KINEMATICS OF ROTATION IN PLACE DURING DEFENSE TURNING IN THE CRAYFISH *PROCAMBARUS CLARKII*

NEWTON H. COPP[1],* AND MARC JAMON[2]

[1]*Joint Science Department, The Claremont Colleges, Claremont, CA 91711, USA* and [2]*Laboratoire de Développement et Pathologie du Mouvement, CNRS, 31 Chemin Joseph Aiguier, 13402 Marseille Cedex 20, France*

*e-mail: ncopp@jsd.claremont.edu

Summary

The kinematic patterns of defense turning behavior in freely behaving specimens of the crayfish *Procambarus clarkii* were investigated with the aid of a video-analysis system. Movements of the body and all pereiopods, except the chelipeds, were analyzed. Because this behavior approximates to a rotation in place, this analysis extends previous studies on straight and curve walking in crustaceans.

Specimens of *P. clarkii* responded to a tactile stimulus on a walking leg by turning accurately to face the source of the stimulation. Angular velocity profiles of the movement of the animal's carapace suggest that defense turn responses are executed in two phases: an initial stereotyped phase, in which the body twists on its legs and undergoes a rapid angular acceleration, followed by a more erratic phase of generally decreasing angular velocity that leads to the final orientation. Comparisons of contralateral members of each pair of legs reveal that defense turns are affected by changes in step geometry, rather than by changes in the timing parameters of leg motion, although inner legs 3 and 4 tend to take more steps than their outer counterparts during the course of a response. During the initial phase, outer legs 3 and 4 exhibit larger stance amplitudes than their inner partners, and all the outer legs produce larger stance amplitudes than their inner counterparts during the second stage of the response. Also, the net vectors of the initial stances, particularly, are angled with respect to the body, with the power strokes of the inner legs produced during promotion and those of the outer legs produced during remotion. Unlike straight and curve walking in the crayfish, there is no discernible pattern of contralateral leg coordination during defense turns. Similarities and differences between defense turns and curve walking are discussed. It is apparent that rotation in place, as in defense turns, is not a simple variation on straight or curve walking but a distinct locomotor pattern.

Key words: crayfish, *Procambarus clarkii*, locomotion, kinematics, turning, orientation, defence.

Introduction

The biomechanics of walking locomotion have been extensively studied in various species of arthropod. The majority of these studies concern straight walking on treadmills (e.g. crayfish, Clarac and Barnes, 1985; Cruse and Müller, 1986; Müller, 1990; lobster, Clarac, 1981; Chasserat and Clarac, 1983; rock lobster, Clarac and Barnes, 1985; fiddler crab, Barnes, 1975; cockroach, Delcomyn, 1971; Hughes, 1951; stick insect, Cruse, 1985; Cruse, 1990; Bässler, 1993). These investigations have delimited the characteristics of the neural control system underlying the observed patterns of leg movement (e.g. Bässler, 1993; Clarac, 1984; Delcomyn, 1980; Müller and Cruse, 1991). The types of leg coordination observed in straight walking and the implications for the neural basis of this coordination have been expanded, however, by more recent studies of freely moving specimens of the crayfish *Procambarus clarkii* (Jamon and Clarac, 1995). It is important to compare freely behaving animals with animals walking on a treadmill and to investigate patterns of locomotion other than straight walking to extend our understanding of arthropod locomotion. The few existing studies on rotational locomotion in crustaceans, for example, indicate patterns of leg coordination that could not be anticipated from studies of straight walking (e.g. Cruse and Silva Saavedra, 1996; Domenici et al., 1999).

Rotational locomotion involves a variety of situations differing in the assumed level of the rotational component. One case is that in which animals exhibit a single change in heading at the beginning of or during a walking sequence (e.g. Camhi and Levy, 1988; Graham, 1972; Strauß and Heisenberg, 1990; Zolotov et al., 1975). In this case, a rotational component is temporarily added to the translational movement of the body. A second case is that of curve walking, in which both rotational and translational components are continuously present while the animal walks along a curved path (e.g. Cruse and Silva Saavedra, 1996; Jander, 1985; Jindrich and Full, 1999; Zollikofer, 1994; Zolotov et al., 1975). At one extreme lies

pure rotational locomotion, corresponding to rotation in place (e.g. Land, 1972; Zolotov et al., 1975; Bell and Schal, 1980; Franklin et al., 1981; Copp and Watson, 1988).

Rotation results from changes in the timing and/or geometry of the stepping pattern of one or more walking legs (Franklin et al., 1981). Changes in step geometry rather than changes in the timing or frequency of steps usually produce gently curved trajectories. In particular, the legs on the inner and outer sides of the curve frequently differ in the relative amplitude of their steps (stick insect, Jander, 1985; fruit flies, Strauß and Heisenberg, 1990; ants, Zollikofer, 1994) and also in the direction of the leg's thrust (Zolotov et al., 1975). Changes in step frequency have been observed in curve-walking insects (honeybees, Zolotov et al., 1975; stick insect, Jander, 1985), but are apparently not common. The same strategies may come into play as specimens turn through increasingly small radii or rotate in place (e.g. cockroaches, Franklin et al., 1981), but other features, such as backward walking (e.g. fruit flies, Strauß and Heisenberg, 1990) or oppositely directed power strokes of contralateral legs (e.g. honeybees, Zolotov et al., 1975), may be added. In the extreme case, when the animal simply rotates in place, rotation can be achieved without making fundamental changes in the pattern of limb movement from that employed in straight walking; the legs on the outside of the turn simply walk forwards while those on the inside walk backwards. Land (Land, 1972), for example, showed that jumping spiders use this pattern while turning to face prey.

The degree to which crustaceans resemble insects in the mechanics of rotational locomotion behavior is unclear because there have been very few studies of such behavior in this group. Analyses of leg movements during curve walking by the crayfish (Cruse and Silva Saavedra, 1996; Müller, 1990) have revealed that, as in insects, changes in step geometry (the trajectory of stance phases and step amplitude) predominate in causing changes in the direction of locomotion. These results were obtained from restrained animals walking on a treadmill in a simulated curve-walking pattern triggered by an optomotor reflex. The results of a more recent kinematic analysis of curve walking in freely behaving crayfish (Domenici et al., 1998) are generally consistent with the earlier findings but differ in several specific and interesting ways. In freely behaving crayfish, leg geometry changes in a systematic way during a curve-walking sequence. Furthermore, rotation is discontinuous. Increases in angular velocity are correlated with the coordinated stances of inner legs 2 and 5 and outer leg 4 acting in a tripod. Apparently, all the legs do not contribute equally to the rotational component of curve walking, and outer leg 4 probably plays the major role in producing the rotation (Domenici et al., 1999).

We report here a series of observations on rotational locomotion in freely behaving specimens of *Procambarus clarkii*. Specimens of the crayfish *P. clarkii* respond to a pinch to a pereiopod with a yaw motion that approximately realigns the body to face the point in space at which the stimulus was delivered. This type of response has been termed a 'defense turn' because it often precedes and serves to direct a defense

posture towards a potential opponent (Copp and Jamon, 2000). Defense turns are not free from translational elements, but the radius of curvature is much smaller than in curve walking such that it approaches rotation in place. The reduction of the translational element in this behavior compared with curve walking means that defense turns need not be accompanied by the same mechanics or utilize exactly the same neural control system as curve walking. Indeed, our results show that rotation in place by the crayfish features different leg geometry, altered step timing and weak leg coordination compared with curve walking. The implications of these differences for the underlying neural control system are discussed.

Materials and methods

Specimens of the crayfish *Procambarus clarkii* (Girard) were obtained from a local supplier, maintained collectively in freshwater tanks at approximately 15 °C, and fed with pieces of dried dog food twice per week. Males and females were used at random. The specimens averaged 44.3±4.7 mm (mean ± s.e.m., N=4) in carapace length. Only animals with all pereiopods intact were used in this study.

Prior to a videotaping session, each specimen was decorated with small dots of white paint to highlight 12 selected anatomical points for digitizing: the dactyl of each pereiopod including the chelipeds, and two points to define the dorsal midline of the carapace (one on the rostrum and one at a position projected dorsally from the midpoint between the bases of the fourth pereiopods). Each leg was treated as a single line segment connecting the tip of the leg with the designated 'center', i.e. the more posterior of the two points on the dorsal carapace (Fig. 1B,E). This method introduced an error in the determination of changes in leg angle because the designated 'center' does not project vertically onto the thoraco-coxopodite (T-C) joints, where the movement of interest is occurring, but is medial to them and at slightly varying distances from them. This error is not considered serious given that the major comparisons of leg motions made in this study are between contralateral members of a pair in which the magnitude of the error is the same. Treatment of the leg as a single line segment focuses attention on promotion and remotion of the leg, but ignores flexion and extension movements about the meropodite–carpopodite (M-C) joint. This is reasonable because promotion and remotion reflect activity primarily at the T-C joints, and these joints play a significant role in determining the orientation of walking (Clarac, 1984).

Decorated specimens of *P. clarkii* were placed in a plastic, hemispherical arena and covered to a depth of approximately 9 cm with water at room temperature (22 °C). The floor of the arena consisted of a 25 cm diameter plastic disc that had been covered with fine gravel and painted 'flat' black to provide traction for the animal and a favorable background for video recording. The animal's activity was restricted to a circle 18.3 cm in diameter by a short plastic cylinder placed in the center of the arena.

Defense turns are elicited naturally when one specimen of

P. clarkii uses a cheliped to pinch a walking leg of another crayfish. This situation was mimicked by using a pair of long-handled forceps to pinch one of the fifth pereiopods at the M-C joint or on the carpopodite just below the M-C joint. Stimuli were delivered when the animal was standing motionless and, as often as possible, with its pereiopods extended laterally in a normal stance posture. After a pinch had been delivered, the forceps were withdrawn slightly, to avoid further contact with the animal, but otherwise left in position to provide a visual target for the orienting response (Copp and Watson, 1988). Stimuli other than a pinch do not elicit turning responses as reliably. Unfortunately, a pinch stimulus is difficult to standardize completely. Partial standardization was achieved by placing a 'stop' between the blades of the forceps. A stimulus consisted of positioning the forceps on the leg without touching it, closing them to the 'stop' and releasing quickly.

Defense turns were recorded on videotape at $30\,\mathrm{frames\,s^{-1}}$ by a Panasonic AG455 video camera positioned directly above the center of the arena. Illumination was provided by two 60 W incandescent bulbs placed above and on opposite sides of the arena. The field recorded by the camera completely encompassed the field within which the animal could move (circular, approx. 18 cm). An attempt was made to reduce error due to parallax by waiting until the animal was positioned near the center of the field before delivering a stimulus. Any responses in which the animal contacted the wall of the arena were rejected from the analysis.

