

CURVE WALKING IN FREELY MOVING CRAYFISH (*PROCAMBARUS CLARKII*)

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Summary

The curve walking of freely moving crayfish trained to walk along a curved path during homing behaviour was investigated using a video-analysis system. The leg kinematics and leg phase relationships, as well as the relationship between stepping patterns and body axis rotation measured relative to external references, were studied.

The anterior and posterior extreme positions of the power stroke (AEP and PEP, respectively) and step amplitudes were analysed. As in a previous study on crayfish curve walking on a treadmill, PEPs were more posterior in outer legs (the legs on the outside of the turn) than in the inner legs. As a result, outer legs showed larger step amplitudes than inner legs. Leg kinematics varied within each walking sequence. AEP leg angles (the angles between the body and leg axes at the AEP) tended to decrease over time for inner legs and increase for outer legs. This leg angle drift was present mainly in the anterior legs and it suggests that these legs did not completely compensate for the body rotation after each step. In addition, leg angle asymmetries in a direction opposite to

that of leg angle drift were observed at the start of each curve-walking sequence, suggesting that the extensive training (3 weeks) may have allowed crayfish to anticipate the leg angle drift.

The rotational component of curve walking showed a discontinuous pattern, with the animal's body axis turning towards the inside of the curve only periodically. Analysis of cross-correlation functions showed that the angular acceleration of the body axis in the direction of the turn occurred during the power strokes of inner legs 2 and 5 and outer leg 4. While the tripod formed by these three legs showed in-phase relationships, the legs of the corresponding contralateral tripod (outer legs 2 and 5 and inner leg 4) were not in phase. We hypothesize that inner legs 2 and 5 and outer leg 4 act synergically causing the inward body rotation observed in curve-walking crayfish and that some of the asymmetries found in step geometry may be a passive phenomenon due to the body rotation.

Key words: locomotion, walking, behaviour, kinematics, turning, crayfish, *Procambarus clarkii*.

Introduction

The kinematics and physiology of arthropod locomotion have been studied extensively, particularly in restrained conditions such as straight walking on a treadmill (e.g. Delcomyn, 1971; Barnes *et al.* 1972; Clarac, 1981; Clarac and Chasserat, 1986; Cruse and Müller, 1986; Müller and Cruse, 1991*a,b*). The main mechanisms implied in the coordination of leg movements have been established (Delcomyn, 1980; Clarac, 1984; Cruse, 1990) on the basis of video analysis and electromyogram recordings (e.g. Delcomyn, 1971; Clarac, 1981; Clarac and Chasserat, 1986; Cruse and Müller, 1986) and of measurements of the forces exerted by the legs during locomotion (Cruse and Saxler, 1980; Clarac and Cruse, 1982; Klärner and Barnes, 1986; Full *et al.* 1991, 1995).

Studies on the leg coordination of crayfish walking on a treadmill have shown that both ipsi- and contralateral connections are present, with the former being stronger than the latter (Müller and Cruse, 1991*a,b*). Müller and Cruse (1991*a,b*) showed that contralateral legs move out of phase, as

in most decapods studied in treadmill situations (Clarac and Barnes, 1985). However, a recent study on freely walking crayfish has shown that both in- and out-of-phase leg coordination can be present (Jamon and Clarac, 1995).

Along with these findings, the idea that individual legs may act as relaxation oscillators was developed (Bässler, 1983), and Cruse and Dean (1991) suggested that peripherally located networks may be sufficient to maintain the locomotor pattern. These principles were successfully applied to the design of a multilegged robot (Müller-Wilm *et al.* 1992). While this may be the case for straight-walking animals, a continuous change in heading may require continuous sensory feedback in order to produce a particular curved trajectory and therefore central commands may take over the control of locomotion under these conditions. In addition, in natural situations, arthropods are likely to walk along complex paths which involve both translational and rotational components (e.g. Etienne, 1977; Bailey and Thomson, 1977).

While only translational components are present in straight walking, rotational components can be present to different extents in turning. Although there are a number of previous studies investigating body rotation in arthropod locomotion, various experimental situations should be considered separately. At one extreme, there is pure rotational locomotion, which corresponds to rotation in place (e.g. Land, 1972; Zolotov *et al.* 1975; Bell and Schal, 1980; Franklin *et al.* 1981; Copp and Watson, 1988). A second case is that of animals turning at the beginning of or during a walking sequence, resulting in a single change of directional heading (e.g. Graham, 1972; Zolotov *et al.* 1975; Camhi and Levy, 1988; Strauss and Heisenberg, 1990). In this case, rotational locomotion is gradually replaced by translation. A third case is that of curve walking, where both rotational and translational components are continuously present while the animals are walking along a curved path (e.g. Zolotov *et al.* 1975; Jander, 1985; Zollikofer, 1994; Cruse and Silva Saavedra, 1996).

It has been suggested that turning may be due to any of the following leg motion asymmetries between inner legs (on the inside of the curve) and outer legs: (1) a higher step frequency of the outer legs; (2) a larger step amplitude or stride length of the outer legs; and (3) asymmetries in leg trajectories (Franklin *et al.* 1981; Cruse and Silva Saavedra, 1996). However, asymmetries in temporal (e.g. leg periods) or geometrical (e.g. leg amplitudes) components do not necessarily imply a cause-effect relationship with curve walking for all legs. In addition, the lack of any actual body turning in studies on tethered animals walking on a treadmill in open-loop situations (Cruse and Silva Saavedra, 1996) does not allow any prediction to be made of which legs actively turn the body.

Here, we study the curve-walking behaviour of the crayfish *Procambarus clarkii* during homing in unrestrained conditions. Unlike studies on tethered animals, our methods allow us to take into account body and leg movements relative to both external and body-centred coordinates. Such an approach, together with the use of the cross-correlation technique, permits possible relationships between body rotation and all leg movements to be identified simultaneously and therefore to test the hypothesis that all legs contribute similarly to curve walking.

We show that the rotational component of curve-walking crayfish varies in relation to the stepping pattern, suggesting that different legs may contribute differently to turning. Hence, some of the asymmetries observed in leg kinematics may not be due to active forces produced by each leg, but rather to the combined forces from other legs exerted on the body, as suggested by Camhi and Levy (1988) for turning in escaping cockroaches. The results are discussed in relation to previous studies on arthropod manoeuvrability, in particular to two recent studies on crayfish locomotion: a previous study on unrestrained straight-walking crayfish of the same species (Jamon and Clarac, 1995) and a study on curve walking in a different species of crayfish (*Astacus leptodactylus*) during treadmill locomotion (Cruse and Silva Saavedra, 1996). Comparisons with the study of Cruse and Silva Saavedra

(1996) on crayfish curve walking need to take into account the different species used and different methods. In our study, crayfish receive continuous feedback on their actual turn (closed-loop situation), while in the study of Cruse and Silva Saavedra (1996) crayfish were tethered and could not turn, and therefore did not compensate for the optomotor stimulus with which they were presented (open-loop situation).

Materials and methods

Experimental procedure

Crayfish (*Procambarus clarkii* Girard) were obtained from a local supplier 4 months before the experiments. They were kept separately in tanks (40 cm×20 cm×20 cm) filled with fresh water at ambient temperature and fed with pelleted food once a week.

The experimental procedure was similar to that utilized in a previously published study on unrestrained crayfish walking along a straight path while engaged in homing behaviour (Jamon and Clarac, 1995). A hollow piece of cinder block with an aperture of 10 cm×5 cm provided a shelter in a circular pool (1.75 m internal diameter, 40 cm high) filled with fresh water (water depth 20 cm). Access to the shelter was along a curved path (radius 62.5 cm) delimited by a series of bricks (22 cm long, 10 cm high and 5 cm thick) placed on either side of it. The distance between the two rows of bricks (i.e. the path width) was approximately 20 cm throughout the length of the path. A strip of rough-textured black rubber was cut and placed on the bottom of the curved path in order to enhance the contrast for filming and to prevent the animals from slipping. White markers were placed at 10 cm intervals along the mid-line of the path in order to allow measurements of crayfish movements relative to external references. For 3 weeks prior to the experiments, crayfish were trained daily to walk towards the shelter. Training consisted of letting each crayfish walk towards the shelter for a period of approximately 20 min (corresponding to approximately 10–20 walking sequences), in which crayfish did not show any sign of fatigue (Jamon and Clarac, 1995). Crayfish were released first near the shelter and then at progressively increasing distances from it. At first, they showed escape responses or aggressive behaviour, but they soon became familiar with the procedure and learned to return to the shelter along the curved path even if they could not see the shelter from their starting position.

The crayfish were trained to walk along an anticlockwise curve; hence, their turning behaviour was always directed towards the left side. During the experimental phase, 183 sequences were filmed. Of these, 30 sequences (representing a total of approximately 300 steps) from five individual crayfish of uniform size (length 9.0±0.15 cm; mass 23.26±2.2 g, mean ± S.E.M.) were chosen. The selection criteria were that crayfish did not stop walking during the sequence and that they did not touch the path-delimiting bricks with their legs. A minimum of four and a maximum of seven sequences were used for each of the five crayfish. Experimental trials for each individual were collected on a single day.

