full and Tu, 1990). In the fore-aft direction, an initial negative impulse is cancelled out by a positive one in the second half of stance. The amplitude was around 0.2 body weight and thus similar to *F. polyctena*. Similar to our experiments, the vertical component fluctuated bimodal around the body weight, whereby the maximum value was not larger than 1.5. Following the calculations of Andrada and colleagues (2013a) these values are typical for grounded running, while aerial phases, such as in running, do not occur until 1.7 body weight. Finally, the asymmetric curve progression of the total vertical ground reaction force (Fig. 9A) is typical for grounded running, as this also occurs in quails (Andrada et al., 2013a). A further consequence of this context is that grounded running in ants more likely occurs in steady, fast locomotion than in exploratory behaviour.

**Conclusions**

In conclusion, there is evidence that the investigated ants performed grounded running. This type of gait in small insects combines high running speeds with small vertical oscillations of the CoM necessary to ensure ground contact (Blickhan and Full, 1993). Flight phases would drastically increase the risk of falling, since their habitat is typically strongly structured and extremely rough for ants. Furthermore, the ability to perform fast turns is hereby guaranteed. Something which in the wild may be more relevant than energy saving or speed (Gatesy and Biewener, 1991; Daley and Usherwood, 2010). In other words, the fact that a bouncing gait could be identified may be less a matter of efficiency but of control. Even in such a small animal the inertia of the system contributes to the gait pattern, influences its dynamics and may help to improve stability (Ting et al., 1994; Full et al., 2002; Blickhan et al., 2007).

**MATERIALS AND METHODS**

**Animals**

The presented studies were conducted between summer and autumn on workers of the local ant species *Formica polyctena*. All animals were taken from the same nest in a forest near Jena and subsequently kept in a formicarium in the lab under constant and natural conditions (cp. Reinhardt et al., 2009). We used nest material as substrate, wetted it at regular intervals and the ants were fed honey and insects. A part of the formicarium was illuminated by a 60 W daylight lamp in a 12 hours rhythm. For an individual
experiment, a single ant was randomly removed from the formicarium and weighed by a precision balance (ABS 80-4, Kern & Sohn, Germany). The ants were put into a plastic tube (Ø: 12 mm; length: 150 mm) which was repeatedly placed via an adaptor to the start and thereafter to the end of an experimental runway (w/h/d: 25 x 30 x 90 mm; Fig. 11A). In that way the ants regularly continued steady fast locomotion for 5 to 20 “rounds” corresponding to total distances of 1.2 m to 4.8 m. This procedure strongly reduced exploratory behaviour and about 10 runs per hour of successful attempts could be completed. Only runs were selected in which the whole runway has been crossed straight at constant speed and in which the tread of the force plate was entered with a single leg.

Experimental setup

The kinematics were recorded with an extremely light-sensitive high-speed camera (Fastcam SA3, Photron, San Diego, USA) which reduces the need of additional lighting (LED illumination with a total power of 4 Watt). One sidewall of the track was equipped with optical prisms so that the motion sequences could be recorded from the sagittal and the horizontal plane synchronously with a single camera (Fig. 11A). The substrate was covered with graph paper and the tread (4 x 4 mm) of a highly sensitive, three-dimensional, ultra-miniature force plate was embedded in the middle of the runway (Fig. 11B). We used a 3D CAD and simulation software to design the force plate and to calculate its properties by the finite element method. For production of the prototype, we used the stereolithography technology and applied semiconductor strain gages to it (Reinhardt and Blickhan, 2014). Forces as great as 10 µN can be resolved with this sensor and the natural frequencies are above 200 Hz for all directions (cp. Table 5). A digital amplifier system (MGCplus, Hottinger Baldwin Messtechnik, Darmstadt, Germany) was used to detect the signals of the strain gages with a sampling rate of 1200 Hz. All further calculations were made in MATLAB R2010a (The MathWorks, Natick, MA, USA). We selected a zero order Savitzky-Golay smoothing algorithm with a box width of 5 points (4.2 ms). Thus, we were able to measure events until 240 Hz without any loss in signal.

