VARIABILITY OF LEG KINEMATICS IN FREE-WALKING CRAYFISH, *PROCAMBARUS CLARKII*, AND RELATED INTER-JOINT COORDINATION

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Summary

The inter-joint coordination in leg 4 of the crayfish *Procambarus clarkii* was investigated while they travelled freely along straight paths. Video analysis of the kinematics of the leg's three-dimensional motion, combined with a statistical method based on conjugate cross-correlation functions, showed stable inter-joint coordination in the leg kinematics. In particular, the inter-joint coordination involved a strong movement in the distal mero-carpopodite (M-C) joint occurring between the movements of the proximal thoraco-coxopodite and coxo-basal joints; thus, the leg extended during the swing phase and flexed during the stance phase. This synchronisation was mainly independent of global changes in the locomotor pattern induced by variation in speed or contralateral inter-leg coupling which occurred during free walking. The main changes in inter-joint coordination were found to be related to the appearance of a biphasic flexion/extension movement during each stroke of the step cycle when the leg retracted far backwards. This movement was observed more frequently in large animals and was therefore possibly related to changes in postural control. The functional role of this distal M-C joint movement in the leg motion is discussed.

Key words: locomotion, crayfish, *Procambarus clarkii*, inter-joint coordination.

Introduction

Locomotion in crustaceans has been investigated in great detail using tethered animals walking on a treadmill (Clarac, 1982; Cruse *et al.* 1983), and in that situation it has been possible to define inter-leg and intra-leg coordination by studying leg movements, the forces exerted and the electromyographic (EMG) activity of the muscles (Chasserat and Clarac, 1986; Clarac and Chasserat, 1986). In such restrained conditions, a forward and a backward locomotory mode have been defined clearly in the rock lobster *Palinurus vulgaris* (Clarac, 1984): forward walking corresponds, in a given leg, to a simultaneous common output of the levator and the promotor muscles during the return stroke and of the depressor and the remotor muscles during the power stroke; backward walking corresponds to the opposite association of the levator with the remotor during the return stroke and the depressor with the promotor during the power stroke. Similar configurations have been described for other crustaceans with an elongated shape (e.g. crayfish, lobster) where the abdomen is large (Ayers and Davis, 1977; Ayers and Clarac, 1978; Grote, 1981). This coordination was found, *in vitro*, to be sustained by monosynaptic inter-joint reflexes between the C-B (coxo-basal) joint and T-C (thoraco-coxopodite) joints through a chordotonal organ (El Manira *et al.* 1991).

The activity of more distal joints has also been studied. Barnes (1977) recorded alternating EMG activity in the muscles of various distal segments, the mero-carpopodite (M-C) joint, the carpo-propodite (C-P) joint and the dactyl joints, during forward locomotion of the crayfish *Astacus leptodactylus* on a treadmill, but did not find a correlation with the activity of proximal joints. Also using EMG recordings, Ayers and Clarac (1978) studied the role of the M-C joint in the different locomotory modes of the free-walking rock lobster and found evidence that this joint is important in lateral walking and is much less involved during forward and backward walking. In the crab *Carcinus maenas* (Clarac *et al.* 1987), the activity patterns of six muscles have been recorded during free walking under water and on land. The relationships between the activity bursts of the muscles of three joints, T-C (the promotor and remotor), C-B (the levator and depressor) and M-C (the flexor and extensor), were compared in detail in relation to whether the leg was on the leading or on the trailing side. In some studies, the sensory activity of the dactyl was recorded and its role in intra-leg and inter-leg coordination was described (Libersat *et al.* 1987; Müller and Clarac, 1990).

All these experiments have provided little information concerning joint positions and movements and have not specified the coordination level between the three main joints, T-C, C-B and M-C. Only the entire leg movement with its anterior extreme position (AEP) and its posterior extreme position (PEP) have been characterised (Clarac, 1984). Several

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questions remain as to the role of the M-C joint in forward walking, its relationship with the other joints, and especially as to the existence of some special hierarchy between the joints. A local synergy between the joints would allow the leg movement to be organised peripherally and would thus simplify the central command system required, which would need to drive only one joint on each leg in order to control inter-leg coordination. Which is the leader joint that is controlled? The answer to this question is particularly crucial since it has been demonstrated that the different proprioceptors found at the level of each joint control the other joints very precisely through inter-joint reflexes. For example, when the C-B joint chordotonal organ relaxes, mimicking a levation, it facilitates the extensor motoneurones (Clarac et al. 1978). Neurologically, the T-C, C-B and M-C joints are very closely connected (Vedel and Clarac, 1979; Head and Bush, 1992).

The present study provides a detailed investigation of the kinematics and coordination of the three main joints involved in locomotion (the T-C, C-B and M-C joints) during free forward walking in the crayfish. The precise kinematics of intra-leg movement have rarely been studied, because of the three-dimensional complexity of the leg movements. This study utilises a powerful video motion-analysis technique based on perpendicular planes filmed using two synchronised cameras. Previous three-dimensional motion analyses of invertebrates have investigated global variables of the leg (Full and Tu, 1990; Weinstein, 1995), righting activity (Full and Ahn, 1995; Full et al. 1995) and the movement of joint angles in the various legs (Cruse and Bartling, 1995). The present study focused on the coordination between the movements of the various joints of one leg. Leg 4 was chosen as a model for this study, because previous work suggested that it plays a prominent role during locomotion (Jamon and Clarac, 1995). Joint movements in the various spatial planes were investigated using video motion analysis. Inter-joint coordination was defined using a statistical method, based on cross-correlation functions (CCFs), first designed for the study of sensorimotor strategies in human motion (Amblard et al. 1994). One of the interesting properties of this method is that it does not involve the use of a time origin and is therefore suitable for the analysis of sustained and periodic sensorimotor controls. It was thus possible to define the inter-joint synergy during free walking. By combining a kinematic study with the CCF statistical approach, we were able to define the respective participation of the three main locomotor joints in the organisation of the leg movement. The time sequence of movements of the various joints is described, and the possibility of a leader role for the T-C joint under the control of the central command system is discussed.