Filming procedure

Throughout the experiments, crayfish were filmed during their homing behaviour as they walked along the curved path. The filming procedure was similar to that employed by Jamon and Clarac (1995). A wheeled table was fitted with a rotating platform carrying a video monitor and a boom, at the end of which an HI 8 mm camera (Canon EX1 with zoom 5–15 mm) was fixed. The camera was connected to the video display screen, the centre of which was located by means of two intersecting lines drawn on the screen.

Crayfish were filmed from above using a magnification factor of 1.5. The field of view of the camera was 27 cm×20 cm. The camera was displaced along the curved path as the crayfish walked towards the shelter. This ensured that the crayfish position was kept at the approximate centre of the screen. Deviations from this central position were unavoidable owing to the movements of the camera. The movement of each body part was given by its projected position onto the ground and was, therefore, subject to parallax errors depending on the projected position and on small deviations of the body from the screen centre. These errors were estimated in Jamon and Clarac (1995), who used the same apparatus for studying straight walking in crayfish, by following the movements of calibrated objects. Deviations did not cause significant errors, as demonstrated by Jamon and Clarac (1995).

The sequences were analysed using an Ariel performance analysis system (APAS, Ariel Life Systems Inc.) at a frequency of 25 frames s⁻¹, i.e. one frame every 40 ms. Therefore, each step was resolved into 20–30 successive images. Each sequence started at approximately 1 m from the shelter, with the animal placed on the curved path at the start of the sequence. Although animals were walking along the curved path throughout the sequence, the first and last steps of each sequence were systematically excluded from the analysis since they could involve acceleration or deceleration.

Prior to the experiments, various positions on the crayfish body were marked using white plastic beads 2.5 mm in diameter. The following 10 positions were marked: the rostrum, the posterior end of the cephalothorax, and each of the crayfish legs at the end of the propodites, approximately at the tip of each leg. The first pair of appendages, the chelipeds, are not used in underwater locomotion and were excluded from the analysis. Therefore, eight legs were considered, inner legs (the legs on the inside of the curved path (termed *i*) and outer legs (the legs on the outside of the curved path (termed *o*) 2–5 (i.e. L2*i*, L3*i*, L4*i*, L5*i* and L2*o*, L3*o*, L4*o*, L5*o*, respectively).

Data analysis

Digitization was performed using the APAS system, which automatically located each point at the barycentre of each mark. The locations calculated by the APAS system were checked manually for each frame. Twelve points per frame were digitized, i.e. 10 points on the crayfish body plus two markers on the ground. As the animals walked and the camera was displaced accordingly, the ground markers moved from right to left on the screen. The disappearing marker on the left

of the screen was replaced by a new marker appearing on the right. The positions of leg markers with reference to the body axis markers allowed analysis of the leg positions in a body-fixed coordinate system. The positions of body markers with reference to the ground markers allowed the analysis of movement in an external coordinate system.

Ten main variables were measured: (1) leg angle, (2) leg angular drift, (3) time of AEP and PEP, (4) period of the step, (5) stance amplitude, (6) step direction, (7) AEP and PEP coordinates, (8) the phase relationships between legs, (9) body axis angle, (10) cross-correlation functions for the time relationship between the leg angles and the angular acceleration of the body axis.

Leg angle was calculated as the angle between the crayfish *y*-axis and the line connecting the leg marker with the marker on the crayfish thorax (Fig. 1A). Leg angles increased during the power stroke and decreased during the return stroke. At the beginning of the stance phase, a leg is in its anterior extreme position (AEP), and the leg angle (α in Fig. 1A) is at a minimum (Fig. 1B), while the end of the stance phase corresponds to the posterior extreme position (PEP) with the leg angle (β in Fig. 1A) at a maximum (Fig. 1B).

Leg angular drift. In order to investigate whether an angular drift occurred in the leg extreme positions (AEPs and PEPs) during curve-walking behaviour, linear regressions between an index of angular variation (I_{AV}) and normalized time were performed for all sequences pooled. Each sequence was normalized with respect to time in order to standardize sequence duration.

The normalized time (T_N) was derived as:

$$T_N = T_R(100/T_T), \quad (1)$$

where T_R indicates real time (in s), starting at 0 s for the first AEP or PEP, and T_T indicates the total duration of the sequence (in s).

I_{AV} of the AEP and of the PEP was computed for each sequence separately as the difference between each actual AEP (or PEP) angle (α_R) and the mean angle (α_M) of all AEPs (or PEPs). In order to prevent slope distortion (due to the time normalization), I_{AV} was computed with respect to the duration of each sequence, using the same multiplication factor as for time normalization:

$$I_{AV} = (\alpha_R - \alpha_M)(100/T_T). \quad (2)$$

Hence, for each sequence, the zero on the I_{AV} axis was the mean I_{AV} .

Significant slopes of the regression between I_{AV} and normalized time indicate that the extreme leg angles (AEP or PEP angles) either increase (positive slope) or decrease (negative slope) with time, representing a positive or negative angular drift, respectively. A non-significant regression indicates that there is no angular drift.

The time of the AEP and the PEP for each leg was calculated as the time at which minimum and maximum leg angle was reached, respectively (Fig. 1B).

The period of the step was the time elapsed between two

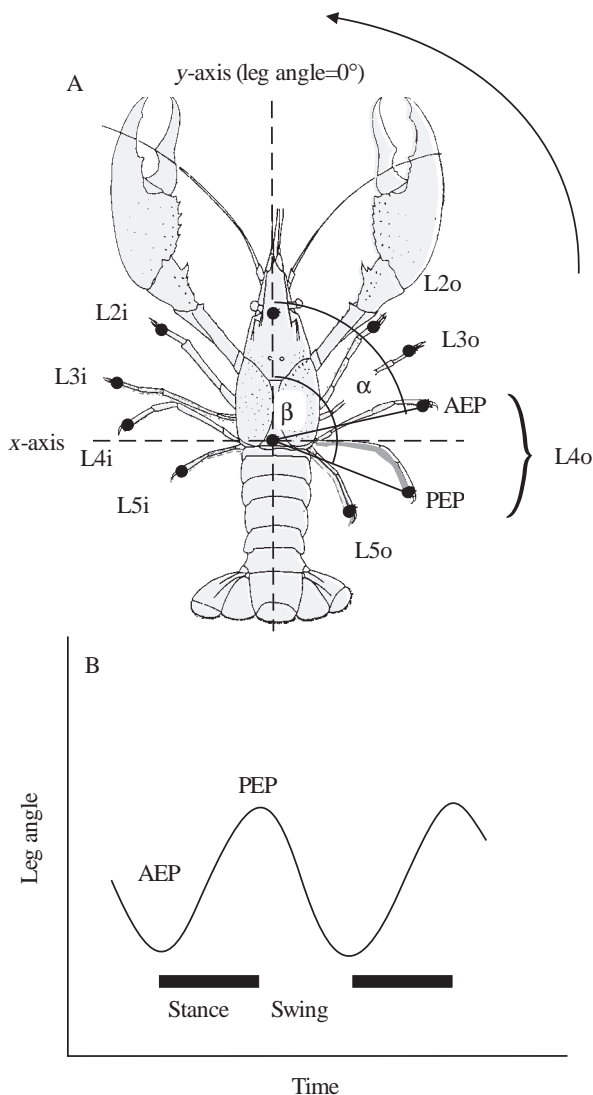


Fig. 1. Geometry of crayfish walking. (A) Top view of crayfish. Filled circles on legs and body show digitized points. The midline (y-axis of the body-centred coordinate system) of the body is shown as a vertical broken line (y-axis, leg angle 0°). The x-axis is shown as a horizontal broken line. The curved arrow shows the direction of the turn. Legs on the inside (i) and outside (o) of the curve are indicated as L2i, L3i, L4i, L5i and L2o, L3o, L4o, L5o, respectively. L4o is shown both in the anterior extreme position (AEP) and the posterior extreme position PEP (shaded). α and β indicate L4o AEP and PEP angles, respectively. (B) The leg angle *versus* time curve, showing the AEP (minimum angle) and PEP (maximum angle). Black bars indicate the stance phase (from AEP to PEP), intervals between bars indicate the swing phase (from PEP to AEP).

successive AEPs. The period consisted of one power stroke (stance phase, starting at the AEP and ending at the PEP) and one return stroke (swing phase, starting at the PEP and ending at the AEP). The relative duration of these two phases was expressed as the duty factor, defined as the ratio between the duration of the power stroke and the whole step period.

The *stance amplitude* for each leg was calculated as the

distance between the AEP and the PEP within each power stroke. Since AEPs and PEPs were measured relative to body-fixed coordinates, and the leg tip is actually in a fixed position during the power stroke when viewed from an external coordinate system, the measured amplitudes corresponded to the forward shift of the body during the power stroke of a given leg viewed from an external coordinate system. Stance velocity (in cm s^{-1}) was measured as the stance amplitude divided by the power stroke duration for each step, while swing velocity (in cm s^{-1}) was measured as the swing amplitude (distance between each PEP and the successive AEP) divided by the return stroke duration for each step.

Step direction was calculated as the angle between the line connecting the AEP and the PEP of each step and the crayfish axis. Positive angles indicate that the PEP is nearer to the body than is the AEP.

