

The behavioural transition from straight to curve walking: kinetics of leg movement parameters and the initiation of turning

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Summary

The control of locomotion requires the ability to adapt movement sequences to the behavioural context of the animal. In hexapod walking, adaptive behavioural transitions require orchestration of at least 18 leg joints and twice as many muscle groups. Although kinematics of locomotion has been studied in several arthropod species and in a range of different behaviours, almost nothing is known about the transition from one behavioural state to another. Implicitly, most studies on context-dependency assume that all parameters that undergo a change during a behavioural transition do so at the same rate. The present study tests this assumption by analysing the sequence of kinematic events during turning of the stick insect *Carausius morosus*, and by measuring how the time courses of the changing parameters differ between legs. Turning was triggered reliably at a known instant in time by means of the optomotor response to large-field visual motion. Thus, knowing the start point of the transition, the kinematic parameters that initiate turning could be ranked according to their time constants.

Kinematics of stick insect walking vary considerably among trials and within trials. As a consequence, the behavioural states of straight walking and curve walking

are described by the distributions of 13 kinematic parameters per leg and of orientation angles of head and antennae. The transitions between the behavioural states are then characterised by the fraction of the variance within states by which these distributions differ, and by the rate of change of the corresponding time courses. The antennal optomotor response leads that of the locomotor system. Visually elicited turning is shown to be initiated by stance direction changes of both front legs. The transition from straight to curve walking in stick insects follows different time courses for different legs, with time constants of kinematic parameters ranging from 1.7 s to more than 3 s. Therefore, turning is a behavioural transition that involves a characteristic orchestration of events rather than synchronous parallel actions with a single time constant.

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Key words: curve walking, turning, behavioural transition, stick insect, *Carausius morosus*.

Introduction

Walking is a context-dependent locomotor behaviour. As an insect moves from the dry, furrowed trunk of a tree to a leaf moist with dew, the environmental conditions that it encounters are marked by gradual changes in surface structure, friction, direction of gravity and the like, and by local discontinuities such as gaps, edges or branching points. The movement pattern of the legs needs to adapt to such changes in locomotor context, as the requirements to the underlying neural controller are altered. Indeed, leg movements in different behavioural contexts are associated with changes in activity patterns in leg motor nerves (e.g. Reingold and Camhi, 1977; Delcomyn, 1987; Duch and Pflüger, 1995), forces and kinematics (e.g. Cruse, 1976; Jindrich and Full, 1999). Interestingly however, transitions between the locomotor states or 'programs' studied are largely subject to speculation or

implicit assumptions, in spite of the often detailed knowledge of the associated changes. One reason for this might be that locomotor patterns have mostly been analysed in situations similar to a steady state, where the observed pattern is stable enough to obtain sufficient data for subsequent analyses.

A prominent example of a well-studied change in locomotor context is curve walking in arthropods, where kinematic changes, gaits in particular, are documented in insects (Zolotov et al., 1975; Franklin et al., 1981; Jander, 1985; Strauss and Heisenberg, 1990; Zollikofer, 1994; Jindrich and Full, 1999), arachnids (Land, 1972) and crustaceans (Cruse and Silva Saavedra, 1996; Domenici et al., 1998, 1999; Copp and Jamon, 2001). Although two of these studies have identified the contribution of individual legs to yaw torque (Domenici et al., 1999) or a lateral component in ground reaction force (Jindrich

and Full, 1999), they do not reveal how a particular event in one leg, e.g. the strong outward push of a front leg, relates to the action sequence of the remaining legs. In other studies where fast turning reactions could be related to triggering sensory events, the subsequent series of actions have either not been analysed in terms of leg kinematics (Camhi and Johnson, 1999; Hedwig and Poulet, 2004) or not as a temporal sequence (Nye and Ritzmann, 1992).

In the stick insect *Carausius morosus*, leg kinematics (Cruse and Bartling, 1995) and gait (Graham, 1972) in straight walking have been well studied. During curve walking, associated changes of leg movements can be related to the curvature of the walked path (Jander, 1982), yielding a rather complete description of stepping pattern for any given steady state of the motor system (where 'state' refers to the set turning tendency). Nevertheless, it remains unclear how the animal actually begins to turn, i.e. how the transition from one of these patterns to another takes place. This behavioural transition, however, is most interesting because steady state curves are not very likely to occur in a real life situation. Rather, it is plausible to assume that any turn performed by a stick insect is a transient change in walking direction, much as in cockroach wall-following (Camhi and Johnson, 1999) or cricket phonotaxis (Hedwig and Poulet, 2004). The objective of this study was to analyse the temporal sequence of leg movements during a transition in walking direction, in order to decide whether the underlying mechanisms act at the same rate in all legs or should be grouped into primary actions that initiate turning and secondary actions that support turning.

A model simulation of the stick insect walking system implemented turning tendency by a course control module that reduced/increased retraction amplitude in legs at the inner/outer side of the curvature (Cruse et al., 1998). Curve walking performance of this controller was quantified for different path curvatures and walking speeds (Kindermann, 2002). It does well for shallow curves but increasingly deviates from observations on real stick insects on tighter curves, suggesting that the implemented mechanism is different in real stick insects. At least two alternative mechanisms are conceivable. Firstly, altering the target touch-down location of the swing movements (leg protraction through the air) of front legs could cause a change in direction of front leg stance movements (leg retraction during ground contact) which, due to a targeting mechanism in middle and rear legs (Cruse, 1979), affects stance direction of the trailing legs. Secondly, a change of stance direction rather than, or additional to, retraction amplitude could act to rotate the resulting ground reaction force vectors to generate a yaw torque. This would be similar to what has been found in cockroaches (Jindrich and Full, 1999). The present study can distinguish between these alternatives.

The experiment was designed as a three-part measurement with two steady state intervals of equal duration, an initial straight walk state and a terminal curve walk state, separated by a transition interval. The analysis was carried out in two steps. First, the kinematic parameters that significantly changed between the two steady states were identified and their

relative magnitude was calculated. In a second step, the time course of each parameter having a statistically significant change between steady states was determined by finding the time constant that best described the kinetics of the observed change. It is shown that the behavioural transition from straight to curve walking is initiated by a primary change in stance direction in both front legs, followed by a number of secondary changes in all other legs. Preliminary results have been published in abstract form (Dürr and Authmann, 2002).

Materials and methods

Experimental procedure and measurement of walked path

Stick insects of the species *Carausius morosus* Brunner 1907 were bred in a predominantly parthenogenetic laboratory culture at the University of Bielefeld. In the experimental set-up, adult females walked on a hollow, thin-walled, low friction Styrofoam ball (diameter, 19.7 cm, mass 11.6 g, moment of inertia $7.5 \times 10^{-2} \text{ g m}^2$) that floated on an air cushion (Fig. 1). The angular moment of inertia of a stick insect is approximately that of a cylinder of equal mass and dimension, rotating around an axis perpendicular to its long axis ($5.4 \times 10^{-4} \text{ g m}^2$). Thus, the sphere increases the natural moment of inertia by a factor of 139 and, therefore, demands larger torques for yaw rotation than during normal walking. As for translational forces, the stick insect requires only 1.23 times more force to accelerate the surface of the sphere in the same way as it would accelerate its own body during straight walking.

Turning was triggered by a large-field visual motion stimulus that reliably caused a strong optomotor response, i.e. a sustained curve walk in the direction of stimulus motion. The visual stimulus was a rotating paper drum with vertical black and white stripes, diffusely illuminated from the outside (drum size: height=32 cm, diameter=27.4 cm; stimulus pattern: $\lambda=24^\circ$, mean luminance=60 cd m⁻², contrast=90%). Following a pre-stimulus period of 7.5 s of straight walking (no visual motion), the drum was rotated for a period of 15 s at an angular velocity of 38.1 deg. s⁻¹, resulting in a contrast frequency of 1.6 Hz. For data analysis, the stimulus period was divided into an early and a late period of equal duration. In terms of walking behaviour, the early stimulus period caused the behavioural transition from straight to curve walking, whereas the late period was characterised by steady state curve walking. Preliminary experiments had shown that stick insects need about four step cycles to complete a transition from straight to curve walking under the given stimulus conditions. To make sure that the transition from straight to curve walking was complete after the early stimulus period, we chose an interval sufficient to comprise six average step cycles, equivalent to 7.5 s. Thus the late stimulus period could be safely considered as a steady state period. Equal duration of all three experimental periods was chosen for convenience of statistical analysis.

Drum rotation and data acquisition was controlled by a PC computer. Direction of visual motion was randomised, but data of counter-clockwise trials were reversed during the analysis to allow pooling of trials. All trials were thus standardised to

clockwise stimulus motion and, therefore, clockwise turning reactions. Accordingly, with respect to the curved path, left legs are always outer legs, right legs are always inner legs.