Movement of the body

A survey of selected aspects of defense turning behavior was assembled from 116 videotaped responses produced by four specimens of *P. clarkii*. Data for this overview were collected by replaying the videotape on a video recorder with stop-motion capabilities and tracing, onto acetate sheets, the position of the dorsal midline segment at the beginning and end of the response. The location at which the pinch was delivered was also recorded. A rotational response was recognized to have begun when the midline of the body as viewed in one video frame was no longer parallel to that midline as viewed in the immediately preceding video frame. The onset of rotational responses was considered to be the frame prior to the one marking this first rotational movement and was thus determined with a resolution of 0.033 s. Rotational responses were defined to have ended when any one of three events occurred: (i) the animal ceased moving for a minimum of approximately 0.1 s (three consecutive video frames); (ii) the animal reversed direction; or (iii) the animal ceased rotating and began translating forwards, backwards or sideways. Twenty-five responses, from four specimens of *P. clarkii*, were selected for more detailed analysis. These responses provided the clearest visibility of the markers throughout most, if not all, of the response.

The accuracy of the turning responses was defined in two ways (Fig. 1). The 'actual error' (α) represents the angle between the final direction of the longitudinal axis (DD) of the animal's body and the line linking the designated center with
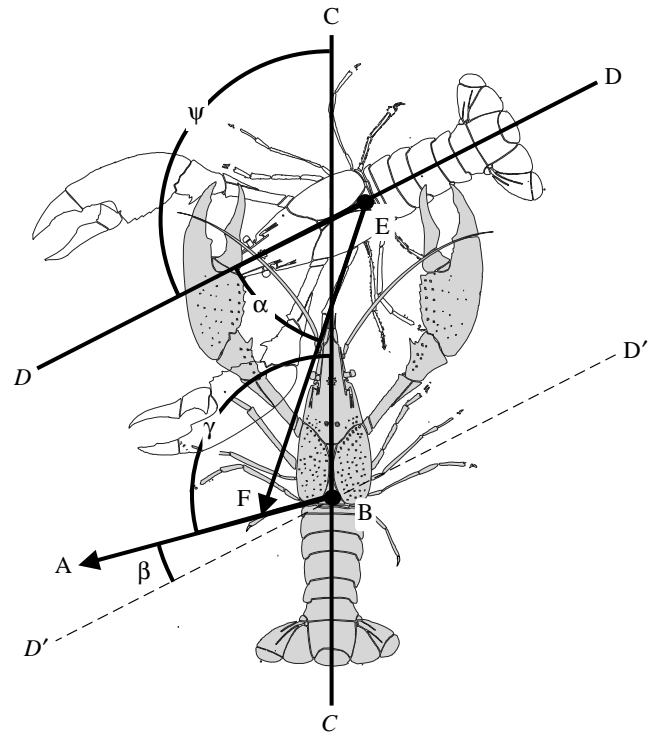

Fig. 1. Schematic diagram of one possible defense turning response to illustrate reference points and angles referred to in the text. A, target direction; CC, midline body axis at initial position (shaded, vertically oriented crayfish); B, posterior marker and assumed center of rotation at initial position; DD, midline body axis at final position (unshaded crayfish); D$'$D$'$, a line parallel to DD, but through B; E, posterior marker and assumed center of rotation at final position; F, point at which stimulus was delivered to fifth walking leg; BE, translation of assumed center of rotation; α, actual error; β, rotational error (response angle minus stimulus angle); γ, stimulus angle; ψ, response angle. In this hypothetical example, the response angle (ψ) exceeds the stimulus angle (γ), producing a positive rotational error (β), while the actual error (α) would be given a negative sign to indicate undershooting of the target by the animal.

the point in space where the stimulus had been applied (EF). The actual error thus includes the effects of axial and lateral translation (BE) of the body. The 'rotational error' (β) indicates the difference between the stimulus angle (γ), defined by the line (AB) through the point of stimulation (F) and the assumed center of rotation (B) and the longitudinal axis of the animal's body immediately before the onset of the turning motion (CC), and the angle (ψ) through which the longitudinal body axis rotated during the response, hereafter referred to as the response angle *(Fig. 1)*. These errors were normalized with respect to the stimulus angle (γ). Negative errors indicate undershooting of the target by the specimen, and positive error values indicate overshooting. The 'average rotation rate' was determined as the response angle (ψ) divided by the response duration in seconds.

The movement of the body during defense turns by specimens of *P. clarkii* approximates to a rotation in place but includes a translational component as well. These two
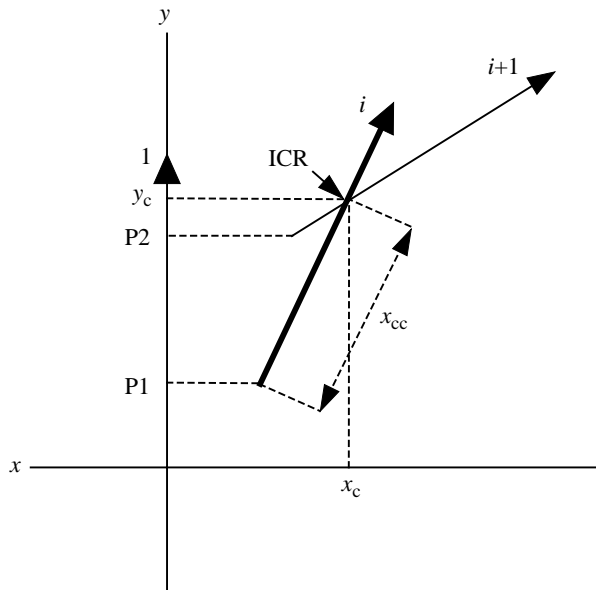
Fig. 2. Schematic diagram to illustrate how the instantaneous center of rotation (ICR) was determined from successive video frames. A system of coordinates is established with reference to the animal's body axis (bold arrow 1) just prior to the onset of a turning response. The y-axis runs along the body's midline with the arrow indicating the animal's carapace (arrowhead, rostrum; origin, the posterior marker as shown in Fig. 1B,E). A line perpendicular to the y-axis through the posterior marker indicates the x-axis. A possible position of the body in a subsequent frame is shown by the bold arrow marked i and the body's position in the subsequent video frame is shown by arrow $i+1$. Changes in the y-axis position of the posterior marker (e.g. P1 to P2) are used to determine whether translation has occurred (see text). The intersection of arrows i and $i+1$ is defined as the ICR with coordinates x_c and y_c. The distance of the ICR from the posterior marker on the carapace of the first of the pair of images is shown as x_{cc}.

components of the overall motion can be difficult to separate. For example, a sideways motion would be pure translation if the lines describing the longitudinal body axis before and after the movement were perfectly parallel. Any slight deviation of these two lines from parallel, however, could be explained either as a translation superimposed on a rotation about a point on the body or as a pure rotation with the center of rotation positioned well anterior or posterior of the animal, depending on the inclination of the lines to each other. We have used the latter interpretation to analyse defense turns by estimating the changes in the 'instantaneous' position of the center of rotation. This was achieved by calculating the position, relative to the carapace in the first of two video frames, of the intersection between the two lines of the long axis of the body in two successive video frames (Fig. 2). First, a frame of reference was defined using the two carapace markers in the initial video field of a response. The position of the more posterior of the two carapace markers was set as the origin of this frame of reference, and the line linking the two markers defined the y axis of this reference frame. The coordinates for the two carapace markers in all subsequent video fields were

determined according to this frame of reference. The carapace was recognized to have moved between two successive video fields if at least one of the two carapace markers moved by at least 0.1 mm. If movement had occurred, the coordinates of the two carapace markers were used to calculate the slopes of the mid-body lines in each of two successive video fields. The movement of the carapace was considered to be a rotation when two successive slopes differed by at least 1°. This value was chosen empirically by comparing the results of the calculations with sequential plots of the mid-body line such as shown in Fig. 5A,D. The intersection between the two lines was defined as the instantaneous center of rotation (ICR). The coordinates of the ICR were determined and used to calculate its position along the line of the body axis in frame i before rotation has occurred as determined by the change in slope of the midline between frame i and frame $i+1$. If movement of the carapace was determined to have occurred but the two slopes did not differ by at least 1°, then the animal was considered to have undergone a translation. An axial translation was distinguished from a lateral translation by comparing the y intercepts of the two slopes. If the y intercepts differed by at least 0.3 mm, then the animal was said to have undergone a lateral translation. Otherwise, an axial translation was said to have occurred. This threshold value was chosen to just exceed the minimum resolution of the digitizing system, as described below. The results of the calculations accorded well with visual inspection of sequential plots of the mid-body lines.

Kinematics of the walking legs

In the more detailed analysis, motion-analysis systems by Ariel Dynamics (Ariel Performance Analysis System, APAS) and Peak Technologies Incorporated (Peak 5) were used to digitize the videotape images manually 'frame by frame'. The computerized systems permitted separate analysis of each of the two fields in a frame, yielding a temporal resolution of 16.7 ms corresponding to an overall rate of 60 fields s^{-1}. The raw data were digitally filtered with the cutoff frequency set at 8 Hz to reduce digitizing error. These data were then transferred to a spreadsheet for further analysis.

The precision and accuracy of the digitization were estimated according to the methods of Walton (Walton, 1986). For the Peak 5 system, multiple digitizations of four points yielded a mean standard deviation of 0.15 mm for both the x and y coordinates. The determination of x,y coordinates was somewhat less precise when the APAS system was used (standard deviations 0.18 mm and 0.27 mm respectively), probably because that software was coupled with a PAL system, although the videotapes had been recorded on an NTSC system, and the adaptation of one to another left a small residual jitter in the displayed image used in digitizing. Repeated digitizations of figures composed of known distances and angles revealed that these parameters were measured with an error of 0.28% and 0.6% respectively.

For the sake of convenience, the geometry of the stance phase of a leg's stepping motion is described below as if the leg were moving with respect to a stationary body. All measurements of

leg motion were made relative to a system of body-centered coordinates. In actuality, however, the body moves while each dactyl during its stance phases remains in a fixed position on the substratum. Thus, the amplitudes and directions shown for each leg represent the movement of the body axis relative to that leg during its stance, potentially indicating, therefore, the amplitude and direction of the force exerted by that leg on the body turn. The chelipeds were ignored in this study because, typically, they do not contact the substratum during a defense turn of a freely behaving specimen but swing towards the stimulated side of the animal. Several parameters of leg motion were determined from the movements of the dactyls: (i) step period; (ii) stance duration; (iii) duty factor; (iv) stance amplitude; and (v) the number of steps taken during a turning response. These parameters are defined below.

