RESEARCH ARTICLE

Inter-leg coordination in the control of walking speed in *Drosophila*

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SUMMARY

Legged locomotion is the most common behavior of terrestrial animals and it is assumed to have become highly optimized during evolution. Quadrupeds, for instance, use distinct gaits that are optimal with regard to metabolic cost and have characteristic kinematic features and patterns of inter-leg coordination. In insects, the situation is not as clear. In general, insects are able to alter inter-leg coordination systematically with locomotion speed, producing a continuum of movement patterns. This notion, however, is based on the study of several insect species, which differ greatly in size and mass. Each of these species tends to walk at a rather narrow range of speeds. We have addressed these issues by examining four strains of *Drosophila*, which are similar in size and mass, but tend to walk at different speed ranges. Our data suggest that *Drosophila* controls its walking speed almost exclusively *via* step frequency. At high walking speeds, we invariably found tripod coordination patterns, the quality of which increased with speed as indicated by a simple measure of tripod coordination strength (TCS). At low speeds, we also observed tetrapod coordination and wave gait-like walking patterns. These findings not only suggest a systematic speed dependence of inter-leg movement patterns but also imply that inter-leg coordination is flexible. This was further supported by amputation experiments in which we examined walking behavior in animals after the removal of a hindleg. These animals show immediate adaptations in body posture, leg kinematics and inter-leg coordination, thereby maintaining their ability to walk.

Key words: walking, motor control, sensory feedback, inter-leg coordination.

INTRODUCTION

In terrestrial animals, legged locomotion is a behavior that is highly optimized (Alexander, 1989). It is also flexible and can be adapted to the external environment and to specific behavioral goals. The locomotor apparatus often has to be used on a variety of substrates such as level surfaces, twigs in a bush or ragged cliffs. Furthermore, the locomotor output can change from slow explorative walking to swift running when it becomes necessary to escape a predator or cross terrain without cover.

Frequently, changes in locomotor output are not restricted to the movements of single legs but also entail changes in the temporal coordination between several or all legs. Many quadrupeds, like cats, dogs or horses, for instance, use specific gaits depending on their movement speed (Alexander, 1989). In these animals, leg coordination changes from walking and pace gaits at slow speeds to trotting gaits at intermediate speeds and, eventually, to gallop at high speeds. The coordination of the frontlegs and hindlegs changes from anti-phase in walking to nearly in-phase during gallop (Orlovsky et al., 1999). The transition from one gait to another is discontinuous and it can be shown that quadrupeds select the energetically optimal gait at a given speed (Hoyt and Taylor, 1981).

In hexapods, i.e. insects, the situation appears, at first glance, to be comparable. However, different patterns of leg coordination can occur. These patterns are typically characterized by the number of legs that are on the substrate during stance. Very slow-walking insects, for example, generate a metachronal wave of leg movements along each side of the body sequentially from back to front while at least five legs are always in stance phase, a coordination pattern called wave gait (Hughes, 1952). For faster walking speeds, coordination is modified accompanied by an apparent reduction in the number of legs that are on the ground simultaneously. At medium speeds, the number of legs is reduced to four, termed tetrapod coordination (Burns, 1973; Graham, 1972; Hughes, 1952; Spirito and Mushrush, 1979; Wendler, 1964; Wendler, 1966), and at high speeds to three, called tripod coordination (Bender et al., 2011; Delcomyn, 1971; Graham, 1985). Interestingly, bipedal anti-phase coordination of insect hindlegs has been reported for the cockroach, *Periplaneta americana*, during top speed running (Full and Tu, 1991). In this situation, the anterior part of the animal is lifted and the front and middle legs no longer touch the ground.

While in quadrupeds the switch between two patterns of inter-leg coordination, or gaits, is distinct and dependent on speed, studies in invertebrates indicate that specific patterns of coordination are part of a larger and speed-dependent continuum and that intermediate forms of coordination exist. In the same speed range, insects can use either tetrapod or tripod coordination, seamlessly transitioning from one to the other by modifying stance duration (Cruse, 1990; Graham, 1985; Wendler, 1966). Several genera of ants (*Cataglyphis*, *Formica*, *Lasius* and *Myrmica*), cockroaches (*P. americana*), fruit flies (*Drosophila melanogaster*) and stick insects (*Carausius morosus*) are known to use tripod coordination during fast locomotion, while at lower speeds leg coordination becomes much more variable, approaching tetrapod coordination (Wendler, 1964; Graham, 1972; Bender et al., 2011; Strauss and Heisenberg, 1990; Zollikofer, 1994).

How is inter-leg coordination achieved? Behavioral studies on four-, six- and eight-legged animals have suggested that sensory signals which reflect the movements of individual legs contribute to the coordination between legs, thereby generating an emergent set of coordination rules (Cruse, 1990; Dürr et al., 2004). Furthermore, the importance of intersegmental neural pathways has also been shown based on studies that reduce or eliminate the mechanical interaction between legs (Graham and Cruse, 1981; Cruse and Epstein, 1982; Gruhn et al., 2006). In normal walking situations, the coordination rules arise from the interplay of mechanical and neural coupling between individual legs during walking. While it is clear that both mechanical and neural influences play important roles, their specific contribution for the generation of leg coordination patterns is not clear, yet. In contrast, there is evidence confirming the importance of central inter-segmental neural pathways for the coordination of local networks controlling leg movements in insect walking, for example. This has been shown for the cockroach *P. americana* (Pearson and Iles, 1973), the locust *Schistocerca americana* (Ryckebusch and Laurent, 1993) and the hawk moth *Manduca sexta* (Johnston and Levine, 2002). However, studies have shown the role of local sensory feedback in establishing inter-leg coordination, e.g. in the hawk moth (Johnston and Levine, 1996; Johnston and Levine, 2002) and the stick insect *C. morosus* (Borgmann et al., 2009; Büschges et al., 1995).

One aspect that has so far hindered further elucidation of the neural mechanisms underlying inter-leg coordination is the fact that insect species at given developmental stages (Graham, 1985) often show a rather narrow range of preferred walking speeds. For example, while it is known that cockroaches can use the full range of inter-leg coordination from metachronal wave gait, in which only one leg is in swing phase at any given time, to tripod coordination (Hughes, 1952), under natural conditions they mostly use tripod coordination (Bender et al., 2011). Adult stick insects also show a preference for a particular coordination pattern. They almost exclusively use tetrapod coordination during level walking, while at high speeds they also use tripod coordination (Graham, 1972). In adult stick insects, tripod coordination is less frequent, though; larval stages tend to use tripod coordination much more frequently (Graham, 1972) but are also much smaller. As a consequence, in the insect groups studied so far only a rather limited continuum of walking speeds could be investigated reliably. This is all the more unsatisfactory as the specifics of inter-leg coordination are often used as important indicators of how the neural mechanisms generating walking behavior are structured (Zollikofer, 1994). It is therefore crucial to determine the full possible range of walking speeds with regard to inter-leg coordination.

In the present study, we used four different *Drosophila* strains in order to address this issue and capture as large a range of walking speeds as possible in a single species. The two wild-type strains *Canton-S* (wt^{CS}) and *Berlin* (wt^{Berlin}) represented the typical behavior in the wild. These two strains have previously been used in studies on inter-leg coordination (Strauss and Heisenberg, 1990; Strauss and Heisenberg, 1993) and global parameters of locomotor activities (Martin, 2004; Martin et al., 1999). In addition, we selected two mutant *Drosophila* strains, *white*^{1118} (w^{1118}) and w^{1118}, Tbh^{nM18} to extend the range of observable walking speeds to lower values. w^{1118} flies have reduced levels of octopamine (Sitaraman et al., 2008), while w^{1118}, Tbh^{nM18} lacks this biogenic amine altogether (Monastirioti et al., 1996). Octopamine is implicated in the high-level control of locomotor activity (Brembs et al., 2007; Gal and Libersat, 2008; Gal and Libersat, 2010) and, as we show here, a reduced level or absence of octopamine seems to induce lower walking speeds in *Drosophila*. Furthermore, the results we present here for w^{1118} flies can also serve as a control for future studies in *Drosophila*, as an extensive amount of transgenic flies have a w^{1118} background. As we show, there are important differences between wild-type flies and w^{1118}, and this might be important for the interpretation of behavioral studies based on transgenic strains.

We show that under relatively unconstrained conditions, individuals of different *Drosophila* strains cover a broad range of speeds during walking. We found that leg coordination patterns change gradually and systematically with walking speed. This suggests that the neural controllers responsible for inter-leg coordination are able to generate a marked flexibility with respect to walking behavior. Furthermore, removing one of the hindlegs revealed that *Drosophila* is capable of adapting its leg coordination immediately, thereby maintaining the ability to propel itself forward even after major biomechanical changes in its walking apparatus.

MATERIALS AND METHODS
Fly strains and breeding
Flies were raised at 25°C and 60% humidity on a 12h/12h light/dark cycle and maintained on standard medium containing cornmeal, molasses, yeast and agar. For the experiments presented here, we used the following *Drosophila melanogaster* strains: wild-type Canton-S (wt^{CS}), wild-type Berlin (wt^{Berlin}), w^{1118}, and w^{1118}, Tbh^{nM18} (Monastirioti et al., 1996). Flies were kindly provided by Dr M. Leptin (wt^{CS}), Dr R. Strauss (wt^{Berlin}) and Dr H. Scholz (w^{1118} and w^{1118}, Tbh^{nM18}).