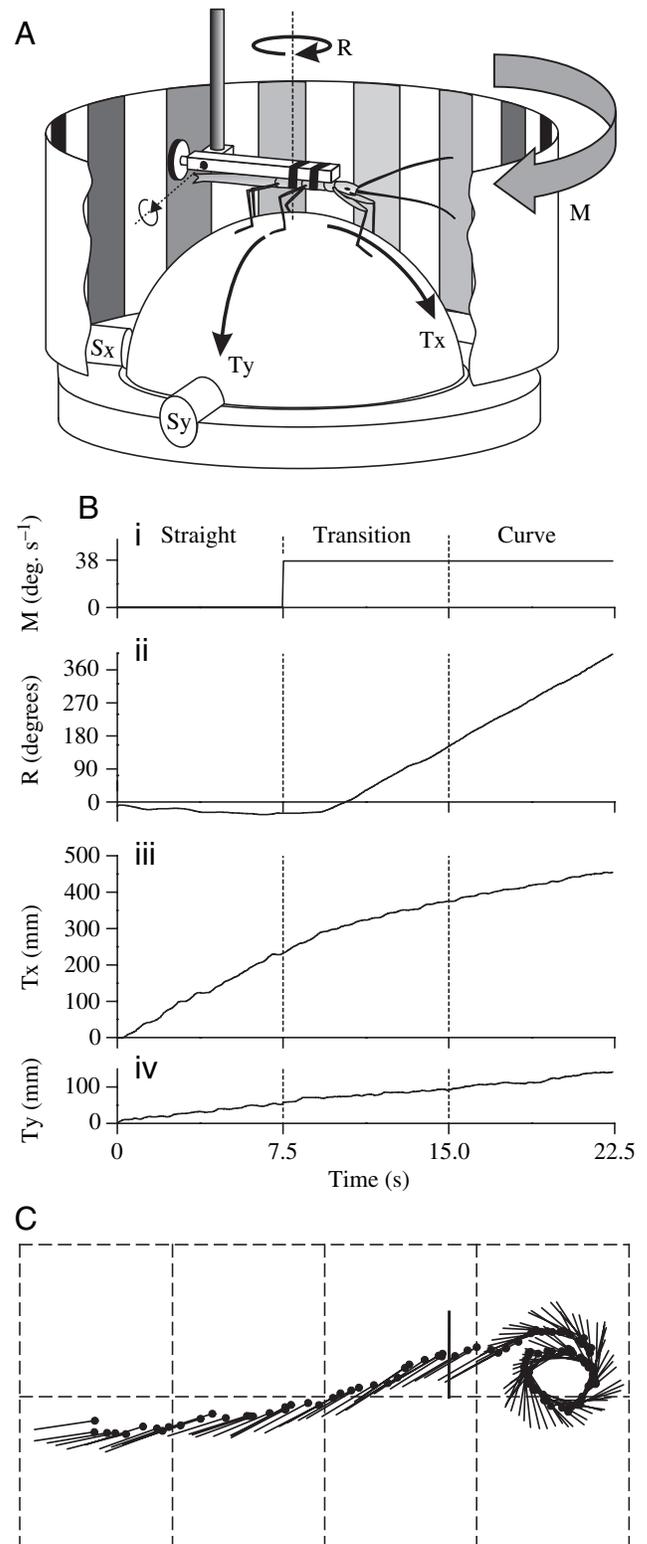
To prohibit sideward and forward translation of the body relative to the set-up without constraining body height, animals were tethered to a 9 cm rod made of balsa wood that was hinged on a horizontal rotation axis. A metal screw at the opposite end of the rod counter-balanced the weight of the lever arm such that the animals only had to carry their own body weight. The surface of the ball was covered by evenly spaced patches of retro-reflective foil (Scotchlite 8850, 3M, Neuss, Germany). As the walking insect caused rotation of the ball, all three degrees of freedom of rotation were monitored by an optical tracking system (custom-made by Dr Hansjürgen Dahmen, Department of Cognitive Neuroscience, University of Tübingen, Germany). Two motion sensors, located at the equator of the sphere (S_x and S_y in Fig. 1A), flashed brief infrared light pulses at the surface of the sphere and recorded the shift of the reflected image by means of a CMOS chip (Arreguit et al., 1996). Sampling rate of the tracker sensors was 832 Hz and angular resolution was approximately 0.15° . Upon receiving a synchronisation trigger pulse from the video system (see below), the computer recorded the summed sensor readings and sent a reset signal. Sensor readings were then converted into measures of intended yaw rotation, and forward and sideward translation of the animal (R , T_x and T_y , respectively, in Fig. 1A). Arrows in Fig. 1A indicate the direction of positive sign of the measurements.

Video analysis of leg movements

Walking animals were filmed by a CCD camera (Fricke GmbH, CCD-7250, Lübecke, Germany; 1 ms shutter speed) located 1.4 m above the sphere. Video films were time-stamped (Magnasonic VTG 200, Spitzer-Mileger, Basel, Switzerland) and recorded on video tape (Panasonic NV-F70).

Fig. 1. Experimental design and automated tracking of the walked path. (A) Schematic drawing of the experimental set-up (not drawn to scale). A stick insect was tethered above a sphere on which it stood or walked. Stationary walking caused rotation of the sphere. Two optic sensors (S_x and S_y) detected all three degrees of freedom of rotation of the sphere, each of which could be attributed to one of three components of walking direction: turning, i.e. yaw rotation around the vertical axis (R), forward translation (T_x) and sideward translation (T_y). Arrows indicate the sign of the measurements taken. A large-field visual motion stimulus (M) elicited an optomotor turning response, causing the animal to walk on a curved path. The stimulus pattern was a vertical grating. (B) Each trial lasted 22.5 s and was divided into three periods of equal duration. A pre-stimulus period without stimulus motion was followed by two periods with constant stimulus motion. Diagrams show representative measurements of R , T_x and T_y in response to a step of stimulus velocity. Movement components were used to reconstruct the walked path (C), visualising the overall walking behaviour (same trial as in B). Each symbol indicates the location and orientation of the body axis. Filled circles label the head. Symbols are plotted every 200 ms. Vertical bar indicates stimulus onset. Grid width, 10 cm.

Spatial and temporal resolution were 0.2 mm and 0.04 s, respectively. For synchronisation with the tracker measurements, LEDs on the stimulus set-up indicated the start and end of data recording. Also, the synchronisation pulse of the video signal was electronically extracted and converted into a TTL trigger pulse at a frequency of 50 Hz.



After the experiments, selected video sequences were captured as AVI files on a PC computer (MiroVideo 30plus, Pinnacle Systems GmbH, Braunschweig, Germany) and analysed manually on a frame-to-frame basis, using a custom-written program. Only those video frames in which a stance–swing transition or swing–stance transition occurred were analysed, i.e. the first and last frame of each stance movement, immediately after a leg touched down or was lifted off the ground. The position of the tibia–tarsus joint was recorded at these instances of transition, equivalent to the Anterior and Posterior Extreme Positions of the step cycle (AEP and PEP, respectively). The coordinates of the tarsus location were then converted into metric measures and transformed into relative coordinates with respect to the coxa of the respective leg. Data sequences of each leg always began with the first AEP and ended with the last transition point (either AEP or PEP) after stop of stimulus motion.

Data analysis

A simple sign test (Hayes, 1988, p. 139) was applied to determine the directional bias of spontaneous turning without stimulus motion. For the kinematic analysis, 13 parameters were determined for each leg and step cycle. These were direction, length, duration and duty cycle of both stance and swing movements, the x - and y -coordinates of the AEP and PEP, and the step period. The experimental period of 21.5 s was divided into 42 time bins. In each bin, data points were pooled from all trials in which either a stance or swing movement began in that bin. Means and standard deviations (S.D.) of all parameters and legs were determined, and a Mann–Whitney test on statistical difference of the median between the pre-stimulus period and the late stimulus period was calculated (Statistics Package for the Social Sciences 10, SPSS Inc.). Tests where $P \leq 0.001$ was reached were considered significant, which seemed appropriate given the large sample sizes of 337–439 steps for the straight walking period and 134–295 steps for the late curve walking period. Typically, this level was reached if the ratio

$$\delta = |R_C - R_S| / \sqrt{\sigma_C^2 + \sigma_S^2}$$

was larger than 0.25, where R_S and R_C are the mean parameter values during straight and curve walking, respectively, and σ_C and σ_S are the corresponding standard deviations of the mean. The ratio δ was also used to rank the magnitude of the observed change during turning, as it standardises the difference between two steady state responses to the natural variance of the parameter (see also text referring to Table 1). Note that a less stringent significance level would have not changed the order of the 49 strongest effects given in Table 1.

To characterise the time course of each kinematic parameter, non-linear curve fits were calculated for all parameters with a statistically significant change between the two steady states. Assuming a low-pass-filtered response to the step of stimulus velocity, the exponential association function

$$f(t) = (R_C - R_S) * [1 - \exp(-t/\tau)] + R_S$$

was used as the theoretical model underlying the time course after stimulus onset. The time constant τ gave a measure of how fast a parameter changed as the animal commenced turning. Non-linear curve fits were calculated using the Levenberg–Marquardt least-square fitting algorithm of Origin 6.0 (MicroCal) with start condition $\tau=1.0$. As R_S and R_C were fixed to the mean values of the pre-stimulus period (straight walk) and late stimulus period (curve walk), respectively, only a single variable was fitted: the time constant τ . Standard deviations (S.D.) of R_S and R_C were used for instrumental weighting during the fitting process, giving more weight to data points with low S.D. than to those with large S.D. Goodness of fit was evaluated through the statistical measures χ^2 and r^2 (see Table 2) and rated as being sufficient if the fit was statistically indistinguishable from the data set at $P > 0.5$ ($\chi^2 < 27.5$, 28 d.f.). The minimum coefficient of determination required was $r^2 > 0.36$, in which case the correlation was significant with $P < 0.001$. A less stringent criterion would have included parameters where the curve-fitting process was very sensitive to minor fluctuations in the time-course data set. Particularly for parameters with small changes in magnitude the resulting time constants would have been very unreliable. Thus, the choice of significance level reflects a trade-off that potentially accepts fast changes of parameters with small statistical effect to go undetected, while reducing the likelihood of incorrect time constants.

