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TWO COUPLING MECHANISMS WHICH DETERMINE THE COORDINATION OF IPSILATERAL LEGS IN THE WALKING CRAYFISH

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SUMMARY

In a crayfish walking on a motor-driven belt at constant speed, the power stroke (stance phase) of an individual leg is experimentally prolonged for a short time; the ways the legs retain their normal coordination are then observed. The results are shown in a modified phase-response curve. Only ipsilateral coupling is considered in detail.

Two coordinating mechanisms are necessary and sufficient to describe the experimental results. (1) The forward-directed influence is only active when the controlling (posterior) leg performs a power stroke. If the controlled leg is in power stroke, this influence leads it to interrupt the power stroke and start a return stroke. If the leg is in return stroke, it is influenced to continue the return stroke, the duration of which is thereby prolonged. The speed of the return stroke is also decreased. (2) The backward-directed influence is active only during the last part of the power stroke and the first part of the return stroke of the controlling (forward) leg. If the controlled leg is in return stroke, it is influenced to interrupt the return stroke and start a power stroke. If it is at the end of its power stroke, it is influenced to continue this stroke.

For legs 3 and 4, each of which possesses both anterior and posterior neighbours, the influences exerted by these two coordinating mechanisms are assumed to be additive. A model calculation shows that the two mechanisms are sufficient to describe the behaviour observed. The results are compared with previous findings from the literature.

INTRODUCTION

The legs of a walking animal perform cyclic movements which consist of two alternating parts. The ‘power stroke’ (PS) takes place when a leg is on the ground, and the ‘return stroke’ (RS) when a leg is lifted off the ground. These cyclic movements of the different legs must be coordinated in order for an animal to produce a proper gait (see Evoy & Fourtner, 1973; Clarac, 1982). This paper considers the nature of the coordinating mechanisms which produce the stable temporal relationship between neighbouring walking legs. The experiments to be

Key words: walking, leg coordination, crayfish.

described involve crayfish and concentrate on the coordination between the four legs positioned on one side of the animal's body.

When two coupled systems oscillate at a constant phase relationship, it is impossible to determine the mechanism coupling the systems together. Neither the nature of such an influence nor its direction can be inferred. That is, the first system may influence the second, or *vice versa*, or the influence may be mutual. Such information can be obtained, however, if the two oscillators are brought into different phase relationships. Effects may then be observed whose strength depends upon the phase between the two oscillators. Usually such effects are plotted against phase, producing a phase-response curve (Pavlidis, 1973; Stein, 1976). Graham (1978) was the first to use a phase-response curve to describe coupling effects between walking legs of insects. In doing so he utilized the fact that in the grasshopper, the front and middle legs can step twice as often as the long hind legs. Thus different phase values can occur between middle and hind legs; changes in the period length (duration of power stroke plus duration of return stroke) were found to depend upon the phase. The results showed that the onset of a return stroke in the middle leg is inhibited as long as the hind leg performs a return stroke.

Phase-response curves have also been used for the investigation of leg coordination in the rock lobster, which during slow walking can also take very long strides with its hind legs (Chasserat & Clarac, 1983). Recently Foth & Bässler (1985) adapted this method as follows to the investigation of leg coordination in stick insects. The animal walked on a treadwheel with five legs, while the remaining hind leg walked on a motor driven belt beside the treadwheel. By changing experimentally the speed of the belt, the hind leg could be made to walk at a rhythm different from that of the other walking legs. This produced different phase values. By measuring the duration of the step period, both Foth & Bässler and Chasserat & Clarac showed that it is possible both to lengthen and shorten the step period of a leg.

In these three studies (Graham, 1978; Chasserat & Clarac, 1983; Foth & Bässler, 1985) the legs investigated walked with different period lengths. In the experiments presented here, in contrast, all the legs of each animal walked with the same rhythm. Different phase values were produced by a short experimental interruption of the movement of a leg during its power stroke. During this interruption, the leg mantained a fixed position relative to the body and slipped on the walking surface. After such a disturbance, the disturbed leg itself and the neighbouring legs appeared to use different strategies to return to the proper phase value. The effects of these strategies were measured. On the basis of the results, we conclude that at least two different coupling mechanisms exist. The two described here are both necessary and sufficient to describe the experimental findings.

METHODS

Adult female and male crayfish (*Astacus leptodactylus*), weighing about 60 g and obtained from the firm of Langbein in Hamburg, were kept in tanks at a temperature of 12–15 °C. For the experiments, individual animals were fixed dorsally by the carapace to a holder and then placed on a motor-driven belt in a water-filled aquarium (Ayers & Davis, 1977; Chasserat & Clarac, 1980). The holder was counterbalanced, allowing the animal to determine its height above the belt. In contrast to the smaller specimens (40–50 g) used in our preliminary experiments, the larger specimens walked well under the conditions imposed by the belt. Although active under normal conditions, the smaller crayfish resisted walking on the belt.