Experimental procedure
For all experiments, 5 day old males were used. At least 2h prior to an experiment, flies were cold anesthetized and put into isolation tubes without food but with water. One fly at a time was then transferred from its isolation tube into the experimental setup were it walked spontaneously back and forth on a 5mm wide transparent walkway (Fig. 1A). Wings were left intact; therefore, to prevent escape by flight, the walkway was enclosed on all sides with acrylic glass. Furthermore, the inner walls of the enclosure were covered with a layer of Fluon (AGC Chemicals Europe, Thornton Cleveleys, UK), which prevented the flies from scaling the walls. To allow for video recordings, a small area (20mm) on one side of the walkway was kept free of Fluon. Beneath this area, we attached a glass prism providing a ventral view of the walkway. Thus, using a single camera we were able to simultaneously record a lateral (Fig. 1B) and a ventral view (Fig. 1C) of the walking fly. Video recordings were taken with a high-speed digital camera (AOS S-PRI High Speed Color 5.2, AOS Technologies AG, Baden Daettwil, Switzerland) at 500 frames s^{-1}, with a shutter time of 200 µs. The setup was illuminated with infrared LEDs (λ=880 nm). The LEDs were externally synchronized to the shutter of the camera in order to provide maximum illumination during the time the camera shutter was open. The camera was controlled *via* AOS Imaging Studio v3 (AOS Technologies AG). After each set of experiments, a 10mm wide marker was recorded with the same settings. This marker was then used to calibrate the analyzed videos.

For the amputation experiments, flies were cold anesthetized followed by the removal of one of the hindlegs at the midpoint of the femur, leaving only a stump consisting of the coxa, trochanter and part of the femur. Flies were then moved to isolation tubes and subsequently treated as described above for the intact animals.

To determine the average mass of the flies, between 9 and 35 flies (3–7 days old) of each sex and strain were collected into separate 1.5 ml plastic tubes (Table 1). The tubes including the flies were
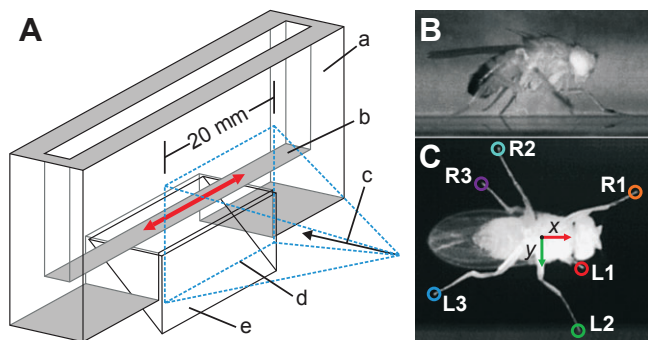
Fig. 1. (A) Schematic diagram of the experimental setup. Flies walked spontaneously back and forth on a walkway as indicated by the red arrow. Walks were recorded through a 20 mm wide window simultaneously from one side and from below (a: acrylic glass, coated on the inside with a layer of Fluon to prevent the flies from scaling the glass; b: 5 mm wide transparent walkway; c: camera viewpoint; d: camera field of view, free of Fluon; e: glass prism, providing a ventral view of the walkway). (B) Exemplary lateral view of a male *Drosophila*, wild-type wt^{CS} strain, during one of the recorded walks. (C) Ventral view of the same fly in the same video frame. The tips of the tarsi are marked with colored circles (R1, R2, R3: right front leg, middle leg and hindleg; L1, L2, L3: left front leg, middle leg and hindleg). Red and green arrows indicate the origin and orientation of the body coordinate system.

then weighed, the mass of the empty tube was subtracted, and the mass of a single fly was calculated. In addition, the body length of each fly recorded during the behavioral experiments was determined by marking the base of the antennae and the tip of the abdomen in the ventral view of the fly, using the same software as for video analysis (ProAnalyst, XCitex, Inc., Cambridge, MA, USA).

Data analysis

During experiments, flies walked spontaneously back and forth on the walkway. We recorded straight walks containing 5–12 complete step cycles per leg. The recorded videos were then evaluated frame-by-frame in a semi-automatic fashion. Body position and axes were determined automatically with ProAnalyst (XCitex, Inc.). The exact times of tarsal lift-off and touchdown events were visually determined in the lateral view of the fly, while the associated tarsus positions were visually determined in the ventral view. Data obtained in this manner were then further processed in MATLAB (MathWorks, Inc., Natick, MA, USA).

The durations of swing and stance phases were calculated as the difference between the time of lift-off and subsequent touchdown of the same leg (swing) or *vice versa* (stance). One cycle period was defined as the time difference between two consecutive lift-off events of the same leg. Onset of swing was used as the reference time for the analysis of temporal coordination of all legs. In trials with intact animals, the reference leg was always the front leg that completed

the most cycles during a given trial. In trials with animals lacking one hindleg, the reference leg was always the front leg contralateral to the lesioned side. Results from the phase analysis of trials in which the right front leg was the reference leg were then flipped in order to combine the results with those in which the left front leg was the reference leg. The CircStat Toolbox for MATLAB was used for phase analyses and the corresponding plots (Berens, 2009).

All positional information with regard to tarsal touchdown and lift-off was transformed into the body-centered *xy*-coordinate system (see also Fig. 1C). Furthermore, in order to compensate for small variations in body size, these body-centered data were then normalized to the respective body length of the fly. Based on these data, we calculated stance trajectories in the body-centered *xy*-coordinate system (Fig. 2B). Step amplitude of a particular step was determined as the distance between the posterior extreme position (PEP) of the tarsus at lift-off and the subsequent touchdown at the anterior extreme position (AEP) in body-centered coordinates. It should be noted that we used step amplitude instead of stride length, which is defined as the distance between two consecutive touchdown positions in floor-fixed coordinates. Stride length is not independent of movement speed and might change even without active changes in the walking motor pattern. This is not true for step amplitude. A change in this measure always necessitates a change in the motor output. Although the two measures are closely related, step amplitude is much more informative when one is interested in kinematic changes the animal has to actively make.

Based on the ventral view, walking speed was calculated for each frame in a trial as the change in position of the fly's body relative to the ground. The resulting speed profile was smoothed with a gliding average of 5 frame width. Based on this complete speed profile, the walking speed associated with a particular swing phase, as used in Fig. 3B,D, for instance, was calculated as follows: we first determined the time interval between the onset and offset of the swing phase and found the section of the complete speed profile associated with this interval. We averaged the speed profile within the interval to obtain a single average speed value. This average speed value was then used as the walking speed associated with a particular swing phase.

Coordination patterns

In hexapod walking, the literature typically distinguishes between three different coordination patterns: tripod coordination, tetrapod coordination and wave gait. The mere existence of these categories implies three distinct gaits, and, in fact, these coordination patterns have often been used synonymously with gaits. The literature, however, also implies that there is a speed-dependent continuum between these prominent patterns (Wendler, 1964; Graham, 1972). Therefore, because they are established, we use these terms; however, we do so in a purely descriptive manner and refer to coordination patterns rather than gaits.

In order to describe the walking patterns that occurred during the recorded trials, we classify these as tripod, tetrapod or undefined coordination according to the following considerations. Tripod coordination is described as the alternating movement of two distinct groups of legs (Hughes, 1952; Wilson, 1966). These tripod groups consist of an ipsilateral front leg and hindleg, and a contralateral middle leg (L1, L3, R2, and R1, R3, L2, respectively). Tripod coordination is typically found in fast-moving animals and therefore constitutes the extreme case at the highest end of the aforementioned speed-dependent continuum. In its ideal form, tripod coordination is characterized as the simultaneous lift-off and touchdown of all legs in one tripod group, while the legs associated with the other tripod group are on the ground. However, using this strict definition of tripod coordination is

Table 1. *Drosophila* strains

	Male				Female	
	Mass (mg)	N	Size (mm)	N	Mass (mg)	N
wt^{CS}	0.70	29	2.06±0.08	6	1.17	29
wt^{Berlin}	0.86	22	2.12±0.01	3	1.32	22
w^{1118}	0.70	27	2.09±0.08	5	1.05	35
w^{1118}, TbH^{nM18}	0.71	12	2.07±0.03	5	1.21	9

Ai

L1
L2
L3
R1
R2
R3

Aii

L1
L2
L3
R1
R2
R3

Aiii

Speed (BL s^{-1})

20
15
10
5
0

0   0.1   0.2   0.3   0.4   0.5

Time (s)

B

Body x dimension (BL)

1
0.5
0
−0.5
−1

L1   R1
L2   R2
L3   R3

x
y

1   0.5   0   −0.5   −1

Body y dimension (BL)