Results

General observations on curve walking in stick insects

Tethered animals walked on a large sphere (Fig. 1A) that they could rotate without any noticeable effort. Once the animal was walking steadily, the experimental protocol was started, consisting of three subsequent periods of equal duration: a straight walk period, a transition period, and a curve walk period. Following the initial period of straight walking in a stationary environment, a large black-and-white grating was rotated around the animal (Fig. 1). When presented to walking animals, this large-field visual motion stimulus reliably caused an optomotor response, in which the stick insect changed its walking direction within a few step periods and turned in the direction of stimulus motion. Bias turning tendency in the stationary environment was 0.6 ± 13.9 deg. s⁻¹. Three out of eight animals showed a statistically significant side preference ($P < 0.05$). During stimulus motion, the distribution of average turning tendencies shifted to -21.6 ± 16.5 deg. s⁻¹ in counterclockwise trials and 17.2 ± 16.4 deg. s⁻¹ in clockwise trials. Occasionally (18/318 trials, 5.7%), animals showed a turning tendency into the wrong direction. 13 of these ‘wrong’ responses were by the three animals with bias turning tendency and consistent with their bias direction. Generally, ‘wrong’ turning tendencies were much weaker than average. Given the large variance of the distribution, they can be considered as outliers of a normal distribution of turning tendencies.

After a transition period, the turning tendency of the insect generally assumed a constant level, resulting in a curve walk

with steady path curvature (Fig. 1C). The change in walking direction was accompanied by obvious kinematic changes in all legs. Also, head orientation and the beating field of both antennae shifted toward the turning direction (see below).

Continuity of walking behaviour and strength of the optomotor response, as indicated by the curvature of the walked path, varied both between and within individual animals. To ensure that parameters were only pooled from qualitatively similar trials, the following two criteria were applied to select a representative and homogenous sample of walking sequences: firstly, forward translation had to be steady throughout the entire measurement; secondly, the diameter of the walked path had to be less than 10 cm. According to these criteria, 33 out of 318 walking sequences from eight animals were selected for further analysis. The steadiness criterion caused us to discard many trials in which the animals transiently stopped walking. This was particularly frequent during the pre-stimulus period, i.e. in a stationary environment. The curvature criterion selected for persistently strong turning responses.

A representative single trial is shown in Fig. 1B, illustrating the three movement components and the walked path. The corresponding tarsus trajectories and stepping pattern are shown in Fig. 2. Prior to stimulus motion, the movement of the animal consisted of steady forward and moderate sideward translation (Fig. 1Biii,iv). A weak bias rotation, as measured in this trial, was also commonly observed. In response to the step of stimulus motion (Fig. 1Bi), the rotation angle reversed sign and increased with constant slope (Fig. 1Bii), indicating steady turning of the animal. During turning, sideward translation remained stable, whereas forward velocity decreased. Reconstruction of the walked path from the movement components reveals a moderately leftward curved trail that enters a nearly perfect circular trail shortly after stimulus onset (Fig. 1C, vertical line indicates stimulus onset).

Although the stepping rhythm during straight walking can be fairly constant, individual steps vary from one to another.

Fig. 2 illustrates this by superimposing the tarsus trajectories of all legs during stance in a body-centred coordinate frame. The orientation of the tethered animal is indicated by the solid circles labeled T1 to T3 that show the location of the thorax-coxa joints. Stance trajectories were weakly curved even during straight walking in a stationary environment

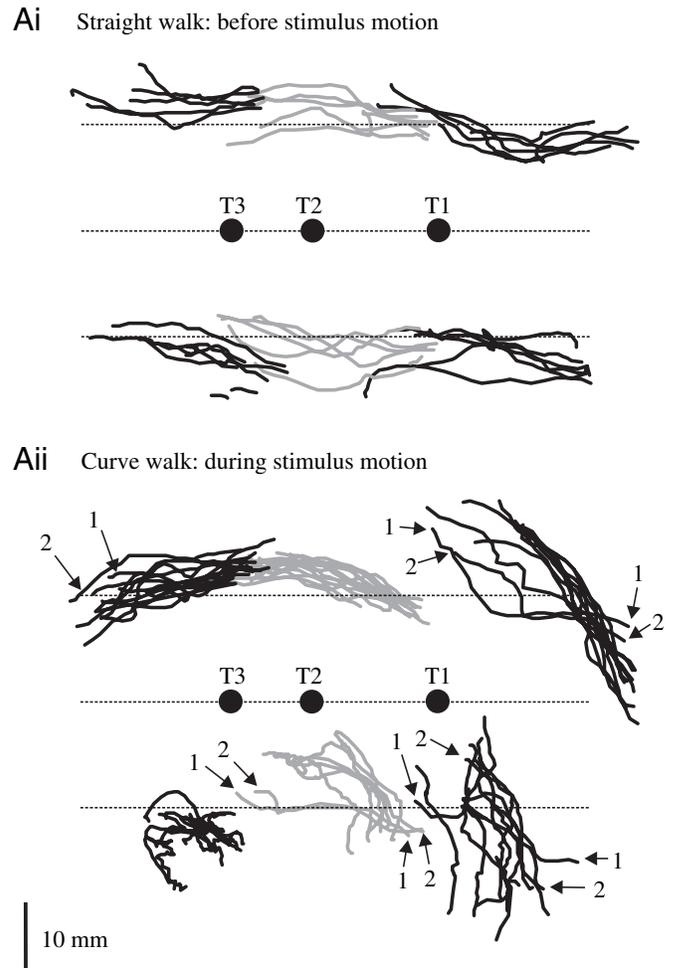


Fig. 2. Stance trajectories and gait pattern change during turning. (A) Stance trajectories of the tibia-tarsus joint on the surface of the sphere, drawn relative to the tethered body. Trajectories are largely parallel to the body axis during straight walking (Ai; before stimulus motion) but of different length and orientation during curve walking (Aii; during stimulus motion). Same trial as in Fig. 1. Each line shows a stance trajectory in body-centred coordinates, with the animal facing to the right. Circles labelled T1, T2 and T3 mark the location of the coxae of the first, second and third thorax segment, respectively. Horizontal dotted reference lines are drawn parallel to the body long axis. Left and right legs are outside and inside the curvature, respectively. For clarity, stance trajectories of middle legs are shown in grey. Numbered arrows on the lower panel indicate selected start- or endpoints of the first and second trajectories after onset of visual motion. (B) The stepping pattern of the same trial as in A. Horizontal line segments depict stance phases of a given leg. O1, O2 to O3 denote outer front, middle and hind legs, respectively (left legs). I1 to I3 denote corresponding inner legs (right legs). Steps that started during the stimulus period are shown in grey.

(Fig. 2Ai), but on average ran parallel to the body long axis. Step lengths varied both between and within legs, and rather short steps could occur in all legs (see right hind leg in Fig. 2A). During curve walking, stance trajectories gradually changed their curvature, length and direction. Numbered arrows in Fig. 2Aii indicate start- or endpoints of the first two stance movements after onset of stimulus motion. In both front legs, the start points of the first two stance movements were located in the same region as during straight walking. The subsequent stance trajectories showed conspicuous bends in the outer front leg (top right), however, and altered orientation in the inner front leg (bottom right). Start points of middle and hind legs changed more subtly. In both hind legs, stance length and the location of trajectory endpoints were clearly discernable soon after onset of stimulus motion. In the inner middle leg, the first two stance trajectories revealed distinct kinks at their ends. In concurrence with the common nomenclature used in the literature, stance trajectory start- and endpoints will be termed Anterior Extreme Position (AEP) and Posterior Extreme Position (PEP), respectively, throughout this paper, despite the fact that during curve walking these labels may not reflect the true location of these points (e.g. AEPs of an inner front leg may be posterior to the PEP).

Apart from the spatial changes of leg movements illustrated in Fig. 2A, temporal coordination between legs also changed during curve walking. This is shown by the sequence of stance phases from the same trial in Fig. 2B. The stepping pattern during the straight walk period (black) is almost a tripod gait, where stance and swing movements (bars and gaps, respectively) of ipsilateral front and hind legs coincide with those of the contralateral middle leg. After start of stimulus motion (grey) the stepping pattern becomes rather asymmetrical, with markedly prolonged stance durations in the inner middle and hind leg. As the current study is concerned with the temporal order of kinematic changes during turning, only the parameters step period, stance and swing phase duration and duty cycle of individual legs are considered here. Leg coordination, i.e. coupling between limbs, is treated separately in the accompanying paper (Dürr, 2005).

Kinetics of overall turning behaviour

As Figs 1 and 2 illustrate the variability of step parameters within a single trial, it is trivial to observe that there is variability between trials, and also between animals. Variability is of central importance to the analyses explained below, not only because it determines whether a given change in magnitude is statistically significant, but also because it is an indicator of consistency among trials that allows ranking of effects irrespective of their unit. For example, consistency of the change in stance direction can be directly compared to the consistency of the change in duty cycle, while their magnitudes cannot be related.

Assuming that there is only a single neural mechanism to elicit visually induced curve walking, this mechanism will directly affect some kinematic parameters (primary parameters), whereas others will be affected by subsequent

indirect, mechanical or neural interactions (secondary parameters). Due to the direct action, visually induced changes of primary parameters are likely to be confounded relatively little by trial-to-trial variability in experimental history, current body posture, neural noise, etc. In contrast, indirect changes of the secondary parameters are likely to be affected more. As the onset of visual stimulus motion causes a change in locomotor context, primary parameters should therefore change more consistently than secondary parameters. This assumption has two implications for the present analysis: (1) primary kinematic parameters that are most important for the initiation of turning, should change most strongly with respect to their variability during the behavioural states before and after the transition; (2) the time course of transition between two behavioural states should be less prone to disturbances or noise and, thus, more reliably detectable in primary than in secondary parameters.

