The Journal of Experimental Biology **198**, 683–700 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

LOCOMOTOR PATTERNS IN FREELY MOVING CRAYFISH (*PROCAMBARUS CLARKII*)

MARC JAMON AND FRANÇOIS CLARAC

Laboratoire de Neurobiologie et Mouvements, CNRS, 31 chemin Joseph Aiguier, 13402 Marseille Cedex, France

Accepted 19 October 1994

Summary

Freely walking crayfish, *Procambarus clarkii*, were studied using a video analysis procedure adapted especially for use with crayfish. The animals were placed in a tank and their homing behaviour was filmed as they returned in a straight line to their shelter. Various sequences were studied at the two following levels. First, the trajectory of each pair of legs (from leg 2 to leg 5) during the step cycle (power stroke and return stroke) was studied to measure stride length and to analyse in detail changes in acceleration. Each leg was found to contribute in a specific manner to locomotion. Second, ipsi- and contralateral leg coordination was investigated. Ipsilateral coordination was found to involve a metachronal organization from front to back in all the walking sequences recorded, whereas contralateral coordination involved, in addition to the weak alternate coupling commonly observed in treadmill walking, another coordination pattern where the legs on each side (legs 3 and 4) are in phase. The results obtained in these free-walking sequences are discussed and compared with those obtained previously, in particular in treadmill situations.

Key words: locomotion, leg coordination, crayfish, *Procambarus clarkii*.

Introduction

The locomotion of animals has been extensively studied for more than a century, particularly in arthropods. Since the studies by Bethe (1897) on crabs and von Holst (1935) on centipedes, in which the various types of inter-leg coordination were described after progressive autotomies had been performed, a large amount of information has been collected showing how insects, spiders and crustaceans walk (see Herreid and Fourtner, 1981). These data have depended closely, however, on the degree of sophistication of the technical apparatus used. Frame-by-frame cine analysis has been one of the most widely used techniques to study the alternating patterns of movement which occur between the three or four pairs of legs. These patterns have been defined in terms of their timing, particularly in terrestrial animals, as consisting of a mostly constant return stroke and a variable power stroke that depends on the duration of the period (Wilson, 1966).

In order to obtain more detailed information, studies have been carried out on restrained animals where walking was limited to a restricted area or even adapted to a treadmill situation. Situations of this kind made it possible to study in detail the various walking variables involved. Electromyogram (EMG) recordings have shown the exact temporal patterns of various leg muscles (Runion and Usherwood, 1966; Barnes *et al.* 1972; Clarac, 1981; Clarac and Chasserat, 1986; Delcomyn, 1971). The force exerted has also been studied to determine the proportion of the propulsive force exerted during the power stroke (Cruse and Saxler, 1980; Clarac and Cruse, 1982). Obstacles or induced perturbations have also been used to investigate some reflex reactions (Evoy and Fourtner, 1973; Barnes, 1977). Recordings have also been made from selected sensory afferents (Libersat *et al.* 1987*b*; Klärner and Barnes, 1986; Müller and Clarac, 1990*a*,*b*), in order to determine when they are triggered during the step cycle. The latter studies showed the importance of the role of the multiple sensory afferents involved in walking and also allowed the determination of their effects on the rhythmic motor output (Libersat *et al.* 1987*a*).

Locomotion studies on crustaceans have focused mainly on the direction of walking, since these animals can walk forwards, backwards and sideways, whatever the general organization of the animal (Burrows and Hoyle, 1973; Ayers and Davis, 1977). Studies on coordination between the legs have demonstrated that, as in insects, the ipsilateral connections are much stronger that the contralateral ones (Müller and Cruse, 1991*a*); however, a rock lobster on a double treadmill is able to maintain a given 1:1 coordination even though the two parts of the belt are moved at different speeds (Clarac, 1984).

Within the step cycle it has emerged that two points are crucial for maintaining coordination. The end of the return stroke, when the leg reaches the ground in forward walking

and starts the power stroke, has been denoted the anterior extreme point (AEP), and the end of the power stroke, just when the leg lifts from the ground, has been called the posterior extreme point (PEP; Cruse, 1979). These are the key points for maintaining the coordination between one leg and another and models have been developed on this basis (Chasserat and Clarac, 1986; Müller and Cruse, 1991*b*).

In all of these studies, fixed or tethered animals have been used, and locomotion has often been only a reaction to a stimulus. Sophisticated techniques have been developed recently with which the kinematics of free locomotion can be studied very closely, but they have so far been rarely used (Full *et al.* 1991). One of the main problems encountered, however, is that of inducing a given animal to perform a series of sequences that can be filmed and analysed in detail.

In the present paper, we describe a kinematic study of free-walking sequences from crayfish. We focused our study on a given behaviour and used a sophisticated commercial software program for frame analysis, adapted for use with crayfish (see Materials and methods).

A shelter was placed in a tank to induce homing behaviour. After several days of adaptation, when the animal was removed from the shelter it returned to it in a straight line. We were then able to film several sequences of active motivated movement on a straight course.

The results of this study show that each of the four pairs of legs seems to have a particular trajectory, that leg 4 is the most strongly involved in the propulsion of the animal and that it is probably the main functional leg. Second, it emerged that the inter-leg pattern commonly observed on a treadmill is not the only pattern that occurs under water during straight-line movements. In this particular situation, legs 4 often operate in phase. Since both in-phase and out-of-phase patterns can be present in the same sequence, the coordination can be said to involve adaptive processes.

Materials and methods

General procedure

The experimental procedure consisted of filming the return paths of unrestrained crayfish, *Procambarus clarkii* Girard, while they were engaged in homing behaviour. The crayfish were placed in a water-filled tank (1.2 m in diameter) containing a shelter. Since they were not constrained in their movements, their locomotion could be studied while they were performing voluntary activities. In this free movement situation, the crayfish actively walked in a straight line to the shelter. This experimental situation, therefore, made it possible to study their leg coordination during free locomotion.

The movement patterns of 17 crayfish were filmed and analysed with respect to their walking speed and phase relationships. The mean length of each walking sequence was approximately 70 cm and contained, on average, 12 steps. 190 sequences were filmed, giving a total of 2264 steps. Of these sequences, 25 (representing a total of 200 steps) were selected on the basis of the length of the straight-line walk (between 9 and 12 steps) and subjected to a detailed video analysis. Ten of the sequences involved one small male crayfish (animal 1; 20 g); another small male crayfish (animal 2; 20 g) and two other large males (animals 3 and 4; 60 g) each featured in five sequences. The number of sequences was necessarily small because of the time constraints of the analysis used, but there was no doubt about the significance of the results obtained, given the accuracy of the measurements obtained and the stereotypy of the individual locomotor behaviour in the behavioural task used.

In addition, the crayfish were filmed from the side when they moved in an aquarium measuring 90 cm×10 cm×20 cm, by means of a fixed camera set in front of the longer side. The narrowness of the tank obliged the animal to walk in a straight line. In this way, the trajectory of the legs could be recorded in the vertical plane. The data obtained with this method were used to draw stick diagrams of the leg movement (see Fig. 3).

Experimental procedure

A strip of rough-textured black rubber 1 m long was placed at the bottom of the experimental tank (Fig. 1A). This black strip enhanced the contrast of the films and its rough texture prevented the animals' legs from slipping. At the end of the strip, a hollow piece of cinder block with an aperture 10 cm×5 cm provided an attractive shelter for the crayfish. A spatial framework was provided by two parallel lines, 10 cm apart, consisting of markers pushed into the ground every 10 cm.

Each crayfish was trained daily to orient in the tank towards the shelter during the week prior to the experiments. Every time the crayfish reached the shelter, it was pulled back by the tail to the other end of the strip. At first, the crayfish escaped by flipping their tail or showed aggressive behaviour, but they soon became familiar with the procedures and quickly learned to return to the shelter in a straight line. This procedure could be repeated for more than 20 min without the crayfish showing any sign of fatigue.

Filming procedure

Throughout the experiments, the crayfish were filmed during their return journeys. The filming device (Fig. 1A) consisted of a wheeled table 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) was fixed. The camera was connected to the video display screen, the centre of which was located by means of two intersecting lines.

The crayfish were filmed (with a magnification factor of 1:1 or 2:1, depending on the size of the crayfish) from above during their homing walks, taking care that they always stayed in the centre of the reticulated control screen by moving the table. For this purpose, we aimed the camera at the central part of the cephalothorax as consistently as possible during each sequence. Deviations from the centre were unavoidable owing to the movement of the camera. The movement of each body part was given by its projected position onto the ground plane and was, therefore, subject to parallax errors depending on the

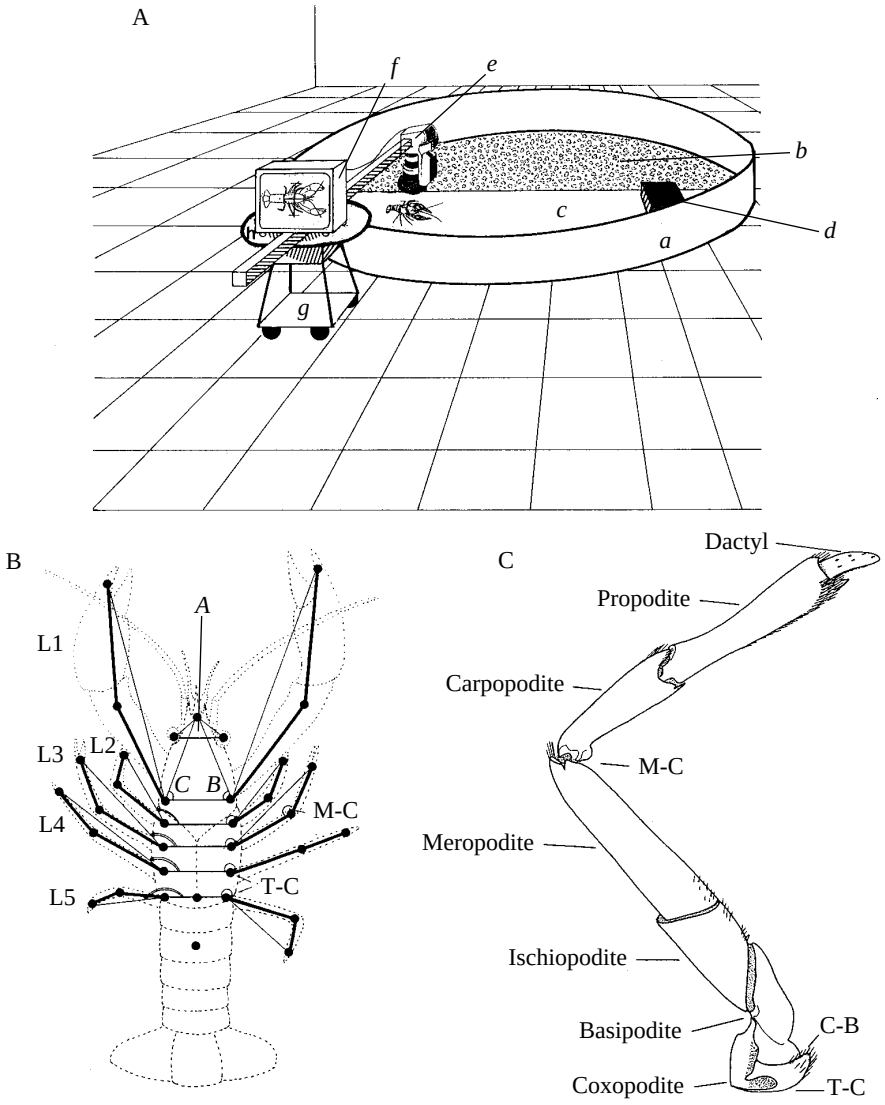

Fig. 1. (A) Experimental arrangement. *a*, swimming pool, 1.2 m in diameter, containing water to a depth of 20 cm; *b*, sandy bottom; *c*, rugged rubber strip; *d*, shelter; *e*, HI 8 mm video camera; *f*, monitor; *g*, rolling table; *h*, rotating plate. Illumination was provided by two 500 W halogen lamps (not shown), on each side of the pool. (B) Distribution of the marks on the crayfish. The marks are indicated by black dots. The lines between the dots are the body segments computed by the APAS. Each of the locomotor appendices L1–L5 is shown by two lines and by an overall leg segment joining the ends of the legs (fine line). All measurements on the leg movements were based on the movements of this overall segment. On the right of the figure, the apparent angles of the T-C and M-C joint are indicated, and on the left side the angles from which angular movements are calculated are shown. Angles *A*, *B* and *C* delimited by the rostrum and the two T-C points of the first pair of legs are used to control body pitching and rolling. (C) Morphology of leg 4 showing the segments and main joints involved in walking. C-B, coxo-basipodite joint; T-C, thoraco-coxopodite joint; M-C, mero-carpopodite joint.

projected position and on small deviations of the body from the screen centre. These errors were estimated by filming moving calibrated objects. This enabled us to estimate the accuracy of the measurements and ensured that the method was satisfactory. For instance, the measurements of a moving square form, 10 cm in size, were quite precise and consistent: the corner angles averaged 89.9±0.7 ° (S.D.; with a maximum range of variation of only ±1.25 °). Likewise, the recomputed side length was 9.9±0.11 cm (S.D.; with a maximum range of variation of only ±0.2 cm). The errors in the *x*,*y*-locations were attenuated in the course of data-processing by smoothing the digitised points by means of a digital filter algorithm, reducing 'noise' above a 2 MHz cut-off frequency. This allowed the exclusion of aberrant localisations and gave a close fit to the actual data.