C

$\pi/2$ $\pi/2$
$\pm\pi$ I1 0 $\pm\pi$ C1 0
$-\pi/2$ n=578 $-\pi/2$

$\pi/2$ $\pi/2$
$\pm\pi$ I2 0 $\pm\pi$ C2 0
$-\pi/2$ $-\pi/2$

$\pi/2$ $\pi/2$
$\pm\pi$ I3 0 $\pm\pi$ C3 0
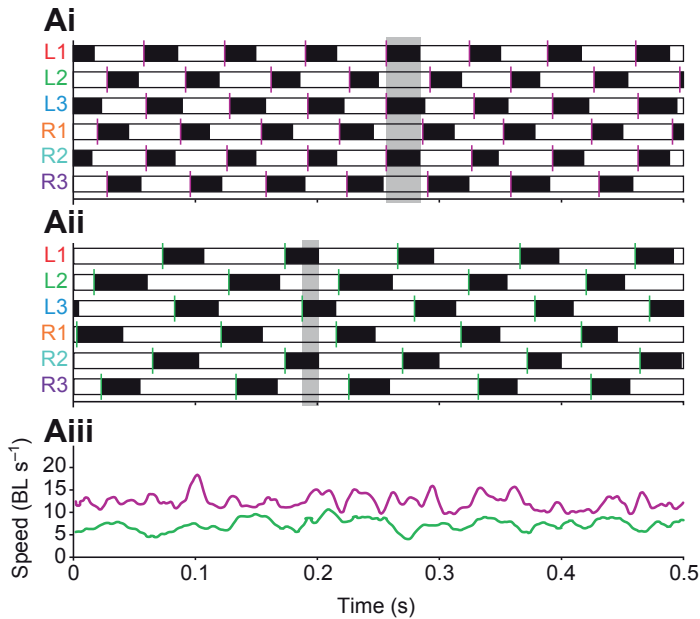$-\pi/2$ $-\pi/2$
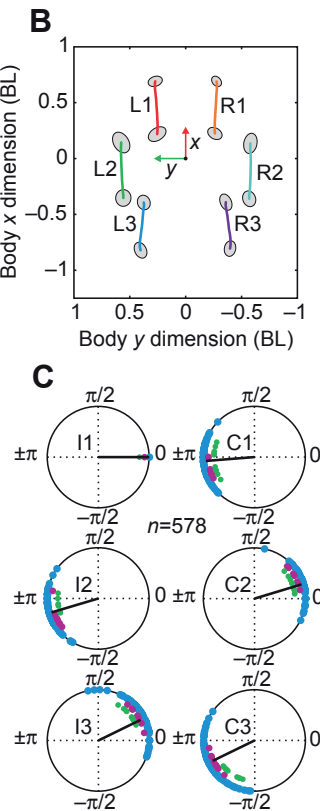
Fig. 2. Walking parameters of wt^{CS}. (Ai) Footfall pattern of all six legs during 0.5 s of one faster trial and (Aii) 0.5 s of one slower trial, and (Aiii) walking speed (BL, body lengths) of the body during the 0.5 s of the trials shown in Ai (magenta graph) and Aii (green graph) (R1, R2, R3: right front leg, middle leg and hindleg; L1, L2, L3: left front leg, middle leg and hindleg). Black bars indicate swing phase, white bars indicate stance phase; magenta lines indicate the onset and end of complete step cycles in the faster trial, green bars indicate those in the slower trial. Shaded areas highlight coordination patterns of interest (see Results). (B) Average stance trajectories of all legs from all trials in relative body coordinates. (C) Phase plots of swing onset of all legs with respect to the left front leg (I, ipsilateral; C, contralateral; blue: data from all trials; magenta: data from Ai; green: data from Aii; black line: mean vector – length indicates variance). n, number of trials.

problematic, for two main reasons. Firstly, in this strict sense, tripod coordination occurs very rarely; even during highly coordinated walking, random fluctuations or small systematic shifts in the phase relationships between legs of one tripod group tend to persist (Bender et al., 2011); in addition, it is known that in most insects the legs of one tripod group are not completely in phase (Hughes, 1952). Secondly, concentrating on this narrow aspect of inter-leg coordination potentially diverts attention from other interesting coordination patterns that do not happen to fall under the tripod definition but might nevertheless be highly regular. In order to address this conceptual problem, we used a more flexible description of tripod coordination: we defined a particular walking pattern as tripod coordination when, during one step, the swing phases of all legs associated with a tripod group concurrently overlapped for at least one frame of recorded video. Here, this is equivalent to 2 ms; for comparison, typical swing durations observed during experiments were in the range 20–40 ms. In addition, once a walking pattern was defined as tripod, we determined the tripod coordination strength (TCS), which we obtained as follows. First, we calculated the time from the earliest swing onset to the latest swing termination. This gave us time t_1, during which at least one of the three legs was in swing phase. Then we determined time t_2, during which all three legs were in swing phase at the same time. The ratio t_2/t_1 then described the TCS. A TCS of 1 indicated perfect tripod coordination; it approached 0 when the temporal relationship of swing phases shifted to other coordination patterns. Tetrapod coordination is defined as a walking pattern in which exactly two legs are lifted off the ground at a particular time (Graham, 1985; Hughes, 1952). Therefore, a walking pattern was defined as tetrapod when, during one step, the swing phases of exactly two legs overlapped for at least one frame of recorded video. Tetrapod coordination constitutes a further special case within the continuum of coordination and is generally associated with intermediate walking speeds. Finally, when a step was neither tripod nor tetrapod we classified it as

undefined. This category is largely identical to what is usually called wave gait, although this was not explicitly tested. It should be noted that we used this classification schema on a step-by-step basis; each step was evaluated separately and could be classified as tripod, tetrapod or undefined, but never as two of these. Although tripod coordination was predominantly found at high speeds, tetrapod coordination was most frequently found at intermediate speeds, and undefined coordination was most common at low speeds, the classification was completely independent from the walking speed during a particular step; each coordination class could have occurred at any speed.

RESULTS

The four different strains of *Drosophila* studied here were similar in size and mass (Table 1). The body lengths of males ranged from 2.06 to 2.12 mm, and their mass ranged from 0.70 to 0.86 mg. In general, the mass of females was higher, ranging from 1.05 to 1.32 mg. Males of the strains wt^{CS}, w^{1118} and w^{1118}, Tbh^{nM18} were almost identical in size and mass, while wt^{Berlin} males were slightly larger (5%) and on average 20% heavier. The same was true for females of wt^{Berlin}. In order to minimize potential age- or sex-related influences on walking behavior, we selected 5 day old males for the present study.

Wild-type wt^{CS}

In the first set of experiments, we studied leg kinematics and inter-leg coordination in wt^{CS} during spontaneous walking. Generally, animals generated walking sequences that were straight with features that were in accordance with previously published findings (Strauss and Heisenberg, 1990; Strauss and Heisenberg, 1993). Legs were coordinated in tripod fashion, as exemplified in the trial displayed in Fig. 2Ai (highlighted area). The features of all further recorded trials of wt^{CS} were qualitatively similar to the one shown in Fig. 2Ai. Movement speed was always relatively constant during each trial;
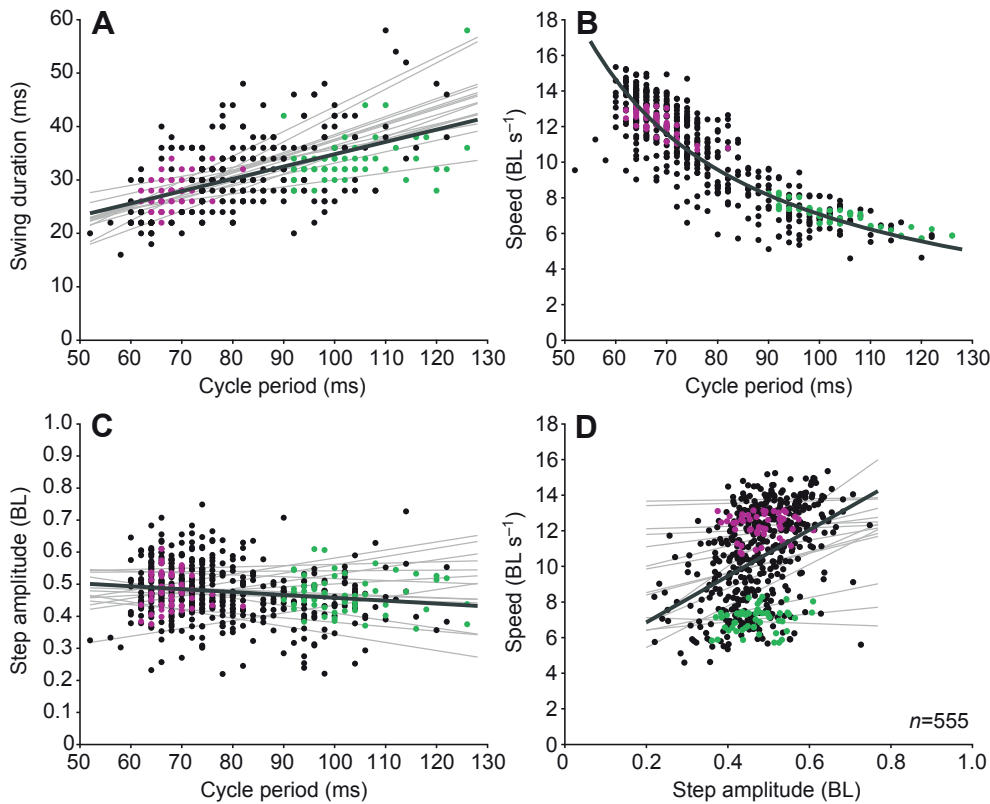
Fig. 3. Evaluation of leg stepping parameters of wt^{CS}. (A) Swing duration as a function of cycle period (black: data from all trials; magenta: data from trial in Fig. 2Ai; green: data from trial in Fig. 2Aii). (B) Walking speed as a function of cycle period (same color coding as in A). (C) Step amplitude as a function of cycle period (same color coding as in A). (D) Walking speed as a function of step amplitude (same color coding as in A). Each panel contains a regression line for the complete data set (black) as well as several further regression lines (gray), each of which is associated with one trial (n=15).