Materials and methods

This study was based on the accurate kinematic analysis of 371 strides recorded from freely walking crayfish *Procambarus clarkii* (Girard) obtained from a commercial supplier. The strides were taken from the paths travelled by four individuals and were typical of the types of movement observed in the crayfish: two were small crayfish, CR1 (total length 9 cm; mass 28 g) and CR2 (10.8 cm; 40 g), and two were larger, CR3 (11.7 cm; 63 g) and CR4 (11.5 cm; 61 g). CR4 used a different type of locomotor pattern to the other three individuals, therefore providing indications about the variability of the locomotor pattern and the effect of this on inter-joint coordination.
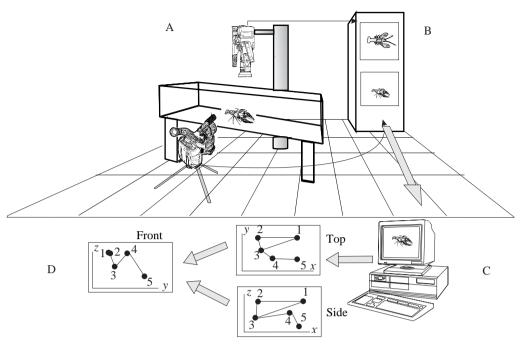
Experimental set-up

During experiments, the crayfish were placed individually into an aquarium 80 cm×20 cm×20 cm (length × width × height). The ground and the sides were covered with a sheet of black rubber. The front was left uncovered for filming. The rubber covers provided a good contrast for filming and prevented the crayfish from slipping on the tank floor. The tank was filled with 10 cm of water. Two fixed video cameras, filming at a rate of 50 frames s^{-1}, simultaneously recorded top and lateral views of a 30 cm wide field of the tank centre (Fig. 1A). The crayfish were trained to walk in a straight line along the tank floor and were filmed for approximately four strides (4.2±1.34 strides, mean ± S.D.) while they passed through the field of view. For each animal, recordings of straight walking without stopping and at a constant speed were used for video analysis. The linearity of the path was assessed by computing two indices: (1) the index of straightness (Batschelet, 1981), i.e. the ratio of the straight-line distance travelled to the actual path length; and (2) the mean amplitude of the successive changes of direction measured for every 10 mm. The values of these indexes were always, respectively, larger than 0.97 and lower than 0.006 rad (see Table 2), indicating movement in a straight line.

Kinematics analysis

Small white beads (diameter 2.5 mm; mass 20 mg) were used as markers: two beads were glued onto the crayfish body (on the rostrum and the back of the thorax) and defined the body axis (x-axis, see Fig. 2); three half-beads were glued to the left leg 4, on the end part of the propodite, the end part of the meropodite and on the basipodite (see Fig. 2). Successive video frames were grabbed into a computer and the coordinates of the markers on each frame were digitized using a commercial video analysis system (Ariel Performance Analysis Systems, Ariel Dynamics, Inc.). The digitized marker position allowed reconstruction of the positions of the body and leg using stick diagrams (see Fig. 1D).

The precision of the digitising technique was assessed using two criteria outlined by Walton (1986). The degree of agreement among repeated observations was quantified using the mean standard deviation of the computed lengths of the various segments defined by marker pairs. The system accuracy was checked by comparing the length of these segments computed from the video analysis with the actual values measured on the animal; it was expressed as the root mean square error (RMSE) between the calculated and

Fig. 1. Apparatus used for recording and analysis of free-walking crayfish. (A) The crayfish were filmed using two cameras oriented at 90° to each other. The recorded image was calibrated by filming a cube frame with marked control points. (B) Film quality was controlled using video monitors. (C) The video sequences were grabbed onto a computer and stored as binary files. The positions of five known markers points were then digitised in an *x, y, z* coordinate system for each synchronised top and side view. (D) A transformation module allowed computation of the positions of the marker points in the third unfilmed view (*y, z* coordinates). The numbered dots represent marker points on the crayfish: (1) head, (2) back of cephalothorax, and (3) proximal, (4) middle and (5) distal parts of leg 4.

measured lengths. This RMSE was less than 0.3 mm for the body and distal leg segments, and 0.58 mm for the proximal segment (Table 1) for which measurements were less accurate because the marker on basipodite was often partly hidden by the leg and was therefore more difficult to locate accurately. The precision of this technique nevertheless was very good, corresponding to a maximum error of 3.2 % (Table 1).

Movement variables

The global movement of the crayfish was described by the movement speed, duty factor and stride amplitude. The movement speed was defined as the distance travelled by the markers on the back, divided by the duration of the travel. The duty factor was the portion of total step cycle period during which the leg was in contact with the ground. The stride amplitude was the angle delimited by the line between the dactyl and the marker on the back (i.e. between markers 2 and 5, see Fig. 1D) during the stride, i.e. between the PEP and AEP.

Measurement of joint angles

The movement of the leg was defined by three axes of rotation termed traction, lift and stretch (see Fig. 2).

Traction is defined as the horizontal antero-posterior movement of the proximal segment (markers 3–4) with reference to the body axis. It was defined as the angle φ (Fig. 2) delimited by the projection of the proximal segment (ischiopodite plus meropodite) and the body axis in the *x,y* plane. Traction movement is related directly to the activity of the thoraco-coxopodite joint (T-C) (Fig. 2). During the swing phase, the proximal segment moved forwards (protraction) to the AEP; during the stance phase, it moved backwards to the PEP (retraction).

Lift describes the up-and-down movement of the proximal segment. This angle, β (Fig. 2), was computed as the arcosine of the projected length of the proximal segment (*p*) in the *x,y* plane divided by its true length (*l*) [$\beta = \arccos(p/l)$]. This angular movement is related to movement of the coxo-basipodite (C-B) joint (Fig. 2) and is described as either a levation or a depression.
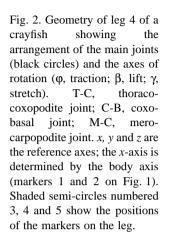
Table 1. *Analysis of the precision of the video analysis system*

Segment	Measured length (mm)	Calculated mean length (mm)	Coefficient of variation	RMSE (mm)	Error (%)
Distal leg (markers 4–5)	17.8	17.883±0.1953	1.095	0.275	1.55
Proximal leg (markers 3–4)	18.0	17.288±0.3116	1.808	0.584	3.24
Body axis (markers 1–2)	27.8	27.884±0.2118	0.76	0.2115	0.76

N=750 frames from three recorded walking sequences by crayfish CR1.