The movement of the legs was recorded by position electrodes as described by Cruse & Müller (1984). A thin copper wire (diameter, 0·1 mm), insulated except at the tip ('recording electrode'), was fixed by small pieces of adhesive tape to each leg to be measured. The tip of the wire was placed at the middle of the dactylopodite. The animal walked in an electrical field running parallel to the long axis of the body. This field was formed by two large grids (40×10 cm) positioned under water at the front and back end of the treadmill. These two field electrodes were connected to a sine wave generator producing alternating current of 1 V and 10 kHz. The recording electrodes on the legs of the animal served as the third electrode of a potentiometer; this third electrode recorded a voltage proportional to its position relative to the grid electrodes. In order to obtain a d.c. signal corresponding to leg position, each recording electrode was connected to a rectifier and a low pass filter (corner frequency 1 kHz) to remove the 10-kHz carrier frequency. This method produced completely linear position signals parallel to the long axis of the body and allowed simultaneous recording with as many electrodes as desired. Position signals from the legs were recorded on a multi-channel pen recorder and then analysed with a graphic tablet connected to an Apple II microcomputer. The experimental procedure allowed measurement accurate to within 40 ms.

The five pereiopods on one side of the body are conventionally numbered from 1 to 5 from front to rear. The first leg (leg 1), the cheliped, is not normally used in walking. Therefore, only legs 2–5 were investigated here. In order to shift one leg out of phase, its movement was interrupted during the power stroke by a vertical stick pressed downwards behind the meropodite. The duration of this interruption was also monitored on the pen recorder. Two examples of such experiments (interruption of leg 4 movement) are shown in Fig. 1. Those steps which follow the end of the interrupted power stroke were analysed.

This paper considers only forward walking, in which the power stroke corresponds to a backward movement of the leg and the return stroke to a forward movement. The position of the leg at the end of its power stroke is called the 'posterior extreme position' (PEP); the end of the return stroke is called the 'anterior extreme position' (AEP). For some cases the slope of the regression line was calculated. Except for those described in the first section of the Results, all experiments were performed with the belt at a constant speed of 9 cm s^{-1}.

When describing the coupling between two rhythmic events, one of the oscillations must be defined as the reference. This can be either the step cycle of the manipulated leg or that of the other leg considered. This definition of one leg as the reference leg is only made for the purpose of description and does not imply that one oscillator is the controlling (influencing) one and the other is the controlled (influenced) one. These

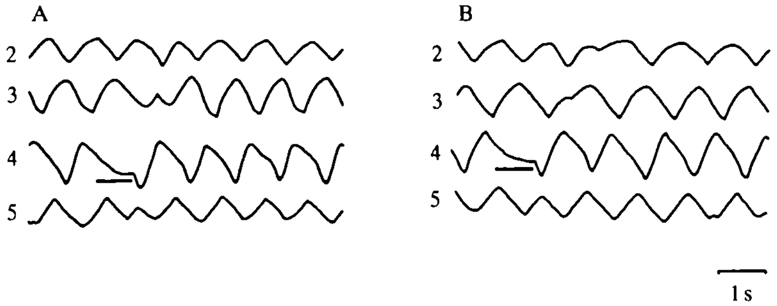

Fig. 1. Two examples of the record of four ipsilateral legs. The abscissa is time. Each trace is labelled with the number of the corresponding leg; leg 2 is the first and leg 5 the last walking leg. Upward deflection means return stroke movement. The power stroke of leg 4 has been interrupted, which is shown by a horizontal bar. Although the oscillation of leg 4 is delayed by this interruption, the other legs retain normal coordination during the subsequent steps.

terms, reference and controlling leg, must not be confused: it is suggestive, but wrong, to assume that the reference leg is the 'sender' and causes the effects described in the other, the 'receiver' leg. In fact, the reference leg could be the receiver or mutual influences may exist. As some analysis is necessary to identify the sender and receiver properties of a leg, the results must be described twice, using each of the two legs in turn as reference and the other as 'test' leg. Identification of descending (from anterior to posterior) or ascending effects will be presented in the Discussion. Here it should be mentioned that the particular leg manipulated is not important. Interruption is only a means to bring two legs into an unusual phase relationship. The same effects as described here can be observed when two legs are brought out of normal phase by any other means, for example irregular stepping. Fig. 1B shows an example where leg 2 made a prolonged return stroke although neither leg 2 nor leg 3 was manipulated.

RESULTS

Inter-leg time delay

Preliminary experiments using different belt speeds showed that power stroke duration increases with the period. The duration of the return stroke was nearly constant for all periods. However, there was a tendency for the more forward legs to show a weak dependence upon period, as has been described for the rock lobster by Clarac & Chasserat (1983). When the crayfish walked on a treadwheel driven by the animal itself (W. J. P. Barnes, in preparation), the duration of the return stroke also increased with the period. This difference may have been due to a difference in load, as was discussed previously by Cruse (1983) and Cruse & Müller (1984).

As is shown in Fig. 1, there is some temporal overlap between the power stroke of one leg and that of its next backward leg. The time between the end of the return stroke of one leg and that of the power stroke of the next forward leg was measured for every pair of neighbouring legs and for different walking speeds and was found to increase with the period. In contrast, in our experiments the inter-leg delay between the end of the return stroke of a leg and the end of the power stroke of the next backward leg was constant for all period values measured, i.e. for the range between 700 and 1600 ms.