Coordinate system

The same system of coordinates was used for the kinematic and dynamic investigations (Fig. 11C). The x-axis is parallel to the animal’s long axis in walking direction. The y-axis is laterally to the left, parallel to the substrate, and the z-axis is perpendicular to the xy-plane. Positive ground reaction forces in the forward direction ($F_x$) accelerate the animal. Positive lateral forces ($F_y$) accelerate the animal to the left, and positive vertical forces ($F_z$) push the animal upwards. We do not distinguish between left and right legs. All results on the right side are mapped to the left. Correspondingly, a positive forward directed reaction force observed for the front leg implies pulling and negative force implies pushing. For the left middle leg a positive lateral force implies pulling inwards, and for the hind leg a positive forward component implies pushing outwards.

Kinematic and dynamic analysis

Our entire data set for the dynamic and kinematic analysis included 61 individual runs from 31 different ants. The studied ants had a mean body weight of 21.0±3.7 mg and moved with velocities from 7.8 to 20.8 cm/s. From this data set we analysed 59 single leg ground reaction force measurements (speed: 13.5±3.6 cm/s, weight: 20.9±3.9 mg) consisting of 21 steps of the front leg, 21 steps of the middle leg...
and 17 steps of the hind leg pair. As confirmed by our investigations (cp. section three-dimensional body
kinematics) the CoM was located near the petiolus. Therefore, the joint between petiolus and gaster
(B3, Fig. 12) was digitized three-dimensionally in 85 gait cycles of 60 runs (speed: 12.5±3.2 cm/s, weight:
21.0±3.7 mg). The complex 3D body kinematics and the stride pattern were studied in 20 gait cycles of
10 runs (speed: 11.0±0.7 cm/s, weight: 21.2±2.0 mg). Pseudo replication was avoided, since we
obtained similar results when mean values for individual trials ($N = 61$) instead of individual
measurements ($N = 85$) were analyzed with the same method (data not shown).

Gait cycle

One finding was that the animals touched the ground first with the middle leg of one tripod (cp. section
stride pattern). A step cycle has therefore been normalised to the contact and the following swing phase
of the middle leg. The contact phase starts with the touchdown of the tarsus and ends with the active
lifting of the leg. In the hind legs the tarsus is regularly dragged over the substrate without a significant
raise. For this reason, the start of the swing phase was here defined by the moment when the tarsus
was moved away from the contact point. For the other two pairs of legs the swing phase started with the
release of the tarsus from the ground. All information in the description of the CoM kinematics are related
to the tripod L1/R2/L3 (left front, right middle and left hind leg).

Digitisation

In order to describe the spatial position of the body segments the following points (B1-B4, Fig. 12) were
digitized three-dimensionally with the software WINalyze 3D (Version 2.1.1, Mikromak, Berlin,
Germany): (i) distal mandible tip (B1), (ii) the cervix (B2), (iii) the joint between petiolus and gaster (B3)
and (iv) the posterior end of the gaster (B4). The field of vision was about 60 mm wide. Camera
resolution was 768 x 512 pixels, so minimum tracking accuracy was below 0.1 mm. An accuracy of at
least 0.05 mm can be assumed since WINalyze, is able to compute sub-pixel resolutions via
interpolating algorithms (Frischholz and Spinnler, 1993). We used the graph paper on the tread to
calibrate the video sequences.

Body model and centre of mass determination

Due to the low leg masses, (the sum of all legs is approximately 10% of the body weight, cp. section
mass distribution) it is assumed that their position has only a small impact on the CoM position. The
same applies for the mandibles, antennae and other small appendages (McMeeking et al., 2012).
Hence, they were not included in the calculations. The remaining body segments of the ant were
separated into three major parts, whereby the gaster (abdomen) and caput (head) each represented
one segment. The third segment that we designated as thorax (alitrunk) consists of the remaining parts
(mesosoma, petiolus and coxae). This simplification was justified through kinematic preliminary
investigations, which indicated that their position to each other did not change.

In order to calculate the location of the total CoM, we needed the relative mass and the position of the
centre of gravity of each body segment. As we have demonstrated, the mass of each segment correlates
with the total mass of the ant and can therefore be calculated as a percentage of the body weight (cp.
section mass distribution). Under the assumption of a homogeneous density distribution the centroid of
a volume is equivalent to the centre of gravity, in each segment (cp. McMeeking et al., 2012 and Fig.
12). Thus, we took a single frame of a typical video sequence to estimate the position of these points and to formulate a geometric relationship concerning the marker points (B1-B4). Accordingly, we determined the gaster centre at a normal distance of 0.35 mm at 4/9 of the distance between B4 and B3. The caput centre was calculated at a normal distance of 0.35 mm at 1/3 of the distance between B2 and B1. The thorax had its centre of gravity in the middle between B2 and B3. Finally, the total CoM position was calculated via the weighted sum of the coordinates of the three segment centres.