See Figs 1 and 2 for positions of markers.

Coefficient of variation is the standard deviation expressed as a percentage of the mean calculated segment length; RMSE, root mean square error between measured and calculated segment length; error is the RMSE expressed as a percentage of the measured length.

Values for calculated length are means ± S.D. (*N*=750).

Fig. 2. Geometry of leg 4 of a crayfish showing the arrangement of the main joints (black circles) and the axes of rotation (φ, traction; β, lift; γ, stretch). T-C, thoraco-coxopodite joint; C-B, coxo-basal joint; M-C, mero-carpopodite joint. *x, y* and *z* are the reference axes; the *x*-axis is determined by the body axis (markers 1 and 2 on Fig. 1). Shaded semi-circles numbered 3, 4 and 5 show the positions of the markers on the leg.

Stretch of the leg was defined as the angle γ (Fig. 2) delimited by the proximal (markers 3–4) and distal (carpopodite plus propodite) (markers 4–5) segments of the leg and corresponded to movement of the mero-carpopodite (M-C) joint, with the leg being either in extension or in flexion. This angle was defined in the plane containing the two segments and was calculated using the three-dimensional analysis routines of the video-based motion-analysis system.

The joints linking the ischiopodite and meropodite on the proximal segment and the carpopodite and propodite on the distal segment are kept stiff (Clarac, 1982) during underwater locomotion and, as they showed negligible movement during walking, they were not considered in this study.

Description of the joint movements was carried out separately for the return stroke and the power stroke of the step cycle. The leg was considered to be in the power stroke of a step cycle for as long as the change in dactyl position between two successive frames was within the precision threshold of the system (0.3 mm).

Statistical analysis of inter-joint coordination

The inter-joint coordination of leg 4 was analysed using a general method based on cross-correlation functions first applied to the study of human movement (Amblard *et al.* 1994). The kinematic variables used were the three main components of the leg movement (traction, lift and stretch), which describe the movements of the T-C, C-B and M-C joints respectively. For each walking sequence and for each pair of joints, the correlation functions estimated the correlation between one joint angular series at time *t* and the other joint angular series at time *t+k* as a function of the lag *k*. The functions were computed over a range of *k* values between −1 s and 1 s with increments of 20 ms. Significance levels of correlation peaks were tested using the Z-transformation of the correlation coefficients by testing 50 equally spaced lags. To

avoid the emergence of false positive results due to multiple *t*-tests, significance thresholds were adjusted on the basis of the number of lags (Amblard *et al.* 1994). Thus, significance level was considered at $P=0.00002$ instead of $P=0.001$, and $P=0.001$ instead of $P=0.05$.

The possible existence of a coherent kinematic strategy implying the related movements of three joints was verified by drawing the latency plane of movement (Fig. 3). In this graphical representation, the time lags between the movements produced by the three pairs of joints are drawn as straight lines on a two-axis diagram: vertical lines cut the abscissa at a distance *d1* corresponding to the time lags between the traction–lift correlation peaks. Horizontal lines cut the ordinate axis at distance *d2* corresponding to the time lag between the lift–stretch peaks. Finally, oblique lines cut the *x* and *y* axes at distance *d3* corresponding to the time lags between the traction–stretch peaks. Theoretically, a coherent combination of movements should satisfy the requirements of both temporal consistency and sign compatibility: temporal consistency implies that the three horizontal, vertical and oblique lines intersect at a single point (owing to the system background noise, lines actually intersect in a small area the size of which depends on the accuracy of the time-lag estimates and inter-individual variability; Amblard *et al.* 1994); sign compatibility implies that the crossing lines correspond either to three positive correlation peaks or to one positive and two negative peaks. If such requirements are satisfied, a three-level movement strategy can be assumed to exist. Furthermore, the positions of the vertices in the various sectors of the latency plane provide information on the inter-joint temporal sequence and thus on the type of strategy used (Lekhel, 1994) (see Fig. 3). Note that 'motor strategy' here refers to the existence of coordinated movements between the various segments of the leg (Amblard *et al.* 1994) incorporating their biomechanical constraints, but does not assume neuronal synergy.

Results

Main features of the movement pattern

The crayfish moved freely in the tank and were able to adjust their locomotor parameters without constraints. General parameters of their movement, such as walking speed, stride amplitude and duty factor, could modify inter-joint coordination at the leg level, and were therefore measured (Table 2). The mean walking speed varied from 5.6 to 8.7 cm s^{-1} between individuals, while the speeds recorded in the various bouts ranged between 4.5 and 13.5 cm s^{-1}, varying
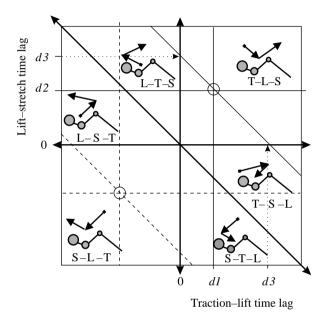

Fig. 3. Example of a three-level representation of inter-joint coordination using a latency plane. The long lines with arrowheads represent the reference axes of the latency plane. They divide the diagram into six sectors corresponding to various levels of inter-joint synergy. In each sector, a schematic leg represents the movements as circles of various sizes (large, traction, T; medium, lift, L; small, stretch, S) and small arrows show the corresponding time sequence of the joint movements. Ascending strategies are in the upper right of the diagram; descending strategies are in the lower left of the diagram; mixed strategies are shown in the remaining parts (adapted from Lekhel, 1994). The time lags between the movements produced by the three pairs of joints are represented by lines intersecting the axes of the latency plane. Continuous lines correspond to positive correlation peaks and broken lines to negative correlation peaks. The *x*-axis here represents the time lag between traction and lift: the solid vertical line cuts the *x*-axis at a distance *d1* where a significant correlation peak exists between traction and lift; the *y*-axis represents the time lag between lift and stretch: the solid horizontal line cuts the *y*-axis at distance *d2* corresponding to a significant correlation peak for this delay. The oblique lines cut both axes at a distance *d3* corresponding to the delay for which a significant correlation peak exists between traction and stretch. Intersections between the horizontal, vertical and oblique lines correspond to compatible inter-joint synergy; they are indicated by circles. In this diagram, the circle in the upper right sector represents a coherent combination (three positive correlations), while the circle in the lower left sector represents an incoherent combination (three negative correlations). This example does not correspond to actual recorded data.