Following these considerations, Fig. 3 describes the behavioural transition from straight to curve walking by the fraction of variability by which each locomotion component changes, and by its rate of change. As the behavioural transition was induced by visual input, the onset of stimulus motion could serve as a reference time for the start of transition. Also, because locomotion was steady before and after the transition period, the steady state magnitude of each locomotion component was measured as the average value in the straight walk and curve walk interval, respectively. The average angular velocity of rotation was close to zero during straight walking (-1°). During turning, it increased by 34.1° with a time constant of 2.4 s. The steady state magnitude changed by 114% of its variability ($\delta=1.14$) and the time course yielded a statistically significant curve fit ($\chi^2=0.8$, $r^2=0.986$). Translation components changed considerably less with respect to their steady state variability, resulting in $\delta=0.33$ and $\delta=0.18$ for sideward and forward speed, respectively. This corresponded to a sideward acceleration by 3.6 mm s^{-1} per 7.5 s and forward deceleration by 2.8 mm s^{-1} per 7.5 s. As the latter difference was not statistically significant, the average turning response to the visual motion stimulus consisted of a large increase in angular velocity, accompanied by a weak increase in sideward velocity.

The behavioural transition from straight to curve walking was observable not only in leg kinematics, but also in corresponding changes of the head and antennal movement pattern (Fig. 4). Stick insects continuously move their antennae during straight walking (Dürr et al., 2001) and also during curve walking, but with a marked shift of their beating field with respect to the body axis (Fig. 4A,B). Whereas the horizontal beating field during curve walking had the same width as during straight walking (σ_S : $19.0\text{--}20.5^\circ$; σ_C : $18.2\text{--}19.1^\circ$), the centre of the beating field shifted from 33.7° to -2.4° for the outer antenna, and from -32.7° to -70.5° for the inner antenna (Fig. 4B). δ -values and time constants associated with this shift were $\delta=1.32$ and $\tau=1.8$ s, respectively, for the outer antenna, and $\delta=1.42$ and $\tau=2.0$ s for the inner antenna. Thus, the shift in both antennal beating

fields leads the change in body yaw rotation. The shift of antennal beating field comprised shifts of the head movement range with respect to the body (Fig. 4C) and of the antennal

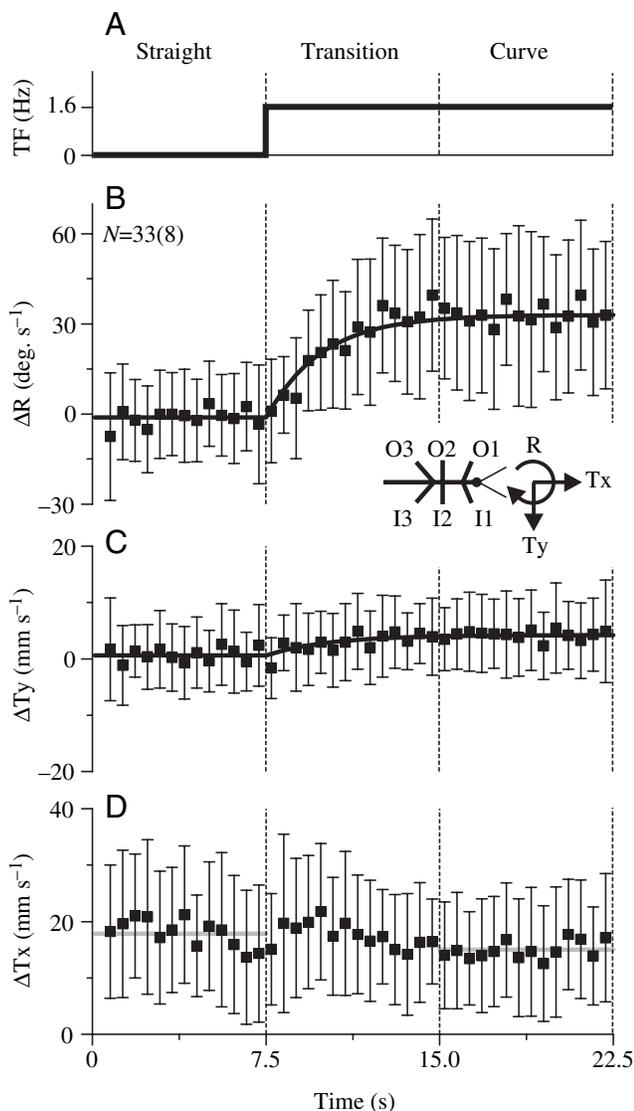


Fig. 3. Kinetics of rotation and translation components during turning. (A) In response to the onset of a visual motion stimulus, i.e. a step in the temporal frequency (TF), stick insects change their locomotor behaviour from a steady straight walk to a steady curve walk. (B–D) The three degrees of freedom of horizontal locomotion were analysed separately and illustrated by their average rate of change per 0.54 s (14 time bins per 7.5 s) among 33 trials from eight animals (square symbols). (B) Angular velocity of yaw rotation (ΔR), (C) speed of sideward translation (ΔT_y) and (D) speed of forward translation (ΔT_x). Values are means \pm S.D., indicating the variability among trials. Arrows in the insert show the sign convention. In response to the visual motion stimulus, angular velocity increases with a time constant of 2.4 s. The speed of sideward translation also increases significantly with a time constant of 2.7 s. Forward speed does not change significantly. Steady state velocities during straight and curve walking are indicated by horizontal solid lines in the first and third measurement interval. Exponential curve fits link significantly different steady states.

movement range with respect to the head (Fig. 4D). The shift of head movement range from 0.5° to -28.1° contributed most strongly to the observed shift in antennal beating field ($\delta=1.50$ and $\tau=2.3$ s). The shifts of the antennal movement ranges were slightly asymmetrical, as the outer antenna did not cross the midline. Whereas the movement range of the outer antenna shifted from 33.1° to 25.7° ($\delta=0.42$, curve fit not significant, $r^2=0.186$), the range of the inner antenna shifted from -33.3° to -42.9° ($\delta=0.51$ and $\tau=1.5$ s). In summary, the time course of the change in head movement range was as fast as the change in body yaw rotation (Fig. 3), and head movement contributed the most to the observed shift in antennal beating field. As both antennal beating fields shifted faster than the head movement range, antennal movement relative to the head must have accounted for this greater speed. This is corroborated by a rather low time constant for the shift in movement range of the inner antenna (black line in Fig. 4D).

Changes of step frequency, stride length and stance direction

Previous studies on curve walking have often been motivated by alternative hypotheses stating that curve walking requires an asymmetry in either step frequencies or step lengths or both on the outside and inside of the curve. Figs 5 and 6 show that, indeed, both step frequency and step length changed, but these changes were not simply due to unilateral increases or decreases in magnitude. Rather, each leg underwent a typical change during the transition to curve walking. Fig. 5 shows the average step frequencies among trials for all six legs. Wilcoxon's matched-pair test was applied to compare step frequencies of the straight walk interval with those of the subsequent curve walk interval on a trial-by-trial basis. This test is insensitive to differences in walking speed among trials, as it only considers sign and size of frequency changes rather than the difference of the sample median. The result reveals that step frequency significantly increased for all outer legs and inner front legs. Note that this significant increase in step frequency was not paralleled by an increase in forward walking speed (see Fig. 3). Interestingly, the apparent reduction of step frequency by the inner hind leg was too variable to be statistically significant. During curve walking, step frequencies significantly differed between contralateral neighbours such that each outer leg stepped at a higher rate than its inner neighbour ($P \leq 0.002$). During straight walking, there was no difference between contralateral neighbours ($P > 0.3$).

Investigation of the second classical curve walk hypothesis, stating relative increase and decrease of step length on the outside and inside, respectively, also proved true for the stick insect. Fig. 6 shows the average location of AEPs and PEPs and indicates the average stance trajectories by black arrows. Fig. 6A,B shows the situation during straight and curve walking, respectively. Stance length of all outer legs significantly increased, whereas that of inner legs significantly decreased during curve walking (compare black and grey arrows in Fig. 6B). In addition to the change in trajectory

length, the direction of stance significantly rotated outward for both front legs and middle legs. The outer hind leg significantly rotated its stance direction to the opposite side, counteracting what otherwise would have resulted in a strong sideward translation. The inner hind leg acted somewhat as a pivot around which the animal turned. Note that the short stance

length, which is defined as the distance between AEP and PEP, does not mean that this leg does not move. On the contrary, it was frequently observed that the inner hind leg appeared to push and pull continuously, as can be seen in the representative trajectories of Fig. 2A and in the video in the supplementary material. Virtually all kinematic parameters of this leg could