The sequences were analysed using the Ariel Performance Analysis System (APAS, Ariel Life Systems Inc.) at a frequency of 25 frames s^{-1}, i.e. one frame every 40 ms. This means that each step was resolved into 20–30 successive images.

The first and last steps were systematically excluded from the analysis since they involved increasing and decreasing speeds and only the linear parts of the paths were analysed.

Marking the crayfish

Various positions on the crayfish bodies were accurately marked using 35 white dots placed dorsally. Each mark was approximately 3 mm in diameter. The APAS automatically located each point at the barycentre of the white mark. The locations calculated by the APAS were then manually checked frame-by-frame.

The marks were distributed on the body as shown in Fig. 1B. The legs of crayfish consist of seven segments and seven joints (Fig. 1C), but the movements of the legs during locomotion mainly involve three joints: antero-posterior movements involve the use of the thoraco-coxopodite joint (T-C), and leg-raising involves the coxo-basipodite joint (C-B). The mero-carpopodite joint (M-C) between the proximal and distal segments makes it possible to perform lateral extension/flexion movements. Three dots on each thoracic appendix (L1–L5) on

each side of the body marked the distal part of the leg (dactyl), the end of the proximal segment (M-C) and the body joint (T-C; Fig. 1B,C). As T-C was ventral and not visible from above, the dots were painted on its projected position on the top of the shell. These points were systematically digitised and analysed in the course of the video analysis, but the present study deals only with the movements of the leg segment connecting the dactyl to the body attachment site (T-C; Fig. 1B,C). Interpretations based on the movement of this segment alone are obviously subject to limitations due to M-C bending and possible changes in the leg extension, but these measurements correspond with the other techniques commonly used in classical treadmill experiments (Clarac, 1984), making comparisons with other published studies possible. A complete analysis of the segmental organization of the step will be dealt with in a subsequent paper. Legs 1 (the chelipeds) mainly remained immobile during locomotion and therefore are not included here.

Three points along the exoskeleton longitudinal axis (rostrum, back of the cephalothorax and second segment of the abdomen) were used to determine the body orientation in relation to the external reference axis provided by the two marked lines. The angles (*A*, *B*, *C*; Fig. 1B) of the fixed triangle delimited by the rostrum and the two T-C points of the first pair of legs also provided a control for rolling (comparative variations of *B* and *C*) and pitching (comparative variations of *A*, *B* and *C*) components of the movement.

Treatment of data

The global parameters of the movement, such as walking speed and path straightness, were determined by analysing successive positions of the dots on the thorax with reference to the ground markers. As the camera tracked the crayfish, the positions of the markers could not be measured in relation to any fixed external cues. All the other measurements were computed in terms of the crayfish's body axis. The following variables were measured.

The angular movement of the leg was approximated by the vertical projection of the angle delimited by the dactyl, the T-C joint between the leg and the body, and the T-C of the contralateral leg (Fig. 1B). This angle was equal to 180 ° when the leg was perpendicular to the cephalo-caudal axis of the body and decreased when the leg moved forwards during the return stroke: the minimum angle was therefore defined as that corresponding to the anterior extreme point (AEP). The angle increased when the leg moved backwards during the power stroke and the largest angle defined the posterior extreme point (PEP).

The leg extension was the length of the vertical projection of the line segment connecting the dactyl and the body attachment site (Fig. 1B).

The leg excursion was the distance covered by the leg during the return stroke. This length was computed from the leg extension at PEP and at AEP and from the amplitude of the angular movement between these extreme points. This was the same parameter as that commonly used in treadmill experiments and should not be confused with the average distance travelled per step: while the leg was swinging, the body itself was pulled forward by the other legs during the power stroke, so that the distance actually travelled by the leg was its own leg excursion plus this body shift (see Fig. 3). No such difference exists in treadmill experiments, where the crayfish is fixed and it is the belt that moves. The distances between the successive landing points corresponded to the distance travelled per step; they were equivalent in the various legs. The anterior point was reached before the legs landed, as can easily be seen from the stick diagrams giving the data on the legs 2 and 3.

The period of the step was the time elapsing between two successive AEPs. It could only be measured to within 40 ms because of the film speed. The period consisted of one return stroke and one power stroke, and the relative durations of these two phases were expressed as the swing ratio, defined as the swing duration divided by the whole period.

The phase of each leg$_n$ relative to any 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 ($\phi_{n \text{ in } n'}$) was therefore computed as follows: $\phi_{n \text{ in } n'}=(\text{AEP}_n-\text{AEP}_{n'})/P_{n'}$.

In addition to these measurements, which are those classically performed in locomotion studies, the horizontal angular accelerations of the legs were analysed as a dynamic parameter. Both the return and power strokes were split into successive acceleration and deceleration phases (Fig. 2). The amplitude of the acceleration curve depended on the time interval between successive frames. This parameter was therefore of limited interest as an absolute value, because the interval of 40 ms between frames smoothed out the acceleration peaks considerably. Nevertheless, the comparative values of acceleration in the different legs provided valuable information because the legs were located simultaneously and with the same frequency at their successive positions. Furthermore, the exact timing of the acceleration and deceleration phases of the return and power strokes provided a very accurate description of the stepping patterns. Moreover, the dynamics of the pattern of acceleration not only described the movement accurately but also had a biological significance, because they mainly corresponded to changes in the forces applied to the movement depending on the recruitment or release of motor units.

Results

Individual characteristics of the legs

Leg specificity

The legs were collected from ten dead crayfish and each joint was measured. Legs 2, 4 and 5 were found to be of the same length, averaging 42±1.9 mm (S.D.) in five small individuals (weighing less than 30 g) and 60±1.5 mm (S.D.) in five large ones (weighing more than 60 g); leg 3 was consistently longer at 47±0.9 mm (S.D.) in the smaller and 70±1.8 mm (S.D.) in the larger animals. The proximal and distal

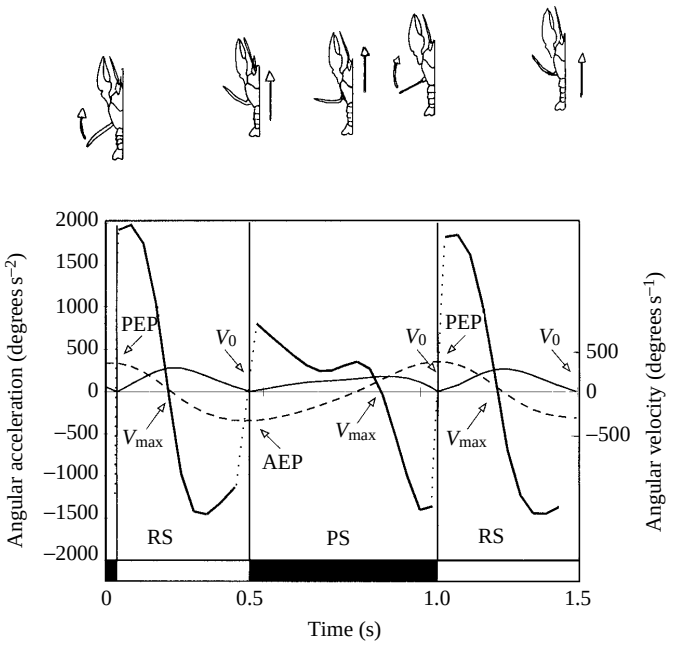

Fig. 2. Kinematics of a typical leg movement. The continuous curve shows the speed variation during each stroke phase; since the direction of the speed vector was not considered here (in order to simplify the acceleration curves), the speed curve was always positive. The speed reaches zero (V_0) at the anterior extreme point (AEP) and the posterior extreme point (PEP). The acceleration curve (heavy lines) reached zero when the speed is at its maximum value (V_{max}) and splits the movement into an acceleration phase (positive acceleration) and a deceleration phase (negative acceleration). As the absolute value of the speed vector is considered here, successive accelerations are isolated events. This is represented by the dotted transition lines joining successive accelerations centred at the PEP or AEP. The fact that these lines are oblique results from the 40 ms frame duration and is artificial. The acceleration was assumed to begin at the zero values corresponding to the PEP or AEP. The acceleration pattern was studied in all the legs except legs 2, because this leg was often hidden by the claws of legs 1 during part of its movement. As an indication, the angular leg movement (dashed curve) is also shown, with the PEP at the upper extreme and the AEP at the lower extreme. The return stroke (RS) ranged from PEP to AEP, the power stroke (PS) from AEP to PEP. At the top of the figure, the corresponding movements of leg 4 and the body are presented in alignment with the AEP and PEP. The arrows indicate the direction of the leg and body movements. Note that the return stroke involves the movement of the leg only, whereas the power stroke includes the body push and is therefore a complex movement resulting from all the forces applied concomitantly by all the legs during the power stroke.

segments were found to be the same length in all legs apart from leg 5, which had a shorter proximal segment.

Besides their morphology, the legs are functionally different: for example, legs 4 and 5 end in a digit (dactyl) and are purely locomotory appendages, whereas the propodites of legs 2 and 3 have a protuberance forming, with the dactyl, two branches of small chelae. These legs are prehensile appendages. This functional specialisation of the legs may correspond to differences in the ways in which the legs contribute to locomotion.

An example of leg specialisation can be seen from the lateral view of a crayfish moving in an aquarium (Fig. 3). The vertical double-headed arrows on the figure indicate the exact time during the return stroke when the distal segment (carpopropodite; Fig. 1C) projected forwards. The anterior legs 2 and 3 projected forward at the beginning of the return stroke. This simultaneous elevation and forward tilt of the leg led to a very typical upward movement of the M-C joint. Leg 4 tilted forward later, when at least one-third of the return stroke had been accomplished. Leg 5 was backward-oriented during the whole return stroke and only tilted at the end of the return stroke. The differences observed between the leg trajectories suggest that each of the legs participated in different ways in locomotion.

Walking speed

The walking speed measured in 17 individuals during a total of 184 sequences averaged 6.2±1.05 cm s^{-1} (S.D.) and ranged between 3.8 and 9.3 cm s^{-1}. Each individual walked at approximately the same speed during each sequence. No correlation was found to exist, however, between the size or mass of the animal and the speed at which it moved.