in the sequence shown in Fig. 2Ai, for instance, movement speed was approximately 13 body lengths per second ($BL\,s^{-1}$) on average. However, over all trials, average walking speed ranged from 5 to $16\,BL\,s^{-1}$. This was equivalent to absolute values of $11-32\,mm\,s^{-1}$ (6 individuals, 555 steps). Average stance phase trajectories of all six legs were relatively straight and almost parallel to the longitudinal body axis (Fig. 2B). The length of stance trajectories was similar for all legs and in the range of half the body length. With regard to temporal coordination, each of the three leg pairs showed anti-phase swing activity on average (Fig. 2C). Legs were generally coordinated in tripod fashion; however, the front leg of a tripod group tended to initiate swing phase first, followed by the middle leg with a phase shift of approximately 15 deg. The middle leg was in turn followed by the hindleg with a further phase shift of 15 deg (Fig. 2C).
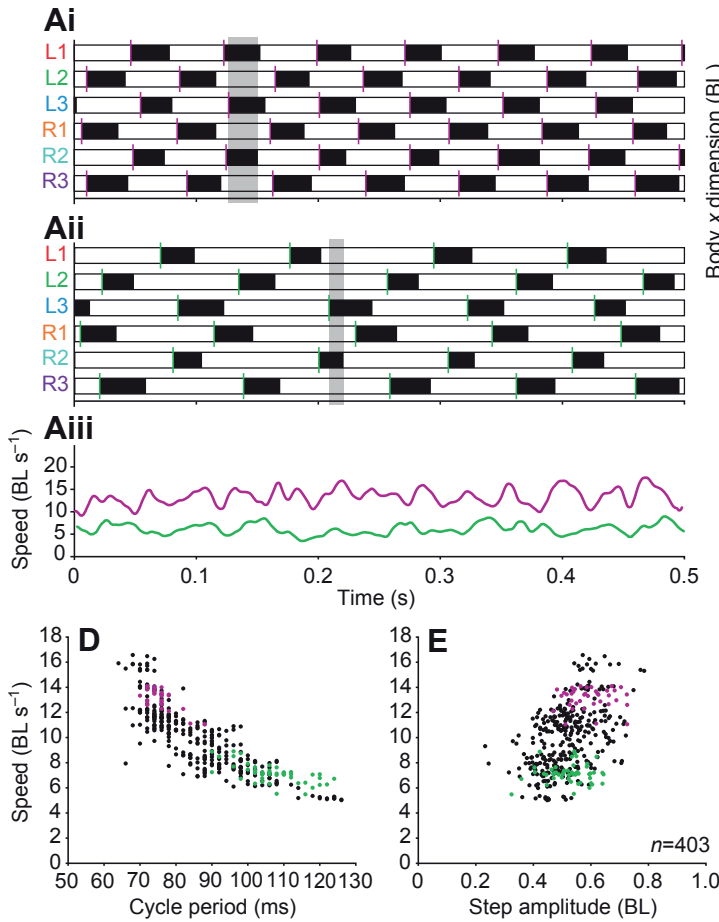
Tripod coordination was more variable only during particularly slow walking sequences. An example of this is shown in Fig. 2Aii. Here, a section of 0.5 s from one of the slower trials in wt^{CS} is shown (approximately $7\,BL\,s^{-1}$ on average). However, even during these slowest walking sequences, coordination was still tripod, according to our conservative definition (see highlighted area in Fig. 2Aii), and phase relationships were similar to those of the faster trials (Fig. 2C, green points). In contrast to a tripod group, in which the temporal succession of swing onset was directed posteriorly, the order of swing onsets on each body side was always directed anteriorly, beginning with the hindleg, followed by the middle leg and finally the front leg, after which the next series started again with the hindleg.

As it is known that insects walking in tripod coordination adapt swing duration depending on step cycle period (Graham, 1985), we examined this relationship for wt^{CS}. We found that swing duration indeed moderately correlated with cycle period (Fig. 3A); this was true for the complete data set (Fig. 3A, black regression line, coefficient of determination R^2=0.37), as well as for individual trials

(Fig. 3A, gray regression lines). Another parameter that more strongly depended on cycle period was walking speed; we modeled this dependence as a hyperbolic relationship over the complete range of cycle periods (Fig. 3B, black line, pseudo R^2=0.76). At the same time, cycle period did not correlate with step amplitude (Fig. 3C, black regression line, R^2=0.03). Although step amplitude contributes weakly to walking speed when we examine the complete range of step amplitudes (Fig. 3D, black regression line, R^2=0.16), this relationship cannot be shown reliably for individual trials (Fig. 3D, gray regression lines).

Wild-type wt^{Berlin}

We then collected data for the wt^{Berlin} strain (Fig. 4). Similar to wt^{CS} flies, wt^{Berlin} flies almost exclusively used tripod coordination during all recorded trials. As an example of comparatively strict tripod leg coordination in this strain, Fig. 4Ai shows a 0.5 s long section of a fast walking trial. Overall, average walking speed ranged from 5 to $15\,BL\,s^{-1}$, which was equivalent to absolute speeds of $11-34\,mm\,s^{-1}$ (3 individuals, 403 steps). Stance trajectories in wt^{Berlin} were on average straight and almost parallel to the longitudinal body axis (Fig. 4B). Each of the three leg pairs showed clear anti-phase swing activity during tripod coordination (Fig. 4Ai, highlighted area; Fig. 4C, magenta points for the sequence shown in Fig. 4Ai). Analogous to wt^{CS}, we found that the front legs of a tripod group initiated swing first, followed by the middle legs, which in turn were followed by the hindlegs (Fig. 4C, blue data points). Only during very slow walking sequences did tripod coordination become more variable and we also found intermittent tetrapod coordination (Fig. 4Aii, highlighted area); this was also reflected in the phase relationship, which started to deviate in a more pronounced way from the typical tripod pattern (Fig. 4C, green data points). These shifts to tetrapod coordination were, however, rare. The succession of swing onset on each body side was always directed

Fig. 4. Walking parameters of wild-type strain *wt^Berlin*. (Ai) Footfall pattern of all six legs during 0.5 s of one faster trial and (Aii) 0.5 s of one slower trial, and (Aiii) walking speed of the body during the 0.5 s of the trials shown in Ai (magenta graph) and Aii (green graph) (R1, R2, R3: right front leg, middle leg and hindleg; L1, L2, L3: left front leg, middle leg and hindleg). Black bars indicate swing phase, white bars indicate stance phase; magenta lines indicate the onset and end of complete step cycles in the faster trial, green bars indicate those in the slower trial. Shaded areas highlight coordination patterns of interest (see Results). (B) Average stance trajectories of all legs of all trials in relative body coordinates. (C) Phase plots of swing onset of all legs with respect to the left front leg (I, ipslateral; C, contralateral; blue: data from all trials; magenta: data from Ai; green: data from Aii; black line: mean vector – length indicates variance). (D) Cycle period as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii). (E) Step amplitude as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii).

anteriorly. Analogous to that in *wt^CS*, walking speed in *wt^Berlin* was clearly correlated with cycle period (Fig. 4D), while it did not depend on step amplitude (Fig. 4E).
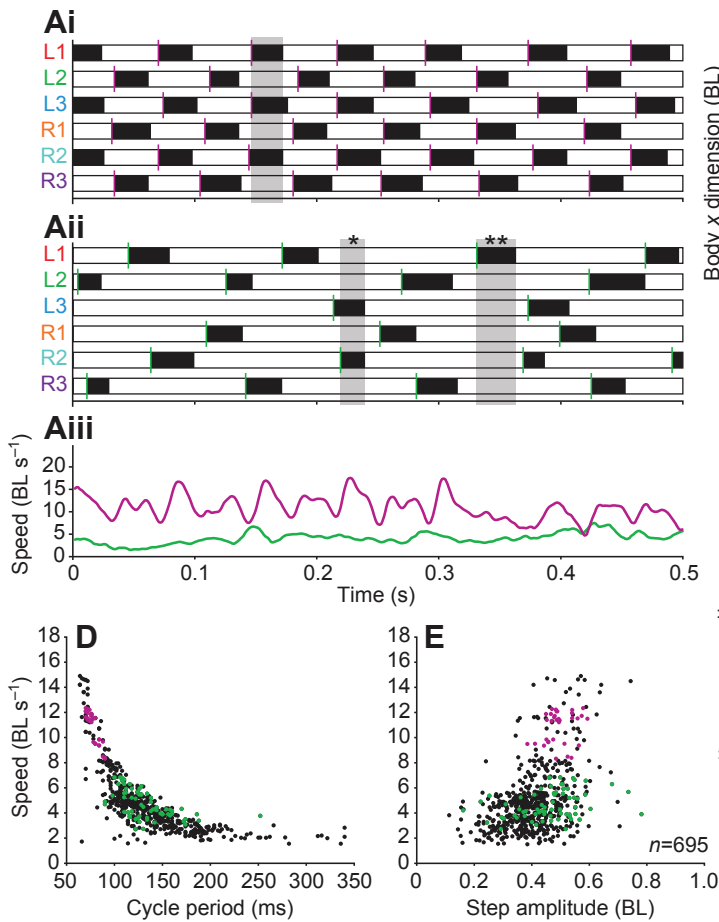
Mutant strain *w^1118*