The stance phase of the motion of a particular leg was determined from changes in angle between the line segment defining that leg (hereafter referred to simply as the leg) and the midline of the body (hereafter referred to simply as the body) (Fig. 1). The anterior extreme point (AEP) is defined as the position of the dactyl at the moment that the angle between the leg and the body reached a minimum in a particular step. The posterior extreme point (PEP) for a particular step is defined as the position of the dactyl when the leg reached the maximum angle to the body in that step. A survey of turning sequences recorded from restrained specimens standing on a turntable revealed only three instances in 214 steps in which the legs ipsilateral to the pinch (hereafter referred to as the inner legs) were not unambiguously in contact with the substratum during promotion. Thus, during rotation by the crayfish, the inner legs are in the power stroke during promotion (i.e. from PEP to AEP), while the legs contralateral to the pinch (hereafter referred to as the outer legs) exert their power stroke during remotion (i.e. from AEP to PEP). This situation could lead to confusion in interpretation of the terms AEP and PEP. In forward walking, the AEP indicates the onset of the stance phase as well as denoting the position of the leg. The AEP has been recognized as a key point for the coordination of ipsilateral legs during forward walking (Clarac, 1984; Müller, 1990; Müller and Clarac, 1990; Müller and Cruse, 1991). In defense turns by specimens of *P. clarkii*, the AEP does not indicate the onset of stance phase in both sets of legs, but the acronyms AEP and PEP will continue to be used because they remain convenient and widely used descriptions of leg position.

Step period constitutes the duration of one complete step cycle measured from the onset of the power stroke (Müller and Clarac, 1990), i.e. from AEP to AEP in the outer legs and from PEP to PEP in the inner legs. Stance duration was determined as the time from the onset of a stance to the onset of the return stroke, as viewed from above. The duty factor is the ratio of the stance duration to the step period. The direction and amplitude of the apparent motion (see above) of each leg during its stance phases were summarized in a net vector calculated from the Cartesian coordinates (relative to the animal's body) of the two extreme points (Cruse and Silva Saavedra, 1996), i.e. AEP to

PEP for outer legs and PEP to AEP for inner legs. Stance amplitude is defined as the length of this net vector. The motion of the animal's carapace was determined in relation to a stationary reference point in the camera's field. The x,y coordinates for the two points on a specimen's dorsal midline were measured with reference to the 'external' coordinate system defined for the motion-analysis system.

An 'index of leg synchrony' (ILS) was calculated as a measure of coordination among the walking legs at the outset of a response. Presumably, the initial acceleration of the body would be related to the number of legs that entered into a prolonged stance phase at the onset of the rotation. The index was calculated as the mean latency from the onset of the rotational response to the onset of the first 'prolonged' stance phase of all legs. Because there was some imprecision in using an overhead camera view of leg angle to determine when a leg was in stance, a leg was required to be defined as in stance phase for three video fields or 0.05 s to count as being in a prolonged stance. The smaller the mean latency, the more synchronous the onset of the first prolonged stance by the various legs.

Values are presented as means ± S.E.M.

Results

Turn accuracy

On average, the forceps used to deliver the pinch stimulus remained in contact with the leg for 0.15 ± 0.01 s ($N=116$), as estimated from the video recordings with a resolution of 0.033 s. The average latency between the onset of the pinch and the onset of rotation of the animal's body was 0.13 ± 0.01 s ($N=116$; also estimated from video recordings with a resolution of 0.033 s). The pinch stimulus therefore overlapped the early part of the rotational response by 0.02 s on average, an overlap that is approximately 2 % of the duration of the typical response (0.99 ± 0.07 s, $N=116$). The angle of the stimulated leg to the body just prior to the onset of the stimulus varied somewhat from trial to trial such that the stimulus angle ranged approximately from 80 to 125 °. The mean stimulus angle was 100.4 ± 2.4 ° ($N=116$, Table 1).

Specimens of *P. clarkii* responded to the pinch by rotating through an average response angle of 106.0 ± 9.4 ° within approximately 1 s to give an average rotation rate of 110 ± 2.5 ° s^{-1} (Table 1). These responses occurred with an average normalized actual error (Fig. 1, α/γ) of -0.10 ± 0.11 and an average normalized rotational error (Fig. 1, β/γ) of 0.07 ± 0.11 (Table 1). These values are significantly different ($P<0.0001$, paired *t*-test). The two types of error differ because, although the orienting motion is dominated by rotation (see below), it also includes a translational component. The timing, amount and direction of translational movement of the body undoubtedly influenced the magnitude of the two measures of error. It is clear, however, that the rotational component makes a significant contribution to the accuracy of the defense turn, as shown by the strong correlation between the rotational error and the actual error (Fig. 3). The difference between the two average errors

Table 1. *Descriptors of defense turns by specimens of the crayfish* Procambarus clarkii

Specimen	Stimulus angle, γ (degrees)	Response Angle, ψ (degrees)	Duration (s)	Mean rotation rate (°s⁻¹)	Normalized actual error, α/γ	Normalized rotational error, β/γ	N
A	103.4±2.0	77.8±2.9	0.79±0.04	103.5±5.0	−0.407±0.035	−0.242±0.03	26
B	104.8±1.7	114.7±2.9	1.04±0.04	112.8±3.2	−0.093±0.046	0.105±0.035	30
C	94.0±1.7	115.0±3.5	1.05±0.05	114.9±4.5	−0.042±0.035	0.230±0.040	30
D	99.2±2.4	116.4±3.7	1.08±0.03	109.9±4.5	0.134±0.034	0.187±0.041	30
Mean of means	100.4±2.4	106.0±9.4	0.99±0.07	110±2.5	−0.102±0.113	0.070±0.107	

Values are given as means ± S.E.M. (*N*).
See the text and Fig. 1 for an explanation of the terms and symbols.
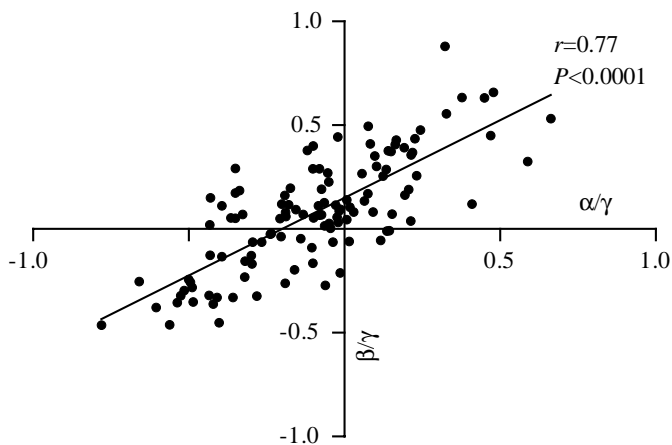

Fig. 3. Mean normalized rotation error (β/γ) *versus* mean normalized actual error (α/γ) of defense turns by specimens of *Procambarus clarkii* (*N*=116). See text and caption to Fig. 1 for explanations of terms and symbols.

Fig. 4. Stimulus angle (unfilled symbols, γ in Fig. 1) and response angle (filled symbols, ψ in Fig. 1) *versus* normalized rotation error (β/γ, see Fig. 1) of defense turns by specimens of *Procambarus clarkii*. Positive values of normalized rotational error indicate rotation through an angle larger than the stimulus angle. The linear regression for each data set is shown: stimulus angle (lighter line), *r*=0.46, *P*<0.0001; response angle (darker line), *r*=0.89, *P*<0.0001 (*N*=116 in each case). The horizontal dashed line indicates the mean stimulus angle.

indicates that the translational components do not improve the accuracy of the orienting response and may make it worse.

Across all responses, response angle (Fig. 1, ψ) was not correlated with stimulus angle (Fig. 1, γ) (*r*=0.009), and the coefficient of variation (CV) for the response angle was twice as large as that for the stimulus angle (CV=0.22 *versus* 0.11 respectively, *N*=116). Furthermore, the average response angle of specimen C was not significantly smaller than that of other specimens despite experiencing a significantly smaller stimulus angle than animal A (ANOVA followed by Tukey's test for multiple comparisons, *P*<0.01) and animal B (ANOVA followed by Tukey's test for multiple comparisons, *P*<0.001).

The magnitude of the rotational error increased when either the stimulus or the response angle departed from approximately 100° (Fig. 4), an angle that corresponds closely to the average position of the pinched leg 5 at rest. The rotational error, β, and response angle, ψ, tended to increase with rotation rate when these measures were correlated across all 116 videotaped responses (*r*=0.345, *P*<0.001 for β; and *r*=0.30, *P*=0.001 for ψ). Variation in rotation rate does not account for differences in rotational error among animals, however, because there were no significant differences among
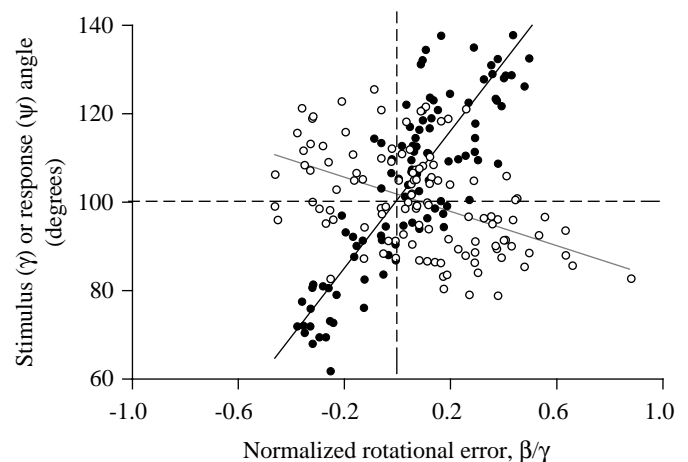
the specimens in average rotation rate (Table 1, ANOVA, *P*>0.05). Variation in rotation rate might, however, account for at least some of the observed variation in response angle.

Animal A differed in rotational error from each of the other animals; it tended to undershoot the target, and each of the other specimens tended to overshoot it (Table 1; ANOVA followed by Tukey's test for multiple comparisons, *P*<0.001). The tendency of animal A to undershoot the target position, as reflected in large negative values for both actual and rotational errors, can be attributed to significantly shorter-duration responses than exhibited by the other specimens (Table 1; ANOVA, *P*<0.001).