Changes in the duration of the return stroke

When the stimulus interrupting the power stroke of a leg ended, the leg usually continued its power stroke. The leg was then generally out of phase with its neighbours. Two examples of this situation are given in Fig. 1. The subsequent behaviour of the interrupted leg and of its direct neighbours depended upon the phase relationship between the legs. Fig. 2A–F gives an overview of the possible outcomes. Each part of this figure shows original measurements of a particular reference leg, together with six traces showing the typical behaviour of one neighbouring leg when in different phase situations relative to the reference leg. The uppermost traces (I) represent the situation of normal coordination. As can be seen, in some cases the duration of the return stroke is prolonged (Fig. 2A, IV, V; Fig. 2B, IV, V; Fig. 2C, IV; Fig. 2D, V). However, there seems to be a continuous range of possible outcomes: the return stroke duration may be prolonged but at a somewhat decreased speed (e.g. Fig. 2D, V), or it may be interrupted by a very short power stroke (e.g. Fig. 2D, IV), or it may be shorter but of variable duration (e.g. Fig. 2D, III).

First preliminary evaluation of these data was done by plotting all effects (shortening and prolongation of return stroke and power stroke duration), using each neighbouring leg as reference and as test leg. Because of reasons which will become clear in the Discussion, the final evaluation presented here uses only the forward leg as reference for the description of the shortening of the return stroke duration (Fig. 3). The prolongation of the return stroke duration is only described using the rearward leg as reference (Fig. 5).

Changes of the return stroke duration are shown in Fig. 3. In Stage I of the evaluation only those return strokes were measured which were prolonged with no intervening retraction movement. In Stage II of the evaluation, return strokes which had been interrupted by a short 'power stroke' (e.g. Fig. 2D, IV) were also included in the data shown in Fig. 3. In these measurements the return stroke duration includes the two protraction movements as well as the short power stroke (retraction movement) by which they are separated.

Changes of the power stroke duration are shown in Fig. 5. Only shortened return strokes, i.e. the duration of the first protraction movement, were measured and these are plotted using the forward leg as reference. Because there is no discontinuity between shortened and lengthened return strokes, no clear separation of these two effects is possible. Therefore the values of some measurements appear in both Fig. 3 and Fig. 5. Plotting the prolongation of the return stroke relative to the backward leg and the shortening of the return stroke relative to the forward leg is justified by the finding described below (see Discussion) that prolongation of the return stroke is influenced by the backward leg, whereas shortening of the return stroke is influenced by the forward leg.

The situations in which prolongation of a return stroke occurs are presented in Fig. 3 in a form similar to a phase-response curve for each pair of legs investigated: the sketch below the abscissa symbolizes the rhythmic movement of the two legs in a normally coordinated walk (solid line). In Fig. 3, the values on the abscissa are given as absolute values rather than as the relative phase values usual in a phase-response curve. The ordinate does not show the absolute duration but rather the difference relative to the return stroke duration of a normal step. Thus, the zero value corresponds to an unchanged return stroke duration.

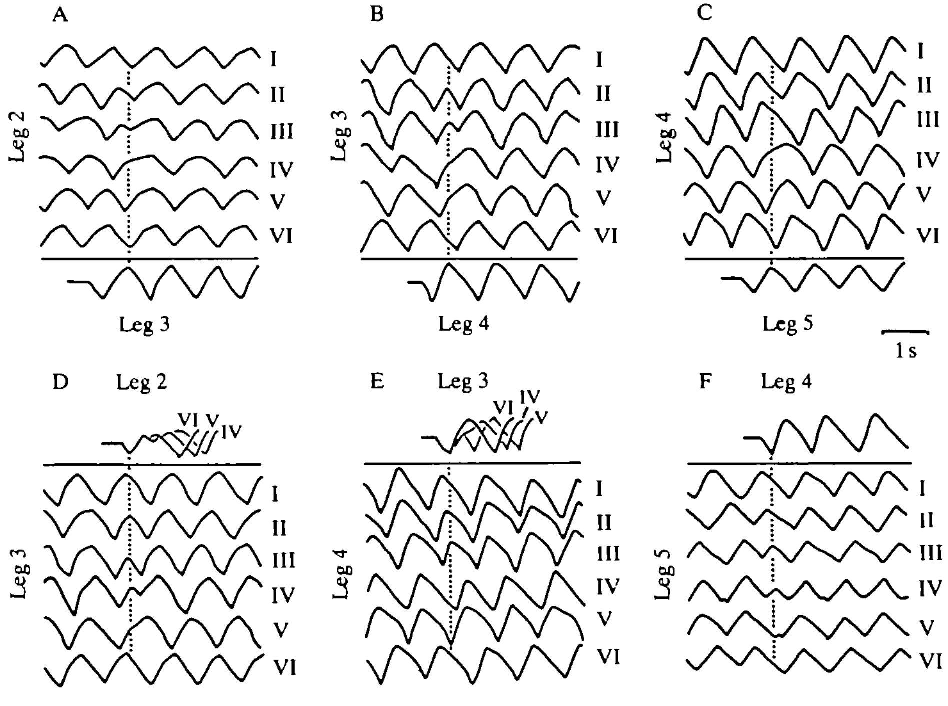

Fig. 2. Some original measurements showing that the legs retain normal coordination after a disturbance stimulus has been applied. For each pair of neighbouring legs, either the backward leg (A,B,C) or the forward leg (D,E,F) is used as the reference leg. In each part of the figure, the single trace is that of the reference leg; the six traces I–VI illustrate movement of the adjacent leg for six values relative to the reference leg. Trace I represents the situation of normal coordination. The end of the return stroke (A,B,C) or of the power stroke (D,E,F) of the reference leg is marked by a dotted line to demonstrate the different phase values of the second leg. Because of the mutual influence between neighbouring legs, the movement of the reference leg is not independent of the phase of the second leg. This is most obvious for leg 2 (D) and leg 3 (E). In these cases, therefore, the deviating movements of the reference leg are indicated by three different traces, which are also marked by their numbers.