Body angles

For describing the sagittal plane kinematics we calculated five angles (Fig. 12). Those were the angles between thorax and substrate (α), thorax and caput (β), caput und substrate (γ), thorax and gaster (δ) as well as the angle between gaster und substrate (ε). In addition, the angle between the thorax and the x-axis (ζ) was calculated in the transverse plane.

Centre of mass mechanics

To classify the gait into existing models of locomotion, it is imperative to calculate the fluctuations of gravitational potential energy (\(E_{pot}\)) and kinetic energy (\(E_{kin}\)) of the CoM. As the marker B3 is in very good agreement with the position of the CoM (cp. section three-dimensional body kinematics), we used its kinematics to calculate the CoM mechanics during the gait cycle. \(E_{pot}\) was calculated using the formula: \(E_{pot} = m g z\), where \(m\) is the mass of the ant, \(g\) is the acceleration due to gravity and \(z\) is the vertical position of the CoM. For each direction, \(E_{kin}\) was calculated via: \(E_{kin} = 0.5 m v^2\). Whereby \(v\) is the velocity in the respective direction, which was determined by temporal derivation of the coordinates. The sum of these energies corresponded to the total energy (\(E_{tot}\)) of the CoM. We relied on the phase relationship of \(E_{pot}\) and \(E_{kin}\) to discriminate running from walking (Ahn et al., 2004). A phase shift of 180° between both energies indicates a walking gait and one of 0° a running gait (Cavagna et al., 1977). The phase shift is also reflected in the %Recovery and in the %Congruity (Cavagna et al., 1976; Ahn et al., 2004). Ideally, %Recovery is 100% in a walking trial and 0% in a running trial, while the opposite is true for the %Congruity. We calculated both values for our data and set a border at 50% congruity to categorise our trials as walking or (grounded) running, following Ahn et al. (2004).

Mass distribution

To determine the sub-segment masses ten animals were selected, which were similar in size and weight to the animals in the dynamic and kinematic studies. The measurements were performed with an electronic precision balance (ABS 80-4, Kern & Sohn, Germany) with an accuracy of 0.1 mg. After weighing the animals \textit{in vivo}, they were killed by freezing (temperature: -18°C). A few hours later, they were individually taken from the freezer and sliced into the segments. Thus, it could be ensured that no weight loss was caused by escaping fluids. The legs were removed distally of the coxae and weighed together.

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FOOTNOTES

Author contributions
L.R. made significant and substantial contributions to the conception, design, execution and interpretation of the findings being published, and drafting and revising the article. R.B. made significant and substantial contributions to the design, execution and interpretation of the findings being published.

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REFERENCES


### TABLES

#### Table 1. Average segment masses ($N = 10$, mean ± s.d.)

<table>
<thead>
<tr>
<th>Segment</th>
<th>Gaster</th>
<th>Caput</th>
<th>Thorax</th>
<th>Σ Legs</th>
<th>Cumulative</th>
<th>Total (in vivo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (mg)</td>
<td>13.00±3.08</td>
<td>3.47±0.23</td>
<td>3.38±0.26</td>
<td>2.21±0.22</td>
<td>22.06±3.12</td>
<td>21.77±3.32</td>
</tr>
<tr>
<td>Relative mass</td>
<td>0.58</td>
<td>0.16</td>
<td>0.16</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
</tbody>
</table>

#### Table 2. Selected stride parameters for velocities from 9.5 cm/s to 12.5 cm/s ($N = 10$, mean ± s.d.)

<table>
<thead>
<tr>
<th></th>
<th>Contact duration ($t_{co}$)</th>
<th>Swing duration ($t_{sw}$)</th>
<th>Stride duration ($t_s$)</th>
<th>Duty factor ($du=t_{co}/t_s$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Front leg</td>
<td>44.7±4.2 ms</td>
<td>40.9±3.5 ms</td>
<td>85.6±3.4 ms</td>
<td>0.52±0.04</td>
</tr>
<tr>
<td>Middle leg</td>
<td>53.0±3.1 ms</td>
<td>33.0±2.2 ms</td>
<td>86.0±2.6 ms</td>
<td>0.62±0.03</td>
</tr>
<tr>
<td>Hind leg</td>
<td>46.1±2.6 ms</td>
<td>40.0±3.8 ms</td>
<td>85.9±3.7 ms</td>
<td>0.54±0.03</td>
</tr>
</tbody>
</table>