AEP and PEP x- and y-coordinates were calculated relative to the crayfish body. The y-axis was defined as the crayfish axis, with the zero point coinciding with the thorax marker.

All the variables described above were analysed using parametric tests (e.g. *t*-tests) if the data distribution was normal (Kolmogorov–Smirnov test; $P > 0.05$). In the case of non-normal distributions, non-parametric tests (e.g. Mann–Whitney *U*-test) were used.

The *phase relationship* of each leg n relative to any other leg n' ($\Phi_{n \text{ in } n'}$) was defined as the occurrence of the AEP of the chosen leg n (AEP_n) within the period of the given reference leg n' ($P_{n'}$). The value of the phase was calculated as:

$$\Phi_{n \text{ in } n'} = (\text{AEP}_n - \text{AEP}_{n'}) / P_{n'}. \quad (3)$$

Phase relationships were calculated in degrees, with 0° representing an in-phase relationship and 180° representing alternation. Circular statistics (Batschelet, 1981) were used for the treatment of phase relationship data. By convention, the reference leg was always the anterior leg in ipsilateral and diagonal pairs, and the inner leg in contralateral pairs.

The *body axis angle* was measured as the angle between the crayfish axis at any given frame and at the first frame of each sequence. This angle was calculated with reference to the ground markers. Angular velocity and acceleration were derived and smoothed using a five-point moving-average method. The average of each value of the unsmoothed data plus two values preceding it and two values following it was calculated. This procedure was repeated for each angular velocity and acceleration value, starting from the third value of a sequence for angular velocity, and from the fifth value for angular acceleration. The loss of these first and last two or four values for angular velocity and acceleration, respectively, corresponded to a loss of less than 3% of each sequence and should not have affected our results. Positive angular velocity and acceleration indicate angular velocity and acceleration in the direction of the turn. Deriving acceleration from kinematic data can result in various sources of error, as reviewed by Domenici and Blake (1997), but such errors concern mainly the determination of peak values. In our case, the relationship

between the acceleration period and the leg period (see below), rather than peak performance, was the relevant parameter. In addition, each negative and positive peak of acceleration consisted of approximately 10–15 points, suggesting that minimum and maximum values were not the result of noise due to measurement errors.

Cross-correlation functions (CCF). The time relationship between the leg angles and the angular acceleration of the body axis was computed using cross-correlation functions, as used previously by Jamon and Clarac (1997) for angular leg movements in crayfish. For each sequence, the estimated CCF between each leg angle and the body axis angular acceleration was computed and the span of time lags or leads analysed within a range from -1 s to 1 s in increments of 40 ms. The CCFs were computed for each sequence and averaged using the procedure developed by Amblard *et al.* (1994). This procedure was consistent with the fact that individual CCFs showed similar profiles. The correlation peaks of the averaged CCF were tested for a significant difference from zero using *t*-tests (see Amblard *et al.* 1994). The *x*-values of the resulting peaks provided an estimate of the time delay between the leg angle cycle and the body axis angular acceleration. Various possible cases are illustrated in Fig. 2.

Results

Temporal and kinematic variables

Crayfish walked along a curved path for a mean duration of 12.87 ± 0.79 s (mean \pm S.E.M., $N=30$) using 9.9 ± 0.27 steps. Mean forward walking speed was 5.9 ± 0.2 cm s $^{-1}$ (range 3.7–7.8 cm s $^{-1}$). At the end of each sequence, the crayfish body axis had rotated by an angle of $64.6 \pm 3.3^\circ$ (range 31.1–106.5 $^\circ$), at a mean angular velocity of $5.2 \pm 0.2^\circ$ s $^{-1}$ (range 2.6–7.4 $^\circ$ s $^{-1}$). The temporal and kinematic variables of leg motion in a body-fixed coordinate system were analysed.

Step directions and stance amplitudes for all legs are shown in Fig. 3. The step directions of contralateral leg pairs 3 and 4 were oriented at significantly different angles ($P < 0.0001$ in both cases; Mann–Whitney *U*-test). No differences were found between the step directions of contralateral leg pairs 2 and 5 ($P > 0.25$ and $P > 0.1$, respectively; Mann–Whitney *U*-test). The stance amplitude of outer legs 2, 3 and 4 were significantly larger than those of their inner counterparts ($P < 0.005$; $P < 0.0005$; $P < 0.01$, respectively; Mann–Whitney *U*-tests), whereas the stance amplitudes of legs 5 were not significantly different (albeit with a low *P* value of 0.06) despite the presence of double steps in L5i (i.e. two consecutive small steps within the duration of one normal step), which represented approximately 15% of the total steps of L5i. Removing the double steps from the analysis yielded an even higher *P* value of 0.1.

The mean timing of the leg periods is shown in Table 1 for inner and outer legs for all sequences combined. Each leg had a period of approximately 1 s, although there were significant differences between the inner and outer legs. The periods of inner legs 3 and 4 were significantly longer than those of their

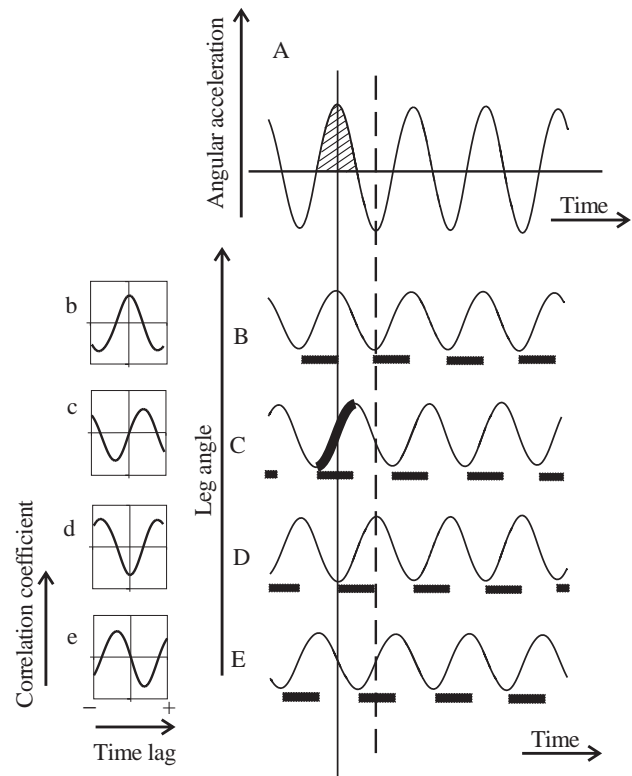


Fig. 2. Theoretical relationships between phase coupling and cross-correlation functions (CCFs). (A) Theoretical curve for body axis angular acceleration *versus* time. The horizontal line indicates zero acceleration. The continuous and discontinuous vertical lines indicate positive and negative acceleration peaks, respectively. (B) Curve for leg angle in phase with A. (C) Leg angle curve lagging 90 $^\circ$ behind A. (D) Leg angle curve 180 $^\circ$ out of phase with A. (E) Leg angle curve leading A by 90 $^\circ$. The panel on the left shows the corresponding CCF curves for B–E, with time lag as the *x*-axis and correlation coefficient as the *y*-axis. Black bars below sinusoidal curves in B–E indicate power strokes. In the CCF curves, positive peaks with 0 $^\circ$ lag (b) indicate that maximum body axis angular acceleration occurs simultaneously with the PEP; positive peaks with 90 $^\circ$ lag (c) indicate that the positive body axis angular acceleration (hatched area on A) occurs during the leg stance phase (thick line on curve C). This is the relationship found if the leg causes the body axis angular acceleration. Negative peaks near 0 $^\circ$ (d) indicate that the body axis angular acceleration occurs at the end of the swing phase. A positive peak with negative lag -90° (e) indicates that the leg angle is 180 $^\circ$ out of phase with the body axis angular acceleration cycle.

contralateral legs, while the period of inner leg 5 was significantly shorter than that of outer leg 5 (Table 1). The difference between contralateral legs 5 was due to the presence of double steps in L5i, which were not observed in legs 3 and 4 and therefore cannot explain differences in their mean period. No differences were found between the periods of legs 2. Duty factors were all approximately 0.5, and no significant differences were found when contralateral legs were compared (Table 1).

Mean stance and swing velocities of outer legs 3 and 4 were

Table 1. Mean period, duty factor and swing and stance velocity of each step for different legs

Leg	Period (s)	Duty factor	Mean stance velocity (m s ⁻¹)	Mean swing velocity (m s ⁻¹)
2 Inner	1.05±0.02 (309)	0.462±0.0003 (309)	2.78±0.07 (339)	2.43±0.06 (320)
Outer	1.05±0.02 (316)	0.467±0.0003 (316)	2.88±0.06 (346)	2.56±0.06 (325)
3 Inner	1.16±0.01 (279)	0.559±0.0002 (279)	3.18±0.05 (309)	4.15±0.06 (294)
Outer	1.12±0.01 (289) *	0.550±0.0002 (289)	3.73±0.06 (319) ***	4.68±0.07 (302) ***
4 Inner	1.20±0.01 (271)	0.533±0.0002 (271)	4.46±0.07 (301)	5.24±0.07 (284)
Outer	1.15±0.01 (285) ***	0.525±0.0001 (285)	4.93±0.07 (315) ***	5.55±0.08 (298) *
5 Inner	1.03±0.02 (325)	0.496±0.0002 (325)	4.01±0.07 (355)	3.94±0.06 (336)
Outer	1.12±0.01 (291) **	0.496±0.0002 (291)	4.00±0.07 (321)	3.96±0.06 (302)

Values are means ± S.E.M. *N* is given in parentheses. Asterisks indicate significant differences between contralateral legs; **P*<0.005, ***P*<0.001, ****P*<0.0005.