Consider first the effect on leg 2 when leg 3 is blocked. This is shown in Fig. 3A,D. The changes in return stroke duration are very large when the point where the return stroke would have ended had it not been influenced occurs just after the AEP of the backward leg (this timing corresponds to a value of −500 ms). The prolongation of the return stroke duration decreases the later the return stroke starts relative to the reference point; the prolongation goes to zero when the start of the return stroke approaches the phase value of normal coordination. Had the slope of the points shown in Fig. 3A reached −1·0, then the prolongation would have completely compensated for the wrong phase value. In this case Δt (the time difference between the end of the return stroke and the end of the power stroke of the backward leg) would have been constant for all phase values. The slope is −0·79 (measured for the range −600 ms to 300 ms). The Δt values (Fig. 3D) show only small deviations from those present in normal coordination. Thus, the compensation during the return stroke is nearly complete.

Qualitatively the same results occur between legs 3 and 4 (Figs 2B, 3B,E). However, the slope in Fig. 3B is even smaller (−0·67 measured in the range from −500 ms to 0 ms), meaning that the compensation during return stroke in leg 3 is less than for leg 2. In the most extreme cases the power stroke of leg 3 starts about 200 ms too early (Fig. 3E). Corresponding effects between legs 4 and 5 (Fig. 3C) may also exist; however, as few data could be obtained in the relevant range of phase values (Fig. 2C, IV), these effects cannot be proved. The reason for the rarity of such phase positions will be explained in the Discussion.

The amplitude of the return stroke increases only to a small extent (Fig. 4 for leg 2 and 3), consistent with the observation that the speed of the second part of the return stroke movement is decreased (see Fig. 2). This most probably shifts the AEP of the leg forward and increases the amplitude of the following power stroke as well, thus completing the process of compensating for the disturbing stimulus.

Fig. 5 depicts the results in which the duration of the return stroke either shortened or remained almost the same. The reference point is the end of the power stroke of the forward leg (t_0). For all three pairs of legs, the duration of the return stroke seems to be prolonged to some extent when the backward leg starts its protraction 'too early'; the duration of the return stroke is clearly shortened when it is started 'too late' relative to the normally coordinated step. The values shown in Fig. 5A are scattered around a line with a slope of −0·73 (measured for the range between −1000 ms and 0 ms); i.e. the compensation is relatively good compared to the slopes found in Fig. 5B,C, which are −0·55 and −0·51 respectively (ranges are between −1000 ms and 400 ms for Fig. 5B and between −1000 ms and −100 ms for Fig. 5C). The remainder of the compensation is accomplished by changing the duration of the following power stroke (see below).

Changes in the duration of the power stroke

As was done above in the case of the return stroke, the influences on the duration of the power stroke will be shown first using the backward leg as the reference and then using the forward leg. However, the results are not split as was the case for the return