In the third set of experiments, we analyzed walking in *w^1118* flies (Fig. 5). The total range of walking speeds in this strain was similar to that of *wt^CS* and *wt^Berlin* flies, with values ranging from 2 to 15 BL s^{-1}, i.e. from 4 to 31 mm s^{-1} (5 individuals, 695 steps), as exemplified for a single trial in Fig. 5Ai. However, *w^1118* flies walked at lower speeds more frequently. In general, speed appeared to be somewhat more variable within single walking sequences compared with that for *wt^CS* and *wt^Berlin* (cf. Fig. 2A, Fig. 4A, Fig. 5A). Stance trajectories were parallel to the longitudinal body axis for all three pairs of legs. On average, step amplitude was slightly shorter than 0.5 BL and was thus shorter than for the other two strains (Fig. 5B). Individuals of *w^1118* often used tripod coordination (e.g. Fig. 5Ai, highlighted area), although the variability of inter-leg coordination seemed to be relatively high (blue points in Fig. 5C; 5 individuals, 713 steps). Nevertheless, according to our conservative definition, inter-leg coordination was still tripod on average (black lines in Fig. 5C). This variability can partially be attributed to the fact that at lower speeds animals no longer used tripod coordination but instead used tetrapod coordination (Fig. 5Aii, *) or even wave gait-like coordination (Fig. 5Aii, **). Similar to *wt^CS* and *wt^Berlin* flies, average swing phase onset of *w^1118* posterior legs in a tripod group trailed front legs (Fig. 5C, magenta points for the trial in Fig. 5Ai, blue points for all data). Still, even in the slowest trial, the succession of swing phase onsets on a body side was directed anteriorly. The
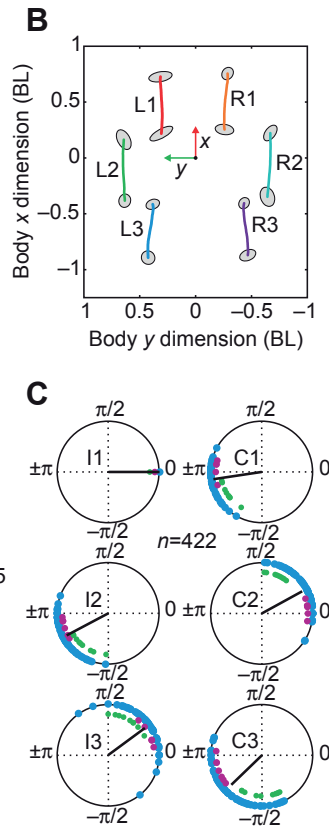
walking speed of *w^1118* flies was strongly correlated with cycle period (Fig. 5D). We found only a weak correlation between walking speed and step amplitude (Fig. 5E, R^2=0.17).

Mutant strain *w^1118, Tbh^nM18*

The octopaminergic neurotransmitter system has been implicated in the regulation of walking in stick insects, cockroaches and crabs. *w^1118, Tbh^nM18* mutants lacking the enzyme tyramine β-hydroxylase necessary for the conversion of tyramine into octopamine have deficiencies in locomotor performance compared with wild-type flies (Brembs et al., 2007; Scholz, 2005). We found that this offered the chance to extend the range of movement speeds studied here to even lower values. For *w^1118, Tbh^nM18* flies, movement speed ranged from 3 to 14 mm s^{-1} (5 individuals, 681 steps), i.e. from 1.5 to 7 BL s^{-1}. *w^1118, Tbh^nM18* flies only rarely walked at higher speeds, as exemplified for a single trial in Fig. 6Ai (see highlighted area for an instance of tripod coordination). Again, average stance trajectories were parallel to the longitudinal body axis and were slightly shorter than those in the *w^1118* strain (Fig. 6B). However, average phase relationships of swing onset were no longer typical for tripod coordination: for example, phase values for R1, L2 and R3 relative to L1 were 175, 120 and 140 deg, respectively. Phase plots show a substantial variability of inter-leg coordination (Fig. 6C; magenta points for the sequence shown in Fig. 6Ai, blue points for all steps; 5 individuals, 713 steps). At low speeds (<5 BL s^{-1}), *w^1118, Tbh^nM18* flies often used tetrapod coordination; during the slowest trials (2–3 BL s^{-1}), coordination resembled wave gait (Fig. 6Aii: see highlighted areas: *tetrapod; **wave gait-like coordination; Fig. 6C, green points). Analogous to the other strains examined here, the

Fig. 5. Walking parameters of mutant strain w^{1118}. (Ai) Footfall pattern of all six legs during 0.5 s of one faster trial and (Aii) 0.5 s of one slower trial, and (iii) walking speed of the body during the 0.5 s of the trials shown in Ai (magenta graph) and Aii (green graph) (R1, R2, R3: right front leg, middle leg and hindleg; L1, L2, L3: left front leg, middle leg and hindleg). Black bars indicate swing phase, white bars indicate stance phase; magenta lines indicate onset and end of complete step cycles in the faster trial, green bars indicate those in the slower trial. Shaded areas highlight coordination patterns of interest (see Results). (B) Average stance trajectories of all legs of all trials in relative body coordinates. (C) Phase plots of swing onset of all legs with respect to the left front leg (I, ipslateral; C, contralateral; blue: data from all trials; magenta: data from Ai; green: data from Aii; black line: mean vector – length indicates variance). (D) Cycle period as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii). (E) Step amplitude as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii).

succession of swing onset on each body side was directed anteriorly. Only small deviations from this pattern could be observed during very slow trials (cf. third swing of R3 in Fig. 6Aii). Again, walking speed in w^{1118}, Tbh^{nM18} was strongly correlated with cycle period (Fig. 6D). The correlation between walking speed and step amplitude was weak (Fig. 6E, R^2=0.15).

Inter-leg coordination depends on movement speed

While all strains used tripod coordination during fast walking, at lower speeds inter-leg coordination became more variable or changed to other patterns such as tetrapod coordination. Based on this observation, we wanted to know whether inter-leg coordination depends systematically on walking speed. Therefore, we first determined the relative frequency of occurrence of tripod, tetrapod and undefined coordination in all four fly strains. We found that wt^{CS} and wt^{Berlin} flies almost exclusively used tripod coordination, while in w^{1118} and w^{1118}, Tbh^{nM18} flies tetrapod and undefined coordination patterns represented almost one-third of all patterns (Fig. 7A). When we pooled the data from all strains and plotted the relative frequency of occurrence of coordination types in three different speed ranges we found that tetrapod and undefined coordination patterns occurred almost exclusively at speeds below $5\,BL\,s^{-1}$ (Fig. 7B). Because we chose a rather conservative tripod definition, we frequently found this coordination type in all four strains. To further flesh out the relationship between tripod coordination and walking speed, we examined the TCS as a function of speed in all four strains (Fig. 7C–F). Fig. 7G shows five exemplary footfall patterns illustrating TCS ranging from 0.8 to 0.1. Generally, in all four strains TCS was variable, but depended systematically
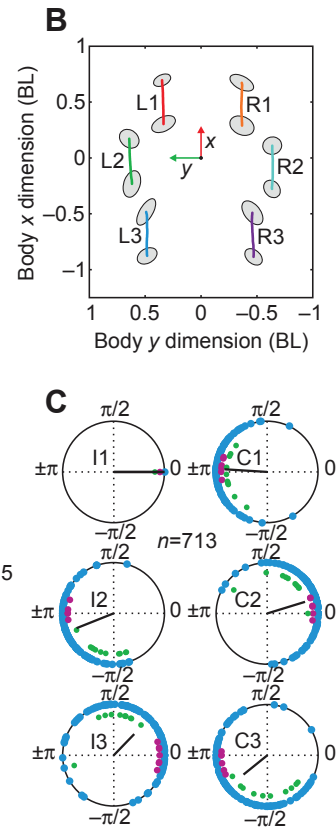
on movement speed. While we did not expect TCS to reach 1.0 because of the aforementioned phase lags within a tripod group, at speeds higher than $10\,BL\,s^{-1}$ it reached maximal values of up to 0.85 (see Fig. 7G). Below $10\,BL\,s^{-1}$, TCS ranged from 0.02 to 0.8. In general, at speeds higher than $10\,BL\,s^{-1}$, inter-leg coordination was tripod. Its variability increased noticeably towards lower speeds, as indicated by lower TCS values. In the range of low walking speeds (<$10\,BL\,s^{-1}$), *Drosophila* seems to be able to also use tetrapod coordination or even wave gait.

Inter-leg coordination changes after the loss of one hindleg

The results presented here suggest that *Drosophila*'s walking system does not generate a fixed motor output. Instead, it seems to be able to flexibly produce inter-leg coordination patterns that change in a systematic and gradual fashion with walking speed. At very slow walking speeds, *Drosophila* uses wave gait; with an increase in speed, inter-leg coordination then transitions to tetrapod and finally becomes tripod at the highest speeds. In order to further study the basis of this apparent flexibility, in a final set of experiments we examined walking in wt^{CS} flies shortly after the removal of one hindleg (Fig. 8). The loss of a leg drastically changes the body geometry and if the animal wants to continue walking successfully it has to adapt its movement pattern to this new geometry. One necessary prerequisite for such an adaptation is that sensory information originating in the legs is taken into account by the neural system that generates walking behavior.