Values are compared using *t*-tests for all variables except for periods (Mann–Whitney *U*-test).

significantly higher than that of their contralateral legs, while no significant difference was found between inner and outer legs 2 and 5 (Table 1). Therefore, the higher stance amplitudes found in outer legs 3 and 4 (Fig. 3B) was associated with higher stance velocity and not with longer periods (in fact, the period of outer legs 3 and 4 is shorter than that of the corresponding inner legs). Higher outer leg stance velocity can be associated with turning (it could be the cause of turning or be caused by turning), since it implies that the outer side of the animal moves faster than the inner side due to the rotational component in the direction of the turn. Further information on the relationship between the stepping patterns and body rotation is necessary (see below) to gain insight into the active or passive character of the asymmetries of such kinematic variables.

Step geometry

The observed asymmetries in stance amplitudes and step directions were associated with asymmetries in AEPs and PEPs. The mean *x* (transverse axis) and *y* (long axis) positions of each leg's AEP and PEP relative to the crayfish body (see Fig. 1A) are shown in Fig. 4. The PEPs of all contralateral pairs were significantly different for both the *x* and *y* coordinates (Table 2). The PEP of each outer leg was posterior to that of the inner legs in all cases and it was farther away from the body axis than the PEP of the corresponding inner leg for all legs except legs 5, where the inner leg PEP was farther away from the body axis than that of the contralateral outer leg. The *x* coordinates of the AEP differed in contralateral pairs 3, 4 and 5, and the *y* coordinates differed only between contralateral legs 5. The AEP was farther away from the body axis in L3o, L4o and L5i than in their contralateral legs, and it was more posterior in L5o than in L5i. Therefore, in addition to their longer stride length, outer legs 3 and 4 were farther away from the body axis during their power strokes, with the PEP shifted outwards, and both the AEP and PEP of L5o were

closer to the body axis than those of L5i. These positional differences may depend on the position of the leg tip with reference to the centre of rotation of the body, which for leg 5 is likely to be posterior.

Fig. 5 shows the leg angles for the inner and outer legs during a typical curve-walking sequence. The minima and maxima for each leg angle indicate the AEP and PEP, respectively. The angular amplitude of each step increased posteriorly. In addition, the AEP and PEP angles for inner legs 2 and 3 tended to decrease during the sequence shown in Fig. 5, while they increased for outer legs 2 and 3. This drift in leg angles during curve walking was investigated using linear regression of standardized variables (see Materials and

Table 2. Differences in anterior (AEP) and posterior extreme position (PEP) *x*- and *y*-coordinates between mean values of inner and outer legs (in cm)

Leg	Transverse axis, <i>x</i>	Long axis, <i>y</i>
2 AEP	0.01	0.05
PEP	0.12***	-0.07*
3 AEP	0.10**	0.07
PEP	0.36***	-0.15**
4 AEP	0.05*	0.05
PEP	0.24***	-0.12*
5 AEP	-0.07**	-0.02*
PEP	-0.12***	-0.16*

The *y*-axis is along the long axis of the animal, the *x*-axis is along the transverse axis (see Fig. 1A).

Asterisks indicate significance differences (Mann–Whitney *U*-test) between values for contralateral legs: **P*<0.05, ***P*<0.01, ****P*<0.0001.

Negative values indicate that the position of the outer legs is posterior to that of the inner legs (long axis) or closer to the body (transverse axis).

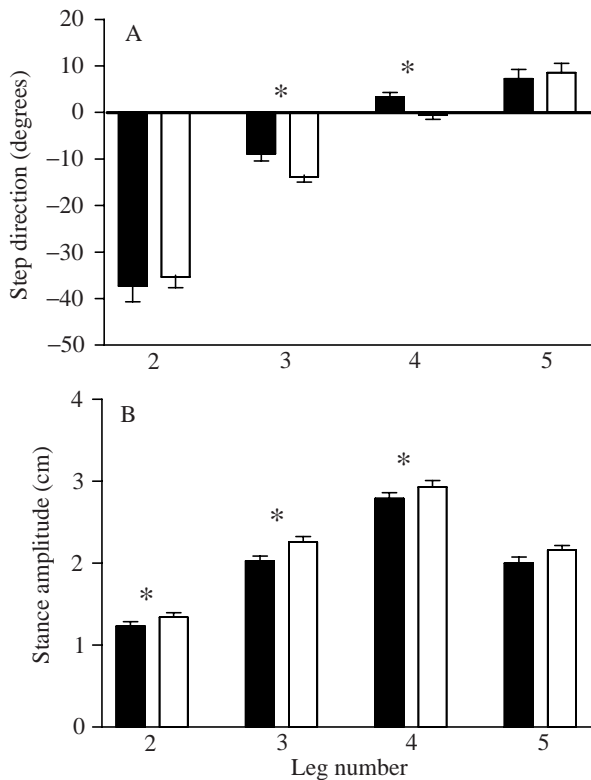


Fig. 3. Mean values and 95% confidence intervals ($N=300-350$; single values for each leg correspond to those for mean stance velocity in Table 1) of step direction (A) and stance amplitude (B) for inner (filled bars) and outer (open bars) legs. Asterisks indicate significant differences between inner and outer legs (P values are indicated in the text).

methods) for all sequences pooled for AEPs and PEPs (Fig. 6). As a general rule, inner legs showed a negative drift ($P<0.0001$ in all cases) except for leg 5. The situation was less clear for outer legs. There was a positive drift in the AEP of L2o and L3o ($P<0.0001$ in both cases) and no drift in L4o and L5o (Fig. 6A). There was no PEP angular drift in L2o and L4o, and a negative drift in L3o and L5o (Fig. 6B); in these two cases, the significance level was lower than for the other legs ($P<0.001$ and $P<0.01$, for L3o and L5o, respectively) and the absolute difference in the mean angle between the beginning and the end of all sequences pooled was quite small (Table 3).

The angular drift observed could be due to an active process, in which changing the leg angles through time would result in curve walking, or to a passive effect, such as incomplete compensation for body turning by the various legs. Let us suppose that, during the power stroke of a given inner leg, the body showed a translation and an inward rotation. As a result, the final PEP angle may be reduced by a small amount, due to the body rotational angle. If the swing amplitude is not reduced accordingly, the AEP angle will also be smaller than the previous one. This may explain the gradual decrease in AEP and PEP inner leg angles. A similar and opposite mechanism (i.e. the swing amplitude failing to increase in order to compensate for the inward body rotation) may explain the gradual increase observed in outer leg AEP angles.

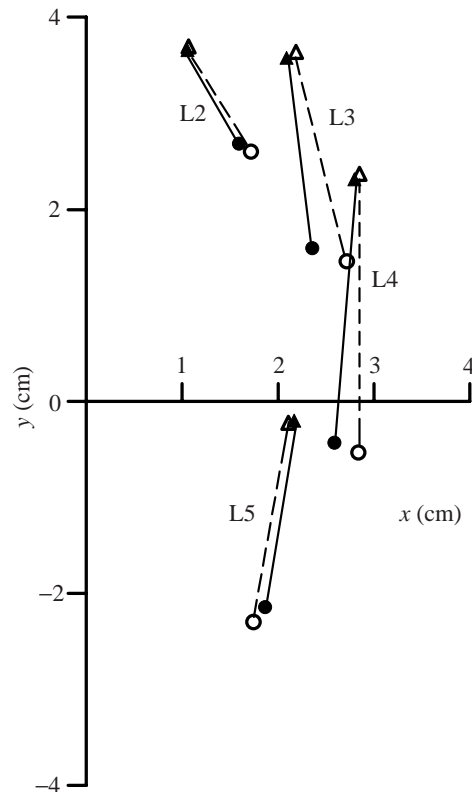


Fig. 4. Geometry of crayfish leg position, with inner and outer leg positions. Mean outer leg anterior extreme positions (AEPs) (open triangles) and posterior extreme positions (PEPs) (open circles) and inner leg AEPs (filled triangles) and PEPs (filled circles) are shown with respect to a body-fixed coordinate system (top view, see Fig. 1A). The origin represents the digitized point at the posterior end of the crayfish cephalothorax. The ordinate represents the crayfish body midline. All values are means (in cm) from all steps of all sequences pooled. The AEP and PEP of each leg are connected by a dotted line (outer legs) or a solid line (inner legs). Significant differences between inner and outer AEP and PEP x - and y -coordinates are given in Table 2.