both between individuals and between various sequences for the same individual. The duty factor D (% period) (and therefore the step period) was inversely related to speed V (in cm s^{-1}) ($D=-0.04V+0.76$; $r=0.75$; $P<0.0001$). This confirmed that an increase in speed is first achieved by a reduction in stance duration, as was shown previously for rock lobsters walking on a treadmill (Chasserat and Clarac, 1986). Stride amplitude was linearly related to speed, although there was individual variation. The regression lines of amplitude A (in degrees) *versus* speed V (in cm s^{-1}) for the four individuals were: CR1, $A=3.07V+20.5$ ($r=0.58$; $P=0.007$); CR2, $A=1.4V+32$ ($r=0.57$; $P=0.043$); CR3, $A=0.85V+35$ ($r=0.20$; $P=0.45$, not significant); and CR4, $A=3V+33$ ($r=0.57$; $P=0.0265$). CR1 walked at a mean ($\pm$ S.E.M.) speed of 5.61 ± 0.18 cm s^{-1}, using small strides and spending over half of the step period in the stance phase. CR2 and CR3 moved faster with larger strides and spent only approximately 40 % of the step period in the stance phase (Table 2).

The pattern of movement was very different in CR4: this crayfish had the largest stride amplitude, but moved relatively slowly owing to a long step period, more than half of which was spent in the stance phase. This crayfish also differed from the other three in the PEP distance (defined as the projection of the extreme backward position of the dactyl onto the body axis with reference to the position of the body–leg insertion point). The increased stride amplitude in this individual resulted from an increase in both the AEP and PEP distances, as seen in Fig. 4. The slopes of the regression lines for PEP distance *versus* stride amplitude were similar for all the crayfish (see Fig. 4, common regression slope=−0.34). As a consequence, the PEP was on average in a forward position relative to the insertion point of the leg in CR1 (owing to its small stride amplitude), and it was at the same level as or just behind the leg insertion point in CR2 and CR3, which had longer strides. In CR4, the regression line had the same slope as for the other individuals, but a lower intercept: in this crayfish, therefore, the leg always moved farther backwards, irrespective of the stride amplitude, and the PEP was on average of 16.6 mm behind the body–leg insertion point. This strong backward extension of the stance greatly affected the movement patterns of the various joints.

In a previous experiment, Jamon and Clarac (1995) showed that freely moving crayfish walk using two different patterns of contralateral coupling: in one, legs 4 moved in phase, while in the other they alternated. The phase relationships between contralateral legs 4 were therefore tested in this study using the Rayleigh test for circular distribution (Batschelet, 1981) to control for a possible effect on inter-joint coordination. The distribution of left leg 4 in right leg 4 phase was significantly alternate in CR1 and CR3 (Table 2) while it was bimodal in CR2 (after doubling the angles, the length of the mean vector $\mathbf{r}^2=0.204$, $N=63$, $P=0.05$). Bimodality occurred in CR2 because legs 4 consistently moved in phase in six out of the 15 sequences considered (these 'in-phase' sequences had a mean phase relationship greater than 0.9 or less than 0.1 and contained 80 % of the steps whose phase value was greater than

Table 2. *General characteristics of recorded sequences of crayfish walking used for the analysis*

Animal (number of bouts)	Speed (cm s⁻¹)	Duty factor (% of period)	Period duration (ms)	Stride amplitude (degrees)	Mean change of direction (rad)	Path straightness index	Phase relationship	Phase vector length (number of steps)
CR1 (30)	5.61±0.18	0.525±0.009	788±12.7	37.4±0.99	0.005±0.0058	0.98±0.0024	0.667	0.28* (74)
CR2 (15)	8.72±0.52	0.412±0.016	737±21.9	45.66±1.62	0.006±0.0067	0.985±0.0023	0.612	0.116 (63)
CR3 (16)	7.49±0.37	0.442±0.022	885±30.7	40.36±1.160	0.006±0.007	0.982±0.002	0.386	0.403* (66)
CR4 (16)	6.92±0.33	0.558±0.014	1035±26.05	52.05±1.64	0.003±0.012	0.969±0.0028	0.93	0.205 (48)

Values are means ± S.E.M.

Phase coupling is represented by means of polar coordinates: phase relationship is the mean phase value; the phase vector length shows the concentration parameter of the phase value.

Significance level was tested using the Rayleigh test.

*Significant level at $P<0.05$.

0.8 or less than 0.2; the nine 'alternate' sequences had a mean phase between 0.4 and 0.6, with 87 % of the steps having a phase value between 0.3 and 0.7). Phase distribution was not significant in CR4, but detailed investigation of these sequences showed eight bouts to be in phase, while in three bouts legs 4 alternated (in-phase sequences had a mean phase
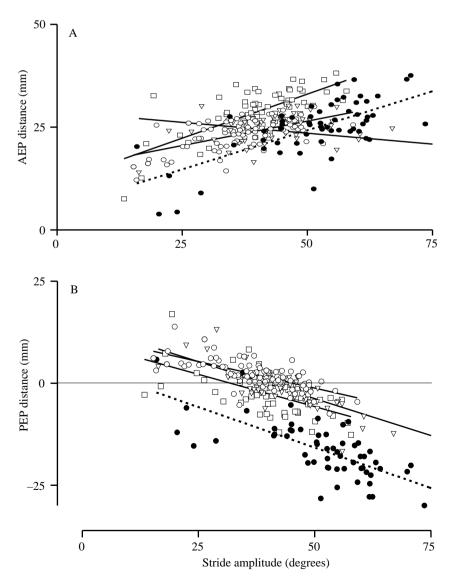