We observed five changes in the walking behavior of flies after the loss of one hindleg: (i) wt^{CS} flies with a missing hindleg walked on average slower than intact animals of the same strain (Fig. 8A;
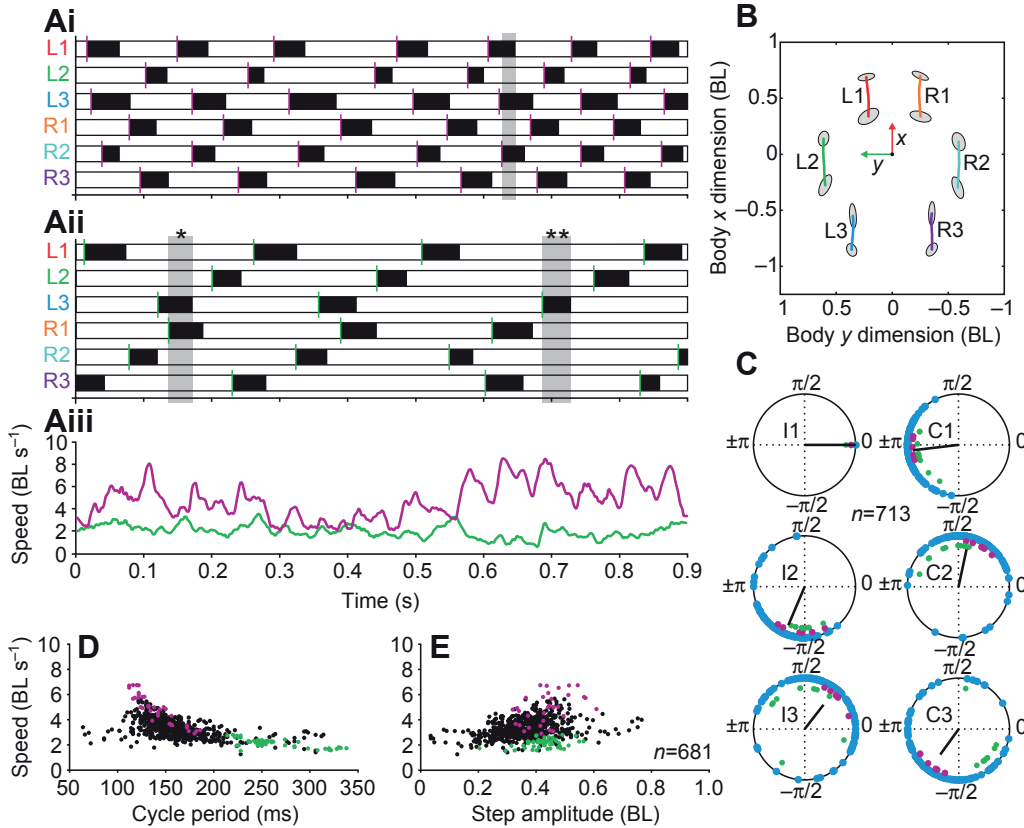
Fig. 6. Walking parameters of mutant strain *w*^{1118}, *Tbh*^{nM18}. (Ai) Footfall pattern of all six legs during 0.5 s of one faster trial and (Aii) 0.5 s of one slower trial, and (iii) walking speed of the body during the 0.5 s of the trials shown in Ai (magenta graph) and Aii (green graph) (R1, R2, R3: right front leg, middle leg and hindleg; L1, L2, L3: left front leg, middle leg and hindleg). Black bars indicate swing phase, white bars indicate stance phase; magenta lines indicate onset and end of complete step cycles in the faster trial, green bars indicate those in the slower trial. Shaded areas highlight coordination patterns of interest (see Results). (B) Average stance trajectories of all legs of all trials in relative body coordinates. (C) Phase plots of swing onset of all legs with respect to the left front leg (blue: data from all trials; magenta: data from Ai; green: data from Aii; black line: mean vector – length indicates variance). (D) Cycle period as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii). (E) Step amplitude as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii).

cf. Fig. 2A). Average walking speeds ranged from 1 to 13 BL s^{-1}, which is equivalent to approximately 2–26 mm s^{-1} (5 individuals, 664 steps), compared with a range of 4 to 18 BL s^{-1} in intact animals. (ii) The shape of stance trajectories changed after amputation of one hindleg and showed distinct curvature. (iii) In all legs, AEPs and PEPs changed within the body coordinate system (Fig. 8B). Generally, we found an outward shift of AEPs and PEPs. In addition, especially in the remaining middle legs and hindlegs, these positions were also shifted caudally. (iv) The average stance trajectories of the remaining hindleg and of both middle legs became noticeably longer. Stance trajectory length increased in the remaining hindleg from 0.43 to 0.47 BL, and in the middle leg contralateral to the lesion from 0.50 to 0.53 BL. The most noticeable increase was found in the middle leg ipsilateral to the lesion. Here, average stance trajectory length increased from 0.50 to 0.60 BL. (v) Phase relationships of both the contralateral middle leg and the remaining hindleg were altered. The hindleg contralateral to the lesion (leg I3) was, on average, no longer in phase with the ipsilateral middle leg (C2); it increased its phase with regard to I3 to 0.85 rad (Fig. 8C) as compared with the intact animal in which the phase of C2 with regard to I3 was 0.16 on average (Fig. 2C). Furthermore, the contralateral middle leg showed an increase in phase with regard to the contralateral front leg (Fig. 8C; cf. Fig. 2C). As a consequence, generally three to four legs were simultaneously on the ground. Slow-walking individuals used either tetrapod or wave gait coordination (Fig. 8Aii). The correlation between walking speed and cycle period was still present though, and step amplitude was not correlated with speed (Fig. 8D,E).

DISCUSSION
We have shown that the walking system of *Drosophila* is able to generate a broad range of locomotion speeds and different strains

walked at preferred parts of this complete range. *wt*^{CS} flies tended to walk faster than both *wt*^{Berlin} and *w*^{1118} individuals. Mutant *w*^{1118}, *Tbh*^{nM18} individuals walked at the lowest speeds. At high speeds, all individuals walked in tripod coordination. With decreasing walking speed, TCS decreased as well (Fig. 7C–F) and animals also used tetrapod coordination more frequently (Fig. 7B). Finally, at very low speeds, walking was often accomplished by simultaneous stance phases of five legs while only a single leg was in swing phase at a time. These findings imply that *Drosophila*'s walking behavior is more flexible than previously thought (Strauss and Heisenberg, 1990): there are no clearly separable gaits and, more specifically, the neural controller producing inter-leg coordination is not restricted to a fixed tripod pattern.

This notion is substantiated by amputation experiments, in which we examined the walking behavior of animals after the loss of one hindleg. These experiments were carried out with individuals of *wt*^{CS}, which is the strain that showed the most robust tripod coordination when intact. Removal of a hindleg in these flies resulted in an immediate reorganization of overall posture, single leg kinematics and inter-leg coordination: the legs of the animals were positioned in a broader frame, the stance trajectories of the remaining middle legs and hindlegs were elongated while the phase of these legs was increased.