Angular drift may produce increasing asymmetries in contralateral leg angles of each sequence. For example, a steady decrease and increase in L2i and L2o angles, respectively, will result in larger L2o angles at the end of a sequence than L2i angles, if the initial angles of these contralateral legs were similar. Therefore, we have also investigated possible asymmetries in the absolute values of contralateral leg angles at the beginning and end of each sequence (i.e. considering only the first and last AEP or PEP angles recorded during a sequence) (Table 3). In agreement with our hypothesis, the final AEP and PEP leg angles were found to be smaller for all inner legs than outer legs, except for legs 5 for which no significant difference was found. Contrary to our expectations, we also found differences between the initial angles of some contralateral legs. The initial AEP angles of inner legs 2 and the initial PEP angles of inner legs 2 and 3 were significantly larger than for the outer legs.

Ipsilateral and contralateral phase relationships

Phase relationships were analysed for ipsilateral and contralateral pairs of legs. All ipsilateral phase relationships resulted in non-uniform distributions (Rayleigh test; $P < 0.0001$ in all cases). Mean values of the phase relationships are shown in Table 4. The distributions of the phase values for corresponding ipsilateral legs (e.g. leg pairs 3i2i and 3o2o) were compared in order to investigate the symmetry of the stepping pattern. Differences were found between all pairs except for 3i2i *versus* 3o2o (Table 4). The ipsilateral phase relationships in outer legs are similar to those observed previously in unrestrained straight-walking crayfish (Jamon and Clarac, 1995), in which the phase relationship values of ipsilateral legs increased posteriorly from the 3–2 pair to the 5–4 pair, while inner legs showed similar phase relationships in leg pairs 5i4i and 4i3i (133° and 130° , respectively).

All contralateral phase relationships resulted in non-uniform distributions (Rayleigh test; $P < 0.0001$ in all cases; Table 4). Although non-uniform distributions suggest some interleg

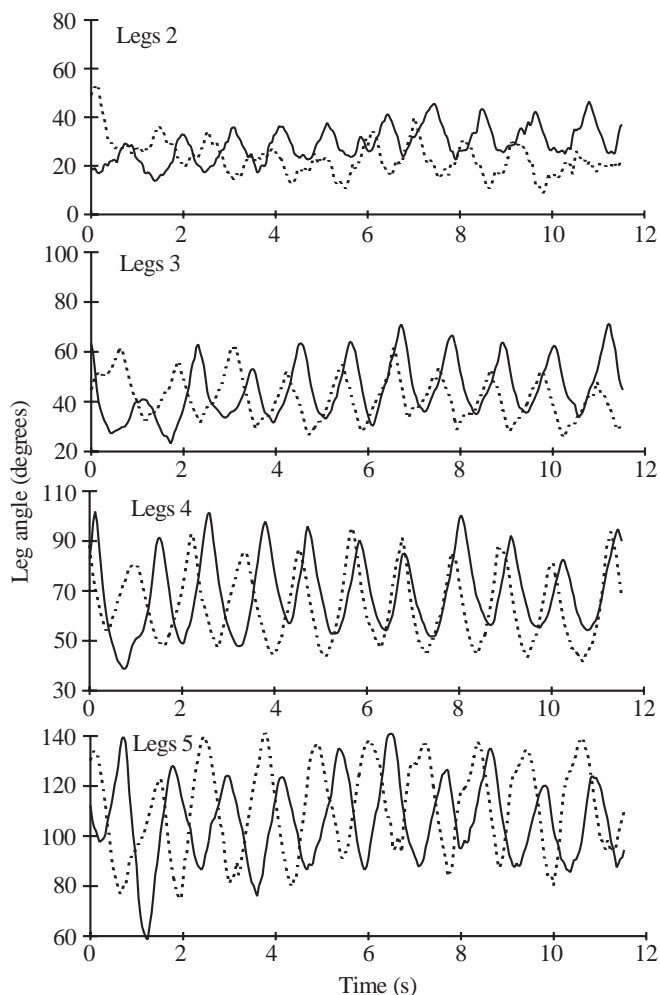


Fig. 5. Leg angles for inner (broken lines) and outer (solid lines) legs 2–5 during a curve-walking sequence. Minima correspond to anterior extreme positions (AEPs) and maxima correspond to posterior extreme positions (PEPs).

coordination, asymmetries in step periods imply that these coordinations are relative and not absolute (absolute coordination implies strictly inter-dependent rhythms with stable phase relationships, while relative coordination implies two weakly coupled rhythms with slightly different frequencies exerting an accompanying quantitative influence on one another; von Holst, 1973). Contralateral phase relationships showed mean values of 143° , 142° , 112° and 166° for leg pairs 2, 3, 4 and 5, respectively, and were significantly different from in-phase or alternate patterns (95 % confidence intervals test; Batschelet, 1981).

Turning behaviour

Turning was determined as the body axis rotation with reference to an external coordinate system. Although the body axis angle *versus* time graph (Fig. 7) showed an overall increase, the turning behaviour was discontinuous, as shown by the body axis angular velocity *versus* time data (Fig. 7), in which angular velocity minima include negative values (i.e. turns in the opposite direction to that of the curved path). In order to investigate the relationship between stepping patterns and turning behaviour, we calculated the body axis angular acceleration during the power stroke (i.e. active phase) of each leg, with the assumption that any leg with a direct role in rotating the body axis in the direction of the turn would cause positive angular acceleration of the body axis during most of its power stroke. The angular acceleration of the body axis (Fig. 8) was positive throughout most of the power strokes of inner legs 2 and 5 and outer leg 4. Therefore, the torque generating the rotational component of curve walking was presumably exerted during the power strokes of these three legs. In order to investigate the possibility of synchrony between legs 2i, 4o and 5i, the phase relationships between them were calculated and compared with the phase relationships between their contralateral legs (i.e. L2o, L4i, L5o) (Table 4). The distributions of the phase values for opposite leg pairs (e.g. legs 5i4o and 5o4i) were compared in order to investigate the symmetry of the stepping pattern. Differences were found between all pairs (Table 4): phase relationships of the tripod 2i4o5i were close to 360° , with leg pair 5i4o showing significant in-phase patterns (tested using 95 % confidence intervals of the mean phase; Batschelet, 1981) and the other two leg pairs showing small phase shifts consistent with synchronicity during most of the cycle. Phase relationship values linking leg 2i with the other two legs (337° for 5i2i and 324° for 4o2i) of the tripod showed that leg 2i followed both leg 5i and 4o by only approximately one-tenth of the step cycle. This implies that, on average, the three legs were on the ground simultaneously during most of each leg's power stroke. In contrast, the phase relationships of the opposite tripod varied considerably, from 75° (4i2o) to 269° (5o4i) and 342° (5o2o) (Table 4).

Three-dimensional representations of the frequency distribution of phase relationships within each tripod are shown in Fig. 9, in which the coordination of leg 4 with the two other legs of the tripod is considered. Fig. 9A shows the frequency