Fig. 4. Distribution of the anterior extreme position (AEP; A) and posterior extreme position (PEP; B) distances in relation to stride amplitude. The distances of the extreme positions of the leg are calculated from the projections of the dactyl position onto the body axis with reference to the position of the body–leg insertion point (i.e. negative *y* values occur when the PEP is in a backward position with reference to the leg insertion point). Open circles, CR1; open triangles, CR2; open squares, CR3; filled circles, CR4. Regression lines are shown as continuous lines for CR1, CR2 and CR3 and as broken lines for CR4. Regression equations for PEP are: CR1, $y=-0.27x+11.8$ ($r=-0.69$, $P<0.0001$); CR2, $y=-0.36x+14.4$ ($r=-0.84$, $P<0.0001$); CR3, $y=-0.32x+10.1$ ($r=-0.59$, $P<0.0001$); CR4, $y=-0.39x+4.07$ ($r=-0.72$, $P<0.0001$). Regression equations for AEP are: CR1, $y=0.23x+14.6$ ($r=0.61$, $P<0.0001$); CR2, $y=-0.11x+28.8$ ($r=-0.31$, $P<0.0137$); CR3, $y=0.43x+11.6$ ($r=0.72$, $P<0.0001$); CR4, $y=0.38x+5.25$ ($r=0.69$, $P<0.0001$).
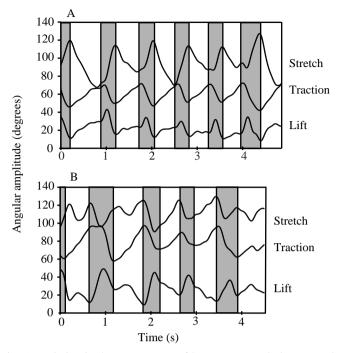
Fig. 5. Variation in the components of leg movement during general step cycles. (A) Typical movement pattern for a small crayfish (CR1). (B) Typical movement pattern for a large crayfish (CR4). Shaded areas delimit the return stroke; unshaded areas delimit the power stroke. The three components of leg movement (stretch, traction and lift) are defined in Fig. 2.

value greater than 0.8 or less than 0.2, and 77 % of their steps had a phase value in this range). The predominance of the in-phase pattern therefore distinguished CR4 from the other individuals. Since legs 4 of CR1 and CR3 moved mainly alternately, those of CR4 moved mainly in phase and those of CR2 moved either in phase or alternately, it was possible to test for a relationship between contralateral coordination and movement speed or related movement parameters. No significant relationships were found between speed or other general movement characteristics and contralateral coordination. Therefore, we could not determine whether the different joint movement characteristics observed in CR4 were directly related to the maintenance of an in-phase pattern.

Inter-joint movement during the step cycle

All three joints contributed to the movement of the whole leg during the step cycle (Table 3). There were inter-individual differences in amplitude, but the crayfish all showed the same tendencies, in spite of the large traction and lift amplitudes for CR4. When the four animals were considered together; the amplitude of the traction movement of the proximal segment averaged 24.2°. This value is approximately half that of the stride amplitude measured at the dactyl (see Table 2). The difference between these values must be accounted for by amplification of the movement by the distal segment. As the joints are monoaxial, such distal amplification of the leg

movement can only be achieved by rotating the proximal segment around its longitudinal axis. However, as no socket joint exists at the base of the proximal segment, this rotation supposedly results both from a small inclination in the axis of rotation of the T-C joint with respect to the horizontal plane and from the loose attachments of the joints. Lift amplitude was similar to that of traction, averaging 25.6°, while stretch movement had the largest amplitude, with an average of 34.5°.

Fig. 5 shows examples of the two leg movement patterns recorded in the present study. Fig. 5A shows a sequence recorded from CR1; this pattern was also found for CR2 and CR3. Each joint shows a stable oscillatory pattern associated with the step cycle: During the return stroke, there is synchronised protraction and extension, together with levation followed by depression. The power stroke is achieved by retraction and flexion, while the lift remains approximately constant (no levation or depression).

Fig. 5B shows a different leg movement pattern, which was characteristic of CR4. In addition to the variability of the pattern between different step cycles (which was also observed frequently in the other crayfish), two main differences were found in the stretch and lift movements relative to the pattern shown in Fig. 5A. First, the leg mainly flexed during the return stroke and extended during the power stroke, sometimes showing a biphasic pattern of flexion/extension when strides were of large amplitude. Second, the lift movement amplitude oscillated to a greater extent and its value was relatively low at the end of the power stroke. Therefore, the lift angle was minimal at the beginning of the return stroke, and levation took a larger proportion of the return stroke.

The precise timing of the leg movements relative to the step cycle is shown in Fig. 6. The return stroke (swing phase) and power stroke (stance phase) were treated separately. To facilitate comparison, despite the presence of inter-individual variability in speed and angular amplitude, each stroke was normalised relative to time and to amplitude.

The stance phase

During the power stroke, the proximal segment showed a remarkably linear retraction: half of the traction amplitude was reached at half of the stance phase (Fig. 6A). This was the most regular component of the movement both between and within individuals, and it did not seem to be influenced by the movement of the other joints. While the proximal segment retracted, the leg flexed at the M-C joint, as shown by the

Table 3. *Mean amplitude of angular joint movement*

	CR1 (N=185)	CR2 (N=62)	CR3 (N=64)	CR4 (N=60)
Traction	20.7±0.4	25.2±1.05	25.3±0.97	32.75±1.14
Lift	22.43±0.48	28.9±0.97	25.08±0.91	34.23±1.11
Stretch	38.53±0.96	31.06±0.32	32.34±1.41	34.87±1.35

See Fig. 2 for definition of joint movements.
Values are in degrees and are given as means ± S.E.M.

reduction in leg stretch (Fig. 6B). Forward movement of the body was therefore achieved by a two-level movement involving both retraction of the T-C joint and flexion of the M-C joint. Differences in this pattern occurred between small and large individuals: in the former, the leg progressively flexed during the whole stance phase (CR2) or extended only slightly at the end of the stance phase (CR1), whereas in the larger individuals the leg extended strongly at the end of the stance phase (CR4). In CR4, this extension began at 60% of the stance phase and the leg was on average slightly more extended at the end of stance than at the beginning. This final extension was related to the retraction of the leg much farther backwards (see Fig. 4). The variation in lift amplitude during the stance phase (Fig. 5) was symmetrical with that seen for the stretch movement, resulting in the leg levating while flexed and being depressed while extended (Fig. 6C). The levator muscles are known to be inactive during the power stroke, whereas the depressors are active. We can therefore assume that this apparent upward movement of the proximal segment during the stance phase resulted mechanically from the leg flexion.