Changes in inter-leg coordination related to walking speed
In the first part of the present study we analyzed walking in the *Drosophila* strains *wt*^{CS} and *wt*^{Berlin} as well as the mutant strains *w*^{1118} and *w*^{1118}, *Tbh*^{nM18} with respect to single leg kinematics and inter-leg coordination. Walking speed differed noticeably between strains, with that of *wt*^{CS} and *wt*^{Berlin} ranging from 5 to 16 BL s^{-1} (11–32 mm s^{-1}), *w*^{1118} speed ranging from 2 to 15 BL s^{-1} (3.5–31 mm s^{-1}), and *w*^{1118}, *Tbh*^{nM18} speed ranging from 1.5 to
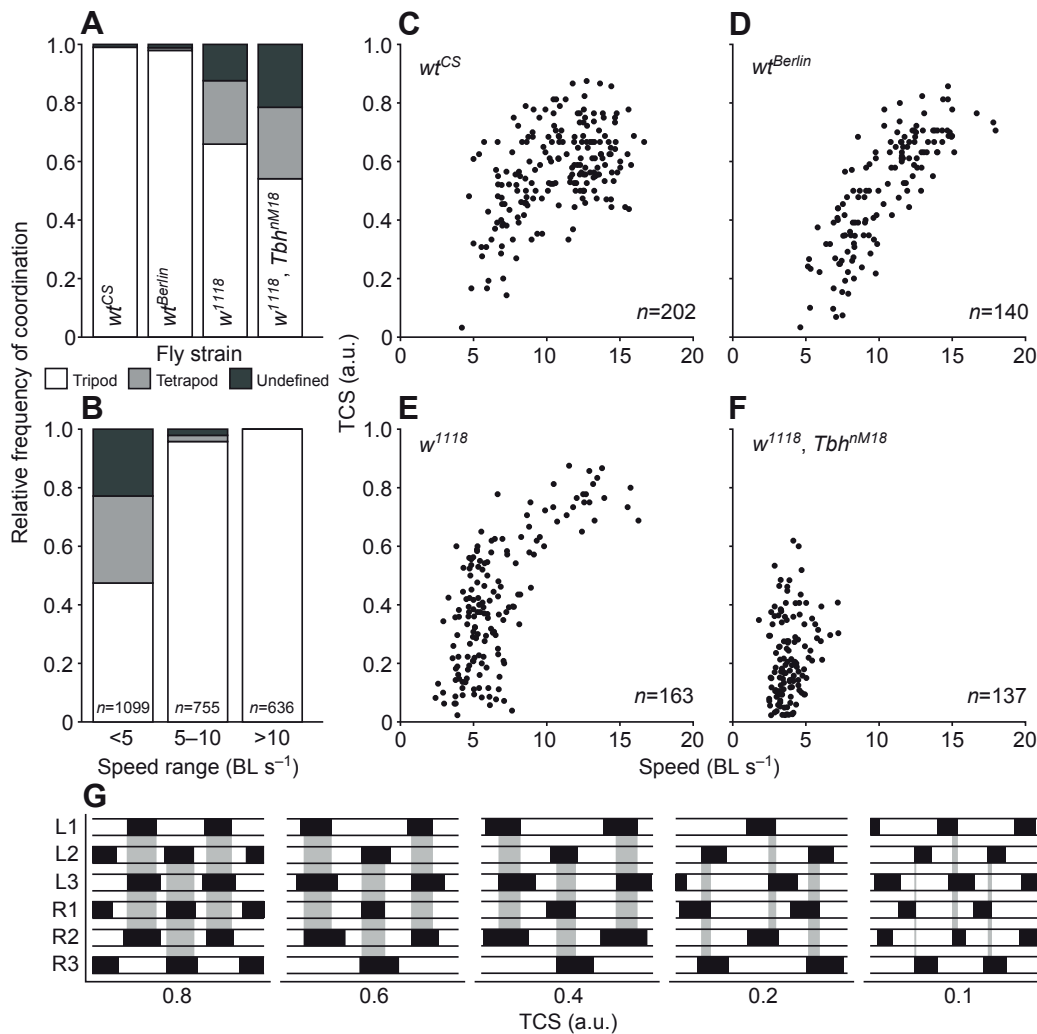
Fig. 7. Analysis of inter-leg coordination. (A) Relative frequency of tripod, tetrapod and undefined coordination in the four different strains (for definition of coordination types see Materials and methods). (B) Relative frequency of tripod, tetrapod and undefined coordination at slow (<5 BL s^{-1}), medium (5–10 BL s^{-1}) and high walking speeds (>10 BL s^{-1}). (C–F) Tripod coordination strength (TCS, for definition see Materials and methods) as a function of walking speed for the different strains: C, wt^{CS}; D, wt^{Berlin}; E, w^{1118}; and F, w^{1118}, Tbh^{nM18}. (G) Five exemplary footfall patterns with a TCS of 0.8, 0.6, 0.4, 0.2 and 0.1 taken from footfall patterns of five different flies. Shaded areas highlight the concurrent overlap of swing phases in the legs of one tripod group.

7 BL s^{-1} (3–14 mm s^{-1}). For the strains wt^{CS} and wt^{Berlin}, the reported average walking speeds in the literature range from 2.2 and 2–3 mm s^{-1} (Serway et al., 2009) to 15 and 21 mm s^{-1} (Poeck et al., 2008; Strauss and Heisenberg, 1993), respectively. Average walking speed for w^{1118} was reported to be approximately 2 mm s^{-1} and for w^{1118}, Tbh^{nM18} it was 4 mm s^{-1} (Scholz, 2005). More detailed data concerning the range of walking speeds are only available for the strain wt^{Berlin}, for which speeds of 12–40 mm s^{-1} were found (Strauss and Heisenberg, 1990). These values correspond with our data in which we found only slightly lower speeds for wt^{Berlin} (11–34 mm s^{-1}). It should be noted, though, that we used a different behavioral protocol from that in previous studies. Some of these used Buridan's paradigm (Bülthoff et al., 1982; Götz, 1980) to elicit straight walks on level ground (Poeck et al., 2008; Serway et al., 2009; Strauss and Heisenberg, 1990; Strauss and Heisenberg, 1993), while others studied walking in *Drosophila* under ambient light conditions without the presentation of visual cues (Scholz, 2005; Wolf et al., 2002).

For all strains examined here, we found that walking speed is controlled *via* changes in step cycle period and stance duration. Over the complete range of walking speeds we found only moderate changes with regard to swing duration, and no systematic modification of step amplitude could be detected. This complements and extends a previous study in which *Drosophila* altered not only its cycle period but also its stride length over the range of walking

speeds (Strauss and Heisenberg, 1990). These authors, however, examined stride length, while the present study focused on step amplitude (see also Materials and methods). The findings presented here do not contradict the previous ones; here, however, we wanted to dissociate the effect body translation during swing phases has on stride length from actual adaptations in leg kinematics during a step cycle. As a consequence, our findings indicate that *Drosophila* controls walking speed solely by adjusting step cycle period while it keeps step amplitude mostly constant.

Strauss and Heisenberg reported that *Drosophila* uses tripod coordination for a large part of the observed speed range (Strauss and Heisenberg, 1990). They found tetrapod coordination only during 'deceleration episodes prior to turns or to a complete stop'. In general, we can confirm these findings. However, in the present study wt^{Berlin} flies also spontaneously generated relatively slow walking bouts. In these trials we found that inter-leg coordination deviated from a strong tripod pattern, as indicated by low TCS values. Comparing this result with the data for wt^{CS} and w^{1118} revealed that this change in coordination is indeed systematically found when *Drosophila* walks more slowly. At walking speeds higher than 10 BL s^{-1}, inter-leg coordination was always tripod. At lower speeds, TCS decreased and within this speed domain we also observed tetrapod coordination. This analysis suggests that the kinematics of the movement pattern generally change systematically and continuously with walking speed.
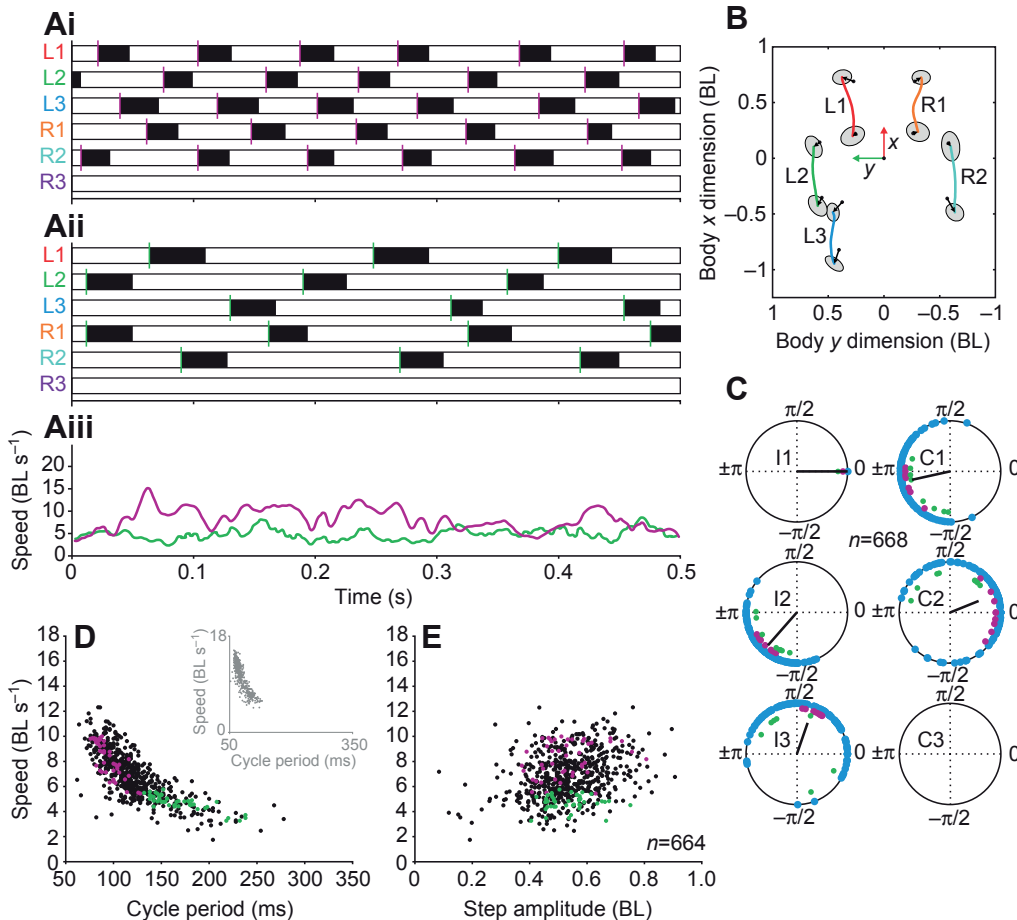
Fig. 8. Walking parameters of *wt*^{CS} after removal of one hindleg. (Ai) Footfall pattern of all six legs during 0.5 s of one faster trial and (Aii) 0.5 s of one slower trial, and (Aiii) walking speed of the body during the 0.5 s of the trials shown in Ai (magenta graph) and Aii (green graph) (R1, R2, R3: right front leg, middle leg and hindleg; L1, L2, L3: left front leg, middle leg and hindleg). Black bars indicate swing phase, white bars indicate stance phase; magenta lines indicate onset and end of complete step cycles in the faster trial, green bars indicate those in the slower trial. (B) Average stance trajectories of all legs of all trials in relative body coordinates. Black arrows indicate shifts of the anterior extreme position (AEP) and posterior extreme position (PEP) (cf. Fig. 2B). (C) Phase plots of swing onset of all legs with respect to the left front leg (blue: data from all trials; magenta: data from Ai; green: data from Aii; black line: mean vector – length indicates variance). (D) Cycle period as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii). For comparison, gray inset shows rescaled data from Fig. 3B. (E) Step amplitude as a function of walking speed (black: data from all trials; magenta: data from Ai; green: data from Aii).