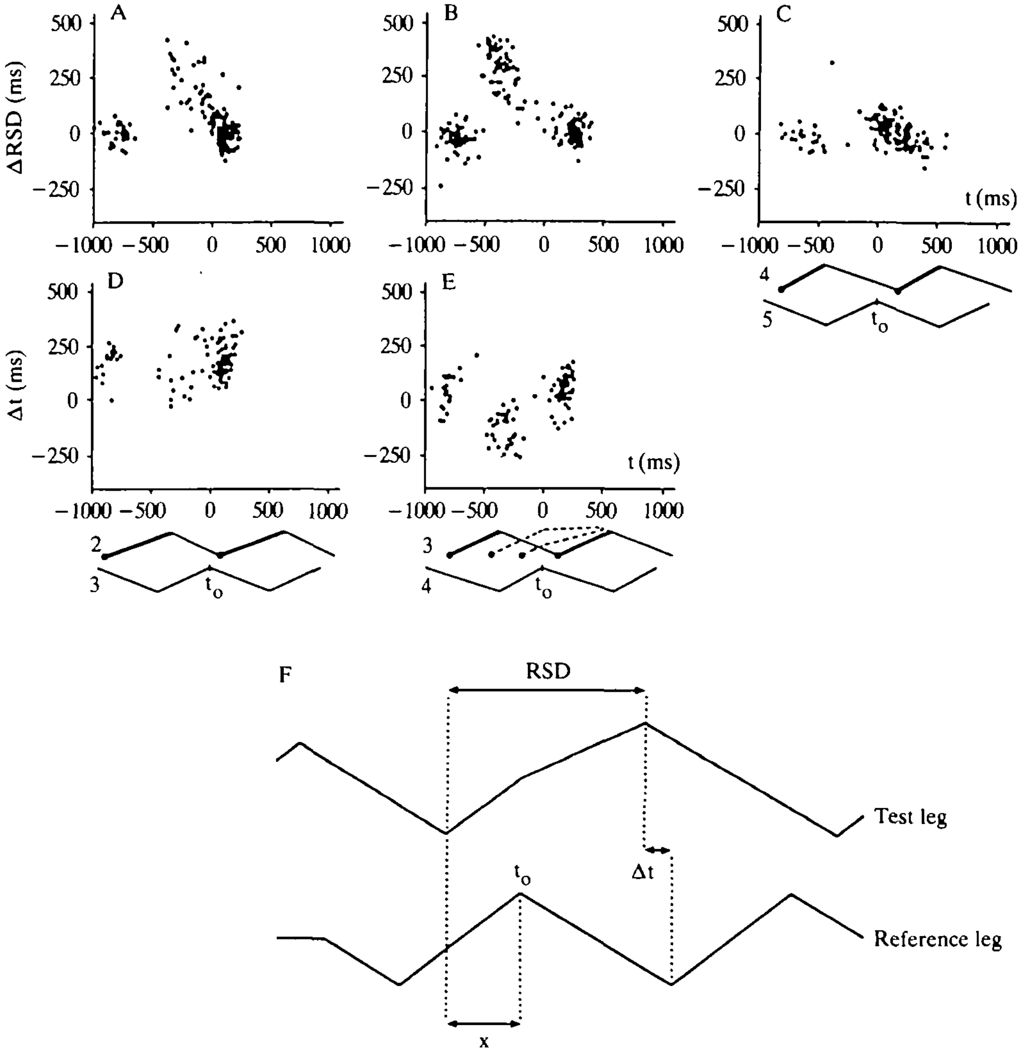

Fig. 3. Changes in return stroke duration and Δt-values for different phase relationships between the (posterior) reference leg and the (anterior) test leg. The abscissa gives the temporal differences (in ms) between the reference point t_0 (the end of the return stroke of the reference leg, shown by a small vertical bar in the step schema below the abscissa) and the start of the return stroke of the test leg (shown by solid circles in the schema). The schema shows the normally coordinated situation of the two legs, which are indicated by their numbers. The schema of Fig. 3E also includes some return strokes (shown as dashed lines) for abnormal, induced phase values. In Fig. 3A,B,C, the ordinate shows the difference between the duration of the actual return stroke (RSD, indicated by a thick line in the schema) and the RSD of the normally coordinated situation (obtained as the mean value from at least 25 steps of an undisturbed walk). Positive values indicate prolongation of the return stroke. In Fig. 3D,E, the ordinate shows the temporal difference Δt between the occurrence of the end of the power stroke of the backward leg and that of the return stroke of the test (forward) leg. Positive Δt values show that the end of the power stroke of the test leg occurs after the reference point. The measured parameters are also illustrated in the sketch of Fig. 3F.

stroke: where possible – i.e. for leg 3 (Figs 6B,E and 7A,D) and leg 4 (Figs 6C,F and 7B,E) – all values are presented relative to both reference legs. In Fig. 6 the end of the return stroke of the backward leg is the reference point. For legs 2 and 3, the power stroke is prolonged to some extent when it has started very early. The power stroke is clearly shortened if it has started later than it would have in normal coordination. The results show that, as long as leg 2 starts its power stroke before the reference point, this stroke ends not more than 100 ms after that of the normally coordinated leg (Fig. 6D). The same holds for leg 3 (Fig. 6E). The end of the power stroke of leg 4 seems not to be influenced by the beginning of the power stroke of leg 5 (Fig. 6F).

In Fig. 7, the duration of the power stroke is considered with reference to the forward leg. The reference point is the end of the return stroke of the forward leg. The results illustrate that the power stroke may be prolonged to some extent if it starts too early. This is very obvious in the case of leg 5 (Fig. 7C). In all three legs the duration of the power stroke is shortened if it starts too late. The Δt value (time difference between the reference point and the end of the power stroke of the backward leg) shows a small amount of scatter, which again indicates that compensation is nearly complete after the end of the power stroke.

The results from both measurements of the duration of the power stroke (Figs 6, 7) can be summed up as follows. The duration of the power stroke can be shortened in two ways. (1) When the power stroke starts at the normal AEP, shortening it causes it to terminate anterior to the normal PEP as the speed remains constant. This implies the existence of a coordinating influence. (2) The power stroke can also start at a position posterior to the normal AEP if the amplitude of the former return stroke was shortened (presumably by a coordinating influence). In this case, the power stroke may end at a position corresponding to the normal PEP; such

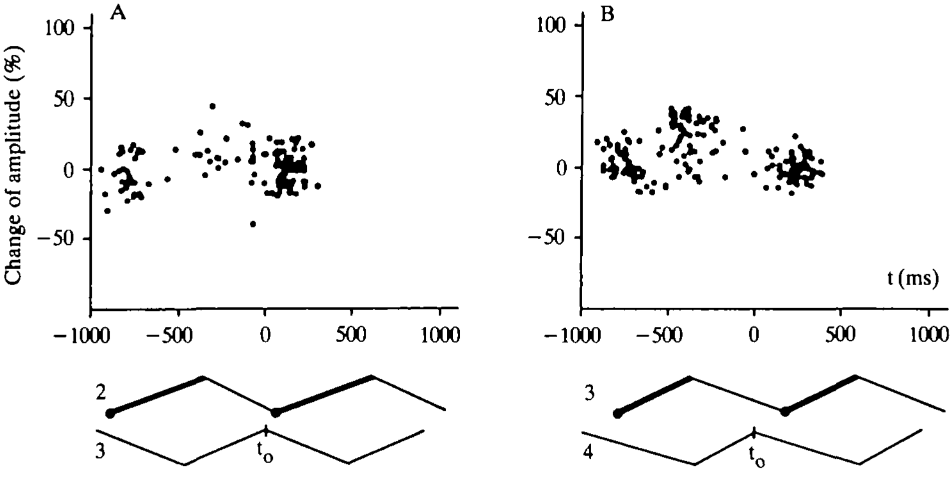

Fig. 4. Changes in amplitude. Abscissa as in Fig. 3. The ordinate shows the changes in return stroke amplitude as a percentage of the return stroke amplitude of normally coordinated steps.