Turn kinematics

Movement of the body

The variable mixture of rotational and translational movements that makes up a defense turn by the crayfish is
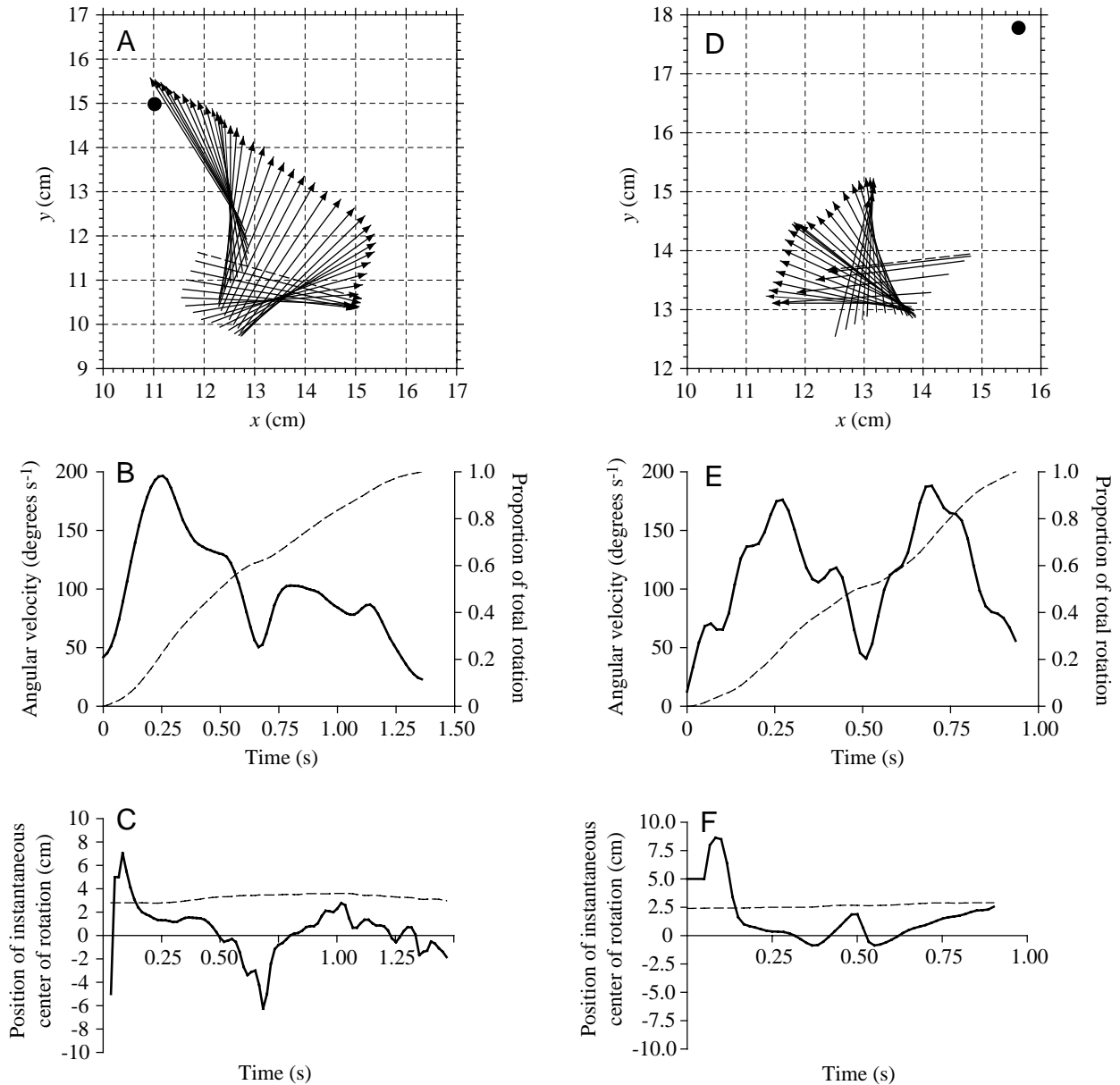
Fig. 5. Two examples (A–C, D–F) of defense turns by *Procambarus clarkii*. (A,D) Arrows represent the approximate length of the carapace (arrowhead=anterior) and show changes in the orientation of the animal's carapace, relative to the video frame of reference, in response to a pinch delivered to one of the fifth walking legs. Filled circles show the position of the dactyl of the stimulated leg at the moment of stimulus onset. The time between successive arrows is 0.033 s. The dashed arrow indicates the orientation of the animal just before it began to rotate. (B,E) Angular velocity of the body (solid line) and progress towards the final orientation angle (dotted line) of the animal producing the turns shown in A and D respectively. (C,F) Position of the instantaneous center of rotation (solid line) relative to the carapace during the turns shown in A and D respectively. The *y*-axis represents a line extending along the length of the animal's carapace. Zero on the *y*-axis indicates the posterior marker on the carapace (see Fig. 1B,E). The broken line indicates the position of the anterior marker at the base of the rostrum. The vertical distance between the *x*-axis and the broken line therefore indicates the approximate length of the carapace. (It changes slightly because the animal was videotaped from directly overhead and the projected length varies with the tilt of the body.) The position of the instantaneous center of rotation was calculated as the intersection of the mid-longitudinal lines indicating the animal's orientation in two successive video fields (interval 0.017 s; see text and Fig. 2 for additional explanation).

illustrated qualitatively in Fig. 5A,D. Commonly, as shown in Fig. 5A, a defense turn begins with a brief lateral roll to the side contralateral to the stimulus. This initial, mostly translatory, motion is quickly followed by an increase in rotation. Translation towards the stimulus point dominates near the end of the turn depicted in Fig. 5A, but the other example
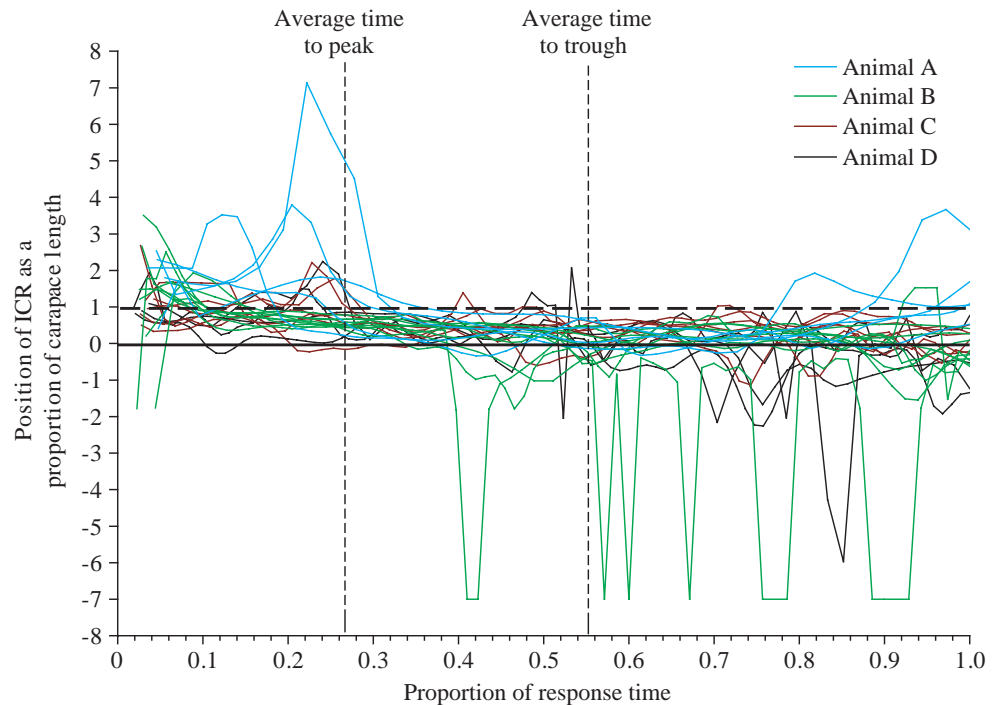
Fig. 6. Changes in the position of the instantaneous center of rotation (ICR), normalized for position on the carapace and response time for 25 responses in four crayfish. The left-hand vertical broken line indicates the proportion of the response time at which the peak angular velocity of the body's motion occurs, on average (see Fig. 7). The right-hand vertical broken line indicates the proportion of the response time at which the trough in angular velocity of the body occurs, on average (see Fig. 7). Values of −7 for the position of the ICR indicate extreme posterior positions of the ICR and times when translation predominates in the body's movement.

closes with rotation about a point near the anterior end (Fig. 5D). It is clear in the two examples shown (and in others not shown) that the rotational component makes the main contribution to this directed behavior.

This conclusion is further supported by data concerning the position of the instantaneous center of rotation (ICR; Figs 5C,F, 6). The position of the ICR is quite variable within the first 200–300 ms (0.2–0.3 of the average response time; Fig. 6) but tends to be in an anterior position either on or in front of the carapace. Despite the uncertainty associated with distinguishing rotation from translation, the translational component of the carapace movement can be considered to increase as the ICR moves further from the carapace, either in front of or behind it. By this measure, Fig. 6 shows that early movements often contain significant translational components (see also Fig. 5A,D). Rotation soon comes to dominate, however, as the ICR moves onto the carapace (Fig. 6). Thereafter, the ICR tends to remain on the carapace, although its position again becomes quite variable, often falling to points well behind the carapace, late in the response. Because the ICR remains on, or within 1 cm of either end of, the carapace for approximately 80 % of the duration of the response, defense turns are considered to be dominated by rotation.

Defense turns begin with a rapid angular acceleration to a peak angular velocity (e.g. Fig. 5B,E). The angular velocity profiles of defense turns become more variable subsequent to the initial acceleration. The turn depicted in Fig. 5B, for example, shows a general decline in angular velocity until the end of the response while the other turn (Fig. 5E) features a second major peak in angular velocity. In the first response (Fig. 5A–C), the response angle was achieved with a single rotational motion. In the other response (Fig. 5D–F), the

animal made two nearly identical turning motions in orienting towards the target position; an initial one that carried it approximately half-way, and a subsequent motion to the final position. (The point of minimal angular velocity between the two peaks is evident in Fig. 5D as the small cluster of arrows pointing approximately to the 10 o'clock position.)