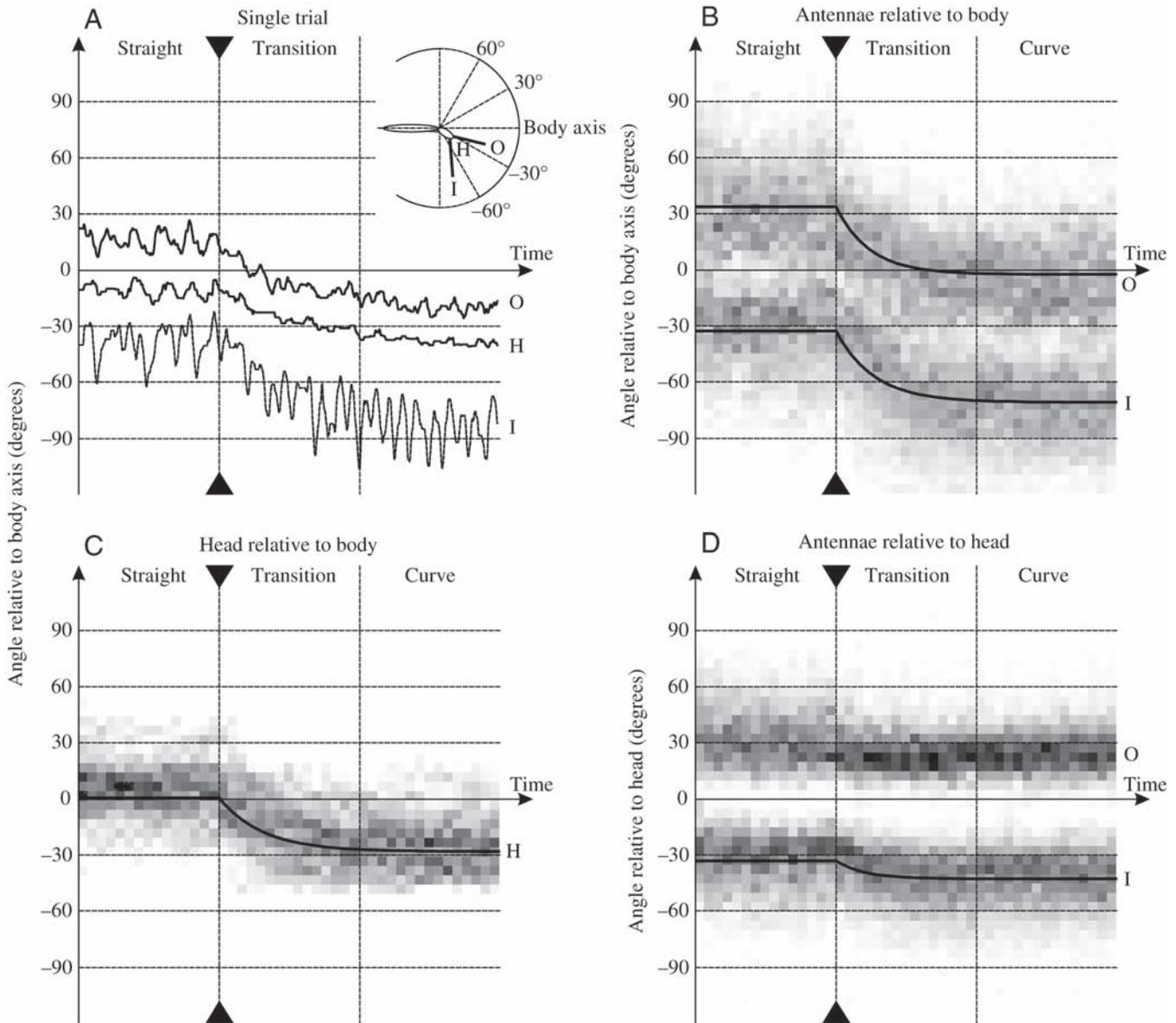


Fig. 4. Antennal beating field and head orientation shift during curve walking. (A) A representative sequence of head and antennal movements during curve walking. Vertical grid lines mark the three experimental periods (straight, transition, curve). Orientation angles of the head (H) and outer (O) and inner (I) antenna relative to the body axis (see insert for a schematic top view) alternate rhythmically during straight walking. The mean orientation and amplitude of oscillations change after the start of visual motion (line marked with arrowheads). (B) Distribution of antennal orientation angles relative to the body axis over time. Greyscale codes the probability (darker, more probable) of the left (top range) or right (bottom range) antenna to point into a given sector (width 5°). Solid lines indicate mean angles during straight and curve walking, connected by best-fit exponential time course. During straight walking, both antennae are most likely to point at $\pm 33^\circ$. After stimulus onset, antennal beating fields shift into turning direction. The shift is faster than for body yaw rotation. (C) As B, but for head angle relative to body axis. Head orientation shifts into turning direction, but slower than antennal beating field. (D) As B, but for antennal angle relative to head. The outer antenna moves closer to the midline but does not cross it. The beating field of the inner antenna shifts into turning direction with a shorter time constant than that of the head.

change drastically on a step to step basis, yet the large variability precluded statistical significance.

What changes the most?

The exact magnitude of all kinematic parameters that undergo a statistically significant change are listed in Table 1. As has been argued above, parameters that are important for the initiation of turning need not necessarily change their magnitude by a large percentage, but are likely to change reliably, i.e. by a significant fraction of their standard deviation in the previous behavioural state. This fraction is expressed by the value δ , which is related to the t -test statistic. Since the number of observations will increase the chance of any statistical test to yield a significant result, it is useful to choose a threshold value of δ , that coincides well with a given significance level. In this study, $\delta < 0.25$ coincided well with $P < 0.001$ of a Mann–Whitney test or, in the case of angular measures, a circular statistics test for common median (Fisher, 1993). Out of 54 parameters with $\delta \geq 0.25$ or $P < 0.001$, two reached the significance level with $\delta < 0.25$, and three were not significant despite $\delta > 0.25$, leaving 49 that satisfied both criteria (Table 1). As δ is a dimensionless ratio, it is suitable for ranking and comparing magnitude changes of all parameters, irrespective of their unit.

Four of the five strongest changes occurred in the inner front leg, affecting step direction and AEP coordinates. Further

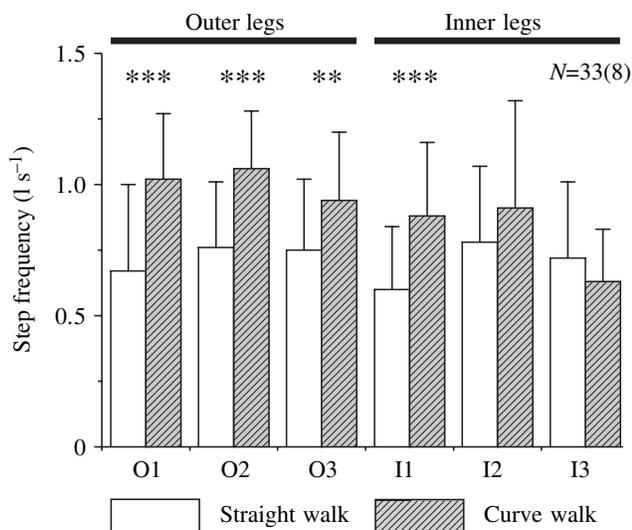


Fig. 5. Step frequency (f) changes during curve walking, but not in a unilateral way. Average step frequencies of each leg during straight walking (white bars, first 7.5 s interval) and curve walking (grey hatched bars, third 7.5 s interval). Values are means \pm s.d. of 33 trials from eight animals. Outer front, middle and hind legs (O1 to O3, respectively) significantly increase their step frequency during curve walking. So does the inner front leg (I1). Inner middle and hind legs (I2 and I3) show no statistically significant change. Wilcoxon test for matched pairs with $H_0: f_{\text{straight}} = f_{\text{curve}}$; $N=33$; *** $P \leq 0.001$; ** $P \leq 0.01$. All contralateral leg pairs step at the same frequency during straight walking ($P > 0.3$), but at different frequencies during curve walking ($P \leq 0.002$).

strong changes occurred in the outer front leg and inner middle leg, also concerning step direction and coordinates of extreme points. Generally, step direction, step length and extreme point coordinates revealed larger δ values than measures of duration or duty cycle. Timing of lift-off or touch-down is more variable and, thus, less likely to be directly affected by a descending turning command than are positional and directional parameters.

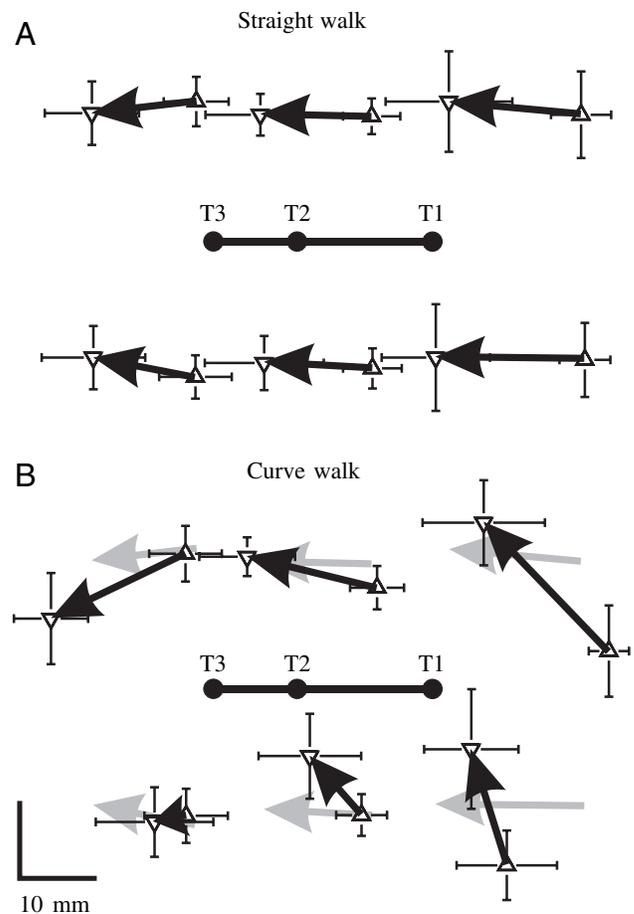


Fig. 6. Stance direction, stance length, and location of extreme positions change differently for each leg. (A) Average stance trajectories during the straight walk interval were nearly parallel to the body long axis (33 trials from 8 animals). Mean positions of touch-down (triangles) and lift-off (inverted triangles) of each leg are drawn in a body-centred coordinate system with the front of the animal facing to the right. T1–T3 label the location of coxae of the three thorax segments. Arrows mark the average stance direction, pointing in the direction of movement. Values are means \pm s.d. of the average positional x - and y -components. (B) During curve walking, stance directions of front and middle legs rotate outward with respect to the curve (towards the top of the panel), and stance direction of the outer hind leg rotates inward. Symbols and black arrows as in A. For comparison, grey arrows duplicate the straight walk condition. Stance length significantly increases for all outer legs and decreases for all inner legs. Front legs undergo the strongest changes during turning, the outer middle leg shows the least difference. Movements of the inner hind leg are the most variable.