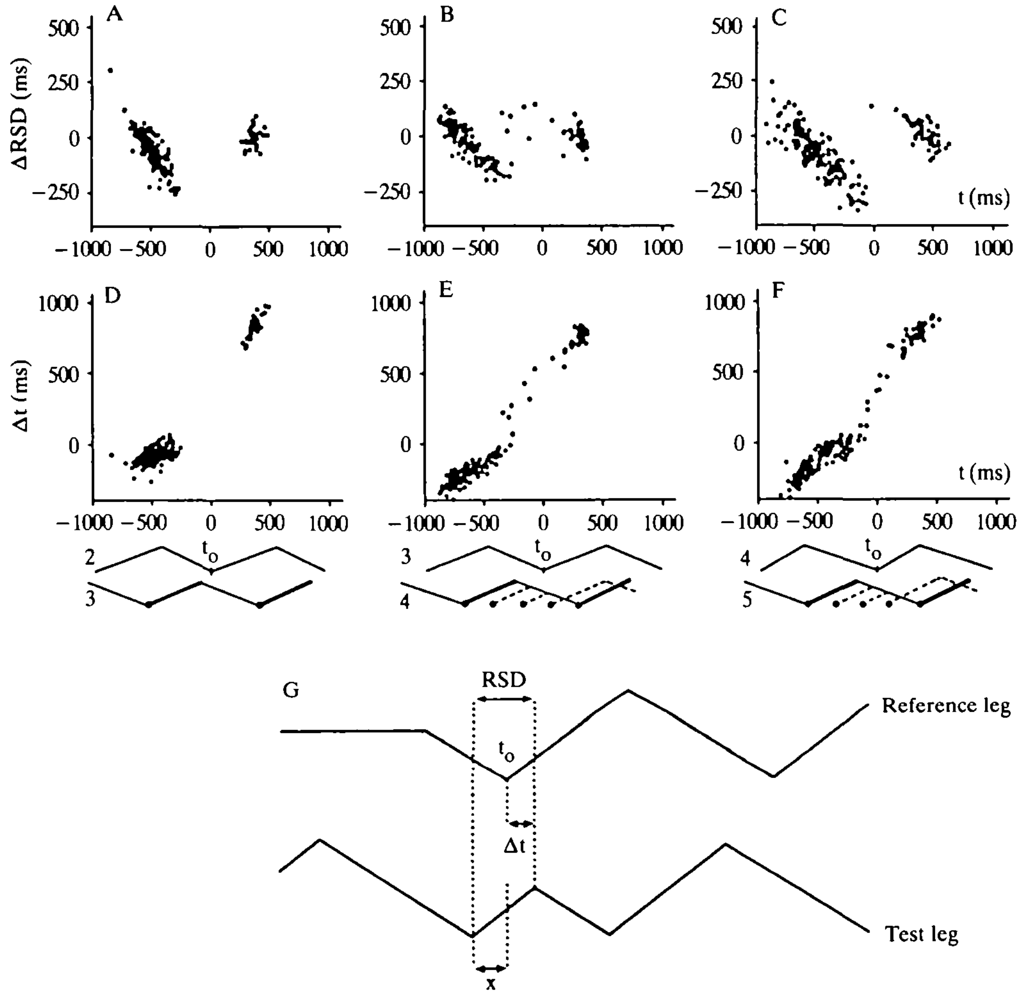

Fig. 5. Changes in return stroke duration and Δt-values for different phase relationships between the (anterior) reference leg and the (posterior) test leg. The abscissa gives the temporal differences (in ms) between the reference point t_0 (the end of the power stroke of the reference leg, shown by a small vertical bar in the step schema below the abscissa) and the start of the return stroke of the test leg (shown by solid circles in the schema). The schema shows the normally coordinated situation of the two legs, which are indicated by their numbers. The schema of Fig. 5E also includes some return strokes (shown as dashed lines) for abnormal, induced phase values. In Fig. 5A,B,C, the ordinate shows the difference between the duration of the actual return stroke (RSD, indicated by a thick line in the schema) and the RSD of the normally coordinated situation (obtained as the mean value from at least 25 steps of an undisturbed walk). Positive values indicate prolongation of the return stroke. In Fig. 5D,E,F, the ordinate shows the temporal difference Δt between the reference point and the end of the return stroke of the backward leg. Positive values show that the end of the return stroke of the test leg occurs after the reference point. The measured parameters are also illustrated in the sketch of Fig. 5G.