The angular velocity profiles suggest that defense turning responses of the crayfish can be divided into two stages, at least for the purposes of description. The first stage features an initial sharp angular acceleration to a peak angular velocity that tends strongly to occur at approximately 0.26 ± 0.011 s ($N=25$) (Fig. 7; see also Fig. 5C,D). This stage reflects the effects on the body of the first stance phases of the walking legs. The low variability of this temporal landmark indicates the stereotypy of the initial acceleration, a conclusion strengthened by the observation that the latency to this peak is independent of the animal's average rotation rate (and hence position) to that point ($r=0.175$, $P>0.05$). The subsequent minimum in angular velocity tends to occur approximately 0.54 ± 0.036 s ($N=24$) after response onset (Fig. 7). The timing of this event is more variable than that of the latency to the initial peak irrespective of whether the latency to the 'trough' is measured from the onset of the response or from the time of the peak angular velocity. Nevertheless, it remains a useful demarcation of the end of the first stage. Approximately 25 % of the total rotation of a turning response is completed by the time the initial peak is reached at 0.26 s, and 60 % by the average latency to the minimum at 0.54 s.

The average duration of first stances (see Fig. 9) approximates, but is slightly less than, the mean latency to the initial peak in body angular velocity, indicating that the initial angular acceleration is driven primarily by the first stances. Although all
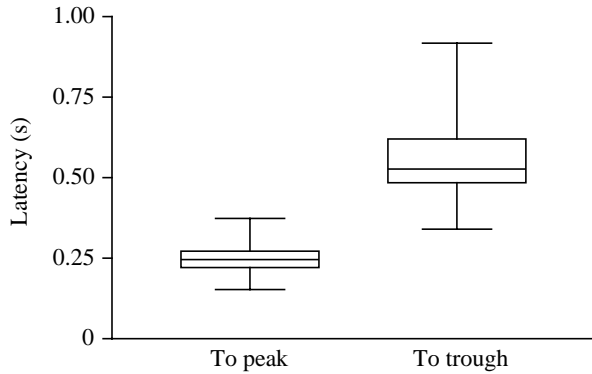
Fig. 7. Latency between the onset of a response and the initial maximum angular velocity ('peak') and subsequent minimum angular velocity ('trough'). The top and bottom of a box mark the 75th and 25th percentiles respectively. The horizontal line within each box indicates the median value. Vertical bars indicate the range of the data. $N=25$ for peak and 24 for trough. One response had no discernible trough.
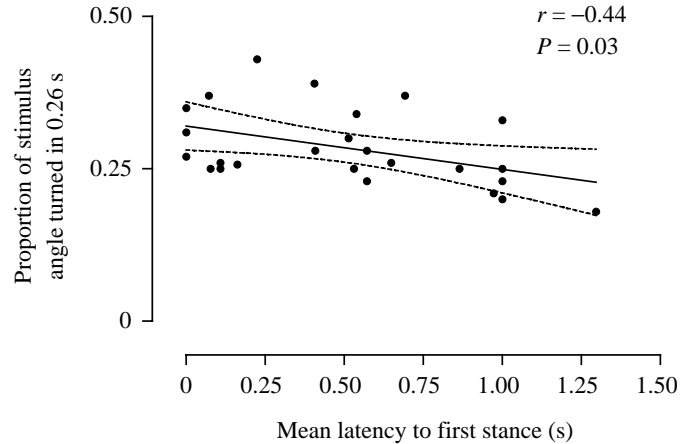

Fig. 8. Relationship between the amount of rotation early in the response and the degree of synchrony of onset of the first stances. The amount of rotation is given as the proportion of the stimulus angle turned within 0.26 s of the onset of the turning response, i.e. within the average time of the initial angular acceleration. The degree of stance synchrony is given as the mean latency between the onset of the turning response and the onset of the first stances of the various legs. A short mean latency indicates a high degree of synchrony of stance onset among the legs. The regression line (solid line) is shown with 95 % confidence intervals (broken lines) ($N=25$).

the legs appeared to be in contact with the substratum immediately prior to the delivery of the pinch stimulus, not all the legs entered into a prolonged stance phase as the body began to rotate. Occasionally, some legs lifted and moved in a swing phase very shortly after the onset of rotation of the body.

The possibility that the initial acceleration is influenced by the degree of stance coordination among the walking legs was examined by plotting the proportion of the stimulus angle turned during the initial phase against the index of leg synchrony, i.e. the mean latency from the onset of the rotational response to the onset of the first stance phase of all legs (Fig. 8). When many legs participate together, the angle subtended by the body in the first 0.26 s is large, and the initial acceleration of the body is smooth. When one or more legs either fail to produce a power stroke or shift quickly from power stroke to return stroke during the initial period, the angle subtended is reduced and the smooth increase in angular velocity of the body is interrupted. Certain walking legs may make larger contributions than others to the torque that initiates rotation of the body, however. The duration of the initial stage of turning might depend, for example, on the stance duration of a particular leg, but no such correlation has yet been found.

The second stage of defense turns in the crayfish, in which the animal achieves the final orientation of the body relative to the stimulus, varied considerably both among trials within a single specimen and among specimens. Some responses featured a generally smooth deceleration to the end point of the response, others included a strong secondary acceleration and still others proceeded at a relatively constant angular velocity before decelerating sharply to the end point. There is gradation among these types of response, making it difficult to distinguish clearly categories of defense turning responses according to the angular velocity profile of the second stage of rotation.

Timing parameters of leg motion

Kinematic comparisons were made between steps taken by

inner and outer legs during defense turns. The kinematics of the first steps were analyzed separately from that of all subsequent steps because the first stances drive the initial acceleration of a defense turn response while subsequent steps produce the decrease in the body's angular velocity following the initial acceleration and the final orienting movements. The two groups of steps do not, therefore, coincide completely with the two stages of body motion as defined above.

With one exception, contralateral members of each pair of legs did not differ, during their first steps, in step period, stance duration or duty factor (Fig. 9A–C). The exception was inner leg 5, which showed a larger duty factor than its outer partner. This difference may have resulted from an effect of the pinch stimulus on the motion of inner leg 5. After the first steps, however, outer leg 3 steps with a larger stance duration but without a concomitant increase in period. Hence, outer leg 3 exhibits a larger duty factor than its inner counterpart, indicating that the swing phase is shortened in outer leg 3 compared with inner leg 3 during the second stage of rotation (Fig. 9D–F). Outer legs 2 and 5 also exhibit larger stance durations than their inner counterparts during the second stage of the response but, unexpectedly, these differences are not accompanied by differences in step period or duty factor (Fig. 9D–F). Finally, inner legs 3 and 4 take a greater number of steps than do their outer counterparts during a defense response (Fig. 9G). The observed differences in timing parameters between the members of some pairs of legs, coupled with differences in stance amplitude (see below), suggest that coordination among opposing legs is weak. There is considerable variability in step period within any one leg from step to step, leaving no
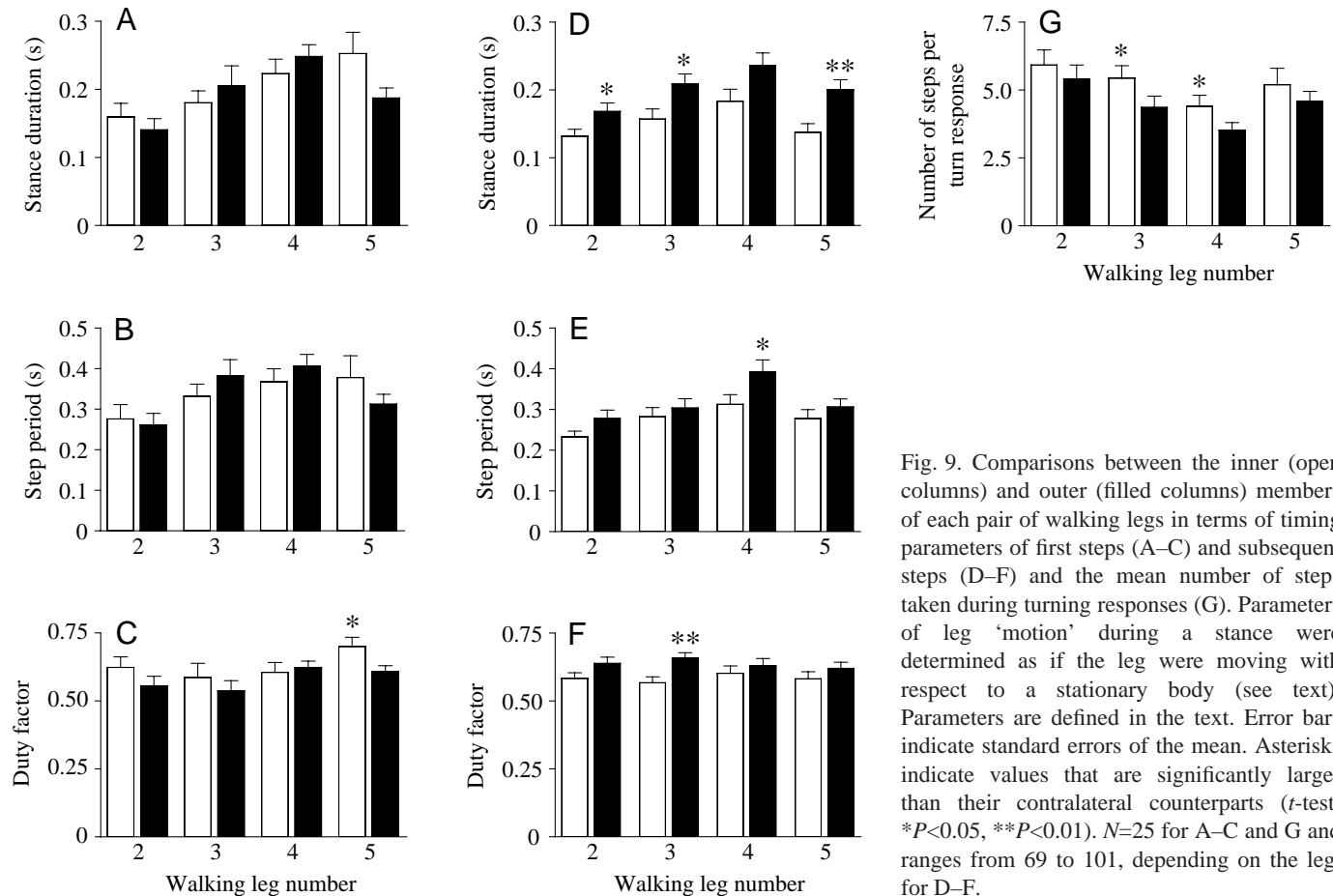
Fig. 9. Comparisons between the inner (open columns) and outer (filled columns) members of each pair of walking legs in terms of timing parameters of first steps (A–C) and subsequent steps (D–F) and the mean number of steps taken during turning responses (G). Parameters of leg 'motion' during a stance were determined as if the leg were moving with respect to a stationary body (see text). Parameters are defined in the text. Error bars indicate standard errors of the mean. Asterisks indicate values that are significantly larger than their contralateral counterparts (*t*-test; *$P<0.05$, **$P<0.01$). $N=25$ for A–C and G and ranges from 69 to 101, depending on the leg, for D–F.

obviously stable phase relationships between pairs of ipsilateral or contralateral legs.