In the four crayfish studied in detail, the walking speeds averaged 6.4±0.52, 5.8±0.53 and 6.44±0.41 cm s^{-1} (mean ± S.D.) for small animal 1 and large animals 3 and 4, respectively. Animal 2 moved faster (7.8±0.8 cm s^{-1}). It was also the fastest animal among all the 17 tested.

Timing of the swing and stance phases

The mean timing of the period is shown in Table 1 for the left legs (the legs on the right side had similar values). These data were computed from the combined steps of all of the sequences analysed. This regrouping was possible because the movements in each animal were consistent from one sequence to the other. All the legs had approximately the same timing characteristics, for both the period duration and the swing ratio (Table 1).

The mean period duration of each leg was approximately 1 s in all the animals (Table 1), except for the fast-moving animal 2, whose mean period approximated 0.88 s (see details in Table 1) and was significantly shorter than that of all the others (P<0.01; except for leg 5 of animal 1). Although no significant differences were observed in the mean period durations between small animal 1 and large animals 3 and 4, the range of variation of the latter two was larger: 80 % of the steps of the smaller animals 1 and 2 lasted between 0.8 and 1.2 s, whereas they ranged between 0.8 and 1.8 s in the larger animals 3 and 4.

At the walking speed at which the animals moved spontaneously, their swing ratio was very close to 0.5 in all sequences (Table 1), i.e. the stance and swing phases lasted for approximately the same time. We analysed variations in the swing ratio for each animal separately and at different speeds, without observing any consistent effects. However, the small variations in the range of speeds found here, in comparison

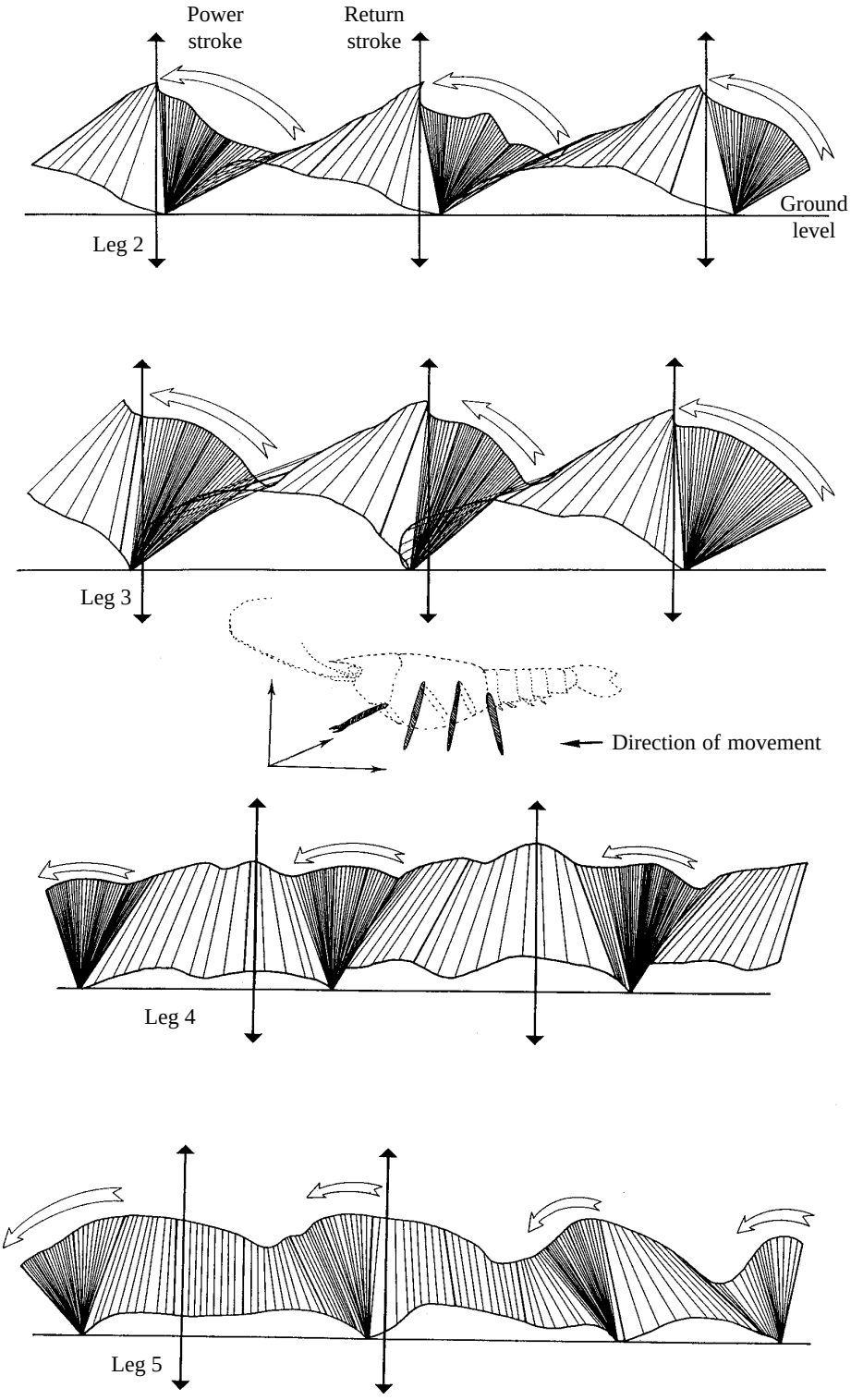

Fig. 3. Lateral view of the leg trajectory in a crayfish stride. A camera was placed in front of an aquarium (90 cm×10 cm×20 cm). The crayfish was filmed from the side during two steps, at a speed of 25 frames s^{-1}. Movement took place from right to left. The successive positions of the distal segment (hatched area in central sketch) of each leg in the *x*,*z*-plane are shown in the stick figures: upper curve, successive positions of the M-C joint; bottom curve, successive positions of the dactyl. In each successive frame, a line connects the M-C joint (top) and the dactyl (bottom); the line becomes heavier every ten frames. Power strokes are indicated by their fan shape with the dactyl on the ground (broad arrows). The vertical double-headed arrows indicate the exact moment when the distal segment projected forward (beyond the vertical axis) during the return stroke.

with treadmill experiments, made it unlikely that any consistent effect of this kind would be observed.

Leg excursion

Fig. 4 shows the distances covered by the legs in each of the four animals, during their return strokes. The larger animals moved with a larger leg excursion, but all showed the same pattern of variation between individual legs. This pattern was therefore characteristic of the movement studied in this investigation. The leg excursion increased from leg 2 to leg 4 but was lower in leg 5. The leg excursion of leg 4 was significantly larger than those of the other legs ($P<0.01$) in all the animals studied. The small values for leg 2 were partly due to the frequent double steps they took, but also reflect the fact

Table 1. *Timing characteristics of the step of walking crayfish* Procambarus clarkii

	Mean period duration (s) Animal				Mean swing ratio Animal			
	1	2	3	4	1	2	3	4
Leg 2	0.98	0.79	0.97	1.0	0.49	0.52	0.49	0.48
S.D.	0.14	0.24	0.33	0.38	0.05	0.13	0.11	0.14
Leg 3	1.01	0.88	1.11	1.23	0.48	0.5	0.44	0.44
S.D.	0.1	0.16	0.24	0.32	0.04	0.06	0.06	0.07
Leg 4	1.0	0.89	1.1	0.99	0.48	0.46	0.43	0.46
S.D.	0.09	0.1	0.2	0.11	0.05	0.05	0.07	0.1
Leg 5	0.87	0.88	0.98	1.03	0.55	0.52	0.45	0.47
S.D.	0.18	0.15	0.26	0.18	0.1	0.09	0.1	0.11

The period duration was the delay between two successive anterior extreme positions; the swing ratio was the ratio of the return stroke duration to the period duration (see Materials and methods).

The total numbers of steps were 65, 50, 50, 45 for animals 1, 2, 3 and 4, respectively.

Values are for left legs only.

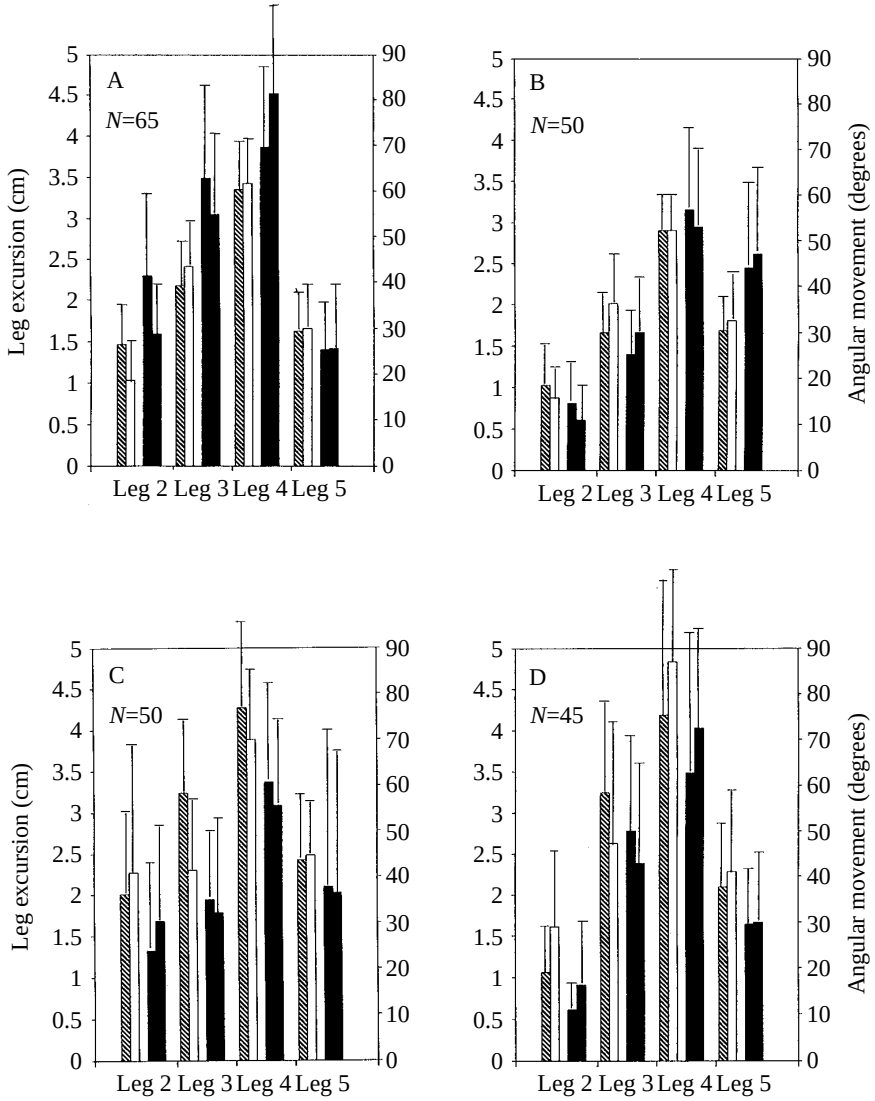

Fig. 4. Mean leg excursion and angular movement in four individual crayfish: (A) animal 1; (B) animal 2; (C) animal 3; and (D) animal 4. The mean values were computed from the accumulated sequences. Error bars are S.D. Hatched bars, left legs; open bars, right legs; filled bars, angular movements of left and right legs.