Table 1. Magnitude of kinematic step parameters that change significantly during turning

Leg no.	Parameter	R_S	R_C-R_S	δ	Leg no.	Parameter	R_S	R_C-R_S	δ
O3					I3				
7*	PEP.y (mm)	-16.6	7.5	1.04	13*	PEP.x (mm)	-15.5	7.9	0.78
16*	PEP.x (mm)	-15.7	-5.3	0.68	18	Stance length (mm)	14.2	-5.4	0.63
17*	Swing length (mm)	14.9	5.5	0.64	23	Swing length (mm)	14.8	-4.2	0.52
22*	Stance length (mm)	14.3	5.7	0.56	31	Step period (s)	1.16	0.68	0.45
24*	Swing direction (degrees)	-3.8	-21.4	0.51	38	PEP.y (mm)	15	2.2	0.36
25	Stance direction (degrees)	171.7	-16.4	0.50	40	Swing direction (degrees)	13.1	-28.9	0.32
					43	Swing duty cycle	0.3	-0.08	0.31
O2					I2				
10*	AEP.y (mm)	-16.2	3.1	0.86	6*	PEP.y (mm)	15.7	-7	1.07
19	Stance direction (degrees)	-181.9	15.6	0.58	9*	Stance direction (degrees)	-170.9	41.6	0.99
37*	Swing direction (degrees)	-3.5	16.7	0.37	12*	Swing direction (degrees)	6.4	45	0.80
38	Step period (s)	1.26	-0.32	0.36	19*	PEP.x (mm)	-4.3	5.9	0.58
43	Stance duration (s)	0.98	-0.27	0.31	40	Swing length (mm)	15.6	-2.8	0.32
46	Swing length (mm)	15.4	2.6	0.29	40*	Stance length (mm)	14.9	-3.1	0.32
49	Stance length (mm)	15.1	2.5	0.25	47	AEP.x (mm)	9.7	-1.4	0.28
O1					I1				
3*	AEP.y (mm)	-16.4	11.5	1.41	1*	Stance direction (degrees)	-177	72.4	1.83
8*	Stance direction (degrees)	-174.7	40.6	1.02	2*	Swing direction (degrees)	3.6	70	1.45
11*	Swing direction (degrees)	5.5	39.9	0.82	4*	AEP.x (mm)	19.7	-10.1	1.39
14*	AEP.x (mm)	19.2	3.6	0.77	5*	AEP.y (mm)	15.3	7.5	1.14
28	Swing length (mm)	19.6	4.9	0.47	15*	PEP.y (mm)	15	-7.2	0.70
30	Step period (s)	1.44	-0.45	0.46	19	Stance duty cycle	0.79	-0.12	0.58
31*	Stance length (mm)	18.8	5.5	0.45	25	PEP.x (mm)	0.4	4.6	0.50
31	Stance duration (s)	1.17	-0.43	0.45	27	Stance duration (s)	1.31	-0.48	0.48
35	PEP.y (mm)	-18.1	-3.4	0.40	28	Swing duty cycle	0.22	0.1	0.47
35	PEP.x (mm)	2.1	4.5	0.40	31	Step period (s)	1.6	-0.47	0.45
					45	Swing length (mm)	21.1	-2.7	0.30
					47	Stance length (mm)	20.3	-2.9	0.28

The list includes all kinematic parameters that change during turning at least by 25% of their steady state s.d. ($\delta > 0.25$ corresponds well to significance level $P < 0.001$ of a Mann-Whitney test).

Parameters are listed separately for each outer (O1–O3, left) and inner leg (I1–I3, right). No., assigned rank; R_S , mean pre-stimulus period; (R_C-R_S), the change of the mean amplitude between steady states; δ , standardised change that determines the rank. AEP, anterior extreme position; PEP, posterior extreme position; .x and .y, components of position vectors.

*Parameters with significant fitting functions for their time course (see Table 2).

What changes the fastest?

Given that the turning response of the whole animal occurred with a time constant of 2.4 s (Fig. 3), it was clear that the primary kinematic parameters, i.e. those that initiate turning, must have changed equally fast or faster than that. Following the same approach as described in relation to Fig. 3, exponential curve fits were calculated to reveal the temporal sequence of all parameters that change significantly as the animal enters a curve. For illustration of the fitting process and results obtained, Fig. 7 shows the time course of the average stance direction for each leg, and the curve fit obtained for the kinetics of the transition. Reliable curve fits were obtained for three out of the five legs that show a significant change in stance direction. These were the two front legs and the inner middle leg. Of these three, the rise time constants of both front legs were both 1.7 s, indicating that they reliably change stance direction some 0.7 s before the effect of this change can be

picked up in the behaviour of the whole animal. The time constant of the stance direction of the inner middle leg is considerably slower (3.3 s), indicating that it is not a primary parameter for the initiation of turning, albeit experiencing one of the strongest changes during the transition. Time courses of the outer middle and hind leg did not allow a significant curve fit ($\chi^2 > 27.5$ and/or $r^2 < 0.36$). No fit was calculated for the inner hind leg, because the steady state values were not significantly different.

Similar to the time courses in Fig. 7, curve fits were calculated for all 49 parameters listed in Table 1. The results of the 23 significant curve fits are given in Table 2. These include the 17 parameters with the strongest change, i.e. the largest δ values, three out of the following ten (see also lines labelled by asterisks in Table 1), stance length of the outer front leg and inner middle leg, and swing direction of the outer middle leg (Nos 31, 40 and 37 by δ value, respectively). All

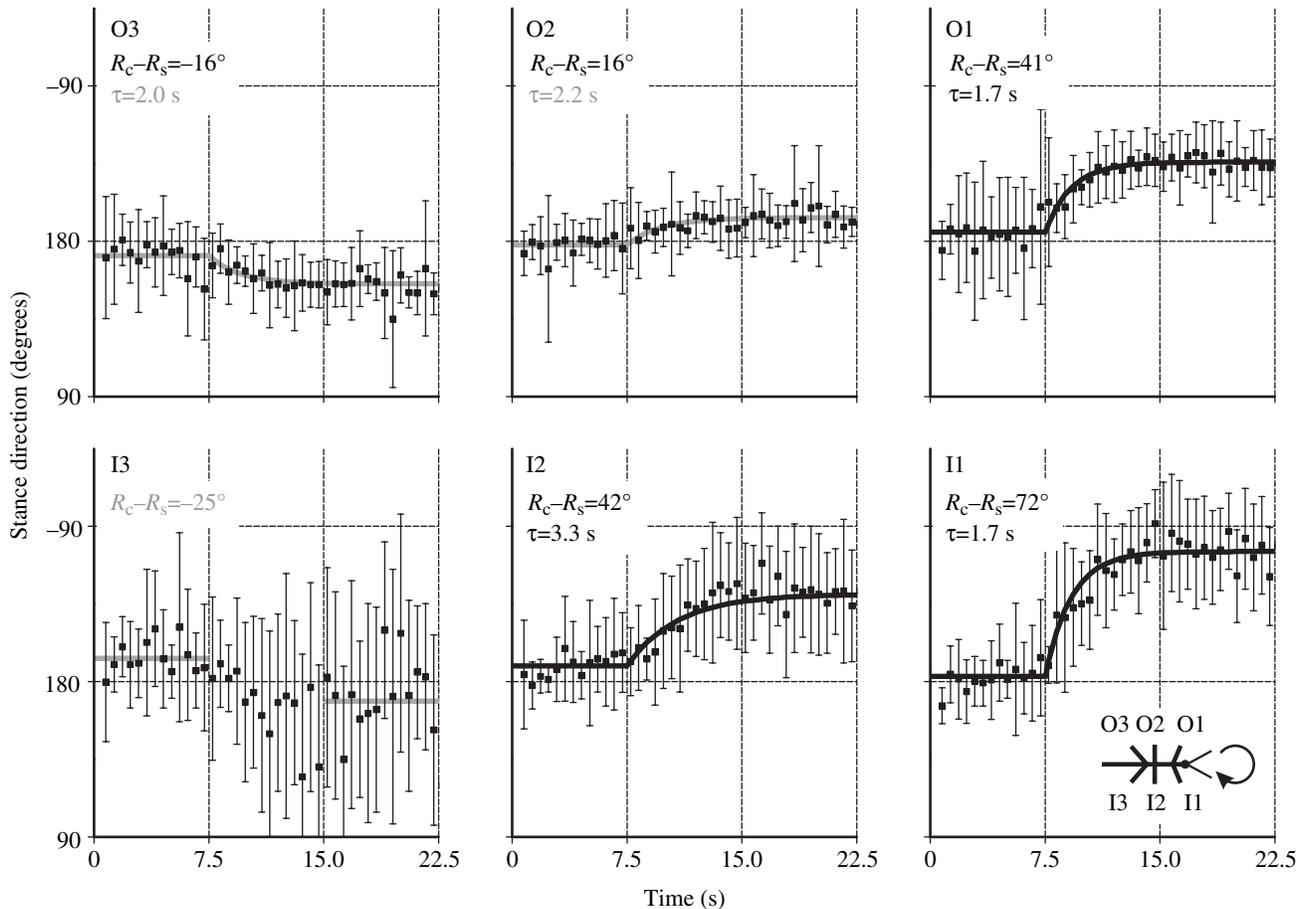


Fig. 7. Kinetics of stance direction is fastest in front legs. The time course of stance direction during turning differs between legs. (O1–O3) Time courses of outer front, middle, and hind leg, respectively, as the animal turns to the right. (I1–I3) Time courses of inner legs (see insert on lower right). Values are means \pm s.d. of 33 trials in eight animals, every 0.54 s (14 bins per 7.5 s interval). Bold solid lines show the time course of the exponential fitting function that explains most of the variance. Differences between the steady state stance directions ($R_C - R_S$) and time constants of the time course (τ) are indicated in the top left corner of each diagram. Values in grey are not statistically significant. Stance direction changes strongest and fastest in front legs. The equally strong change in the inner middle leg is, on average, much slower. Since the change $R_C - R_S$ in the inner hind leg is not significant, no curve fit was calculated for this data set.

of the significant fits concern transitions in stance or swing direction (including AEP or PEP coordinates) and stride length. There were two reasons for a fit result to turn out non-significant: (1) strong variance during the transition interval, resulting in a high χ^2 value (low fit quality), and (2) a low magnitude of change, resulting in low r^2 values (not sufficient sample variance explained). In the first case, the parameter was interpreted as too variable to be of consistent importance to the initiation of turning. In the second case, parameters changed too little to determine their time course. In both cases, parameters were interpreted as being of secondary importance to the initiation of turning. This suggests that they are not directly affected by a descending turning command.