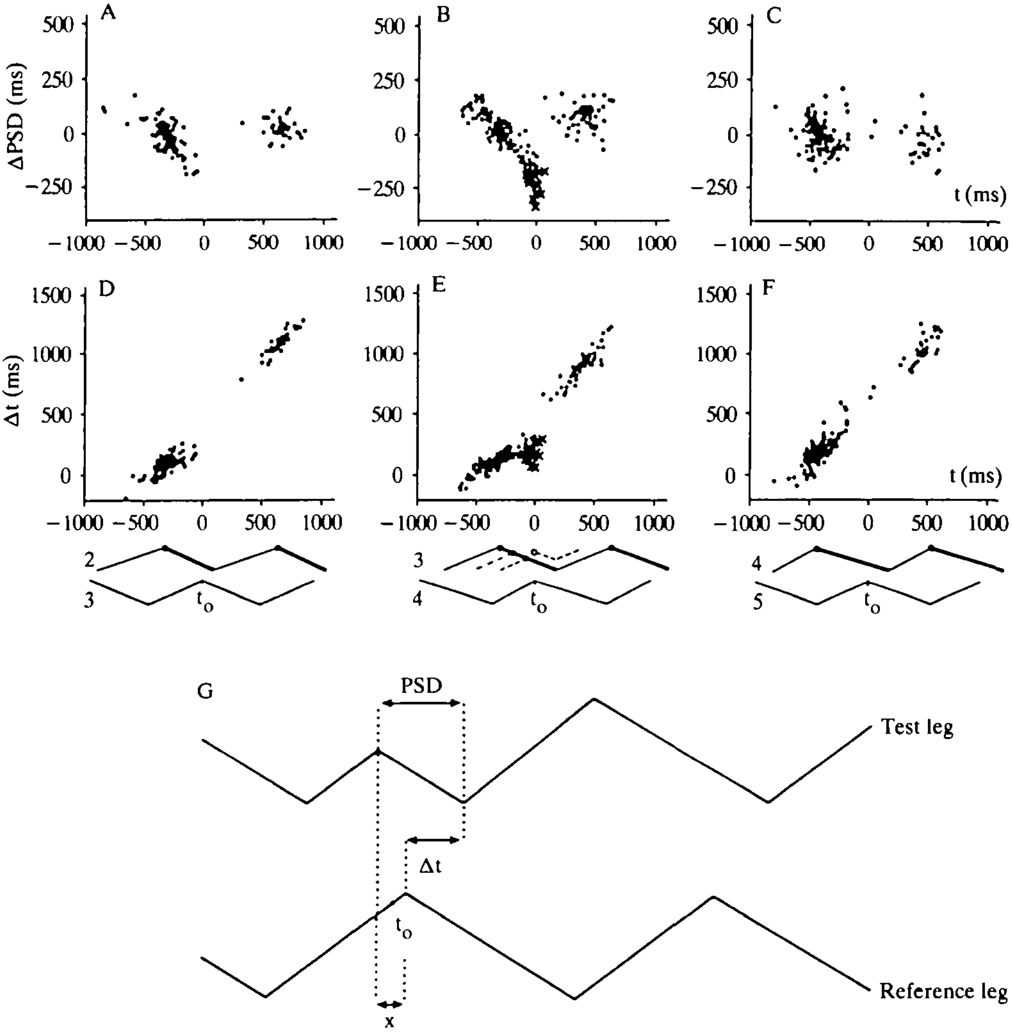

Fig. 6. Changes in power stroke duration and Δt-values for different phase relationships between the (posterior) reference leg and the (anterior) test leg. The abscissa gives the temporal differences (in ms) between the reference point t_0 (the end of the return stroke of the reference leg, shown by a small vertical bar in the step schema below the abscissa) and the start of the power stroke of the test leg (shown by solid circles in the schema). The schema shows the normally coordinated situation of the two legs, which are indicated by their numbers. The schema of Fig. 6E also includes some return strokes (shown as dashed lines) for abnormal, induced phase values. In Fig. 6A,B,C, the ordinate shows the difference between the duration of the actual power stroke (PSD, indicated by a thick line in the schema) and the PSD of the normally coordinated situation (obtained as the mean value from at least 25 steps of an undisturbed walk). A value of zero indicates that the power stroke duration equals that of a normal step and positive values indicate prolongation of the power stroke. In Fig. 6D,E,F the ordinate shows the temporal difference Δt between the reference point and the end of the power stroke of the test (forward) leg. Positive Δt values show that the end of the power stroke of the test leg occurs after the reference point. For the meaning of the crosses, see text. The measured parameters are also illustrated in the sketch of Fig. 6G.