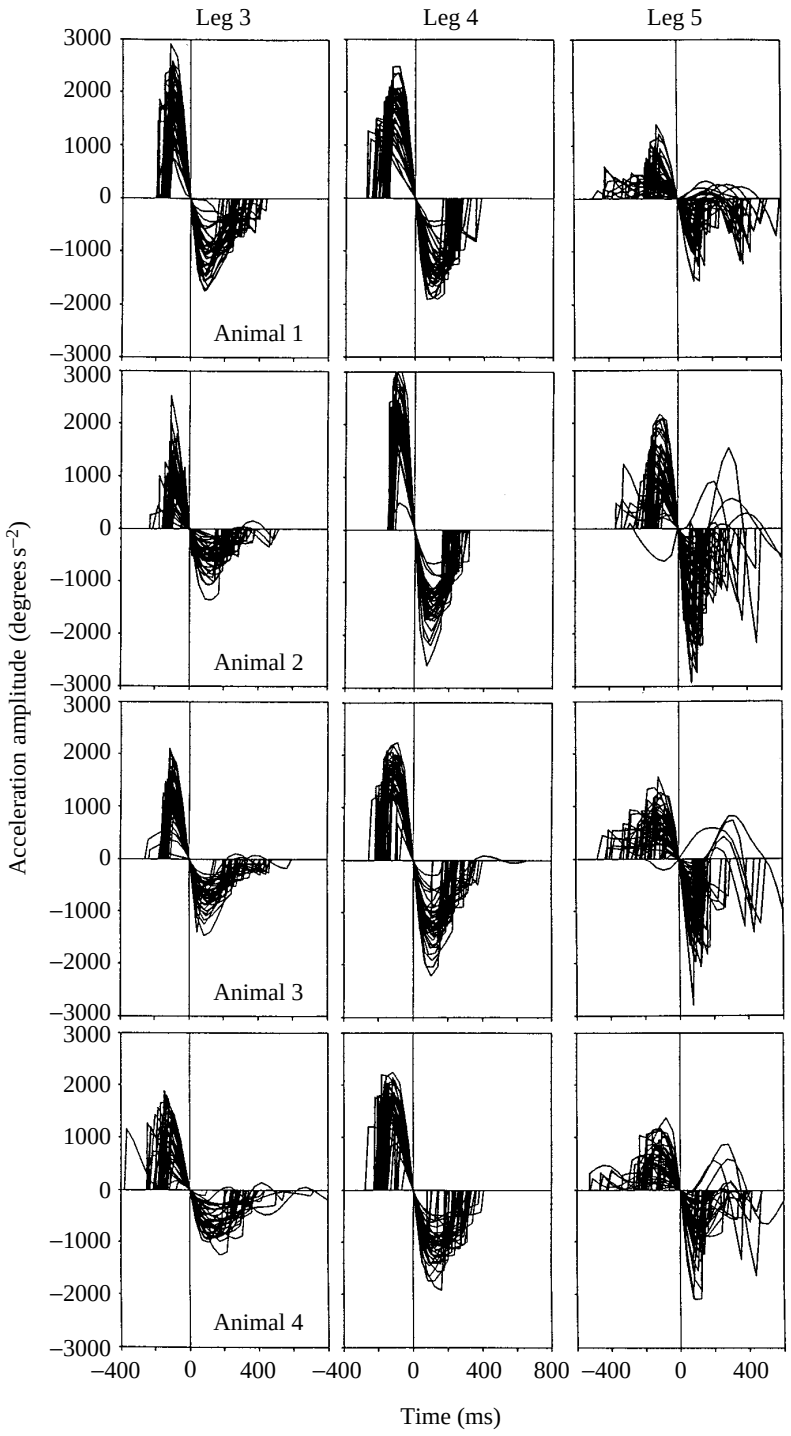

Fig. 5. Variations in the acceleration amplitude during the return stroke. Patterns of acceleration are shown for left legs 3–5 of animals 1–4. Each trace represents a single step, and the steps of all the sequences are superimposed in each figure. Time zero is set to the zero acceleration point corresponding to V_{max}. The positive part of the curves (negative *x*-values) corresponds to the acceleration phase of the stroke. The negative part (positive *x*-values) corresponds to the deceleration phase.

that these stride lengths were small. However, the leg excursion estimates for leg 2 should be treated with caution, because their AEPs were often hidden by the claws and the exact position had to be estimated. Leg 5 tended to make smaller strides than leg 3 in all the animals, except for animal 2; however, this was not significant. One possible reason for the smaller mean amplitudes found for leg 5 may have been the occurrence of double steps, but when investigating the distribution of leg excursions for leg 5, it was noted that, apart from a group of small amplitudes corresponding to double steps, the main cohort was also in a range of smaller amplitudes than for leg 3.

The leg excursion was computed from both the leg extension at PEP and AEP and the angular movement of the leg between these points. It therefore necessarily depended on both variables, but the angular movement was mainly responsible for the measured values as it changed proportionately with leg excursion (Fig. 4), unlike leg extension.

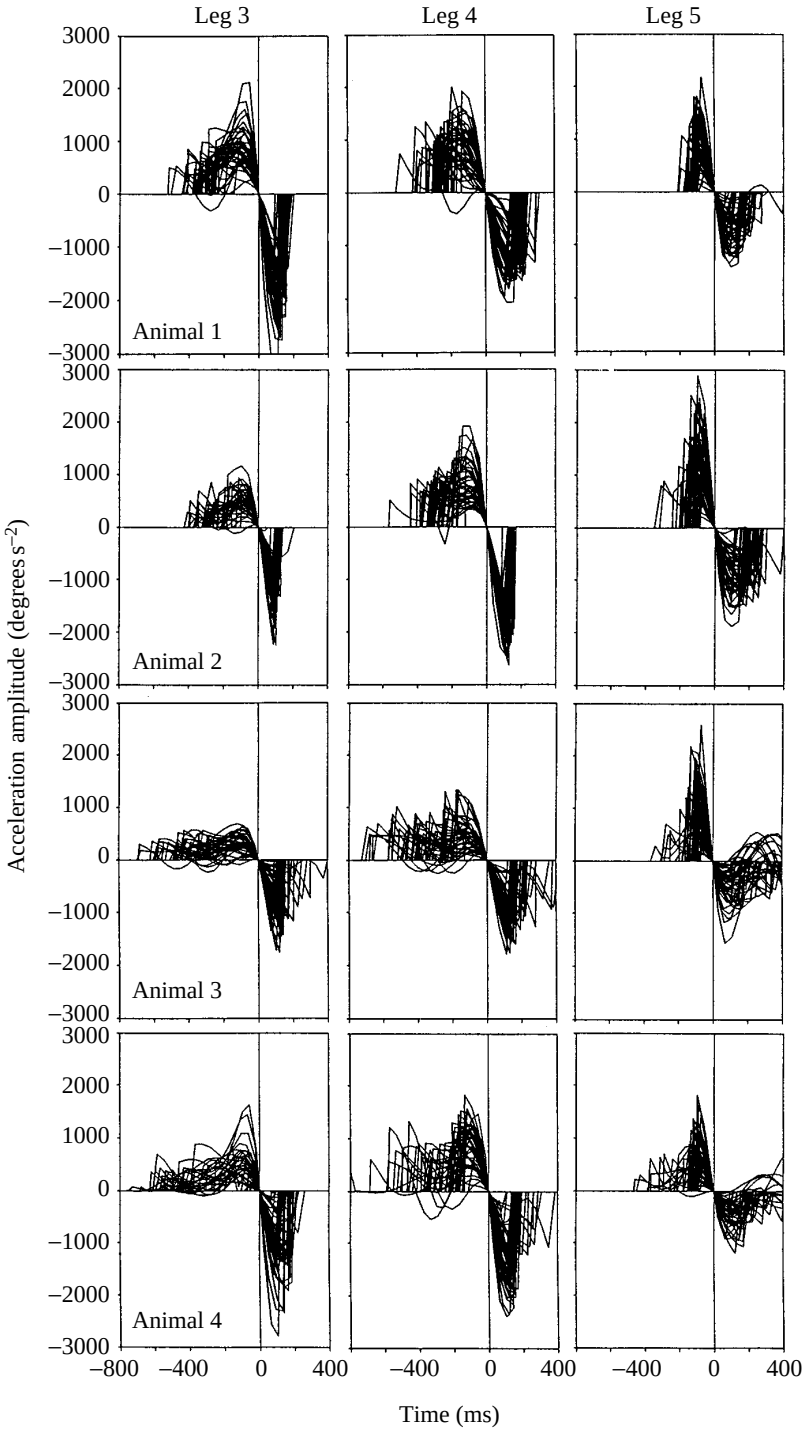

Fig. 6. Variations in the acceleration amplitude during the power stroke. See Fig. 5 for details.

Patterns of acceleration

Figs 5 and 6 show the combined acceleration curves for all the steps of legs 3–5 for each animal. In these figures, the curves were centred on the zero acceleration point, which corresponds to the maximum angular speed of the leg. In each plot, steps with different durations have been combined without a normalising function, because the parameter of interest was time and not relative duration: the exact timing of the acceleration phases, irrespective of the step period, was of interest, and some interesting properties emerged from these diagrams which would have been masked by normalising the time scale. The overlapping of the curves shows that the pattern of acceleration was very stable in the different steps even though they were taken from different movement sequences. The acceleration patterns were also similar between the different animals but differed greatly depending on whether the legs were engaged in a return (Fig. 5) or a power (Fig. 6) stroke.

During the return stroke, legs 3 and 4 showed the same regular pattern. Leg 4 showed the most regular pattern of

Table 2. *Timing of leg acceleration in four individual crayfish* Procambarus clarkii

	Return stroke		Power stroke	
	Acceleration (ms)	Deceleration (ms)	Acceleration (ms)	Deceleration (ms)
Leg 3				
Animal 1	169±40	309±78	351±99	157±53
Animal 2	154±36	275±69	330±89	140±35
Animal 3	167±24	324±89	488±148	166±44
Animal 4	209±68	439±312	495±154	374±93
Leg 4				
Animal 1	208±30	256±53	306±87	217±52
Animal 2	163±16	240±38	311±83	159±32
Animal 3	198±32	270±69	445±161	216±76
Animal 4	199±24	294±59	449±239	184±47
Leg 5				
Animal 1	202±50	238±137	178±62	226±93
Animal 2	220±74	255±130	173±48	242±75
Animal 3	314±125	231±182	145±63	412±166
Animal 4	313±122	154±126	145±58	388±204

Values are mean ± S.D.

acceleration, for both amplitude and duration. This leg accelerated up to 2500 degrees s^{-2} and then decelerated to -2500 degrees s^{-2}. The duration of the acceleration phase was also very constant: its mean value was 201±30 ms (S.D.) in animals 1, 3 and 4; and it was even shorter (163±16 ms) in the faster animal 2 (Table 2). The subsequent deceleration phase lasted 1.5 times longer and varied more. The acceleration pattern in the leg 3 return stroke was similar to that of leg 4 (Fig. 5), although the range of amplitudes was smaller, especially during the deceleration phase. The observation that the amplitude of leg 3 was smaller was not surprising because this leg covered a shorter angular distance than leg 4 during the same period of time. The leg 3 acceleration and deceleration phases tended to last longer and were more variable than those of leg 4 (Table 2), although the difference was not statistically significant.

As shown in Fig. 5, there was a regular deceleration of legs 3 and 4 until they reached their lowest speed. This pattern fits the hypothesis that the return stroke is a ballistic movement initiated by the protraction command at the PEP. The subsequent variation may, therefore, have been specifically due to a change in acceleration at the final stage of the leg movement, i.e. the leg did not land immediately after the end of deceleration, but in most cases, the deceleration slope changed just before landing. This change could not result from an experimental artefact involving the geometrical projection of the descending leg from the three-dimensional movement space to the plane of the ground (on the contrary, this would have increased the apparent deceleration). It was possibly due either to a change in the angular relationship between the proximal and distal parts of the leg, resulting from M-C joint bending, or to a final correction of the leg course, resulting from the action of the distal muscles. This will be clarified in a future analysis of the relative movements of the proximal and distal segments of the leg.

Unlike the return stroke, the acceleration during the power stroke varied irregularly both in magnitude and duration, often showing two small successive acceleration peaks and a short, sharp deceleration (Fig. 6). The acceleration pattern in the power stroke therefore represented the mirror image of that seen for the return stroke. The differences between these acceleration patterns reflected the different processes involved in the two strokes forming the step cycle. During the return stroke, the acceleration pattern results from the motor commands sent to the remote leg, whereas during the power stroke, the apparent backward acceleration results from the forces applied concomitantly by all the legs in drawing the body forwards. As these legs do not land at the same time, they are not all at the same stage in their movement and therefore produce a complex overall pattern.