Comparisons between first steps and subsequent steps within a leg do not reveal changes in stance duration, step period or duty factor for any leg except inner leg 5, which showed a larger mean stance duration, step period and duty factor during first stances than subsequently. Again, however, these differences may reflect an effect of the pinch stimulus on the motion of that leg.

Geometry of stances

Outer legs 3 and 4 have larger first stance amplitudes than their inner counterparts (Figs 10A, 11). It may seem surprising that this difference is not accompanied by similar differences in stance duration, period or duty factor (Fig. 9A–C). Outer legs 3 and 4 may be in stance during larger angular accelerations than characterize the times when inner legs 3 and 4 are in stance. It is also possible that the M-C joints of outer legs 3 and 4 become more extended than their counterparts during their first stances. This extension would produce larger-amplitude stances without requiring changes in stance duration. The possibility that movements about the M-C joint contribute to rotational movements by the crayfish, as they do to forward walking (Jamon and Clarac, 1997), will be examined in future studies. Greater flexion of the M-C joint of the inner legs compared with the outer legs would introduce a

lateral translation to the rotational motion. A lateral translational component is evident in the motions depicted in Fig. 5A,D, especially near the beginning of the responses.

All outer legs produce larger-amplitude stances than their inner counterparts during the second stage, rather than just outer legs 3 and 4 as in the initial stage (Fig. 10B). Except for the fourth pair of legs, these differences in stance amplitude are not accompanied by differences in step period (Fig. 9E). In most cases, then, differences between members of a pair of legs in net vector amplitude must be related to differences in one or more of the parameters of stance duration (with a compensating change in swing duration to maintain the period) or to extension of the leg as described above. The longer-duration stances and the larger duty factor of outer leg 3 compared with its inner counterpart indicate that the swing phase is shortened in outer leg 3 compared with inner leg 3 during the second stage of rotation. Differences in net vector amplitude between members of the second as well as the fifth pair of legs are more difficult to explain. The larger amplitudes of subsequent stances of outer legs 2 and 5, compared with their inner counterparts, are accompanied by longer stance durations (Fig. 9D). One would expect similar differences in either step period or duty factor between contralateral legs 2 and 5, but this was not the case (Fig. 9E,F).

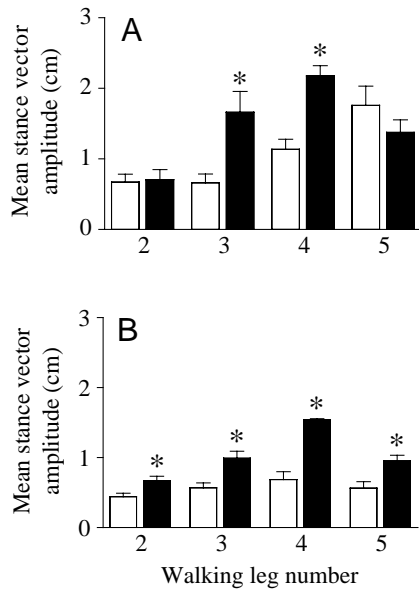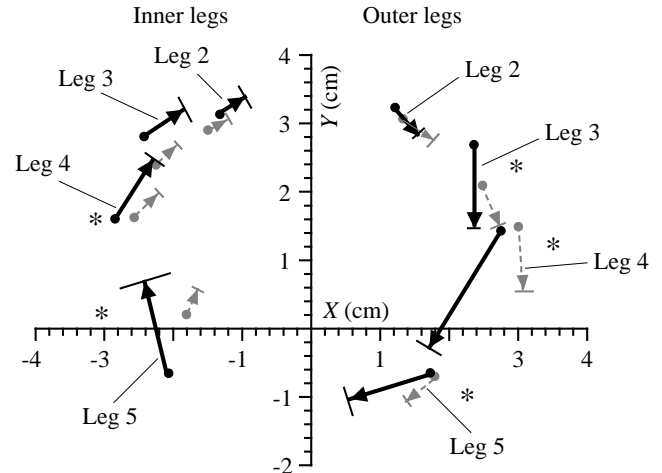For all legs except inner leg 3 and outer leg 2, steps taken

Fig. 10. Comparisons between the inner (open columns) and outer (filled columns) members of each pair of walking legs in terms of mean stance vector amplitudes of initial stances (A) and subsequent stances (B). Stance vectors were determined as if the leg were moving with respect to a stationary body (see text). Error bars indicate standard errors of the mean. Asterisks indicate values that are significantly larger than their contralateral counterparts (*t*-test; $P<0.01$ in all cases). $N=25$ in A and ranges from 69 to 101 in B, depending on the leg.

Fig. 11. Mean net stance vectors for first stances (darker symbols) and subsequent stances (lighter symbols) by inner and outer legs 2–5 during defense turns by specimens of *Procambarus clarkii*. Stance vectors were determined as if the leg were moving with respect to a stationary body (see text). Vertical (*Y*) and horizontal (*X*) axes indicate body-centered coordinates with the vertical axis as the animal's midline (anterior is up). The intersection between the horizontal and vertical axes represents the posterior marker on the carapace (see Fig. 1B,E). Each vector was determined as the line connecting the average position, relative to the body, of a dactyl at stance onset and the average position, relative to the body, of the dactyl at the end of the stance. The line drawn at the tip of each vector represents the 95 % confidence intervals about the average vector angle calculated using circular statistics (Batschelet, 1981). Asterisks indicate cases in which the angle of the mean first stance vector differs significantly from the angle of the mean vector for the corresponding stances of a particular leg (circular statistics, Watson–Williams test, $P<0.05$). $N=25$ for all first stance vectors and ranges from 69 to 101 for the vectors representing subsequent stances.

by a particular leg during the initial stage have larger stance amplitudes than subsequent stances taken by the same leg (see Fig. 11; *t*-test, $P<0.05$, $P<0.01$, $P<0.001$, $P<0.05$, $P<0.05$, $P<0.01$ for inner legs 2, 4 and 5 and outer legs 5, 4 and 3, respectively). Differences in stance amplitude between the first and subsequent steps could be related to a change in the translational component of the body movement.

Walking legs differ systematically in the trajectory of their stance phases both during the initial acceleration and during the second stage of rotation. Fig. 11 shows the net vector of each dactyl's motion, as drawn from the position of the dactyl at the onset of the stance phase (AEP for outer legs, and PEP for inner legs) to the position of the dactyl at the end of the stance phase. The legs on the outside of the arc show posteriorly directed stances, while the stances of the inner legs are directed anteriorly, as in forward and backward walking respectively. The net trajectory of each leg's stance is canted to the body's midline axis such that, collectively, these trajectories describe a circle around the animal. This more-or-less circular pattern suggests that the crayfish is twisting on its legs and is consistent with the data presented above showing that the center of rotation lies on or near the body axis throughout most of the response.

The stances of outer legs 3, 4 and 5 and inner legs 4 and 5 are directed differently during the second phase of rotation from during their first stances (Fig. 11). Comparisons of the value of the *X* coordinate of the dactyl at stance onset reveal that inner

legs 4 and 5 begin their subsequent stances significantly closer to the body's midline than their first stances (paired *t*-test, $P<0.01$ in both cases; see Fig. 11). The *X* coordinates of the dactyl's position at stance onset for the other legs do not change significantly from the first to subsequent stances.

Discussion
Accuracy of defense turns

The average 'actual' error of 0.10, normalized to the stimulus angle (Table 1), indicates that defense turns in the crayfish are sufficiently accurate to bring the target well within the angle subtended by the outstretched chelipeds, which is approximately 90 °, i.e. 45 ° either side of the animal's midline. The observations (i) that the rotational error, normalized to stimulus angle, is positively correlated with the response angle as if the 'effective' stimulus angle were fixed (Fig. 3), (ii) that the magnitude of the rotational error increases as the stimulus

or response angle departs in either direction from the average angular position of the pinched leg 5 at rest (Fig. 4), and (iii) that animal C experiences a smaller average stimulus angle than animals B and D, but that its average response angle does not differ from that of these other two specimens (Table 1), indicated that the turn response was more-or-less fixed. Breithaupt et al. (Breithaupt et al., 1995) also observed that temporarily blinded crayfish turning in response to the water flow provoked by a swimming fish increasingly undershoot as the stimulus angle increases beyond approximately 100°, although the opposite is not true at smaller stimulus angles. These results suggest that the magnitude of the rotation in defense turns by the crayfish appears to be determined according to the leg that was pinched rather than according to more precise proprioceptive information about leg position. Copp and Watson (Copp and Watson, 1988) reported that the angle through which a restrained specimen of *P. clarkii* rotates a turntable increases as more posterior legs are pinched, but they did not isolate changes in leg angle from changes in leg number.

Angular velocity profiles

In unrestrained specimens stimulated by a pinch to a walking leg, defense turns proceed in two phases: (i) an initial phase, featuring a more-or-less stereotyped acceleration to a peak angular velocity, followed by (ii) a more variable period during which the angular velocity decreases and the final orientation is achieved (Fig. 5). The stereotypy of the initial acceleration, based on the relatively low variability of the elapsed time between the onset of rotation and the initial peak angular velocity (Fig. 7), is supported by the additional observation that the timing of the initial peak is independent of the body's rotation rate and angular position.

The magnitude of the turn achieved during the initial acceleration of the body is related to the coordination of stance phases among the legs (Fig. 8). The failure to observe a correlation between the duration of the stance of any one leg and the duration of the initial phase suggests that explanations for the stereotypy of the initial phase will not be found in the behavior of one leg. Rather, the duration of that first stage might depend on the duration of a central command or on the delay for initiating the second stage of the turn. That is, the initial acceleration may itself be a programmed act or appear stable because it is a consequence of another act that is itself stable.

During the second stage of a defense turn, the legs exhibit highly variable and poorly coordinated bouts of stepping that continue the orienting response. Coupling between inner and outer legs is weak, as demonstrated by the lack of coordination patterns such as those frequently observed in forward (Jamon and Clarac, 1995; Müller, 1990; Müller and Cruse, 1991) and curve (Domenici et al., 1998) walking by the crayfish. The amplitude of the stances of all legs decreases during this phase relative to the initial phase (Fig. 10), which contributes to an erratic, but generally decreasing, pattern of changes in the angular velocity of the body.