The swing phase

During the return stroke, all the crayfish showed the same pattern of protraction (Fig. 6D): traction amplitude was a sigmoidal curve centred on 0.5, with a plateau occurring during the first and the final part of the swing. This pattern was found consistently in all the crayfish. The leg did not protract immediately and sometimes continued to retract at the beginning of the swing phase. This delay before protraction of the leg could result from the inertia of the pushing movement
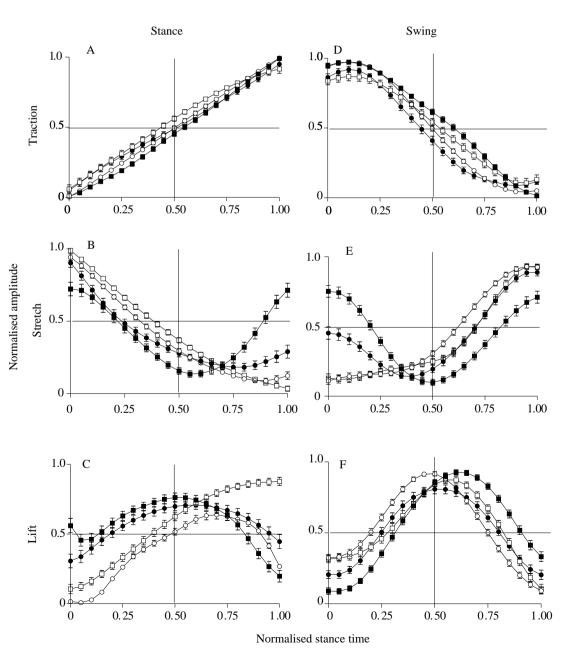

Fig. 6. Mean variation in movement amplitude at the three joints (traction, stretch and lift, see Fig. 2) during the power stroke (A,B,C) and return stroke (D,E,F). Swing and stance phases are treated separately. Amplitude values are normalised first relative to time, taking the beginning of the stroke as 0 and the end of the stroke as 1, and then relative to the angular amplitude, taking the smallest measured amplitude as 0 and the largest as 1. Each point represents the mean (±S.E.M.) normalised amplitude at a point in the step cycle (CR1, N=183; CR2, N=67; CR3, N=65; CR4, N=56). Open circles, CR1; open squares, CR2; filled circles, CR3; filled squares, CR4.
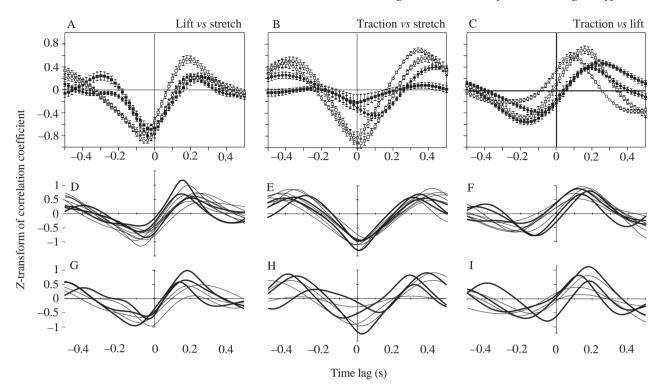
Fig. 7. Cross-correlation functions between angular leg movements. The leg movements (lift, stretch and traction) are defined in Fig. 2. (A,B,C) Averaged cross-correlation functions between the different movement combinations. Values are means ± S.E.M. of the Z-transform of the correlation coefficients (CR1, $N=30$; CR2, $N=17$; CR3, $N=16$; CR4, $N=16$). Time lags are shown on the abscissa. Open squares, CR1; open circles, CR2; filled circles, CR3; filled squares, CR4. (D–I) Overlapping of cross-correlation functions for the individual walking bouts by CR2. (D,E,F) Sequences travelled in which legs 4 moved alternately. (G,H,I) Sequences travelled in which legs 4 were in phase. Heavy lines show sequences in which walking speeds exceeded $9\,\mathrm{cm\,s^{-1}}$.
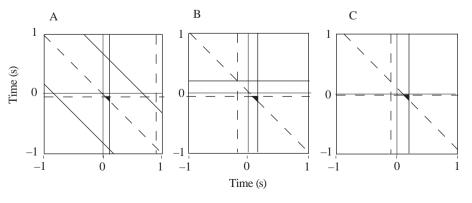
of the preceding power stroke or could be associated with the forward rotation of the leg. The leg ceased to protract before landing and, in most cases, had already begun retraction before the end of the swing phase. This premature end of protraction could be interpreted as a preparation for the pushing movement of the leg during the following power stroke.

Extension of the leg occurred only when almost 50 % of the return stroke was completed (Fig. 6E), and consistently ended before the end of the return stroke, as did protraction. During the first half of the swing, either the extension movement was

small (only 10 % of the extension was achieved in small individuals) or the leg was flexed strongly (e.g. the large crayfish). This size-related difference resulted from the pattern during the previous stance phase: when the leg was in an extended position at the end of the power stroke, it bent before extending again. This did not occur in the smaller crayfish in which the leg did not become extended at the end of the stance phase and thus began the return stroke in a flexed position.

The levation of the proximal segment varied regularly with a typical sine-wave-shaped pattern (Fig. 6F). In all animals,

Fig. 8. Latency planes of conjugate cross-correlation functions (CCF) for individual crayfish. (A) CR1; (B) CR2; (C) CR3. Vertical lines represent time lags of significant CCF peaks between traction and lift; horizontal lines represent time lags of significant CCF peaks between lift and stretch; oblique lines represent time lags of significant CCF peaks between traction and stretch. Continuous lines represent positive correlations and broken lines represent negative correlations. Coordinated movements between the three joints level are shown by the dark triangular areas. Note that they are all clustered in a sector corresponding to a stable sequence of traction–stretch–lift movements.

levation increased only slowly at the onset of the swing phase in a similar manner to the small change in protraction (Fig. 6D). The maximum of levation was reached at mid swing phase in CR1 and occurred slightly later in the other crayfish. Depression of the leg began shortly after it began to extend (Fig. 6E), and the lift angle decreased at a constant rate during the second part of the swing phase, until landing. The major size-related difference observed was that levation was minimal at the beginning of the swing phase in large animals, while it was minimal at the end of the swing phase in small animals. This difference was particularly clear in CR4, but was also found in CR3; it resulted from the fact that the smaller extension during the power stroke (Fig. 6E) caused a reduction in the lift position in the large animals.