The acceleration patterns between the return and power strokes of leg 5 also differed considerably, but they were mainly the opposite of those observed in legs 3 and 4: the acceleration phase of the return stroke varied greatly and looked like the characteristic power stroke pattern of the other legs (Fig. 5), whereas the power stroke showed a strong acceleration followed by a fluctuating deceleration phase. The mechanism by which leg 5 contributed to the overall movement was responsible for this different pattern. While legs 2, 3 and 4 projected far ahead of the body joint for each stroke, and then pulled the body with a large antero-posterior movement, leg 5, in contrast, remained behind its body joint even at the AEP and, on landing, pushed backwards strongly to propel the body. These movements can be seen in Fig. 3. Leg 5, therefore, had a shorter distance to cover during the return stroke and was mainly disturbed by additional uncoordinated small landings, serving either to complete propulsion or to correct body roll.

Relationships between acceleration and position

Fig. 7 shows the relationships between the speeds and the angular positions of legs 3, 4 and 5 for animals 1 and 2. This representation of the leg movements in their phase plane confirmed the consistency of the movements of each leg and usefully completed the analysis of temporal stability of leg movement, showing that each individual had a characteristic pattern of leg movement.

The phase portrait (Fig. 7) included several overlapping sequences of steps. The stability of leg 4 is apparent, but it should be pointed out that the variability of the phase portraits of legs 3 and 5 did not result from the accumulated movements performed over the course of different sequences of several steps (see Materials and methods), but was found to exist at the level of each sequence, which indicates that this pattern was reproduced from one sequence to another. Furthermore, the pattern of leg movements differed markedly between animals 1 and 2. The phase portraits were also different in animals 3 and 4. The locomotion of each animal could

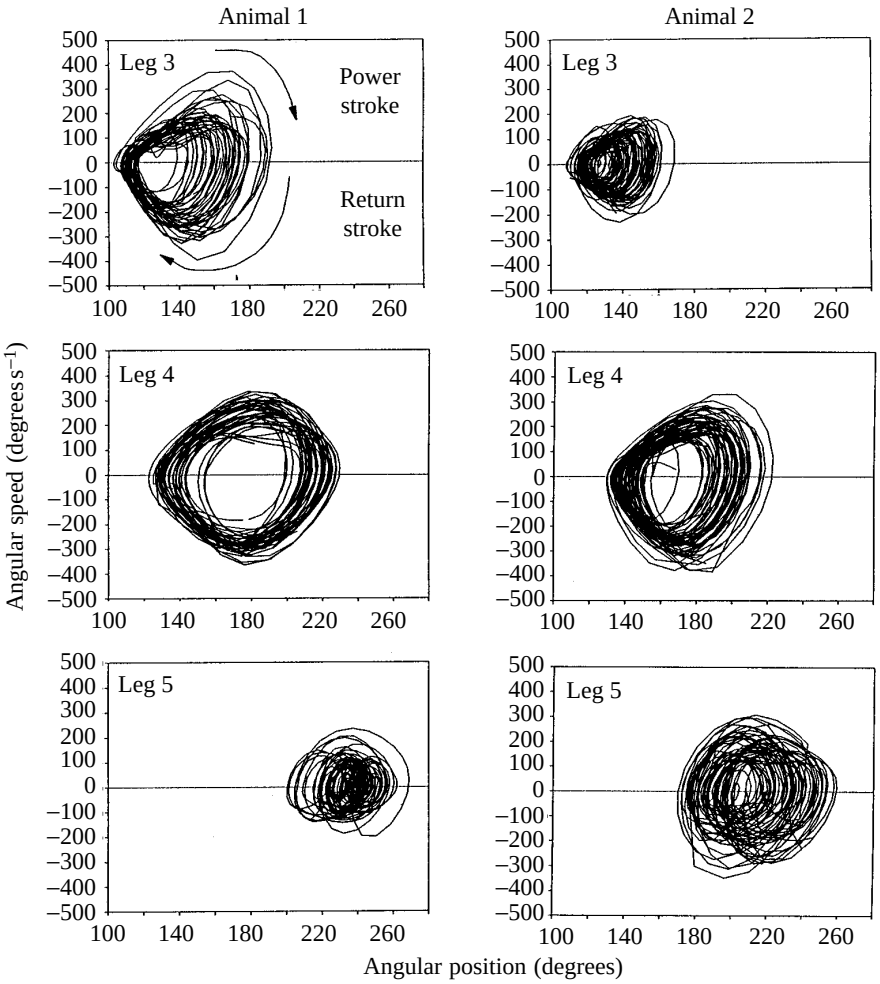

Fig. 7. Movements of the legs in their phase plane. Angular speeds plotted as a function of angular positions. The 180 ˚ value on the *x*-axis shows the position in which the leg was in line with its segmental axis. Steps occurring during five sequences (42 for animal 1 and 50 for animal 2) have been superimposed.

therefore be characterized by the cyclic movement of its legs onto their phase plane. Note that, as shown by the amplitudes of the cycles, animal 1 mainly walked with legs 3 and 4 (this was also true for animals 3 and 4), whereas animal 2 walked with legs 4 and 5 (Fig. 7). This was confirmed by the angular variations in Fig. 4. It is possible that the greater role of leg 5 in animal 2 resulted from its faster travelling speed.

The legs differed considerably in their phase portraits. Leg 4 movements were symmetrically distributed on both sides of the body attachment axis (180 ˚), while leg 3 remained in front of the corresponding axis and leg 5 behind it. This spatial range of the leg movements was in agreement with measurements from the lateral view (Fig. 3) and can be explained in terms of the angles at which the legs are joined to the body.

The stable cycling movement of legs 4 in their phase plane suggested that this movement was not affected to any great extent by the other legs. Legs 3 and 5, in contrast, varied considerably and were probably influenced by the other legs. Leg 5 was very unstable as regards both its position and its speed. Leg 3 values were also variable, but in this case the variations mainly involved the PEP, whereas the AEP was quite stable. This can be seen particularly clearly for animal 1 (Fig. 7) and is also apparent for animal 2 in spite of the greater variability of leg 3. This suggested that landing depended only on the leg position, but that the beginning of the return stroke depended on the position of the adjacent leg 4. This can be easily understood because leg 3 protraction consistently followed leg 4 landing, in agreement with Hughes' rule (1952), and thus occurred even when the power stroke was not completed. In contrast, the exact landing position probably depended only on the angular configuration of the leg, which could be monitored using stress detectors.

These results, as well as the results for leg excursion (Fig. 4), suggest that leg 4 played a prominent role in step organization and was probably the main functional leg during locomotion.

Inter-leg coordination

During preliminary experiments on freely moving crayfish, two main gait patterns were observed, depending on whether the crayfish moved their contralateral legs alternately or in phase. This alternative mode of locomotion was subsequently confirmed upon analysing a set of 190 filmed sequences from 17 different animals (incorporating a total of 2264 steps). In this analysis, we computed the phase value at the AEP of the fourth pair of legs on the assumption that they played a leading role in free locomotion. The general histogram of Fig. 8A shows the distribution of these phase values. There was a broad

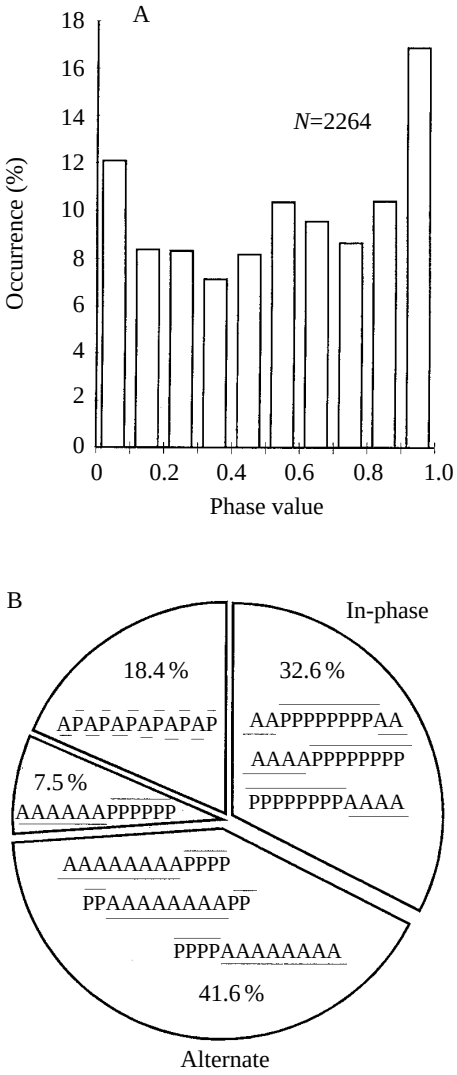

Fig. 8. Phase distributions in contralateral legs 4. (A) Histograms showing the distribution of phase values in the sequences walked by 17 animals. (B) Frequency of sequences with stable patterns showing either in-phase or alternate coupling. A sequence was considered to be in phase (or alternate) when more than 66% of successive steps were in phase (or alternate). Examples of the different possible successions of coupling are given in the sectors. P, in-phase (ϕ>0.75 or <0.25); A, alternate (0.25<ϕ<0.75).

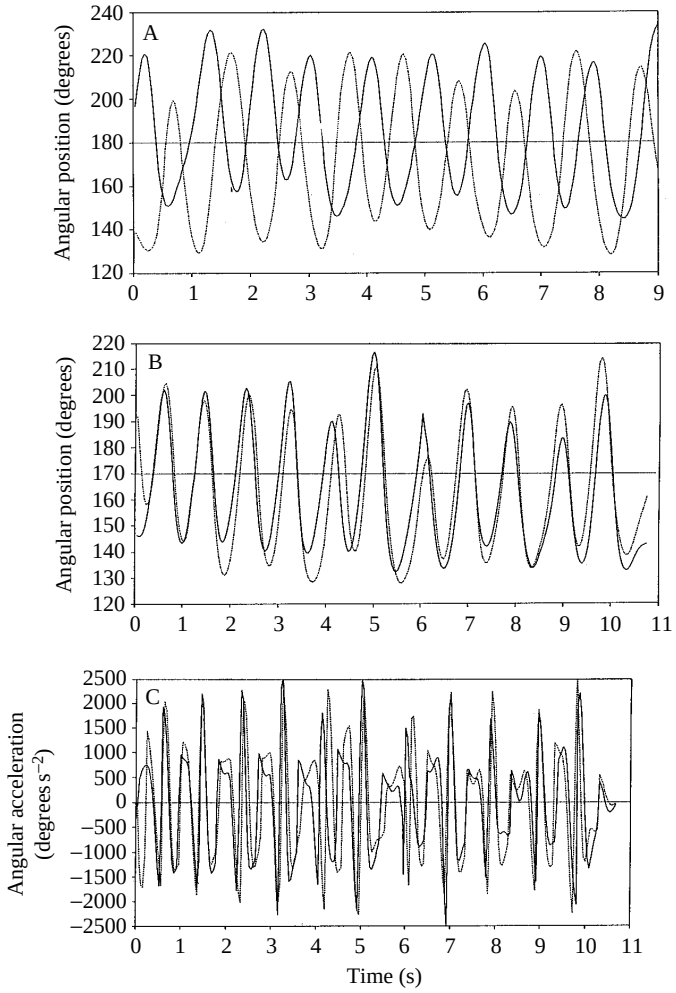

Fig. 9. Coordinated movements of contralateral legs 4. Two examples of coordination are shown with the legs moving alternately (A) or in phase (B). The minimum angular values are at the AEP, maximum values are at the PEP. (C) Acceleration curve of the sequence shown in (B). Sharp upward peaks correspond to the acceleration peaks recorded during the return stroke; those with a lower amplitude are peaks occurring during the power stroke. Continuous line, left leg; dotted line, right leg.

distribution of all the phase values with a small peak at 0.5 and a preference for in-phase coupling: 29% of the phase values were in the range 0±0.1; this was significantly higher than the predicted rate (20%) if all phases were equally probable (χ^2=108; d.f.=1; P<0.001). It appeared, therefore that, in addition to the weak alternate coupling commonly observed in treadmill walking, freely walking crayfish frequently moved their contralateral legs 4 in phase.