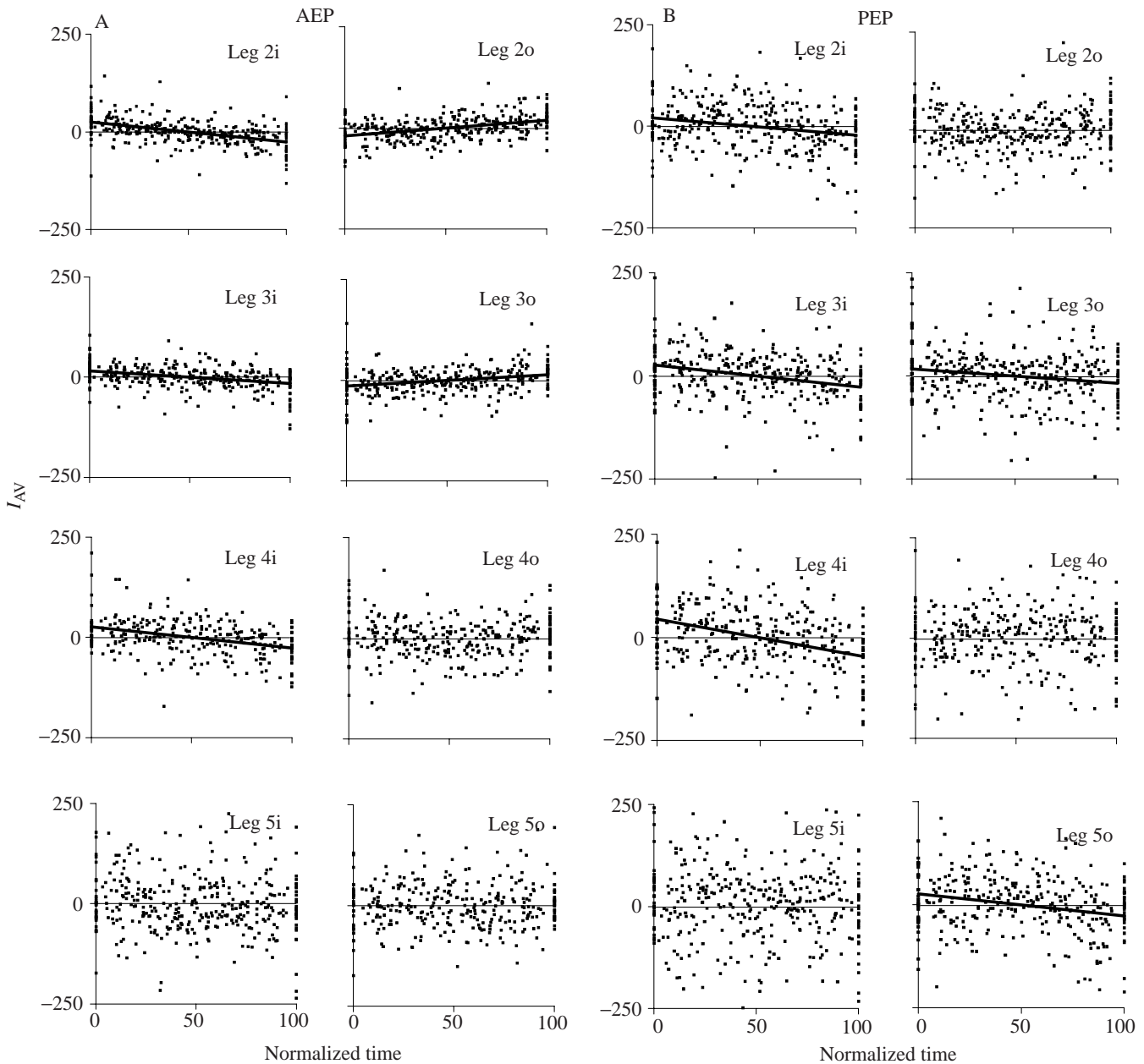


Fig. 6. The relationship between the index of angular variation (I_{AV}) (see Materials and methods) and normalized time for the anterior extreme positions (AEPs, A) and posterior extreme positions (PEPs, B) of each leg. Only significant ($P < 0.05$) regression lines are shown. i, inner leg; o, outer leg.

distributions of the phase relationships between L4o and L2i and L5i. Consistent with the suggested tendency of these three legs to be synchronous, a peak is present when L4o is in phase with both L5i and L2i, with high values also occurring when L4o has a relationship of 270° with L2i. This is because L2i follows L4o by approximately one-tenth of a cycle, as shown in Table 4 (324° corresponding to -36° or a 10% lag); therefore, the peak in the L4o–L2i relationship should lie between 270° and 360° . The three-dimensional graph for the contralateral tripod 2o4i5o shows a peak when L4i is 90° out of phase with both L5o and L2o (Fig. 9B). High frequencies occur diagonally across the horizontal plane of the graph in

sectors of equal phase relationship values of L4i–L5o and L4i–L2o. This was expected, because L5o and L2o tended to be in phase (Table 4). Therefore, the coordination found within tripod 2i4o5i was not found in the opposite tripod (2o4i5o), and thus was not the result of simple symmetrical step patterns.

After determining the degree of synchronicity within the tripod 2i4o5i, we investigated its coordination with the body axis rotational movement. A leg's power stroke is delimited by a leg angle minimum (AEP) and a leg angle maximum (PEP). Therefore, if peak angular acceleration of the body axis were to occur during a leg's power stroke, the phase relationship between the body axis angular acceleration and that leg's angle

Table 3. Mean initial and final anterior extreme position (AEP) and posterior extreme position (PEP) angles for all sequences pooled

Leg	AEP		PEP	
	Initial angle (degrees)	Final angle (degrees)	Initial angle (degrees)	Final angle (degrees)
2 Inner	19.5±0.8	13.6±0.9	37.2±1.5	29.8±1.3
2 Outer	14.7±1.0 ***	18.8±1.0 **	33.1±1.3 *	35.8±1.5 **
3 Inner	30.6±1.3	28.7±1.1	66.1±2.2	52.5±2.3
3 Outer	32.9±0.9	33.4±0.9 ***	58.9±2.2 ***	60.6±0.9 ***
4 Inner	52.2±1.8	48.3±1.6	100.1±3.0	93.1±2.9
4 Outer	53.8±1.6	52.8±1.1 *	104.3±3.1	100.0±3.3 ***
5 Inner	96.4±2.2	95.0±3.6	144.1±2.1	136.6±2.7
5 Outer	96.8±3.0	97.0±2.3	142.4±1.9	140.9±1.6

Values are means ± S.E.M. (N=30).

Significant differences between contralateral legs (paired *t*-tests) are indicated by asterisks: **P*<0.05; ***P*<0.005; ****P*<0.001.

should lie between 0 and 180°. Since the maximum force exerted by a leg is not likely to occur at the beginning (AEP) or at the end (PEP) of the power stroke, only intermediate values of phase relationship would suggest that the given leg is involved in contributing to the body axis angular acceleration peak (see Fig. 2). Indeed, Klärner and Barnes (1986) showed for the crayfish *Astacus leptodactylus* that both peak forces propelling the animal forward and lateral forces occurred approximately half-way through the power stroke.

Using cross-correlation function (CCF) analysis (Fig. 10),

we found that legs 5i, 2i and 4o were the only legs whose lag corresponded to a phase relationship with the body axis angular acceleration that was intermediate between 0 and 180°, i.e. with the body angular acceleration peak occurring during the leg's power stroke (Fig. 2A,C). The other legs were either in phase with the body axis angular acceleration (leg 3o and leg 4i) or showed a negative lag. Therefore, for legs 3o and 4i, the peak in body axis angular acceleration occurred close to their PEP (i.e. at the end of the power stroke), while for legs 5o, 2o and 3i, it occurred during their return stroke.

Table 4. Phase relationships of ipsilateral and contralateral pairs of legs and of leg pairs forming opposite tripods 2i4o5i and 2o4i5o, where *i* is the inner leg and *o* is the outer leg

	Leg pair	Mean phase			Leg pair	Mean phase			Distribution comparison (χ^2 -test)
		(degrees)	Mean vector	<i>N</i>		(degrees)	Mean vector	<i>N</i>	
Ipsilateral	3i2i	83±4	0.58	275	3o2o	75±3	0.63	294	
	4i3i	130±2	0.80	270	4o3o	117±2	0.81	283	*
	5i4i	133±4	0.57	313	5o4o	148±2	0.84	289	**
Contralateral	2o2i	143±7	0.31	310					
	3o3i	142±5	0.44	289					
	4o4i	112±9	0.26	281					
	5o5i	166±6	0.37	296					
Opposite tripods	4o2i	324±7	0.32	280	4i2o	75±9	0.26	277	**
	5i2i	337±6	0.36	308	5o2o	342±3	0.67	286	**
	5i4o	345±8	0.28	313	5o4i	269±7	0.34	278	**

The reference leg is the inner leg in contralateral pairs and the anterior leg in ipsilateral and tripod pairs.

Values are means ± S.E.M.

The mean vector is a measure of concentration around a mean angle, with a maximum value of 1 (maximum concentration) and a minimum value of 0 (Batschelet, 1981).

The distributions of homologue ipsilateral and tripod pairs were compared using a χ^2 -test: **P*<0.001; ***P*<0.0001.

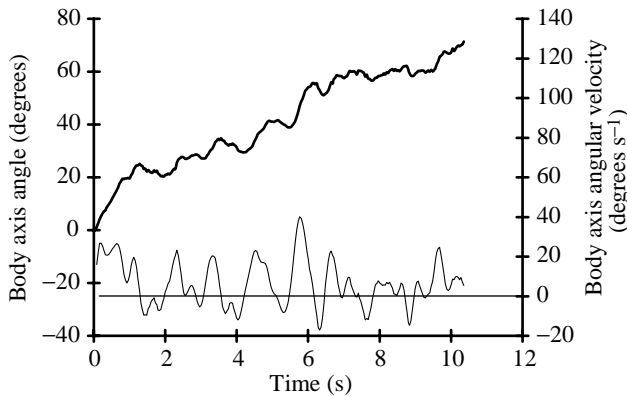


Fig. 7. Body axis angle (heavy line) and angular velocity (fine line) during a typical curve-walking sequence.

These results confirm that inward angular acceleration of the body axis occurred during the power strokes of legs 2i, 5i and 4o. They do not, however, prove that all three legs contributed to the inward rotational force exerted on the body axis during curve walking. For example, two of the three legs may simply tend to be in phase with a single actively contributing leg. There was enough variability in the interleg coordination to test whether the body axis angular acceleration during a leg's power stroke varied as a function of that leg's phase relationship with the other two legs of the tripod. For instance, we can hypothesize that the body axis angular acceleration observed during the power stroke of a single actively contributing leg should be independent of the leg's phase relationship with the other two legs. In order to test this hypothesis, we carried out a two-way analysis of variance (ANOVA) in which we grouped phase relationships into in-phase ($270\text{--}90^\circ$) and out-of-phase ($90\text{--}270^\circ$) classes. We found that, in all three legs, the body axis angular acceleration observed during the power stroke was related statistically to their phase relationship ($\Phi_{n \text{ in } n'}$) with at least one of the other legs. For leg 5i, angular acceleration depended on $\Phi_{L5i \text{ in } L2i}$ ($P < 0.005$) and $\Phi_{L5i \text{ in } L4o}$ ($P < 0.0001$); for leg 2i, it depended on $\Phi_{L2i \text{ in } L4o}$ ($P < 0.0001$); and for leg 4o, it depended on $\Phi_{L4o \text{ in } L5i}$ ($P < 0.05$). In all three legs, the highest body axis angular acceleration was found when in phase with the other two legs and the lowest when out of phase. These results suggest that the rotational component of curve walking is not related to the activity of a single leg, but there must be some synergy between the three legs in question.