The initial phase may correspond to a period when open-loop control predominates, whereas the relatively greater variability of duration and body motion characteristic of the second phase may reflect the influence of sensory feedback in a closed-loop control mode. Escape behaviors in at least two other arthropod species exhibit a two-phase structure in which closed-loop control follows an initial, ballistic response (e.g. cockroach, Camhi and Tom, 1978; crayfish, Wine and Krasne, 1972). Breithaupt et al. (Breithaupt et al., 1995) contrasted the continuous turning motion of crayfish towards swimming fish with the slower, more discontinuous search behavior of crustaceans in odor plumes and suggested that turning towards prey is controlled entirely by an underlying ballistic or open-loop control system. The angular velocity profiles of crayfish turning towards a swimming fish, nevertheless, closely resemble those shown here (as can be shown after recomputation of Fig. 4 in Breithaupt et al., 1995) and could also be consistent with a movement in two phases. An open-loop control element in defense turns is suggested by the finding that restrained specimens of *P. clarkii* are able to rotate a turntable with fair accuracy even when the pinch stimulus ceases before the turning response begins and no specific visual cues of stimulus location are available (Copp and Watson, 1988). Visual feedback from the turning motions increases turn accuracy in restrained specimens both by stimulating accelerated rotation in the early moments of a response, a positive feedback effect also seen in forward walking in the lobster (Davis and Ayers, 1972), and by partially inhibiting further turning when a novel object appears in the visual field late in the response (Copp and Watson, 1988). Feedback control of the second stage of defense turns, in which final orientation is achieved, could account for the observation that the response angle of a defense turn does not closely correlate with the stimulus angle. Further research is needed to determine which type of control system governs defense turning behavior in the crayfish.

The angular velocity profiles of defense responses in the crayfish are usually not smooth, but quite irregular (e.g. Fig. 5B,E). Variability in angular acceleration of the body may indicate the characteristics of the underlying control system. Kelly and Chapple (Kelly and Chapple, 1990) studied cheliped elevation during the defense display of the crayfish and showed that, although the propodite of the cheliped reaches a stereotyped end-point, the movements of the cheliped joints leading to that end-point are quite variable in terms of angular and tangential velocity. There is no discernible inter-joint coordination. They contrasted this variability with the smooth acceleration characteristic of reaching movements by primates. In primates, an underlying system of close coordination among the arm joints permits a high degree of control over the trajectory of the hand (Atkeson and Hollerbach, 1985). Kelley and Chapple (Kelley and Chapple, 1990) concluded that the crayfish apparently lacks analogous mechanisms for inter-joint coordination that would allow control of propodite trajectory in the defense reflex. Instead, the summed effect of independent activity at multiple joints produces the characteristic end position of the propodite (Kelly and

Chapple, 1990). Perhaps the final position of the body of a crayfish in a defense turn more closely reflects the summed activity of more-or-less independently acting legs rather than the outcome of a planned trajectory of the body.

Kinematics of the turn

The rotational component of a defense turn begins with a strong twist of the body on the legs. The smoothness and magnitude of the ensuing angular acceleration of the body depend on the number of legs that remain in stance during this initial stage. All legs may not participate equally, however. In a number of arthropod species, the different walking legs contribute differently to locomotion (e.g. Full et al., 1991; Nye and Ritzmann, 1992; Ward and Humphreys, 1981). The kinematic data reported above suggest that the same is true in defense turns by the crayfish. For example, the large stance amplitudes of outer legs 3 and 4 during the initial stage of a turn (Fig. 10) imply a major active role for them in the initial acceleration. The step amplitude of a leg, measured in kinematic and motion-analysis studies, has been related in some cases to the contribution of that leg to propulsion in locomotion (e.g. Clarac, 1984; Bowerman, 1977). The fourth pair of legs in decapod crustaceans, for example, is primarily responsible for producing thrust in forward walking (crayfish, Jamon and Clarac, 1995; Klärner and Barnes, 1986; Pond, 1975; rock lobster, Clarac, 1984). Other studies on crayfish in which leg motions (relative to the body) have been related either to angular accelerations of the body (Domenici et al., 1998) or yaw torque produced by the legs (Domenici et al., 1999) have shown that outer leg 4 makes a major contribution to the rotational component of curve walking.

During the second stage of a defense turn, outer legs 2 and 5 join outer legs 3 and 4 in exhibiting larger stance amplitudes than their inner counterparts (Fig. 10), again suggesting that the outer legs play the major role in generating torque. The difference in stepping frequency between inner and outer legs 3 and 4 in defense turn behavior (Fig. 9G) emphasizes the possibility of a functional difference between these legs in this behavior. By taking smaller, more frequent steps, inner legs 3 and 4 may be serving more to maintain balance and to act as pivot points than as producers of torque compared with outer legs 3 and 4. This role, at least for inner leg 4, would be consistent with the observed repositioning of the dactyl closer to the body during subsequent stances (Fig. 11). Similar considerations apply to the fifth pair of walking legs. The two fifth legs do not differ in step frequency, but they are distinct in the positions of their stances during the second stage of rotation (Fig. 11). Inner leg 5 begins its 'second stage' stances well forward of the AEP of outer leg 5. This might simply be an artifact of stimulating inner leg 5 to elicit the response. Another explanation is that inner leg 5 might provide balance and a pivot during the rotation. The repositioning of the inner leg 5 dactyl closer to the body and the small amplitude of the stances by inner leg 5 after the first stance are consistent with this interpretation. Cruse and Silva Saavedra (Cruse and Silva Saavedra, 1996) observed in curve walking by the crayfish *Astacus leptodactylus*

that the stance amplitude of inner leg 5 progressively decreased with the radius of simulated curvature and argued that, as in the honeybee (Zolotov et al., 1975), inner leg 5 serves as a pivot. The highly skewed stances of outer leg 5 during defense turns also suggest a supportive role for that leg. It was not unusual to see the stance of outer leg 5 extend all the way under the abdomen such that the dactyl appeared on the other side of the animal briefly before the leg began its return stroke. From this position, outer leg 5 could compensate for an imbalance created by the forward shift of inner leg 5 during rotation.

The inner and outer legs therefore appear to participate in different ways in producing movement of the body during defense turns. As discussed previously (Domenici et al., 1998), however, it is not possible, in the absence of direct force measurements or correlations between particular leg movements and changes in angular acceleration of the body, to separate the active contributions of any particular leg to body motion from the passive effects of forces exerted by other legs. Conclusions from kinematic studies regarding the contributions of various legs to propulsion in arthropod locomotion have been confirmed in some instances by direct measurements of forces exerted by the legs on the substratum (crayfish, Klärner and Barnes, 1986; cockroach, Full et al., 1991; for an exception, see Cruse, 1985). We have not found a clear pattern of correlation between the stances of any leg or group of legs and increases in angular acceleration of the body during defense turns. Measurements of the ground reaction forces exerted by the dactyls of the walking legs will be needed to determine whether differences among legs in stance amplitude indicate differences in their contribution to rotation. Experiments directed to this question are in progress.

Comparison between defense turns and curve walking

Leg motions in defense turns differ significantly from leg motions during curve walking in the crayfish. Curve walking features a prominent forward translational component and stepping patterns in which all the legs produce power strokes in remotion. The tight rotational responses characteristic of defense turning, however, are produced by oppositely directed stances of the inner and outer legs. Also, during curve walking, the stance amplitude depends largely on the amount of body translation during the leg stance, while during defense turns the translation is replaced by a rotational component giving the amplitude of movement of the body another meaning.

In curve-walking behavior by restrained specimens of *A. leptodactylus*, the inner legs step at the same frequency as the outer legs (Cruse and Silva Saavedra, 1996). If, in curve walking, the inner legs stepped at a higher frequency than the outer legs, as observed in defense turns, the effect would be to retard rotation. Indeed, it is the outer legs that step at higher frequency in curve-walking by honeybees (Zolotov et al., 1975) and stick insects (Jander, 1985), and outer legs 3 and 4 of freely behaving, curve-walking specimens of *P. clarkii* step with shorter periods than their inner counterparts (Domenici et al., 1998).

Finally, the time scale for defense turning and curve walking

differ strongly; the complete defense turn is achieved in approximately 1 s, which is approximately the duration of one step in curve walking. The rotation in place characteristic of defense turns therefore represents a distinct type of locomotor pattern in the crayfish.

The leg motions in defense turns, however, share some features with the leg motions during curve walking in the crayfish. During rotation in place by specimens of *P. clarkii*, stance trajectories are not parallel to the longitudinal axis of the body, as in forward and backward walking (e.g. Jamon and Clarac, 1997), but inclined to the body to a degree related to their position (Fig. 11). Similar differences in stance trajectory between the members of all contralateral pairs of legs occur in restrained curve-walking specimens of *A. leptodactylus* (Cruse and Silva Saavedra, 1996), and between the members of the third and fourth pair of legs in freely moving, curve-walking specimens of *P. clarkii* (Dominici et al., 1998).

In both curve walking and defense turns, rotation is effected in part by changes in stance amplitude of selected legs. Cruse and Silva Saavedra (Cruse and Silva Saavedra, 1996) and Domenici et al. (Domenici et al., 1998) documented that outer legs 2, 3 and 4 exhibit larger stance amplitudes than their inner counterparts during curve walking by restrained specimens of *A. leptodactylus* and freely behaving specimens of *P. clarkii* respectively. In defense turns, only outer legs 3 and 4 exhibit larger-amplitude stances than their inner counterparts during the initial acceleration, but all the outer legs exhibit larger stance amplitudes than the inner legs during the second stage of rotation. Changes in stride length are commonly observed in arthropods as they switch from straight walking to curve walking or tight rotation. The stride length of the inner legs is reduced relative to the outer legs in the honeybee (Zolotov et al., 1975), cockroach (Franklin et al., 1981), stick insect (Jander, 1985), ant (Zollikofer, 1994), dung beetle (Frantsevich and Mokrushov, 1980) and fruitfly (Strauß and Heisenberg, 1990).

The kinematics associated with changes in stance amplitude vary depending on the behavior. Increases in stance amplitude in curve-walking specimens of *A. leptodactylus* reflect a prolonged power stroke (Cruse and Silva Saavedra, 1996). Larger stance amplitudes in freely moving specimens of *P. clarkii* during curve walking were not associated with longer power strokes, however, but instead with more rapid power stroke velocities. In defense turns, differences between contralateral legs in stance amplitude during the initial acceleration are not reliably accompanied by differences in stance duration or duty factor, which would indicate a prolonged power stroke. It may be that the outer legs, with larger stance amplitudes, are in stance during periods of greater angular acceleration of the body than occurs when their inner counterparts are in stance. Alternatively, the dactyls of some outer legs may be positioned further from the body as a result of greater extension of the M-C joint than in the inner legs. Stance amplitude differences between contralateral legs during the second stage of rotation, however, do reflect differences in stance duration (legs 3 and 5) and perhaps also in leg extension.