The occurrence of these alternative coordination patterns did not result from random variations from step to step, as shown by the frequency of stable successions of in-phase or alternate coupling (a coupling was assumed to be stable over a sequence when 66% of successive steps were either in phase or alternate, see Fig. 8B). 32.6% of sequences were found to contain mainly a succession of in-phase steps ($\phi_{4L\text{ in }4R}$>0.75 or $\phi_{4L\text{ in }4R}$<0.25) and 41.6% contained mainly alternating steps (0.25<$\phi_{4L\text{ in }4R}$<0.75), whereas 7.5% of the sequences were performed with half of the steps in phase and the other half alternating, and no stable pattern occurred in the remaining 18.4% of the sequences. Some individuals preferentially walked in phase and others walked with alternate coupling in most of their sequences, but in most of the animals both of these patterns occurred.

In the four animals for which a thorough kinematic analysis was performed, we noted whether the steps were made with legs 4 moving alternately or in phase. Animal 2 systematically moved legs 4 in phase throughout the five sequences analysed. Animal 1 moved legs 4 in phase in four sequences but alternately in six others. The larger animals 3 and 4 did not

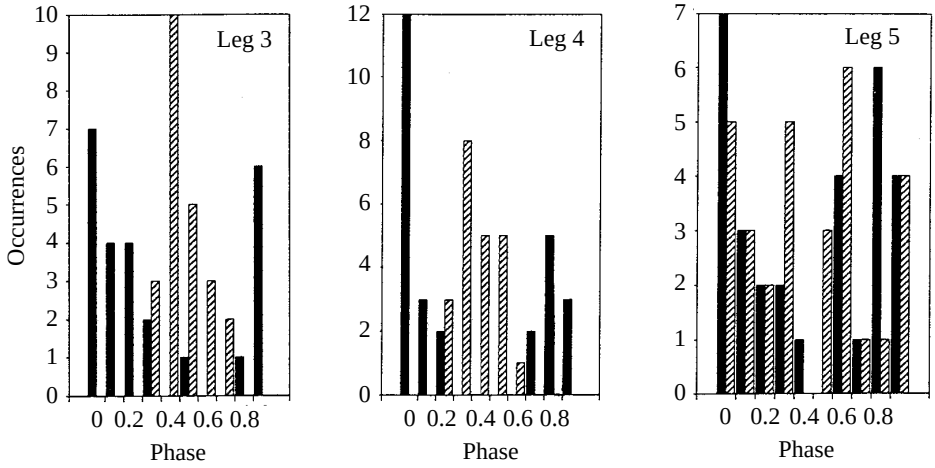

Fig. 10. Phase histograms of contralateral leg coupling. The distribution of phase values is shown for all sequences of animal 1. Each class was split into two, corresponding to the sequences where the locomotor pattern of leg 4 was in phase (hatched bars) or alternate (filled bars). A reference histogram of leg 4 is also given. Alternate movements correspond to phase values ranging between 0.25 and 0.75; in-phase movements are shown by phase values outside this range. Contralateral legs 3 (left side) and 5 (right side) tended to be either in phase or alternate depending on the coupling in leg 4.

show such stable patterns: a consistent in-phase pattern was observed in one sequence only in animal 3, and in all the other sequences both animals moved their legs either in phase or alternately. No stable pattern of coordination emerged for these individuals, although the in-phase pattern occurred more frequently.

Fig. 9 shows two sequences of leg 4 movements with alternate (Fig. 9A) and in-phase (Fig. 9B) patterns. When legs 4 moved in phase, they were coupled throughout their angular movement, and this steady coupling became even more obvious when the acceleration patterns were examined (Fig. 9C): the simultaneous variations occurring in the acceleration curves of contralateral legs 4 suggest the existence of a common control mechanism.

The contralateral coupling in the other legs tended to be either in phase or alternate, in agreement with the pattern found for leg 4 coupling. A good example of this was observed in animal 1. As the legs of this animal moved in phase in almost half of the sequences recorded, the coupling in contralateral legs 3 and 5 could be analysed separately from the sequences with legs 4 in phase and with legs 4 alternating. It emerged quite clearly that whether the coupling between legs 3 and that between legs 5 was in phase or alternate depended on the coupling between legs 4 (Fig. 10). However, the synchronisation between the pairs of legs other than leg 4 was less clear-cut. For instance, in animal 2, whose legs 4 moved in phase in 90 % of the steps, the other legs moved in phase in only 50 % of the steps.

The relationships within the pairs of legs other than leg pair 4 seems to depend mainly on their relationships with the ipsilateral adjacent leg. Adjacent ipsilateral legs were strongly coupled and showed the same phase relationships in the various sequences, irrespective of the contralateral leg coupling, as has been commonly observed in crustaceans. The characteristics of the adjacent leg coupling were identical in all the animals (although greater variability was observed in the larger animals 3 and 4) and the data are summarised in Fig. 11. It is clear that the mean phase value increased from the anterior to the posterior pairs of adjacent ipsilateral legs: legs 2 and 3 mainly landed in phase and the mean phase value for legs 3 and 4 ranged around 0.3, whereas the phase value between legs 5 and 4 was 0.4. In legs 4 and 5, the phase relationship was only a relative one because of the unstable pattern of legs 5 (see Fig. 7).

A possible coupling mechanism responsible for this progressively reinforced alternating pattern between ipsilateral

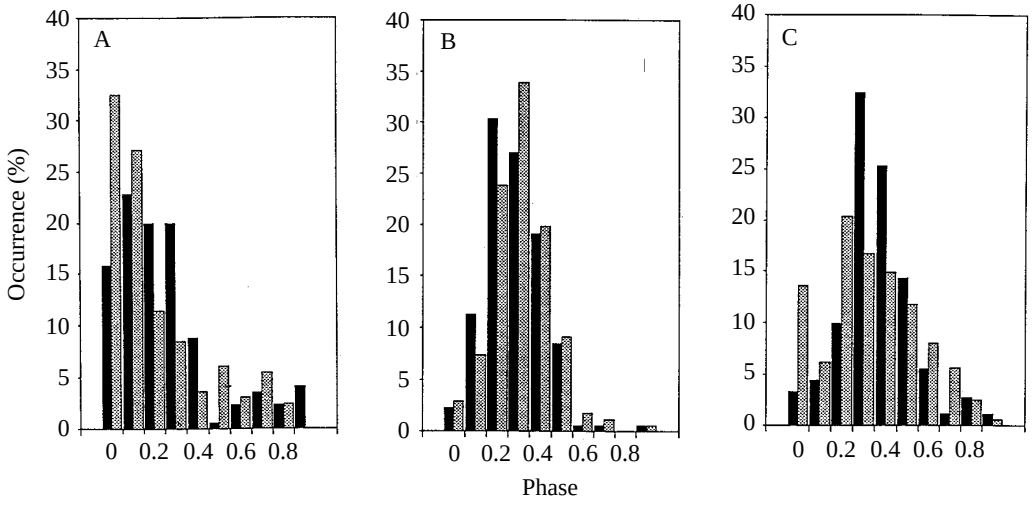

Fig. 11. Phase histograms for ipsilateral legs. (A) Phase value of $leg_{3\ in\ 2}$; (B) Phase value of $leg_{4\ in\ 3}$; (C) Phase value of $leg_{5\ in\ 4}$. The percentage of occurrence was computed for the total sample of all the sequences recorded with the four animals. Filled bars, right legs; shaded bars, left legs.

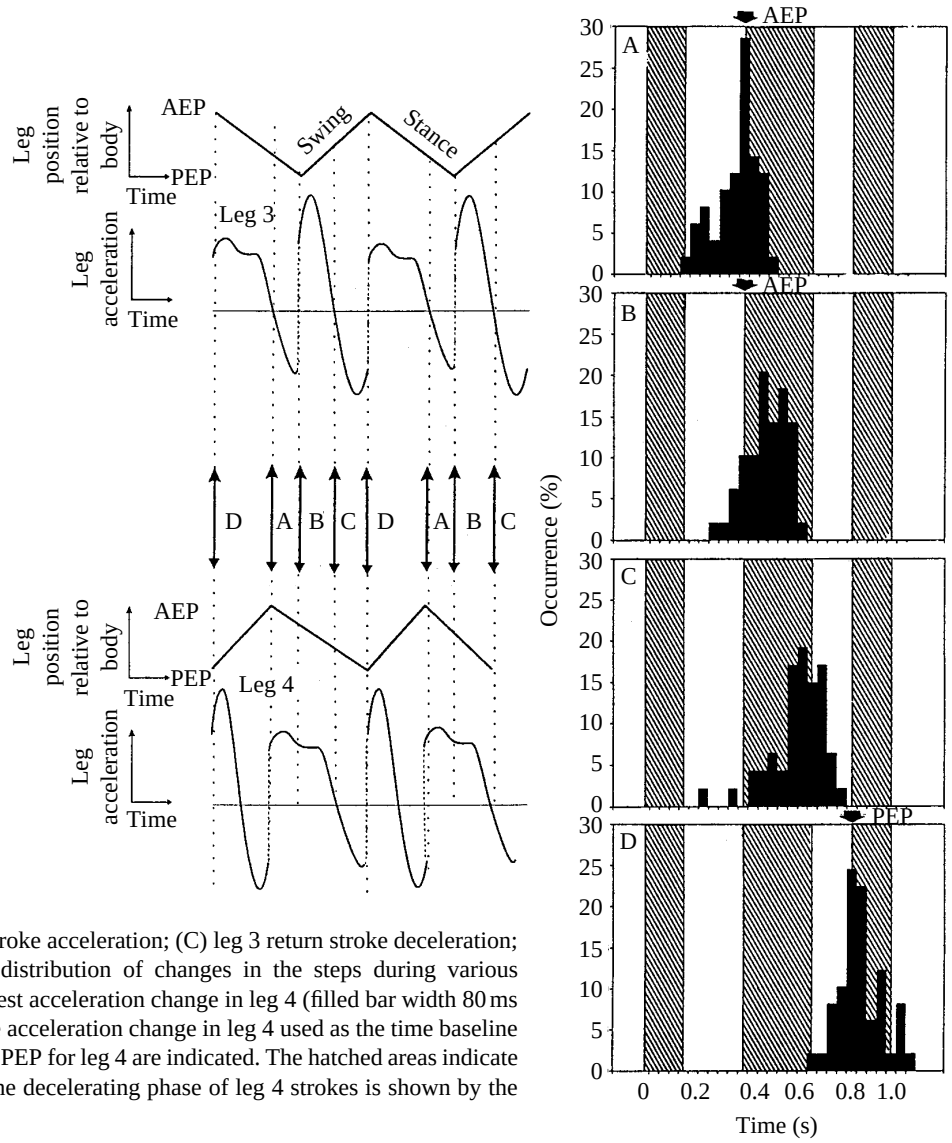

Fig. 12. Synchronization of acceleration changes between adjacent legs 3 and 4. The panel on the left shows typical coupling between legs 3 and 4 in animal 2. Changes in position and acceleration of leg 3 are shown at the top; corresponding changes in leg 4 are shown at the bottom of the panel. The arrows in the central section show the related acceleration changes in legs 3 and 4. The corresponding distributions of these changes (A–D) are given on the right side as histograms. (A) Leg 3 power stroke deceleration; (B) leg 3 return stroke acceleration; (C) leg 3 return stroke deceleration; (D) leg 3 power stroke acceleration. The distribution of changes in the steps during various sequences are given as a function of the nearest acceleration change in leg 4 (filled bar width 80 ms interval). Arrows on the histograms show the acceleration change in leg 4 used as the time baseline for the distribution histograms. The AEP and PEP for leg 4 are indicated. The hatched areas indicate the accelerating phase of leg 4 strokes and the decelerating phase of leg 4 strokes is shown by the white areas.

legs is provided by the now classical Hughes (1952) rule for the timing of leg protraction, which states that no leg protracts until the one behind it is in a supporting position. The crayfish did not depart from this rule, but the mean inter-appendicular delay between the landing of one leg and the protraction of the preceding one was longer for legs 2 and 3 than for legs 4 and 5. In the smaller animals 1 and 2, this delay averaged between 220 and 250 ms in legs 2 and 3 and between 100 and 150 ms in legs 4 and 5. The delay was longer in the larger animals (320–450 ms in legs 2 and 3, and 160–280 ms in legs 4 and 5), but it always differed significantly between legs 2 and 3 and legs 4 and 5 (P<0.01). The delays in legs 3 and 4 were usually intermediate in length.