Discussion

Kinematics

This study is the first on crustacean curve walking to be carried out on unrestrained animals. The most relevant comparisons can be made with previous studies on curve-walking locomotion in arthropods, both tethered and untethered (e.g. Zolotov *et al.* 1975; Jander, 1985; Zollikofer, 1994; Cruse and Silva Saavedra, 1996), where animals perform

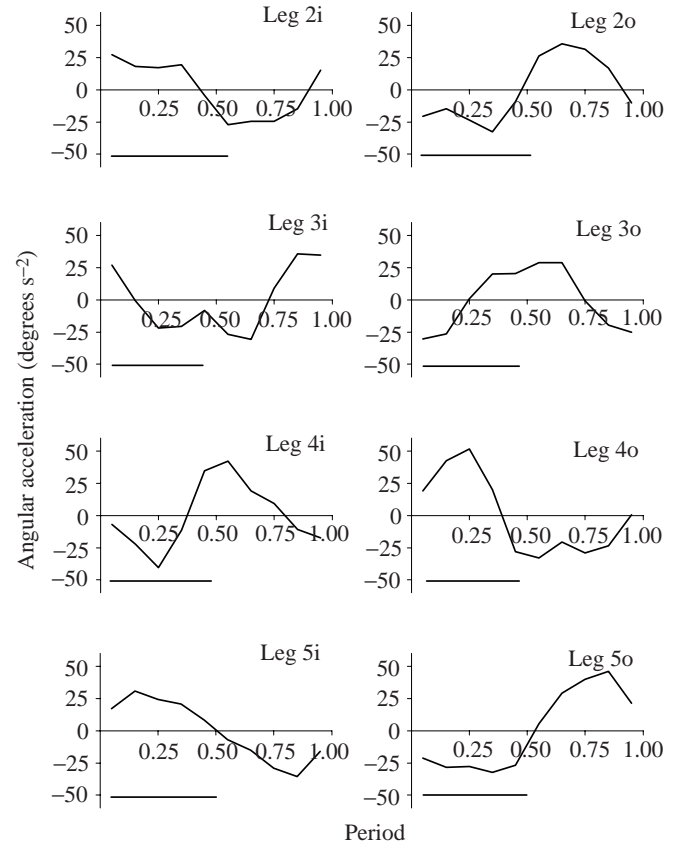


Fig. 8. The angular acceleration of the body axis averaged over intervals of 10% of the period of each leg (mean values of all sequences pooled). Horizontal bars represent the duration of the power stroke within the period of each leg. Angular acceleration of the body axis shows slightly different profiles in each plot because of normalization (0–1) of periods with slightly different overall average values (see Table 1).

a continuous change in heading while walking. Although observations on single turns (e.g. Graham, 1972; Zolotov *et al.* 1975; Camhi and Levy, 1988; Strauss and Heisenberg, 1990) and on rotation in place (e.g. Land, 1972; Zolotov *et al.* 1975; Bell and Schal, 1980; Franklin *et al.* 1981) may provide some parameters for comparison, they mainly deal with simple rotation, which implies different mechanisms from curve walking where rotation of the body axis is associated with translation. For example, rotation in place can derive from power strokes directed forwards in inner legs (Land, 1972; Zolotov *et al.* 1975; Franklin *et al.* 1981): escape turns of cockroaches *Periplaneta americana* (Camhi and Levy, 1988) involved simultaneous stance of five or six (i.e. all) legs, or pure rotation locomotion can involve nearly zero amplitude in the inner hindleg, which acts as a 'pivot' (Zolotov *et al.* 1975; Camhi and Levy, 1988; Strauss and Heisenberg, 1990). None of these features is observed in curve-walking behaviour, where translation is always present and is associated with contributions from both inner and outer legs.

Previous studies on curve walking in arthropods have

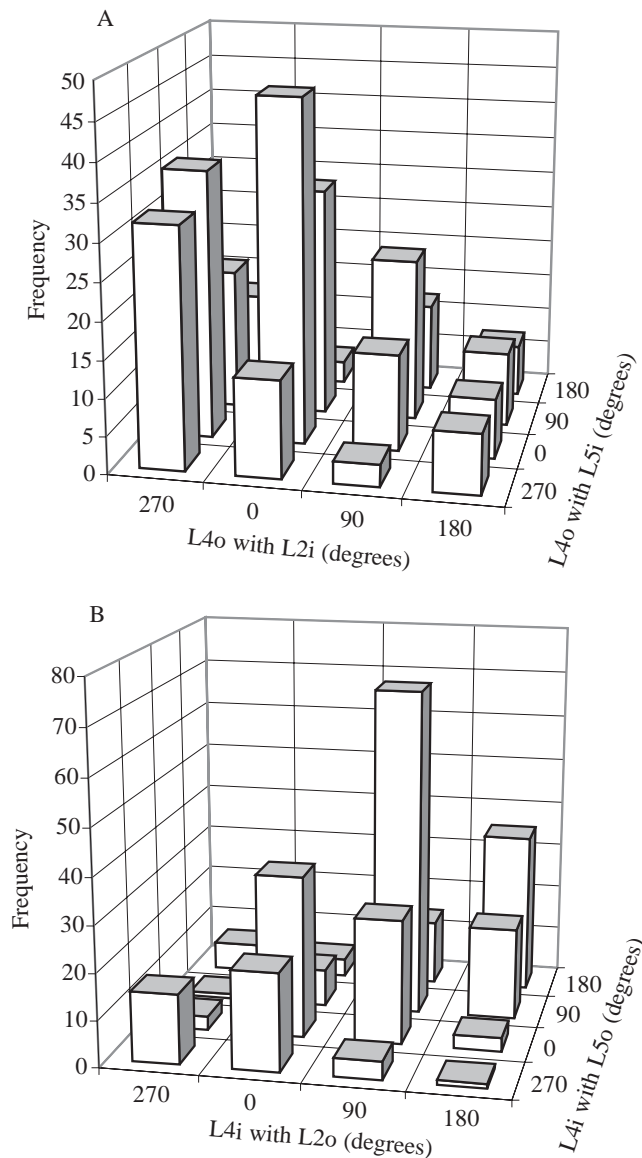


Fig. 9. Three-dimensional frequency distribution of phase relationships for tripods 2i4o5i (A) and 2o4i5o (B), where i is inner leg and o is outer leg. Phase relationships of legs 4 with legs 2 and 5 are represented. Phase relationships are divided into four sectors, with sector 0 centred on in-phase coordination and sector 180° centred on out-of-phase coordination.

suggested that turning is related to asymmetries in footfall positions. In freely moving ants (*Cataglyphis bombycina*), Zollikofer (1994) showed that curve walking was due to a decrease in the stride length of inner legs when compared with straight walking. Zolotov *et al.* (1975) and Jander (1985) found similar results for the step amplitude of curve-walking honeybees (*Apis mellifera*) and stick insects (*Carausius morosus*), respectively, although they also observed lower stepping frequencies in inner legs than in outer legs for sequences with relatively small turning radii. The study of Cruse and Silva Saavedra (1996) is the only previous study on

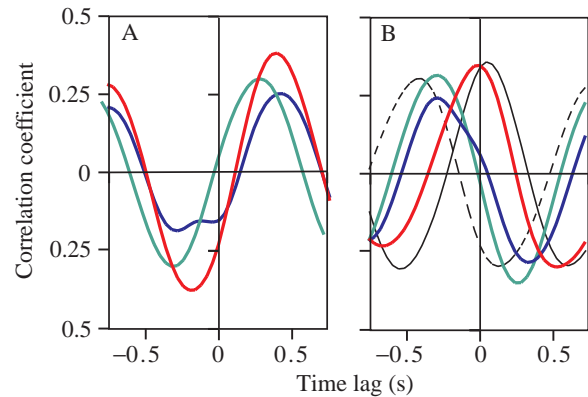


Fig. 10. Averaged cross-correlation functions between leg angles and body axis angular acceleration. y-values are means of the correlation coefficients. (A) Tripod 2i4o5i, where i is inner leg and o is outer leg (L4o red, L5i green, L2i blue). (B) All other legs. Legs of the opposite tripod (2o4i5o) are colour-coded as their contralateral leg in A (i.e. L4i red, L5o green, L2o blue). Legs 3o and 3i are shown as continuous and broken black lines, respectively. All negative and positive peaks in the averaged CCF plot were found to be significantly different from 0 (*t*-tests, $P < 0.05$ in all cases).

curve walking in crustaceans (the crayfish *Astacus leptodactylus*). Using tethered animals, they found that crayfish curve walking was due to asymmetries in the legs' PEPs, causing the amplitudes of the outer legs to be greater than those of the inner legs. In particular, Cruse and Silva Saavedra (1996) found differences among the step amplitudes of all contralateral legs, while the present study found differences among leg pairs 2, 3 and 4, but not 5. Similarly, Cruse and Silva Saavedra (1996) found large asymmetries between the step directions of contralateral legs 2, 4 and 5, while we found differences in the step direction of contralateral pairs 3 and 4 only.