#### Table 3. Average CoM energetics of the contact phase at two speed ranges (mean ± s.d.)

<table>
<thead>
<tr>
<th>Group</th>
<th>Speed range (cm/s)</th>
<th>N</th>
<th>Mean speed (cm/s)</th>
<th>Height CoM (mm)</th>
<th>Energy recovery (%)</th>
<th>Congruity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>&lt;11.5</td>
<td>43</td>
<td>10.31±0.14</td>
<td>2.43±0.39</td>
<td>16.15±14.70</td>
<td>68.98±13.87</td>
</tr>
<tr>
<td>G2</td>
<td>&gt;11.5</td>
<td>42</td>
<td>14.61±0.39</td>
<td>2.62±0.38</td>
<td>18.12±16.75</td>
<td>61.48±14.13</td>
</tr>
</tbody>
</table>

#### Table 4. Mean body angles in the sagittal plane (mean ± s.d.)

<table>
<thead>
<tr>
<th></th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>$\delta$</th>
<th>$\varepsilon$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22.7±2.3°</td>
<td>139.4±1.9°</td>
<td>-17.9±2.7°</td>
<td>161.3±2.3°</td>
<td>41.4±2.3°</td>
</tr>
</tbody>
</table>

#### Table 5. Specification of the force plate based on FEM simulations and measurements

<table>
<thead>
<tr>
<th></th>
<th>$x$-direction</th>
<th>$y$-direction</th>
<th>$z$-direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated (FEM) natural frequency (Hz)</td>
<td>379.16</td>
<td>278.93</td>
<td>200.77</td>
</tr>
<tr>
<td>Calculated (FEM) spring constant (N/mm)</td>
<td>0.1005</td>
<td>0.0691</td>
<td>0.3598</td>
</tr>
<tr>
<td>Sensitivity (V/N)</td>
<td>97.84</td>
<td>197.62</td>
<td>51.05</td>
</tr>
<tr>
<td>Unfiltered residual noise (±s.d., mV)</td>
<td>1.18 ± 0.33</td>
<td>1.34 ± 0.45</td>
<td>1.39 ± 0.31</td>
</tr>
<tr>
<td>Resolvable force after filtering (µN)</td>
<td>5.42</td>
<td>2.87</td>
<td>10.75</td>
</tr>
</tbody>
</table>
**FIGURES**

**SEGMENT MASSES**

![Boxplots results of the segment mass distribution. The upper and lower sides of the blue box represent the interquartile range and the red line is the median. Black bars extending from the box indicate the highest and lowest values, excluding outliers.](image)

**STRIDE PATTERN**

![Stride pattern of *Formica polyctena* at running speeds of 10.9±0.9 cm/s (N = 10, mean ± s.d.). Shown is the average temporal sequence of the ground contacts of all legs (left: L1-L3, right: R1-R3). The measures are normalised to the gait cycle time (black bars, 0-100%) which is defined by the right middle leg (R2). The extrapolated sequences of the previous (-100-0%) and subsequent (100-200%) steps are illustrated by grey bars. Standard deviations are indicated by blue areas.](image)
Fig. 3. Phase durations (A), covered distances (B), duty factor (C), cycle time (D), stride length (E), stepping frequency (F), mean CoM height (G), %Recovery (H) and %Congruity (I) versus running speed. In subplot A and B, black dots symbolize the values of the contact phase and red dots represent the swing phase (N = 85). Linear regressions are designated by solid lines, and the equations are presented for each plot.
Fig. 4. Three-dimensional CoM kinematics in *Formica polyctena*. Average (mean ± s.d.) trajectories of CoM’s position, velocity and acceleration during one gait cycle are shown in the coordinate system defined in Fig. 11C. The areas between the dashed vertical lines (0-12% and 50-62%) indicate phases when legs of both tripods were on the ground. Mean values are shown as solid black lines and s.d. as grey areas. The investigated animals weighed 20.2±3.0 mg and ran with an average speed of 10.9±0.9 cm/s. Presented curves were calculated from all measurements of animals in the velocity range from 9.5 to 12.5 cm/s (N = 50).
Fig. 5. CoM energetics of the contact phase at different speed ranges. The investigated trials ($N = 85$) were divided into two velocity groups, ranging from 7.8 cm/s to 11.5 cm/s ($N = 43$, black), and from 11.5 cm/s to 20.8 cm/s ($N = 42$, red). In the upper graph (A) the mean trajectories of the potential (solid lines) and kinetic (dotted lines) energies are shown. The boxplots below (B-D) compare the average
CoM height, the percentage recovery of mechanical energy (cp. Cavagna et al., 1976) and the percentage congruity (cp. Ahn et al., 2004) between the two groups.