Patterns of acceleration

The study of the phase relationships allowed us to analyse the inter-leg coordination at a key point of the step cycle (the AEP), but it did not give information on coordination throughout the step cycle. However, information on the corresponding changes in acceleration in adjacent legs proved to be of value for investigating coordination during the step cycle. We examined the timing of the acceleration changes in leg 3 in comparison with those occurring in leg 4 over the course of successive sequences performed by each animal. The histograms in Fig. 12 show the distribution of the timing of the changes in the acceleration occurring during successive step cycles in leg 3 compared with the related events in leg 4 of animal 2 (Fig. 12A–D). This figure shows synchronizations at the AEP and PEP (Fig. 12B,D), but two other strong co-occurrences emerged, indicating a possible coupling at other stages of the step cycle.

The distribution of the power stroke deceleration (Fig. 12A) and that of the return stroke acceleration of leg 3 (Fig. 12B) were compared with the power stroke acceleration in leg 4. The latter corresponded to the inter-appendicular delay (Fig. 12B), showing that the power stroke of leg 4 preceded leg 3 protraction, as stipulated in the Hughes rule for leg protraction; but the former (Fig. 12A) indicated that the power stroke of

leg 4 also occurred just before or at the same time as the deceleration of the power stroke in leg 3. This result suggests that the relationship between legs 3 and 4 not only invoked the precedence of the power stroke in leg 4 over the return stroke in leg 3, but that the deceleration of the power stroke of leg 3 was already synchronized with the beginning of the power stroke of leg 4. Such correlations could be the result of mechanical coupling.

As shown in Fig. 12D, the power stroke of leg 3 accelerated concomitantly with the return stroke of leg 4. These events occurred at the AEP of leg 3 and at the PEP of leg 4, respectively. Their correlation therefore confirms the importance of these extreme points in inter-leg coordination. But Fig. 12C also shows a correlation between the deceleration of the return stroke of leg 3 and that of the power stroke of leg 4. A change in the acceleration in one leg must necessarily occur at about this phase value, even if there is no special coupling, but the high frequency of this simultaneous change in acceleration in spite of relative variations in parameters for both legs suggests, nevertheless, that some supplementary coupling may exist.

Interestingly, two of these events were related neither to the AEP nor to the PEP, sustaining the idea that inter-leg coordination is additionally controlled at other stages in the step cycle. However, the fact that the histogram distributions were quite widely scattered (the S.D. of the synchronisation of acceleration changes was about 200 ms for animal 2) rules out the possibility that there is a direct coupling between the events occurring in the different legs. Furthermore, the nature of the concurrent events in the legs was not consistent between animals. It is probable, therefore, that a change in the motor command in one leg does not condition the nature of the command sent to the adjacent leg, but affects the motor command strongly enough for that leg to induce a change. The nature of that change is not determined by the influencing leg but may depend instead on the state of the receiving leg.

Discussion

The aim of our study was twofold: to characterize the various locomotion patterns recorded from 17 animals and to analyse in detail the angular leg positions recorded from four animals (two small and two large). This involved the analysis of a large number of sequences and step cycles. This is the first time that the locomotor activity relating to a given behaviour has been investigated in crustaceans. As mentioned above, the advantage of this procedure was that the animals produced oriented movements. Although our main goal was to characterize crayfish locomotion in general, inducing this behaviour pattern was a means of obtaining rectilinear trajectories. This kinematic study was carried out without invasive procedures to prevent any possible artefacts that could occur with implanted electrodes. We will now compare these data with those obtained previously for the crayfish and other arthropods. We will first review the organization of the step in general and then discuss inter-leg coordination.

Crayfish leg movements

Crayfish locomotion has been extensively studied at the level of the gait in free sequences and, more recently, on a treadmill. The previous studies on gait were largely descriptive, even though they demonstrated the existence of a fundamental alternating pattern occurring at different speeds in different animals (Clarac and Barnes, 1985). Although treadmill conditions differ from those of free locomotion, this approach has been very useful for defining some mechanisms of coordination. Speed, for instance, can be increased over a large range by the experimenter and allows comparison of return stroke and power stroke evolution. The walking speed in our experiment corresponds approximately to the mean speed (6–10 $cm\,s^{-1}$) for animals walking on a treadmill (Müller and Cruse, 1991*a*), but variations in walking speed were too small for systematic investigation of their effects.

On the treadmill, the central state of the animal is not known: it can be either active and exert forces onto the belt or passive, in which case the leg will follow the belt. The animal is often fixed by its back and, although the posture can be adjusted, it is not normal and the visual feedback due to the displacement and the proprioceptive leg responses are unlikely to be the same as in free walking. Contact with the ground is also somewhat hazardous and some authors have used a slippery surface as a stimulus when characterizing leg coordination (Barnes, 1977; Graham, 1985). In the present study, we performed a detailed analysis of a single locomotory behaviour in freely moving crayfish, and not during exploratory behaviour, as in two previous studies (Pond, 1975; Grote, 1981) on freely walking crayfish, or on a treadmill (Cruse and Müller, 1986; Müller and Cruse, 1991*a*). Pond (1975) and Grote (1981) were mainly interested in comparing locomotion in and out of water and in comparing walking patterns before and after amputation. In several studies in which a walking leg was autotomized, it was observed that the legs show great plasticity and can replace each other in locomotion. Although this is true, and provides a nice model of adaptation, the results from our study show that each walking leg has a defined role. The leg trajectories recorded (Fig. 3) show that each leg has its own dynamic pattern, possibly due to the angle of insertion of the leg into the thorax. Under these conditions, only leg 5 has a pushing activity while legs 4, 3 and 2 pull the body forward. Leg 4 was shown to play a major role in locomotion. These results are consistent with the force measurements of Klärner and Barnes (1986), who showed that each leg had a different function during walking in the crayfish: legs 3 lift and stabilise the body and legs 4 share these functions and produce the largest proportion of the propulsive force.

Under our experimental conditions, three main parameters were used to define each leg movement, namely the leg excursion and the timing and direction of the acceleration. Leg 4 appeared to be dominant, owing to its larger strides and precise and reproducible acceleration. Comparing the various strides with data from other authors, we found differences in the amplitude recorded in the different studies. The values presented here are in the same range as those obtained in the

treadmill experiments with rock lobsters (Clarac, 1984) and crayfish (Müller and Cruse, 1991*a*), where the actual stride amplitude was measured. Our values cannot be directly compared with the average distance travelled per step measured for free-moving crayfish (Pond, 1975; Grote, 1981) as, in these studies, the leg position was measured in relation to external cues and included the body shift occurring during the leg swing. As suggested by Clarac (1984), the leg excursion value is quite stable both in the same animal over a single sequence and over different sequences or in different animals. It is striking, however, that legs 4 and 5 can have very different movement amplitudes. A continuous adaptation is required to maintain a 1:1 coordination. Other studies have often reported sequences where relative coordination occurred between legs 4 and 5 and between the two legs 5 (see Clarac and Chasserat, 1983).

The dynamic data confirmed the importance of leg 4 and the quite different role of leg 5. As demonstrated previously, two important points in the step cycle, the AEP and the PEP, were observed. These correspond to possible coordination sites. The pattern of acceleration confirms the hypothesis that they may participate in determining inter-leg synchronisation.

Our data show the stereotypy of the walking pattern and that legs 3 and 4 are the main legs used during locomotion, although legs 4 and 5 were also sometimes used (see Fig. 6). These findings are in agreement with the model presented by Müller and Cruse (1991*b*) and raise the question of possible inter-leg coordination.

Inter-leg coordination

Extensive studies have been carried out recently on the leg coordination of crustaceans (Barnes, 1975, 1977; Clarac and Coulmance, 1971; Clarac, 1981, 1984; Cruse and Müller, 1986; Müller and Cruse, 1991*a*,*b*). In the rock lobster *Jasus lalandii*, it has been demonstrated, using a double treadmill technique, where an animal walks at a different speed on the right and the left sides, that the animal adapts using the return stroke speed and the stride amplitude as an effective mechanism for maintaining 1:1 coordination. It has therefore been demonstrated using several approaches that ipsilateral coordination is stronger than contralateral coordination, although the latter can also be quite efficient.

Ipsilateral relationships

In ipsilateral coordination, rostrally directed and caudally directed connections are responsible for maintaining a delay in the coordination. Ipsilateral coordination has been shown to be based upon a limited number of key events involving proprioceptive feedback associated with the extreme leg positions (i.e. AEP and PEP; Chasserat and Clarac, 1980; Cruse and Müller, 1986). Cruse and Müller (1986) observed two coordinating processes functioning in crayfish. The first was rostrally directed: a leg maintained the preceding one in the swing phase as long as it performed a power stroke. The second was a caudally directed effect: it occurred at the end of the power stroke, lasted for up to 200 ms after the beginning of the swing and influenced the return of the following leg with increasing intensity. This 200 ms delay corresponds approximately to the duration of the acceleration phase of the return stroke in the present study. It therefore seems possible that the return stroke may influence the following leg together with the motor command (see Cattaert *et al*. 1993).

These two mechanisms satisfactorily describe the coordination of treadmill locomotion. They, nevertheless, probably operate in addition to other supplementary (or redundant) mechanisms, such as the inhibitory ascending and excitatory descending influences observed, for instance, in rock lobsters (Chasserat and Clarac, 1983).

Detailed examination of related changes of acceleration in adjacent legs showed that changes often occurred simultaneously in both legs over the course of the step cycle. Although these correlations could arise artificially from mechanical coupling, they could also support the assumption that the synchronisation was maintained throughout the whole step cycle by a mechanism whereby each change in the motor command to one leg influenced the adjacent leg strongly enough to induce a change in that leg also. The dispersion of the distributions precludes the possibility that direct interactions occurred between the legs, but we can nevertheless conclude that the commands sent to motor units in one leg are concomitant with related commands sent to the adjacent legs.

Contralateral relationships

The coupling between contralateral legs is often described as being variable and as having a weaker influence than ipsilateral coupling (for reviews, see Clarac, 1982; Clarac and Barnes, 1985). In most studies on decapods, the contralateral legs have been shown to move alternately with a phase value of about 0.5 (see Table 2 in Clarac and Barnes, 1985), but the inter-leg coupling depends upon the conditions under which the movements were observed. In the crayfish *Astacus leptodactylus* moving on a treadmill, Cruse and Müller (1991*a*) reported alternate contralateral movements, while Pond (1975) reported that freely moving *Austropotamobius pallipes* moved legs 2, 3 and 4 in phase and legs 5 alternately (although this author did not indicate how this was calculated). Barnes (in Clarac, 1982; Clarac and Barnes, 1985) reported that, in *Astacus leptodactylus*, alternating contralateral leg movements occurred during treadmill locomotion, whereas during free movement in a tank, this coupling was very loose, in some cases showing a bimodal distribution. Different coupling types were used by different crayfish and also by the same individuals at different times. Clarac and Barnes (1985) concluded that the coupling was not so much weak as variable, since contralateral pairs of legs were characterized by transient patterns of coordination which differed from one bout of walking to another.