The orchestration of the retrieved primary parameters is illustrated in Fig. 8, where the time constants of the time courses are plotted in the order of their magnitude, indicating the sequence of events in which a given parameter completed 67% of the context-dependent change. Eight parameters have

shorter time constants than yaw rotation (see Fig. 3), indicating that they lead the change in overall turning behaviour of the animal. These are stance directions of the two front legs, PEP of the inner front leg, swing direction of the front legs and the outer middle leg, and swing length and AEP of the outer front leg. All other parameters lag behind the turning of the animal and are, thus, secondary. Furthermore, because stance parameters of front legs change consistently faster than the corresponding swing parameters, we conclude that the front legs initiate a turn by altering their stance direction rather than by altering their swing direction.

Discussion

The objective of the present study was to investigate the behavioural transition during turning, in particular to identify the kinematic parameters that initiate turning in hexapod locomotion. The optomotor response to large-field visual

Table 2. *Temporal sequence of kinematic parameter changes during turning*

Leg no.	Parameter	χ^2	r^2	τ	Leg no.	Parameter	χ^2	r^2	τ
O3					I3				
9	Swing direction (degrees)	0.972	0.577	2.9	21	PEP.x (mm)	0.018	0.375	4.3
14	PEP.y (mm)	0.091	0.811	3.4					
18	PEP.x (mm)	0.004	0.557	3.5					
20	Stance length (mm)	0.355	0.626	4.2					
22	Swing length (mm)	0.007	0.534	4.4					
O2					I2				
4	Swing direction (degrees)	0.015	0.508	2.2	10	PEP.y (mm)	0.070	0.822	3.2
14	AEP.y (mm)	0.070	0.634	3.4	11	Stance direction (degrees)	0.096	0.889	3.3
					11	Stance length (mm)	0.376	0.449	3.3
					14	Swing direction	0.320	0.653	3.4
					23	PEP.x (mm)	0.011	0.603	5.7
O1					I1				
2	Stance direction (degrees)	0.131	0.681	1.7	1	PEP.y (mm)	0.007	0.501	1.5
4	Stance length (mm)	0.595	0.415	2.2	2	Stance direction (degrees)	0.188	0.906	1.7
4	Swing direction (degrees)	0.053	0.798	2.2	4	Swing direction (degrees)	0.115	0.702	2.2
8	AEP.x (mm)	0.079	0.572	2.3	14	AEP.y (mm)	0.005	0.867	3.4
11	AEP.y (mm)	0.002	0.968	3.3	18	AEP.x (mm)	0.006	0.894	3.5

Results of fitting an associative exponential function to the mean time courses of individual kinematic parameters.

Parameters are listed separately for each outer leg (O1–O3, left) and inner leg (I1–I3, right). No., assigned place in the temporal order of all changes; χ^2 , the ratio $\chi^2/\text{d.f.}$ (reduced χ^2) that was minimised during the fitting process; r^2 , coefficient of determination that indicates the fraction of the variance explained by the fit; τ , time constant that determines the temporal order of changes. AEP, anterior extreme position; PEP, posterior extreme position; .x and .y, components of position vectors.

Fits with $r^2 > 0.36$ and $\chi^2 < 27.5$ (28 d.f.) are significantly correlated ($P < 0.001$) and statistically undistinguishable from the data ($P > 0.5$).

motion was used to trigger turning reliably, allowing analysis of the sequential timing of kinematic events. First, it was shown that the kinematics of stick insect stepping movements vary both within trials (Fig. 2) and among trials. As a consequence, the behavioural states of 'straight walking' and 'curve walking' were described by their characteristic distributions of horizontal movement components (Fig. 3) and of kinematic parameters of the head, antennae (Fig. 4) and legs (Figs 5, 6). Accordingly, the transition between the behavioural states was characterised by the fraction of the variance by which the distributions differ (Table 1), and by the rate of change that governs the transition of each kinematic parameter (Figs 3, 4, 7; Table 2). Based on these observations, turning was shown to be initiated by stance direction changes of the front legs, followed by corresponding changes in swing direction (Fig. 8).

Methodological considerations

Various methodological aspects affect curve walking behaviour in arthropods, for instance the behavioural situation, which can be characterised by the experimental method of eliciting a turn. Previous studies have analysed turning during optomotor responses to visual large-field motion (e.g. Zolotov et al., 1975; Jander, 1985; Cruse and Silva Saavedra, 1996), visual tracking of small objects (Land, 1972), spontaneous turns (Jindrich and Full, 1999) or particular behaviours such as mating (Franklin et al., 1981) or defence behaviour (Copp and

Jamon, 2001). Moreover, the locomotor state (static vs dynamic locomotion) and movement constraints (tethered vs free walking, natural vs un-natural inertia) varied among these studies. Three of these aspects need to be discussed in relation to the presented results: (1) effect of tethering, (2) increased inertia and (3) sustained visual stimulation.

Tethering

The tethering method of this study ensured natural height control by the animal, because load was normal and the body height range was virtually un-restricted. Theoretically, the placement of the animal on the sphere could have biased the ratio of forward and sideward translation, for instance causing a pronounced sideward component. However, as turning directions were randomised during the experiments, such a translation bias should have averaged out among trials, resulting in increased variance only. Thus, the significant increase in stimulus-induced sideward translation (Fig. 3) cannot be due to tethering. Rather, sideward translation is a component of turning.

Inertia

Because the moment of inertia of the sphere was markedly larger than that of the stick insect body (by a factor of 139), angular acceleration and deceleration required the insects to produce considerably more force than when walking on a plane surface. However, comparison with Jander's results on stick

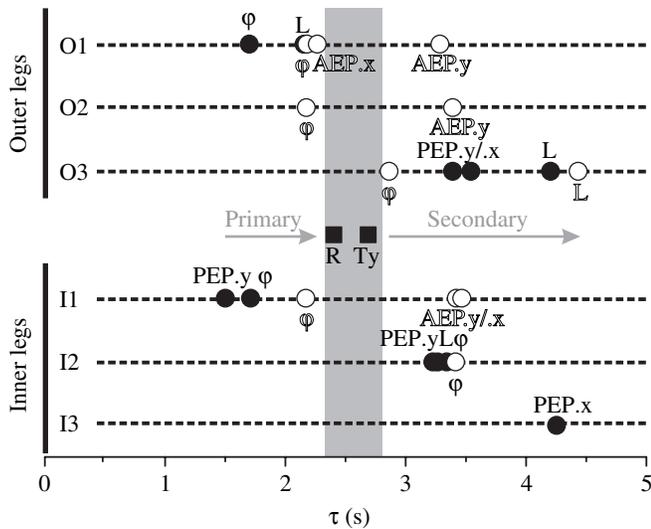


Fig. 8. Orchestration of kinematic changes during turning. As an illustration of the temporal sequence of the kinematic changes, time constants of all reliable curve fits (χ^2 value n.s. at $P > 0.5$, and $r^2 > 0.36$) are drawn in their temporal order from left to right. Time constants of parameters of the same leg are arranged in individual rows: O1–O3 and I1–I3 label outer and inner front, middle and hind legs, respectively. $\tau = 0$ denotes stimulus onset. Filled squares in the centre of the graph mark the time constants of the locomotion parameters, i.e. angular velocity of rotation (R) and speed of sideward translation (Ty). Circles mark time constants of stance phase (filled) and swing phase parameters (open). The identity of a stance phase parameter is indicated above the symbol, that of a swing phase parameter is indicated below (AEP, Anterior Extreme Position; L, length; PEP, Posterior Extreme Position; x and y , components of position vectors; ϕ , direction). The grey bar separates primary parameters that lead the overall locomotor change from those that lag behind (secondary). The leading parameters, i.e. stance direction of both front legs and shifts in PEP of the inner front leg and AEP of outer front leg, initiate turning.

insects that walked on an actively counter-rotating sphere (Jander, 1982), particularly stance length and direction (Fig. 6) show no sign of differences attributable to load. Given the experimental situation of the present study, angular acceleration of the sphere was very low during the steady states, but strong at the onset of turning. Therefore, any significant impact of increased inertia should have shown up immediately after stimulus onset, possibly resulting in an increased variance of the movement components. There was no indication of such effects. Inertia will have affected stance movements more than swing movements. As stance direction showed a faster rate of change compared to swing direction (Fig. 8) in spite of increased force requirement, the observed differences between stance and swing movements must be expected to be the same or even larger under normal walking conditions. Hence, the claims made by this study are not affected by increased inertia.

Prolonged stimulation

The experimental situation ensured that locomotion was

steady over a period of many steps. Qualitatively, the locomotor pattern remained the same, quantitatively it remained within a characteristic distribution. Following a period of steady forward walking, sustained visual motion stimulation caused the locomotor system to assume another steady state after a transition period. Of course, prolonged stimulation causes a rather un-natural situation. However, the two steady locomotor states merely served as a reference to define the bounds of the behavioural transition, which is a prerequisite to determine the rate of change. In other words, the final behavioural state may well be un-natural, nevertheless it can be exploited as a key to determine the sequence of events that occurs during natural short-term course corrections.