Fig. 6. (A) Trajectories of the digitized body markers (B1-B4) from 20 gait cycles of 10 runs in the sagittal plane. Each run was performed by another individual ant and is shown in a different colour. Black lines and dots symbolize the mean body posture of all steps. By a pink dot, the calculated position of the CoM is marked. (B) Position of the tarsi and body markers in the horizontal plane with respect to the CoM (pink dot). The trajectories of the individual experiments are shown as thin grey lines and the mean curves as thick black ones. The animal moves from left to right and the leg tips from right to left.
Fig. 7. (A) Average (mean ± s.d.) body angle trajectories during one gait cycle (N = 20). Note the different scales. (B) Angular velocities of two selected angles. (C) Fluctuations of CoM’s kinetic (in the xy-plane) and potential energy. Presented curves were calculated from all measurements of animals in the velocity range from 9.5 to 12.5 cm/s. Mean values are shown as solid black lines and s.d. as grey areas. The areas between the dashed vertical lines (0-12% and 50-62%) indicate phases when legs of both tripods were on the ground.
Fig. 8. Components of the ground reaction force of the legs. Curves are shown in the coordinate-system defined in Fig. 11C. Mean values are shown as solid black lines and s.d. as grey areas. The ground reaction forces are normalised to body weight. While a value of one corresponds to the weight force of the animal. The investigated animals weighed 20.9±3.9 mg and ran with an average speed of 13.5±3.6 cm/s.
Fig. 9. (A) Components of leg-generated force acting on the CoM, calculated as the sum of the single leg forces (mean curves in Fig. 8) during the gait cycle, taking into consideration the two phases of stance overlap (dashed grey lines). All values are normalised to body weight and gait cycle time. The fore-aft force ($F_x$, blue) and lateral force ($F_y$, green) oscillated around zero and the vertical force ($F_z$, red) was positive throughout the stance and fluctuated around the body weight with a net momentum of one.

(B) Projection of the force vectors of the three legs in the sagittal plane during one stance phase. Front leg (black), middle leg (red), hind leg (blue) black dot (CoM, [0, 2.5]). The animal moved from left to right and the leg tips (bases of the vectors at the x-axis in the xy-plane) from right to left with respect to the CoM. Coloured numbers at the bases define the following time-points: beginning (1), midstance (50) and the end of the contact phase (100).
Fig. 10. Exemplary sequence of the contact phase during level locomotion (running speed: 12.1 cm/s). The selected frames show the posture of *Formica polyctena* at the time of touchdown, midstance and lift-off from the side and from above. In each image a red dot indicates the calculated CoM position. Yellow lines in the sagittal view show the position of the two functional leg segments (femur, tibia-metatarsus-tarsus) with ground contact. Triangles in the top view visualize the support polygon.

Fig. 11. (A) Experimental setup from above. The floor was covered with graph paper and the tread of the miniature force plate was embedded in the middle. One sidewall of the track was equipped with optical prisms for an additional side perspective. At both ends plastic tubes were mounted through which the ants entered and left the experimental runway. (B) Single frame of a video sequence of the locomotion of *Formica polyctena*. The kinematics were recorded at 500 Hz with a resolution of 768 x 512 pixels (0.074 mm/pixel). (C) Coordinate system.

Fig. 12. Schematic representation of the digitized body markers (B1-B4, red), the estimated positions of the individual segment centres of gravity and the calculated angles in the sagittal plane.