In the present study, we report the co-existence of both alternate and in-phase patterns and support the contention that freely moving crayfish are capable of producing different coupling patterns, whereas in constrained situations, involving loading or moving on a treadmill, only one pattern has been

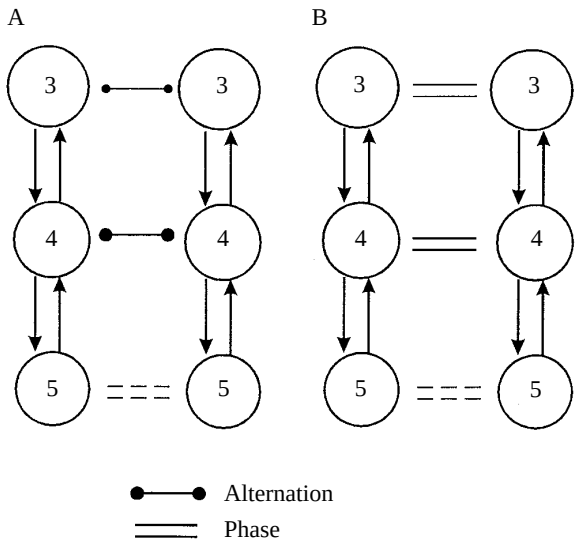

Fig. 13. Two possible coupling mechanisms in the legs of the freely moving crayfish *Procambarus clarkii*. In all cases, an ipsilateral metachronal wave dominated the coordination (arrows). A second strong coupling moved legs 4 either alternately (A) or in phase (B); both patterns are frequent in crayfish moving freely under water. Legs 3 adopted the same pattern of coupling as legs 4 as the result of the ipsilateral coupling between these legs. Legs 5 tended to move in phase, but each was controlled by its ipsilateral neighbour, leg 4. Moreover, legs 5 produced only a relative coordination with their adjacent legs.

described in which they move their legs alternately. The fact that a variety of patterns have been described in several species suggests that the existence of these different patterns is not due to species-related characteristics of locomotion, but results from the locomotion conditions, though the reason for this disparity between free and treadmill walking has not yet been established.

Contralateral coupling has been analysed in the rock lobster *Jasus lalandii* (Clarac, 1984) and the crayfish *Astacus leptodactylus* (Müller and Cruse, 1991*a*) in the course of split-treadmill experiments. The results showed that these animals are capable of compensating, at least partially, for a unilateral change in belt speed. This adjustment can be variable, giving either an absolute or a relative coupling of the legs in the new phase. Müller and Cruse (1991*b*) assumed that the legs on both sides acted like two mutually coupled oscillators with intrinsic frequencies, one side being dominant as a result of the endogenous asymmetry between their mutual coupling. Increasing the belt speed on the side of the dominant leg resulted in absolute coordination, whereas lowering the speed resulted in relative coordination. As shown by Müller and Cruse (1991*a*, their Fig. 4), the normal phase value (approximately 0.5) temporarily stabilised at around 0.8 when a change in the belt speed imposed relative coordination. Thus, it is possible that, in the present study, the legs on both sides may have oscillated with a relative coordination and may therefore have been either temporarily in phase, in opposition or in an unstable state, in relation to their own frequency.

Müller and Cruse (1991*a*) have pointed out that the coupling was stronger in legs 4 and 5. This is consistent with the strong coordination we found in legs 4. We assumed that the contralateral coupling of legs 4 was a strong characteristic of locomotion and that this feature was related to the prominent role of that leg in locomotion. Contralateral coupling of the other legs was assumed to be indirect and probably to depend on ipsilateral coordination of movements. Our data suggest that ipsilateral relationships dominate during free walking. The ipsilateral relationships were similar, whatever those between the contralateral pair; that is, ipsilateral coordinations persist whatever the signal delivered by the contralateral leg.

The two main locomotor patterns found in this study are summarized in Fig. 13. It seems likely that both sides act independently, as chains of coupled oscillators, but are connected at the level of leg 4, so that they can move either in phase or alternately. Whether the coordination between legs 4 is centrally directed or results from mechanical effects arising from locomotion itself is, at present, unknown. Further research is in progress to analyse the leg movements with a view to understanding the switch between the two inter-leg patterns.

References

AYERS, J. L. AND DAVIS, W. J. (1977). Neural control of locomotion in the lobster, *Homarus americanus*. I. Motor programs for forward and backward walking. *J. comp. Physiol.* **115**, 1–27.

BARNES, W. J. P. (1975). Leg co-ordination during walking in the crab, *Uca pugnax*. *J. comp. Physiol.* **96**, 237–256.

BARNES, W. J. P. (1977). Proprioceptive influences on motor output during walking in the crayfish. *J. Physiol., Paris* **73**, 543–564.

BARNES, W. J. P., SPIRITO, C. P. AND EVOY, W. H. (1972). Nervous control of walking in the crab, *Cardisoma guanhumi*. II. Role of resistance reflexes in walking. *Z. vergl. Physiol.* **76**, 16–31.

BETHE, A. (1897). Vergleichende Untersuchungen über die Funktion des Zentral-nervensystems der Arthropoden. *Pflügers Arch.* **68**, 449–545.

BURROWS, M. AND HOYLE, G. (1973). The mechanism of rapid running in the ghost crab *Ocypode ceratophthalma*. *J. exp. Biol.* **58**, 327–349.

CATTAERT, D., BARTHE, J. Y. AND CLARAC, F. (1993). Sensory–motor coordination in crustacean limbs during locomotion. In *Interlimb Coordination: Neural, Dynamical and Cognitive Constraints* (ed. S. Swinnen, H. Heuer, J. Massion and P. Casaer), pp. 49–73. San Diego: Academic Press Inc.

CHASSERAT, C. AND CLARAC, F. (1980). Interlimb coordination factors during driven walking in Crustacea. A comparative study of absolute and relative coordination. *J. comp. Physiol.* **39**, 293–306.

CHASSERAT, C. AND CLARAC, F. (1983). Quantitative analysis of walking in a decapod crustacean, the rock lobster *Jasus lalandii*. II. Spatial and temporal regulation of stepping in driven walking. *J. exp. Biol.* **107**, 219–243.

CHASSERAT, C. AND CLARAC, F. (1986). Basic processes of locomotor coordination in the rock lobster. II. Simulation of leg coupling. *Biol. Cybernetics* **55**, 171–185.

CLARAC, F. (1981). Postural reflexes coordinating walking legs in a rock lobster. *J. exp. Biol.* **90**, 333–337.

CLARAC, F. (1982). Decapod crustacean leg coordination during

walking. In *Locomotion and Energetics in Arthropods* (ed. C. F. Herreid and C. R. Fourtner), pp. 31–71. New York, London: Plenum Publishing Corporation.

CLARAC, F. (1984). Spatial and temporal co-ordination during walking in crustacea. *Trends Neurosci*. **7**, 293–298.

CLARAC, F. AND BARNES, W. J. P. (1985). Peripherical influences on the coordination of the legs during walking in decapod crustaceans. In *Coordination in Motor Behaviour* (ed. B. M. H. Bush and F. Clarac), pp. 249–269. Cambridge: Cambridge University Press.

CLARAC, F. AND CHASSERAT, C. (1983). Quantitative analysis of walking in a decapod crustacean, the rock lobster *Jasus lalandii*. I. Comparative study of free and driven walking. *J. exp. Biol*. **107**, 189–217.

CLARAC, F. AND CHASSERAT, C. (1986). Basic processes of locomotor coordination in the rock lobster. I. Statistical analysis of walking parameters. *Biol. Cybernetics* **55**, 159–170.

CLARAC, F. AND COULMANCE, M. (1971). La marche latérale du crabe (*Carcinus*): coordination des mouvements articulaires et régulation proprioceptive. *Z. vergl. Physiol.* **73**, 408–438.

CLARAC, F. AND CRUSE, H. (1982). Comparison of forces developed by the legs of the rock lobster when walking free on a treadmill. *Biol. Cybernetics* **43**, 109–114.

CRUSE, H. (1979). The control of the anterior extreme position of the hindleg of a walking insect, *Carausius morosus*. *Physiol. Ent.* **4**, 121–124.

CRUSE, H. AND MÜLLER, U. (1986). Two coupling mechanisms which determine the coordination of ipsilateral legs in the walking crayfish. *J. exp. Biol.* **121**, 349–369.

CRUSE, H. AND SAXLER, G. (1980). Oscillations of force in the standing leg of a walking insect, *Carausius morosus*. *Biol. Cybernetics* **36**, 159–163.

DELCOMYN, F. (1971). The locomotion of the cockroach *Periplaneta americana*. *J. exp. Biol.* **54**, 443–452.

EVOY, W. H. AND FOURTNER, C. R. (1973). Nervous control of walking in the crab *Cardisoma guanhumi*. III. Proprioceptive influences on intra- and intersegmental coordination. *J. comp. Physiol*. **83**, 303–318.

FULL, R. J., BLICKHAN, R. AND TING, L. H. (1991). Leg design in hexapedal runners. *J. exp. Biol.* **158**, 369–390.

GRAHAM, D. (1985). Pattern and control of walking in insects. *Adv. Insect Physiol.* **18**, 31–140.

GROTE, J. R. (1981). The effect of load on locomotion in crayfish. *J. exp. Biol*. **92**, 277–288.

HERREID, C. F. AND FOURTNER, D. R. (1981). *Locomotion and Energetics in Arthropods*. London, New York: Plenum Press. 546pp.

HUGHES, G. M. (1952). The co-ordination of insect movements. I. The walking movements of insects. *J. exp. Biol.* **29**, 267–284.

KLÄRNER, D. AND BARNES, W. J. P. (1986). The cuticular stress detector (CSD2) of the crayfish. II. Activity during walking and influences on the leg coordination. *J. exp. Biol*. **122**, 161–175.

LIBERSAT, F., CLARAC, F. AND ZILL, S. (1987*a*). Force-sensitive mechanoreceptors of the dactyl of the crab: single-unit responses during walking and evaluation of function. *J. Neurophysiol*. **57**, 1618–1637.

LIBERSAT, F., ZILL, S. AND CLARAC, F. (1987*b*). Single-unit responses and reflex effects of force sensitive mechanoreceptors of the dactyl of the crab. *J. Neurophysiol*. **57**, 1601–1617.

MÜLLER, U. AND CLARAC, F. (1990*a*). Dactyl sensory influences on rock lobster locomotion. I. Intrasegmental and intersegmental leg reflexes during standing and walking. *J. exp. Biol*. **148**, 89–112.

MÜLLER, U. AND CLARAC, F. (1990*b*). Dactyl sensory influences on rock lobster locomotion. II. Role in interleg coordination. *J. exp. Biol.* **148**, 113–128.

MÜLLER, U. AND CRUSE, H. (1991*a*). The contralateral coordination of walking legs in the crayfish *Astacus leptodactylus*. I. Experimental results. *Biol. Cybernetics* **64**, 429–436.

MÜLLER, U. AND CRUSE, H. (1991*b*). The contralateral coordination of walking legs in the crayfish *Astacus leptodactylus*. II. Model calculations. *Biol. Cybernetics* **64**, 437–446.

POND, C. M. (1975). The role of the walking legs in aquatic and terrestrial locomotion of the crayfish *Austropotamobius pallipes* (Lereboullet). *J. exp. Biol.* **62**, 447–454.

RUNION, H. I. AND USHERWOOD, P. N. R. (1966). A new approach to neuromuscular analysis in the intact free walking insect preparation. *J. Insect Physiol*. **12**, 1255–1263.

VON HOLST, E. (1935). Die Koordination der Bewegung bei den Arthropoden in Abhängigkeit von zentralen und peripheren Bedingungen. *Biol. Rev*. **10**, 234–261.

WILSON, D. M. (1966). Insect walking. *A. Rev. Ent.* **11**, 103–122.