The mean leg movement patterns observed were relatively stable in spite of inter- as well as intra-individual variability in the locomotor pattern. Most of the inter-individual differences were associated with the variable contribution of leg extension to the movement and were clearly size-related. The very different leg extension pattern of CR4 (Fig. 6B,E) was obviously related to the extensive backward movement; the same tendency (although less pronounced) was also observed in CR3. It is possible that this pattern could therefore be due to different postural requirements in heavier animals.

Inter-segmental coordination during the step cycle

The mean leg movement patterns showed a stable oscillatory pattern during the step cycle (Figs 5, 6) and appeared well synchronised at some stages of the step cycle. We measured the level of temporal synergy between them using a statistical method based on cross-correlation functions (Fig. 7). The timing of movement was compared between each pair of joints, and each showed a significant correlation peak with a time lag near 0. These correlation peaks were often bordered by two other peaks with opposite sign and a phase shift of approximately 0.4 s, representing nearly half a step period. These supernumerary inverse peaks resulted from the periodicity of the movement (i.e. two consecutive events appear to be synchronised in the opposite order, with half a period of lag) and were often of lower amplitude owing to the cumulative variability along the step cycle. Therefore, only peaks within lags ranging between −0.4 s and +0.4 s (i.e. less than half a period) were considered. Each pair of joints showed only one significant peak of correlation during this period, indicating a unimodal coordination. The pattern of joint coordination was found to be similar for all the crayfish, although some size-related differences were found between small (CR1, CR2) and large (CR3, CR4) individuals. The significant correlation peaks for each pair of leg joints are described below.

Lift and stretch movements always showed the best correlation (Fig. 7A): levation followed extension in small crayfish after a delay of only 60 ms and this delay was extremely short in the large crayfish (approximately 20 ms). These movements were in opposite directions, as is shown by the negative sign of the correlation peak, i.e. levation of the

proximal segment was associated with leg flexion and depression was associated with leg extension.

Traction and stretch were also almost exactly synchronised in the two small crayfish (CR1, CR2), as is shown by the position of the correlation peaks (−20 and +20 ms), whereas traction consistently preceded stretch in CR3 as is shown by the 100 ms delay of the correlation peak (Fig. 7B). Since the correlation was negative, protraction was associated with leg extension and retraction with leg flexion. No significant correlation was found for CR4, probably because the variable and mainly biphasic variation in the movement of the M-C joint masked any statistical effect.

Traction and lift showed two correlation peaks (Fig. 7C). A negative correlation peak occurred at −200 ms and −180 ms in the two small crayfish and at −100 and −160 ms in the two large animals; a positive correlation peak appeared at 160 ms and 100 ms in the two small animals and at 200 and 260 ms in the two large animals. Since the delay between the two peaks was approximately 400 ms (half a step period), one of these peaks must have resulted from the periodicity of the movement. The correlation was better for the positive peak, suggesting that traction indeed occurred before lift. The combined analysis of the joint pairs in the latency plane (Fig. 8) clearly showed only one coherent joint synergy, associated with a positive lag between protraction and levation, and further confirmed this hypothesis.

The synchronisation patterns shown in Fig. 7A–C represent the mean cross-correlation functions for each individual and do not allow investigation of variations in synchronisation pattern related to changes in walking speed or contralateral coupling in the various sequences. This possibility was investigated for CR2, which walked with legs 4 clearly in phase in six sequences and alternating in eight sequences. In these individual CCF curves (Fig. 7D–I), no clear-cut change in the pattern of inter-joint synchronisation could be found between in-phase or alternating leg 4 stepping patterns. In addition, movement sequences for higher speeds (heavy lines in Fig. 7D–I) did not differ in any detectable way from those for lower speeds. We therefore concluded that the inter-joint coordination patterns described above were not modified by global locomotion parameters such as walking speed or contralateral coupling.

The cross-correlation functions revealed the presence of a stable temporal synchronisation between the three pairs of joints for CR1, CR2 and CR3. In CR4, traction and stretch were found not to be significantly correlated. It was therefore possible to look for global movement synergy in leg 4 of the three other crayfish only. This was carried out by drawing the time lags for the different leg pairs in the latency plane (Fig. 8). In Fig. 8, the vertical, horizontal and oblique lines correspond, respectively, to traction–lift, lift–stretch and traction–stretch time lags. In each animal, the three lines cross in a narrow area, indicating the existence of a coherent global synergy of joint movement. Moreover, the vertices for each animal fall in the same sector of the latency plane (see Fig. 3). This suggests that they used a common strategy (Lekhel, 1994) corresponding to

a stable sequence of traction–stretch–lift movements. A time difference was found in this sequence between the two smaller crayfish (CR1, CR2), which showed a close traction–stretch synchronisation, and the larger CR3 which had a close stretch–lift synchronisation. In addition, the two pairs of joints positioned furthest apart (T-C and M-C or C-B and M-C) appeared to be more closely synchronised than the two proximal joints (T-C and C-B). This result implied that, in addition to the commonly described sequence of activation of the promotor/levator followed by remotor/depressor muscles, there is participation by the extensor/flexor muscles whose activity occurs in narrow synergy with that of the other muscles. The movement of the crayfish leg should therefore be considered to be a three-level joint movement.

Discussion

The present study investigated the movement patterns in leg 4 of a crayfish walking freely along a straight path, using video analysis combined with a statistical approach based on cross-correlation functions. This allowed a detailed description of the movements performed by the main segments of the leg in the various spatial planes and allowed determination of inter-joint coordination in a natural behavioural situation. The use of a three-dimensional video motion-analysis system imposed technical constraints such as filming within a restricted area. However, there were no limitations on the movement characteristics of the crayfish. The recorded step periods and walking speeds were consistent with those recorded in a previous study (Jamon and Clarac, 1995). Although a smaller angular stride amplitude was measured in this study than in that of Jamon and Clarac (1995), this difference resulted from a change in the origin used for this measurement: the body centre was used here instead of the leg insertion point as in the previous study. The leg excursion (the distance between the PEP and the AEP) was within the same range in both studies.