Experimental parameters such as stimulus velocity are known to cause quantitative changes in path curvature and associated kinematic parameters (e.g. Jander, 1982), and to give rise to qualitatively different turning behaviours (Zolotov et al., 1975). Therefore, stimulus conditions were held constant in the present study, and trials were pooled only if the walked path was qualitatively similar (see Fig. 1C). The existence of a descending visual input to motor networks and the reliable strong turning response it mediates suggest that visually elicited turning does play an important role in insect walking. Of course, it is possible that visually induced turning differs from other behavioural situations, e.g. spontaneous turning. Yet, the finding that kinetics of movement parameters differ strongly between legs shows that the functions of the six legs during turning also differ strongly. Moreover, it suggests that descending input causes a stronger drive on the front legs than on the other legs.

A final methodological consideration concerns the difficulty to measure the effect of a continuous descending drive on discrete variables of leg movement, i.e. kinematic parameters of alternating and mutually exclusive swing and stance modes. This problem was solved by fitting a continuous function to discrete sample distributions of each parameter. Theoretically, important effects could have been missed by this approach, provided they were small and/or rather variable, in spite of their importance. Since the transition bounds R_S and R_C , as well as the δ -value, depend on both the average change and on variability, small effects cannot reach a large coefficient of determination r^2 and might have gone undetected (even if χ^2 was very small). However, the existence of fast, strong and consistent effects, particularly on front leg movement, which can explain a strong yaw moment, can hardly be accounted for by any potentially undetected effect in another leg.

What changes during turning?

Many previous studies on curve walking claimed that there were two principal alternatives for negotiating a curve: by changing either step frequency or stride length. This view is based on the assumption that the movements of each leg are caused by a protraction–retraction oscillator with adjustable frequency and amplitude. It implicitly relies on a unilateral change of one of these variables, e.g. by an increase on one side and decrease on the other. There are three reasons why

this view is too simple to understand turning behaviour: Firstly, rhythmic patterns are not particularly stable, compromising the importance of an oscillator. Secondly, the forces on the ground that ultimately determine the movement of the body axis, are not a simple function of stride length or stance duration, but vary considerably between legs (Cruse, 1976; Jindrich and Full, 1999; Domenici et al., 1999). Thirdly, rotation of the body axis requires differing ground force directions among legs. Since stance direction has a strong effect on the ground force direction, it may be most efficient for an animal to alter stance direction rather than stride length or duration. This is in line with the present results, showing that each leg undergoes a specific change of step frequency, stride length, and stance direction (Figs 5, 6; Table 1). In contrast to insects that tend to maintain a tripod coordination during turning (fly: Strauss and Heisenberg, 1990; ant: Zollikofer, 1994), stick insects change their gait during curve walking (Fig. 2B). While stepping becomes seemingly more regular among outer legs, it becomes less regular among inner legs (Dürr, 2005). In particular, the inner hind leg appears to be taken out of the normal leg coordination, executing only infrequent, short swing movements and prolonged stance movements with very complex trajectories. Jander (1985) showed that the stepping behaviour of the inner hind leg in a turning stick insect is tightly linked to the location of the centre of gravity within the stability polygon spanned by the legs on ground. As the centre of gravity of a stick insect is located posterior to the hind leg coxae, Jander suggested that the inner hind leg lifts off the ground so rarely because it seldom is sufficiently unloaded.

Strongest changes were observed in both front legs, the inner middle leg and the outer hind leg (Table 1). This is similar to the cockroach, where the inner front legs show strong kinematic changes in tactile-mediated turning behaviour (Ridgel et al., 2002) and the inner middle legs strongly affect wind-elicited turns at the beginning of escape reactions (Nye and Ritzmann, 1992). Based on model calculations, Jindrich and Full (1999) concluded that the front legs of the cockroach would be most effective to cause turning, although all legs could theoretically rotate the body axis. For the stick insect, Tables 1 and 2 show that the set of kinematic parameters that exhibit the fastest change corresponds well to the set of parameters with the most consistent change, i.e. largest δ -value. Thus, front leg movement appears to be the primary target of the descending visual information that triggers turning. The observed fast and strong change in stance direction requires coordinated action of all leg joints, rather than a simple modulation of retraction amplitude and speed.

Primary vs secondary parameters

Optomotor responses generally resemble low-pass filtered versions of the stimulus function (reviewed in Hengstenberg, 1993). For instance, activation of fly steering muscles in response to descending information about large-field visual motion is low-pass filtered compared to the properties of the visual interneurons of the optic lobe (Egelhaaf, 1989). As the

stimulus function in the present study was a step onset of visual motion, an associative exponential function was assumed to model continuous descending visual input to the motor networks of the thoracic ganglia. The discrete nature of the stepping rhythm was dissolved by calculation of sample distributions of each kinematic parameter over a sequence of 42 brief time bins. Accordingly, the curve fit should be interpreted as the drive of descending inputs on a given kinematic parameter. If it was a stance parameter, a point on the time course would show the most likely magnitude of this parameter for a stance movement elicited at that time.

Behavioural relevance of a given parameter is assessed by determining the consistency of the change observed, which necessitates relating the magnitude of the change to the natural bandwidth during stable behavioural states. Statistical significance by itself is not a very useful indicator of behavioural relevance, as it strongly depends on the sample size, and sample size is not of behavioural relevance in our case. In other words, while the large sample size allows statistical discrimination of context dependency for many parameters (see Table 1), functional importance to the animal may vary strongly, for instance when considering whether a change is active or passive. Due to lack of information about neural activation patterns, we cannot discriminate here between active and passive changes. However, based on differences in the time course, we can discriminate between primary and secondary parameters. It is clear that the fastest changes, which lead the yaw rotation of the whole animal, must be due to an active effort of the animal, as there is nothing else to cause them. The slower time courses of secondary parameters, those that lag behind yaw rotation of the whole animal, could have at least three different causes. Firstly, they could be caused by passive effects, e.g. due to mechanical constraints imposed by other, active effects. Secondly, they could be due to active movements of the animal that are less consistent at the beginning of the transition, resulting in a slow change of the mean. Thirdly, they could be due to active movements that lag behind the actions underlying the primary parameters. Accordingly, the orchestration of kinematic events shown in Fig. 8 begins with a sequence of active events, followed by both active and passive secondary events interspersed.

Intuitively, one might think that all legs in stance should change stance direction with the same time constant, and that both front legs, which typically step alternately, should have different time constants. On a single trial basis this intuitive view is true. However, time constants were determined for a pool of responses, and stance direction changes in a front leg need not always coincide with the same set of corresponding changes in other legs. Since fit functions and corresponding time constants reflect the most consistent time course, stance direction in other legs may well change more slowly than in front legs, despite occasional fast reactions. Similarly, front legs need not step synchronously to achieve the same average time course in stance direction.

Interestingly, the antennal movement pattern undergoes a faster change than any leg, as revealed by time constants between 1.5 and 2.0 s for antennal angle relative to the head or body (Fig. 4). These time constants were not included in Fig. 8 as antennae do not physically contribute to yaw rotation. Nevertheless, these fast reactions suggest that an antennal orienting response leads the response of the locomotor system. This is in line with the results of Dürr et al. (2001) who suggested that stick insects use their antennae for active tactile searching of the locomotor action range.

Swing or stance? Implications for modelling studies on turning behaviour

Since we analysed a set of sample distributions rather than single behavioural chains, the results presented here may not apply to every single trial. Rather, they capture the most likely scenario. If there were different strategies involved, the result would have been dominated by the strategy that is elicited most reliably among trials, i.e. by the set of kinematic changes that are most robust within the sample. For example, stick insects may be able to initiate a turn by targeting swing movements to another AEP, just as they can retarget ongoing swing movements following antennal contact (Dürr and Krause, 2001), but if they had done this consistently, the distribution of swing direction would have shifted earlier than that of stance direction and, as a consequence, the current analysis would have detected a shorter time constant for swing direction than for stance direction. This was not the case. Rather, time constants of stance direction were approximately 0.5 s shorter than those of swing direction. Accordingly, turning was initiated most consistently by a bilateral change in front leg stance direction, followed by corresponding changes in swing direction. Together with the complex sequence of events, revealing different time courses in each leg, the importance of the front leg stance contradicts all current models of curve walking. Cruse et al. (1998) suggested that curve walking is caused by a differential change in angular velocity of the thorax-coxa joint in outer and inner legs. According to their model, a positive displacement feedback mechanism then accounts for adjustments in the femur-tibia joint, while the coxa-trochanter joint is controlled separately by a negative feedback mechanism that adjusts clearance over ground. Curve walking performance in this model is fairly good, but a quantitative description of its stance trajectories (Kindermann, 2002) reveals major differences to the data shown in Fig. 6. The most profound discrepancies concern mediad stance direction of the outer hind leg and shortening of the inner hind leg's stance. Both of these discrepancies might be removed by a model in which descending visual input only affects stance direction in both front legs, leaving all other changes to mechanical coupling and assistance reflexes as implemented by Kindermann (2002). For example, infrequent short steps of the inner hind leg may be simply due to rare unloading of this leg because of its closeness to the centre of gravity (Jander, 1985). It is possible that the kinematic changes described above need to

be accompanied by corresponding changes in coupling strength of leg coordinating mechanisms. Such context-dependent changes in coupling strength are described in the accompanying paper (Dürr, 2005). As yet, it is difficult to judge whether changes in coupling strength by themselves would suffice to cause curve walking, e.g. by causing unequal step frequencies and stride lengths on both sides. Most certainly, however, changes in coupling strength will act to support the kinematic changes described, e.g. by uncoupling the inner hind leg and enhancing coherence of outer stance movements along a single convex trajectory. Due to the large number of interacting parameters, modelling studies will have to unravel the relative contributions of local changes in step kinematics on the one hand, and inter-leg coordination mechanisms on the other.

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