Franklin et al. (Franklin et al., 1981) described three strategies that arthropods could use, individually or in any combination, to effect turning: (i) the legs on one side of the animal could step more frequently than the legs on the other side; (ii) the legs on one side could step through a wider arc than the legs on the other side; and (iii) the legs on one side could change their 'functional length' more than those on the other side (e.g. by flexing or extending). It is also possible, of course, that the legs on opposite sides of the animal could step in opposite directions, as demonstrated by a number of arthropod species (the cockroach *Periplaneta americana*, Bell and Schal, 1990; Camhi and Tom, 1978; dung beetles, Frantsevich and Mokrushov, 1980; jumping spiders, Land, 1972; fruitflies, Strauß and Heisenberg, 1990; ants, Zollikofer, 1994; honeybees, Zolotov et al., 1975). Not every study of turning behavior reports on all four possibilities, but it seems evident that different species use characteristically different combinations to produce rotation. Specimens of *P. clarkii* employ at least three of these strategies in generating defense turns: step frequency, stance amplitude and oppositely directed stances. It seems likely that changes in the functional length of one or more legs also contribute to the rotation of the body.

The degree to which the stepping motions of the various legs are coordinated during rotational motion varies considerably. Ants retain rigid tripod leg coordination even during tight turns (Zollikofer, 1994), but leg coordination in the cockroach becomes quite variable during turning (Franklin et al., 1981). Differences in the strength of coordination among walking legs can reflect differences in mechanical coupling among legs or differences in underlying neural control mechanisms. Relative changes in the stepping parameters of the inner and outer legs, as occurs in specimens of *P. clarkii* during defense turns, indicate a suspension of coupling mechanisms that allows the two sides to behave in more-or-less independent yet adaptive ways to maintain balance while producing rotation. Uncoupling of contralateral legs during tight rotation or curve walking has been reported a number of times (Zolotov et al., 1975; Jander, 1985; Cruse and Silva Saavedra, 1996; Zollikofer, 1994), although it is not required to generate turning responses (e.g. Strauß and Heisenberg, 1990). This uncoupling may reflect a shift from a control system principally dependent on the patterned output of coupled central oscillators to a system more dependent on reflexes directed by peripheral sensors that signal shifting loads on legs during rotation.

Kinematic studies therefore show that defense turning behavior in the crayfish represents neither a simple application of forward and backward stepping patterns to a rotational behavior nor a direct extension of leg coordination patterns in curve walking, but is a distinct locomotor behavior. The neural control system underlying defense turning behavior is likely to differ significantly from that controlling straight or curve walking. The differences are undoubtedly related to the specific characteristics of defense turns, such as the limited translational component of the movement, the short duration of the defense response and the importance of the orienting

component of the movement. Because of the strong constraints on this type of movement in terms of speed and accuracy, the nervous system must deliver optimised commands to produce the goal-oriented movement. The defense turn, however, relies on the same biomechanical constraints of the motor apparatus and the same sensory-motor circuitry as straight or curve-walking behavior, as suggested by similarities in the kinematics of leg motion. Continued investigation of turning behaviors will therefore add to our understanding not only of the kinematics of locomotion but also of how distinct but related locomotor patterns are produced by the same nervous system.

References

Atkeson, C. G. and Hollerbach, J. M. (1985). Kinematic features of unrestrained vertical arm movements. *J. Neurosci.* **5**, 2318–2330.

Barnes, W. J. P. (1975). Leg coordination during walking in the crab *Uca pugnax. J. Comp. Physiol.* A **96**, 237–256.

Bässler, U. (1993). The walking- (and searching-) pattern generator of stick insects, a modular system composed of reflex chains and endogenous oscillators. *Biol. Cybernetics* **69**, 305–317.

Batschelet, E. (1981). *Circular Statistics in Biology.* New York: Academic Press.

Bell, W. J. and Schal, C. (1980). Pattern of turning in courtship orientation of the male German cockroach. *Anim. Behav.* **28**, 86–94.

Bowerman, R. F. (1977). The control of arthropod walking. *Comp. Biochem. Physiol.* A **56**, 231–247.

Breithaupt, T., Schmitz, B. and Tautz, J. (1995). Hydrodynamic orientation of crayfish (*Procambarus clarkii*) to swimming fish prey. *J. Comp. Physiol.* **177**, 481–491.

Camhi, J. M. and Levy, A. (1988). Organization of a complex movement: fixed and variable components of the cockroach escape behaviour. *J. Comp. Physiol.* A **163**, 317–328.

Camhi, J. M. and Tom, W. (1978). The escape behavior of the cockroach *Periplaneta americana.* I. Turning response to wind puffs. *J. Comp. Physiol.* A **128**, 193–201.

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.

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

Clarac, F. (1984). Spatial and temporal coordination during walking in Crustacea. *Trends Neurosci.* **7**, 293–298.

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

Copp, N. H. and Jamon, M. (2000). The defense response in crayfish: behavior and kinematics. In *Biomechanics in Animal Behavior* (ed. P. Domenici and R. W. Blake), pp. 275–290. Oxford: BIOS Scientific Publishers.

Copp, N. H. and Watson, D. (1988). Visual control of turning responses to tactile stimuli in the crayfish *Procambarus clarkii. J. Comp. Physiol.* A **163**, 178–186.

Cruse, H. (1985). Which parameters control the leg movement of a walking insect? I. Velocity control during the stance phase. *J. Exp. Biol.* **116**, 343–355.

Cruse, H. (1990). What mechanisms coordinate leg movement in arthropods? *Trends Neurosci.* **13**, 15–21.

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 Silva Saavedra, M. G. (1996). Curve walking in crayfish. *J. Exp. Biol.* **199**, 1477–1482.

Davis, W. J. and Ayers, J. L. (1972). Locomotion: Control by positive feedback optokinetic responses. *Science* **177**, 183–185.

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

Delcomyn, F. (1980). Neural basis of rhythmic behavior in animals. *Science* **210**, 492–498.

Domenici, P. and Blake, R. W. (2000). *Biomechanics and Animal Behaviour.* Oxford: BIOS Scientific Publishers.

Domenici, P., Jamon, M. and Clarac, F. (1998). Curve walking in freely moving crayfish, *Procambarus clarkii. J. Exp. Biol.* **201**, 1315–1329.

Domenici, P., Schmitz, J. and Jamon, M. (1999). The relationship between leg stepping pattern and yaw torque oscillations in curve walking of two crayfish species. *J. Exp. Biol.* **202**, 3069–3080.

Franklin, R., Bell, W. J. and Jander, R. (1981). Rotational locomotion by the cockroach *Blatella germanica. J. Insect Physiol.* **27**, 249–255.

Frantsevich, L. I. and Mokrushov, P. A. (1980). Turning and righting in *Geotrupes* (Coleoptera, Scarabaeidae). *J. Comp. Physiol.* A **136**, 279–289.

Full, R., Blickhan, R. and Ting, L. H. (1991). Leg design in hexapedal runners. *J. Exp. Biol.* **158**, 369–390.

Graham, D. (1972). A behavioral analysis of the temporal organization of walking movements in the 1st instar and adult stick insect (*Carausius morosus*). *J. Comp. Physiol.* A **81**, 23–52.

Hughes, G. M. (1951). The coordination of insect movements. I. The walking movements of insects. *J. Exp. Biol.* **29**, 267–284.

Jamon, M. and Clarac, F. (1995). Locomotor patterns in freely moving crayfish (*Procambarus clarkii*). *J. Exp. Biol.* **198**, 683–700.

Jamon, M. and Clarac, F. (1997). Variability of leg kinematics in free-walking crayfish *Procambarus clarkii* and related inter-joint coordination. *J. Exp. Biol.* **200**, 1201–1213.

Jander, J. P. (1985). Mechanical stability in stick insects when walking straight and around curves. In *Insect Locomotion* (ed. M. Gewecke and G. Wendler), pp. 33–42. Hamburg: Parey.

Jindrich, D. L. and Full, R. (1999). Many-legged maneuverability: dynamics of turning hexapods. *J. Exp. Biol.* **202**, 1603–1623.

Kelly, T. M. and Chapple, W. D. (1990). Kinematic analysis of the defense response in crayfish. *J. Neurophysiol.* **64**, 64–76.

Klärner, D. and Barnes, W. J. P. (1986). Crayfish cuticular stress detector CSD_2. II. Activity during walking and influences on leg coordination. *J. Exp. Biol.* **122**, 161–175.

Land, M. F. (1972). Stepping movements made by jumping spiders during turns mediated by the lateral eyes. *J. Exp. Biol.* **57**, 15–40.

Müller, U. (1990). Contralateral leg coordination: analysis of curve walking in crayfish. In *Frontiers in Crustacean Neurobiology/*

Advances in Life Sciences (ed. K. Wiese, W.-D. Krenz, J. Tautz, H. Reichert and B. Mulloney), pp. 247–253. Basel, Boston, Berlin: Birkhäuser Verlag.

Müller, U. and Clarac, F. (1990). Dactyl sensory influence in rock lobster locomotion. II. Role in interleg coordination. *J. Exp. Biol.* **148**, 113–128.

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

Nye, S. W. and Ritzmann, R. E. (1992). Motion analysis of leg joints associated with escape turns of the cockroach, *Periplaneta americana*. *J. Comp. Physiol.* A **171**, 183–194.

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.

Strauß, R. and Heisenberg, M. (1990). Coordination of legs during straight walking and turning in *Drosophila melanogaster*. *J. Comp. Physiol.* A **167**, 403–412.

Walton, J. S. (1986). The accuracy and precision of a video-based motion analysis system. *Proceedings of the 30th International Technical Symposium on Optical and Optoelectronic Sciences and Engineering.* **693**, 251–263.

Ward, T. M. and Humphreys, W. F. (1981). Locomotion in burrowing and vagrant wolf spiders (Lycosidae). *J. Exp. Biol.* **92**, 305–321.

Wine, J. and Krasne, F. B. (1972). The organization of escape behavior in the crayfish. *J. Exp. Biol.* **56**, 1–18.

Zollikofer, C. P. E. (1994). Stepping patterns in ants. I. Influence of speed and curvature. *J. Exp. Biol.* **192**, 95–106.

Zolotov, V., Frantsevich, L. and Falk, E. M. (1975). Kinematik der phototaktischen Drehung bei der Honigbiene *Apis melifera* L. *J. Comp. Physiol.* A **97**, 339–353.