This study showed that movement of the M-C joint contributed strongly to the overall leg movement. This is wholly consistent with previous results from EMG recordings (Barnes, 1977), which showed alternating activity in the flexors and extensors of leg 4 in another crayfish, *Astacus leptodactylus*, during walking on a treadmill. It is therefore likely that the observed movement of the M-C joint reflects active participation of this joint in the movement of the whole leg, unlike the situation in other crustaceans, such as the crab *Carcinus maenas* (Clarac *et al.* 1987) and the rock lobster *Palinurus vulgaris* (Ayers and Clarac, 1978), where the M-C joint is active mainly during sideways locomotion, in a similar manner to that in some insects (reviewed in Frantsevitch, 1995).

During underwater locomotion, propulsive and frictional forces are applied not only to the ground contact point but also to the whole water medium during strokes of the step cycle and with an intensity considerably higher than during terrestrial locomotion. Nevertheless, we consider that the power stroke is a dynamic event because the various joints have to produce the forces needed to propel the body forward, whereas the return

stroke corresponds to a kinematic event because the joints move the leg to the AEP and control the leg trajectory. Thus, movements of the T-C and M-C joints perform different functions in each phase of the step cycle. During the power stroke, movement of the M-C joint may act to counterbalance the circular movement of the T-C joint, thus allowing straight translation of the body. Frantsevitch (1995) hypothesised that such a control mechanism producing a straight trajectory may be responsible for the biphasic change in the leg flexion/extension pattern seen in insect middle limbs during walking. However, as proposed by Barnes (1977), this biphasic change in flexion/extension could also be caused by successive pulling and pushing actions of the M-C joint which increase the traction force produced by the T-C joint. The extensor muscles are known to be active during the entire return stroke (Barnes, 1977), but in the present study the leg was strongly extended only while the proximal segment was depressed. During the first half of the swing phase, the leg was only slightly extended or was even strongly flexed. This early swing flexion occurred when the crayfish had successively flexed and extended the leg during the power stroke, often producing a biphasic movement of the M-C joint. This pattern was found mainly in the largest crayfish (CR4), but was sometimes observed in the other crayfish during long strides (especially in the next largest individual, CR3), and possibly could be related to different postural requirements in these heavy animals. Barnes (1977) reported early swing flexion in walking sequences in which the leg accidentally slipped, and interpreted this pattern as a resistance reflex caused by the strong extension that occurred during the skid. In the present study, we observed this movement as a part of the normal inter-joint pattern: during the stance phase, the leg naturally bends while pulling the body forwards until the leg reaches a backward orientation, then it extends while pushing the body forwards. During the following swing phase, the leg either remains flexed during the levation or is actively bent, depending on the extended position reached at the end of the stance phase. It is known that this movement is synchronised locally in crustaceans by reflex actions (Vedel and Clarac, 1979; El Manira *et al.* 1991). We therefore suggest that the leg must be in a flexed position to be levated. The changes observed in the various components of the leg movement during the swing phase may have been related to the control of the dactyl trajectory, the function of the swing being, in this case, not only to propel the leg forward but also to achieve an AEP close to the PEP of the next anterior leg. A similar targeting function of the swing has been described in the stick insect *Carausius morosus* (Cruse, 1979), but has never been described in the crayfish. Alternatively, the flexed leg position could be a mechanism for reducing the drag forces generated when moving under water.

The use of free walking in the present study made movement parameters difficult to control and allowed the animals spontaneously to express a large variety of movement patterns. The use of the CCF statistical method nevertheless allowed some general principles to emerge from the behavioural

variability inherent to the natural conditions. This technique yields statistical estimates of the time intervals corresponding to the maximum degree of correlation between two coordinated movements; the time lag so obtained is thus an average estimate over the sampling period. Although it is robust, this technique makes the assumption that any relationships between the descriptors are linear. Moreover, a time lag may correspond to a repeated lag between correlated movements and/or to a constant lag over a significantly large part of the stride. The synchronisation measured by the CCF technique in this study showed that the joints were synchronised globally over the whole cycle, without the assumption of any causal relationships between the synchronised movements. For example, the CCF technique showed that lift movement followed traction with a time lag ranging between 100 and 260 ms in the various animals, but this does not preclude the existence of a possible additional transient synchronisation between protraction and levation at some stage of swing onset. Such short-term synchronised events would not have a long enough duration to produce a high correlation peak and could therefore be masked by the statistical background noise. Moreover, the nature of the coordination could change during the course of the movement. The synchronised flexion and depression movements, for example, were related to an active association during the return stroke, while during the power stroke they resulted from a change in the leg height in relation to the flexion/extension movement. In spite of the limitations of the method, it appeared that each pair of joints was coordinated in a basic inter-joint motor strategy which implied the sequence of traction–stretch–lift. This pattern was not modified by walking speed or contralateral leg coordination pattern. We suspect therefore that the inter-joint kinematic strategy is driven at the level of the leg independently of the influences of higher-level commands.

Previous studies of arthropod locomotion have focused on inter-leg coordination and have considered the whole leg as a relaxation oscillator (Bässler, 1983) performing alternating cycles of protraction/levation and retraction/depression associations. Assuming that the leg works as a unitary oscillator, control of the leg movement must be carried out locally by means of elementary processes. Proprioceptive information regarding the positions of the various joints would then be important in the control of the leg movement. The overall movement pattern of the leg is achieved by a synchronised movement of the three joints with regard to biomechanical constraints. Further studies should therefore integrate flexion/extension movements into the leg movement.

The assumption that the inter-joint movement of the leg is controlled by local interactions based on proprioceptive reflexes raises the question of which joint is driven by the central nervous system in order to trigger the whole movement. This problem cannot be solved only by kinematic studies of the leg; additional information is needed on the activity of the leg muscles in relation to the movements of the body and the other legs. Nevertheless, the stable linear retraction pattern observed suggests that this component was not influenced by

the others but rather that other joints had to adapt to it. We therefore suggest that overall control of the system is *via* the protraction/retraction movement, which positions the leg during the swing phase and moves the body during the stance phase, while the other joints adjust locally by reflex interactions. This means that inter-leg coordination is controlled *via* the T-C joint. This hypothesis differs from results from *in vitro* experiments which identified the C-B joint as having an important role in triggering the swing and stance phases, and it will therefore be investigated further by studying the relative joint movements in two neighbouring legs, in conjunction with EMG recordings from their promotor and remotor muscles.

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