Despite these differences, possibly due the different methodologies (tethered animals on a treadmill *versus* freely walking animals) and/or the different species used, our results on leg geometry do not differ markedly from those of Cruse and Silva Saavedra (1996), except for their results showing a considerable change in the PEP position of inner leg 5, which almost coincided with its AEP, giving it a very small leg amplitude and negative step direction (Cruse and Silva Saavedra, 1996).

Although previous studies suggest that curve walking is achieved through asymmetries in leg kinematics, sometimes accompanied by asymmetries in stepping frequencies, data on body axis angular velocity or acceleration for comparison with stepping patterns were not provided, so it is not possible to establish whether the rotational component of curve walking in these studies was relatively constant or whether it varied in relation to stepping pattern, as in our study. Therefore, it is possible that the larger step amplitude of all outer legs, whether it is measured from external (e.g. Zollikofer, 1994) or body-centred references (e.g. Jander, 1985; Cruse and Silva

Saavedra, 1996), may in part be a passive phenomenon due to the body rotation caused by only some of the legs. In the present study, we cannot conclude that asymmetry in leg geometry is an active mechanism responsible for curve walking. Other asymmetries were found in our study, i.e. in angular drift, in inter-leg phase relationships and in the phase relationships between leg angles and body axis angular acceleration. Any asymmetry or any difference from straight walking in crayfish found in the present study may be due to active or passive effects as discussed below.

Leg angle drift

An important finding of the present study was that leg geometry varied throughout the walking sequence, particularly for the anterior leg position, for which the most significant effects were found and where the general tendency was for inner legs to show negative angular drift (Figs 5, 6; Table 3). This was probably due to a passive effect, such as incomplete compensation for body turning by the various legs. In addition, we found some asymmetries in leg angle at the beginning of the curve-walking sequences. The inner leg angles for leg 2 AEP and PEP and leg 3 PEP were larger than their corresponding contralateral leg angles at the beginning of the sequence and smaller at the end of it. Therefore, leg angular drift was not due to simple readjustment to a symmetrical situation, since the asymmetries in the final leg angle showed that the drift continued well beyond the point at which symmetry was reached. It is therefore possible that, following the extensive (3 weeks) training, crayfish could anticipate the asymmetrical effects (passive or active) that curve walking would have on their leg angles, particularly for the anterior legs. Therefore, crayfish may have started each curve-walking sequence with larger inner leg angles since they tended to decrease throughout the sequence. This effect may be particularly marked for the most anterior legs (legs 2), because their AEP angles were fairly small (range approximately 5–30°) and their lowest value will be limited mechanically by the crayfish body. Starting the curve-walking sequence with larger AEP inner angles would delay the occurrence of the angular threshold set by mechanical limits.

We hypothesize that, for curve-walking sequences longer than we observed, leg angles might drift slowly until a certain threshold (possibly mechanical) is reached and leg repositioning would occur. Such a mechanism of periodic repositioning may seem more primitive than continuous feedback compensation, but it would simplify the coordination of eight-legged locomotion by using a mechanical threshold as a resetting mechanism. Alternatively, leg angles may decrease gradually until they reach a plateau which may have to be maintained only in prolonged curve-walking sequences. Further detailed studies of leg angular drift in animals engaged in longer curve-walking sequences are needed in order to test these hypotheses.

Timing, periods and phase relationships

Cruse and Silva Saavedra (1996) found no differences in the

step frequencies of the inner and outer legs, and suggested that higher step amplitude in outer legs with the same step frequency as inner legs may be due to prolongation of the power stroke in the outer legs, compensated by a shortening of the duration of the return stroke. In the present study, we found differences in period within contralateral leg pairs 3, 4 and 5; in particular, outer legs 3 and 4 had shorter periods than their contralateral legs. However, since we did not find differences in the duty factors of contralateral legs, prolongation of the power stroke compensated by a shorter return stroke (as suggested by Cruse and Silva Saavedra, 1996) does not apply to our study. Here, outer legs 3 and 4 had larger step amplitudes despite shorter periods due to higher power-stroke and return-stroke velocities. Increased step velocity of the outer legs can be related to turning (Cruse and Silva Saavedra, 1996) because the outer side of the crayfish moves faster than the inner side. Jander (1985) showed that asymmetries in step frequency depend on the turning radius, which may explain the discrepancies between our study and that of Cruse and Silva Saavedra (1996).

The ipsilateral phase relationships of the outer legs were similar to those found in straight-walking *P. clarkii* (Jamon and Clarac, 1995), although in the present study outer leg pairs 5o4o and 4o3o showed different ipsilateral coordinations (albeit by only approximately 15°) when compared with inner leg pairs 5i4i and 4i3i. Contralateral coupling is weaker than ipsilateral coupling in arthropods (Clarac, 1982). Here, we observed significant contralateral coordination which can be considered as relative (von Holst, 1973), since absolute coordination could not be maintained owing to differences in step periods between contralateral legs. Contralateral coordination ranged from approximately 110° (for legs 4) to almost completely out of phase (166° for legs 5). These results differed both from the alternate coupling commonly observed in arthropods (Clarac and Barnes, 1985; Müller and Cruse, 1991a) and the in-phase coupling observed in freely walking crayfish (Jamon and Clarac, 1995). The significance of the difference from the pattern found in straight-walking crayfish may be in maintaining in-phase relationships within the tripod 2i4o5i, which is hypothesized to be the main contributor to turning. Legs 2 and 5 on each side were expected to be almost in phase owing to metachronal organization from front to back. The phase relationships within leg pairs 4o2i and 5i4o, however, are likely to be mainly related to contralateral coordination, because ipsilateral coordination is relatively fixed and mechanically constrained. In addition, we can hypothesize that the double steps observed in leg 5i may be related to two co-occurring, but possibly sometime conflicting, tendencies; the leg's tendency to be in phase with legs 4o and 2i, and its relatively fixed ipsilateral coordination with leg 4i.

Turning behaviour

Our experiments on freely moving crayfish show that the rotational component of curve walking is discontinuous, with the animal's body axis turning towards the inside of the curve only periodically (Fig. 7). The discontinuity of the rotation is

related to the stepping pattern. We can hypothesize that inner legs 2 and 5 and outer leg 4 contribute most to the body axis rotation observed in curve walking. These three legs tend to form a tripod which may produce the torque necessary for the body rotation to occur. In crayfish, this tripod was not as spatially and temporally rigid as in curve-walking insects (Zollikofer, 1994), and this resulted in a variable body axis angular acceleration through time (Fig. 8), related to the level of synchrony between legs 2i, 4o and 5i. Interestingly, the body axis angle during a turn of approximately 40° in *Drosophila melanogaster* seems to change (i.e. the body axis is rotating) during the stance phase of the tripod 1i2o3i, while it remains constant during the stance of the opposite tripod (Fig. 10a in Strauss and Heisenberg, 1990). This tripod arrangement is similar (inner fore and hind leg and outer middle leg) to that of the tripod 2i4o5i, which showed power strokes in phase with the body axis rotational acceleration in the present study. Further studies are needed in order to investigate whether there is a relationship between the rotational component of curve walking and stepping pattern in other species of arthropod.

In the crayfish's tripod, each leg may have its own function, which may be related to its footfall position and its movement pattern. Although it is possible that only two or even one of the three legs contributed actively to curve walking, while the others were approximately in phase, our results based on ANOVA suggest some synergy between the three legs. Jamon and Clarac (1995) showed that legs 2, 3 and 4 pull the body forward and legs 5 have pushing activity only. We therefore hypothesize that the body rotation observed may be caused by a synergy of inner leg 2 pulling the anterior part of the crayfish towards the inner side of the curve, while inner leg 5 pushes and leg 4 pulls the posterior part outwards. These hypotheses will be tested by using simultaneous force measurements, electromyograms and kinematic analysis of curve-walking crayfish.

In conclusion, our study shows a combination of temporal and spatial effects associated with curve walking in freely moving crayfish. We hypothesize that three legs are the principal contributors to curve-walking behaviour, as suggested by the agreement between their power strokes and the inward rotational acceleration of the body axis. The geometrical differences (such as leg positions and leg angle drift) found for some of the other legs may be passive effects. An important difference between contralateral legs in geometrical terms was the larger amplitude of the outer legs, as found by Cruse and Silva Saavedra (1996). Asymmetries in leg amplitudes can be expected if significant body rotation was caused by any of the eight legs during the power stroke of at least one of the legs of a contralateral pair. As the body axis turns towards the inner side of the curve, the inner amplitudes become smaller than the outer amplitudes for a given leg pair. This may be a passive phenomenon due to other legs actively turning the body axis. Similarly, although the body axis showed a rotational acceleration in the direction of the turn during the power stroke of only three legs, leg angular drift was observed in most legs. We hypothesize that the latter

phenomenon was passive, caused by incomplete compensation by the legs for the changes in the body axis orientation through time.

It remains to be established whether the differential contributions of the legs to turning are due to physiological/mechanical constraints on the characteristics of each leg (e.g. turning can occur only during the stance phase of some legs). The relationship between the positions of the legs and the body axis angular acceleration suggests that there must be at least a preferential spatio-temporal arrangement of the legs which is associated with the rotational component of curve walking.

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