It is important to emphasize what a decrease in TCS means with regard to inter-leg coordination: a TCS of 0.5 means that the swing phases of the legs associated with a tripod group overlap 50% of the time during which any of these legs move. For a TCS of 0.4 this decreases to 40%; however, this also means that for 60% of the time these legs are not in swing phase simultaneously. In other words, during this time four or five legs are on the ground. This time only increases with a further decrease in TCS. Consequently, although low TCS levels still indicate tripod coordination (according to our conservative definition), swing phase overlap in these cases might be more consistent with coordination patterns that conventionally have been associated with tetrapod coordination. In addition, examination of the two mutant strains w^{1118} and w^{1118}, *Tbh*^{nM18} shows that at very low walking speeds *Drosophila* no longer uses tetrapod coordination and instead coordinates its legs in a pattern that resembles wave gait, a pattern first described for larger insects (Hughes, 1952; Wilson, 1966).

Interestingly, it appears that inter-leg coordination in *Drosophila* reflects all possible coordination patterns known in insects. Studies on inter-leg coordination in other, much larger insects, including cockroaches and beetles (Hughes, 1952), or grasshoppers (Burns, 1973), showed that inter-leg coordination is tripod only at high walking speeds and short cycle periods. At lower speeds, inter-leg coordination becomes increasingly variable, including tetrapod walking patterns. However, in these studies the examined species often differed noticeably in size and mass. Burns, for instance, studied two orthopteran species, locusts and grasshoppers, which differed in size by a factor of two (Burns, 1973). With respect to a systematic analysis of inter-leg coordination and walking speed, previous insights were derived from studies on ants (Zollikofer, 1994), cockroaches (Delcomyn, 1971) and stick insects (Graham, 1985; Wendler, 1966). Freely walking ants predominantly use tripod coordination in a speed range between 5 and 32 BL s^{-1}; no data, however, are available for slower walking speeds. Unrestrained cockroaches walk at speeds in the range 1–20 BL s^{-1} (Bender et al., 2011; Delcomyn, 1971) and it has been reported that tripod coordination is present across a broad range of speeds, i.e. above 1.2 BL s^{-1}. However, inter-leg coordination in cockroaches becomes more variable with slower speeds. Delcomyn (Delcomyn, 1971) used the term 'uncoupled alternating triangle' for the increasing variability in tripod coordination occurring at slow speeds (Kozacik, 1981). Bender and co-workers (Bender et al., 2011) also reported clear changes in inter-leg coordination related to walking speed. They proposed the term ambling gait for inter-leg coordination that is found during slow walking. It is important to note that although cockroaches tend to move the legs of a tripod group simultaneously at low speeds, the coordination pattern becomes much more variable and there does not seem to be a fixed coupling anymore. When adult stick insects walk on a level surface they mostly do so at speeds well below 1 BL s^{-1}; in this situation, their preferred inter-leg coordination is tetrapod (Cruse et al., 2009; Graham, 1972). At higher speeds, sequences of tripod coordination can also be observed (Graham, 1972). Our results on *Drosophila* show two things: firstly, as has been found in the stick insect, inter-leg coordination in *Drosophila* is not fixed, but changes systematically and gradually as a function of walking speed over a broad speed range; secondly, below walking speeds of 5–6 BL s^{-1}, *Drosophila* seems to be able to choose which coordination type it uses and can walk in tripod, tetrapod or even wave gait-like inter-leg coordination. Importantly, we found that

swing duration was uncorrelated with walking speed. These findings have implications for the organization of the neural structure controlling walking in *Drosophila*: there is no justification for the hypothesis that there is a specific neural tripod generator in *Drosophila*.

This conclusion is corroborated by the changes observed in inter-leg coordination following the loss of one hindleg in wt^{CS}, which is the strain that had the most robust tripod coordination pattern. We found that inter-leg coordination as well as stance kinematics changed after the loss of one hindleg (Fig. 8). In the present study, compensatory changes were observed on two different levels: temporal and kinematic. With regard to temporal coordination, the stepping activity of the remaining legs, specifically the contralateral middle leg and hindleg, was modified such that the absence of support from the missing hindleg was compensated for. Swing phase activity in the contralateral hindleg and middle leg was delayed compared with that in an intact animal. Kinematic changes entail an extended stance trajectory in the ipsilateral middle leg and a general outward shift of AEPs and PEPs, i.e. overall, the animal adopts a broader posture. In particular, this outward shift suggests an overall compensatory modification of body posture. In addition, the extended stance trajectory of the ipsilateral middle leg nicely corresponds to Cruse's coordination rule 1 (Cruse et al., 1998; Dürr et al., 2004). This rule ensures that a leg in swing phase inhibits the transition to swing phase in an anterior neighbor. As the amputated leg in the present study can be interpreted as being locked in swing phase, this would explain the extended stance phase in the ipsilateral middle leg. These findings are interesting as they provide evidence for cooperative interactions (neural and mechanical) between the legs in the generation of propulsion and posture. Similar changes in inter-leg coordination after the loss of one leg have been reported for stick insects (Bässler, 1972; Graham, 1977) and cockroaches (Delcomyn, 1991; Hughes, 1957). Hughes (Hughes, 1957), for instance, found that upon removal of one hindleg in cockroaches, the other legs had extended stance trajectories and the stance trajectories were shifted outward. Our results also parallel findings reported by Delcomyn (Delcomyn, 1991), who showed that inter-leg coordination during walking became more variable after the loss of one hindleg (compare Fig. 2C with Fig. 8C). We note, however, that the postural adaptations we observed, especially the broader placement of the tarsi, might at least in part be due to a relative increase in load, a consequence of the loss of muscle force available to the animal.

Based on the changes in inter-leg coordination with regard to walking speed and upon removal of one leg, we conclude that the neural control system for walking in *Drosophila* allows for a modular control of single-leg stepping in which individual legs are largely independent of each other and are only loosely coupled. We hypothesize that the neural control system for walking in *Drosophila* is similar to that in fast-walking insects, like ants and cockroaches, as well as to that found in insects like the stick insect. The behavior of *Drosophila* agrees well with that found in stick insects (see Introduction). Behavioral studies in stick insects suggest that inter-leg coordination is the result of the interplay of individual leg controllers based on specific rules (Cruse, 1990; Dürr et al., 2004). Although not (yet) studied in *Drosophila*, it is quite conceivable that the 'coordination rules 1–3', as proposed by Cruse (Dürr et al., 2004), would suffice to generate the walking behavior observed here. However, the fact that the output of any locomotor system is shaped by the complex interaction between neural and mechanical influences needs to be taken into account. In order to further substantiate how *Drosophila*'s walking system compares to that of other insects it will be necessary to distinguish between the level of neural control and the level of mechanical coupling. Experimental paradigms for insect locomotion are available that allow for this dissection, e.g. slippery surfaces that reduce or even remove mechanical coupling between the legs (Graham and Cruse, 1981; Gruhn et al., 2006).

Another interesting aspect of the present study is the results for the two mutant strains w^{1118} and w^{1118}, Tbh^{nM18}. Both of these strains exhibited walking speeds that were lower than those of the two wild-type strains, a fact that allowed us to extend the range of speeds that we investigated. Walking speed in w^{1118}, Tbh^{nM18} was lower than that in w^{1118}. It is quite conceivable that w^{1118} flies walk slower because of visual impairment (Kalmus, 1943). The even lower speed range used by w^{1118}, Tbh^{nM18} can likely be attributed to the fact that w^{1118}, Tbh^{nM18} lacks octopamine (Monastirioti et al., 1996), a biogenic amine that plays an important role during various locomotor behaviors in invertebrates. It is known to influence the initiation and maintenance of flight (Brembs et al., 2007) and pre-flight jumps in *Drosophila* (Zumstein et al., 2004), and is also implicated as a modulator of walking behavior in cockroaches, for example (Gal and Libersat, 2008; Gal and Libersat, 2010). Interestingly, in all of these studies octopamine appears to selectively influence high-level aspects of locomotion, while more low-level aspects, such as leg kinematics, for instance, remain unaffected. Although the present study did not focus specifically on the effects of octopamine, our data support the findings of these previous works. Individuals of the w^{1118}, Tbh^{nM18} strain walked noticeably slower and less frequently, while inter-leg coordination and kinematics seemed to be very similar to those of w^{1118}. It is important to note that these low octopamine levels might only explain reduced walking speed in w^{1118}, Tbh^{nM18}. While w^{1118} also has reduced levels of other biogenic amines like dopamine and serotonin (Sitaraman et al., 2008), its octopamine levels are similar to wild-type or are only very slightly reduced (Sitaraman et al., 2008; Yarali et al., 2009). Modifying octopamine levels might be useful in future studies in order to specifically modulate the walking behavior in *Drosophila* mainly with regard to movement speed.

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