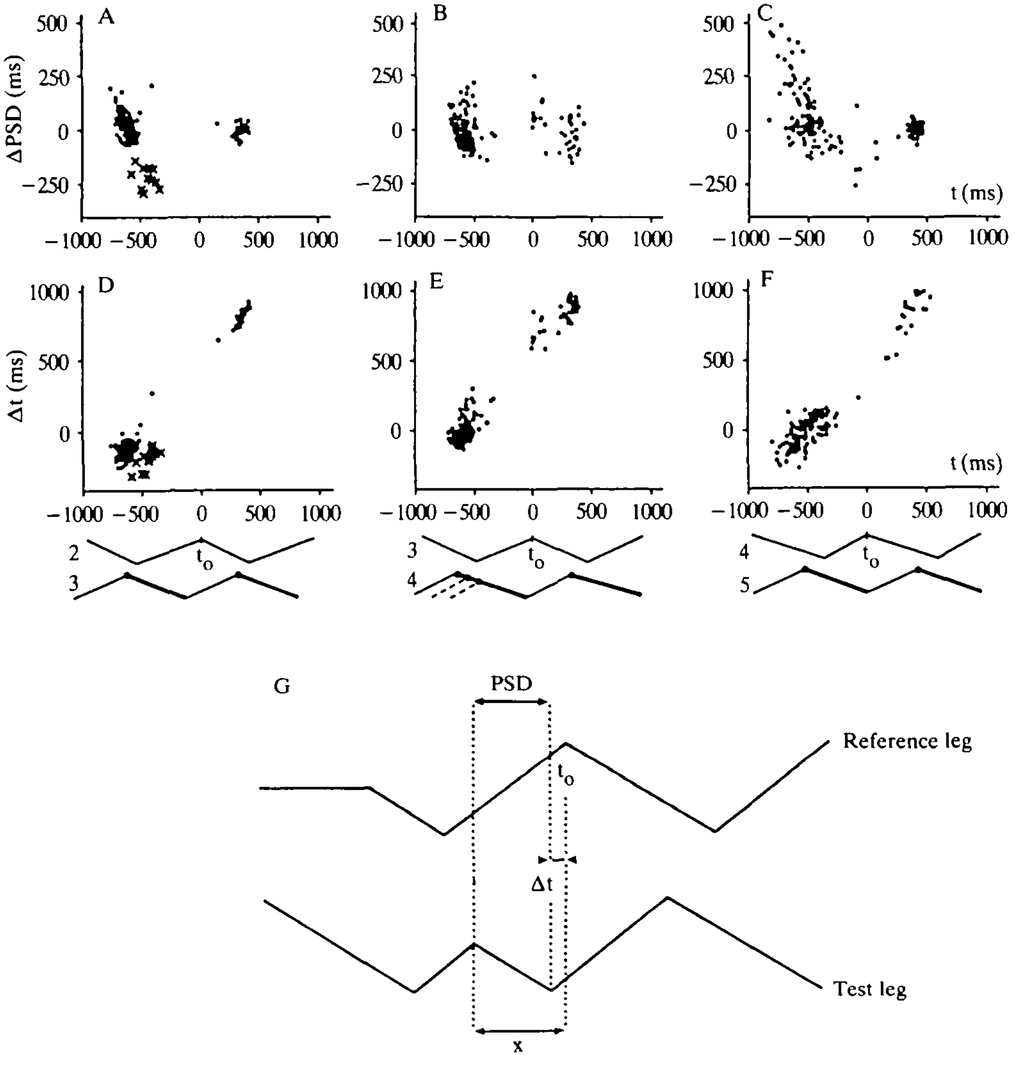

Fig. 7. Changes in power stroke duration and Δt-values for different phase relationships between the (anterior) reference leg and the (posterior) test leg. The abscissa gives the temporal differences (in ms) between the reference point t_0 (the end of the return stroke of the reference leg, shown by a small vertical bar in the step schema below the abscissa) and the start of the power stroke of the test leg (shown by solid circles in the schema). The schema shows the normally coordinated situation of the two legs, which are indicated by their numbers. The schema of Fig. 7E also includes some return strokes (shown as dashed lines) for abnormal, induced phase values. In Fig. 7A,B,C, the ordinate shows the difference between the duration of the actual power stroke (PSD, indicated by a thick line in the schema) and the PSD of the normally coordinated situation (obtained as the mean value from at least 25 steps of an undisturbed walk). Positive values indicate prolongation of the power stroke. In Fig. 7D,E,F the ordinate shows the temporal difference Δt between the reference point and the end of the power stroke of the backward leg. Positive Δt values show that the end of the power stroke of the test leg occurs after the reference point. For the meaning of the crosses, see text. The measured parameters are also shown in the sketch of Fig. 7G.

an outcome may be caused by the mechanism controlling the movement of the leg itself rather than by any coordinating influence. Thus, in this case the shortening of the power stroke may be only an indirect consequence of a coordinating mechanism having influenced the previous return stroke. For leg 2, possibility 1 always occurs, whereas both 1 and 2 may arise for leg 3. Occasions on which the PEP of leg 3 was clearly shifted in the anterior direction are indicated by open circles in Figs 6 and 7.

DISCUSSION

The results show that influences exist between walking legs which are able to bring an out-of-phase leg back into phase within one step. In this section we attempt a more detailed description of these mechanisms on the basis of the experimental results presented above. Such a description needs to address the following questions. Are there different types of influences and, if so, what are their specific properties?

We restrict our considerations by two assumptions. First, we assume that influences from contralateral legs are weak compared to those from ipsilateral legs, so that the former can be neglected for a first approximation. This is supported by our own results (Müller, 1985), by those of Barnes (unpublished, cited in Clarac, 1982), and – for the rock lobster – those of Chasserat & Clarac (1983). Second, we assume that influences from directly neighbouring legs are much stronger than those between next-nearest neighbours, so that the latter are also neglected here. This assumption is justified by the model calculation mentioned below, which shows that such indirect influences need not be postulated.

Ascending influences

Even given these restrictions it is often not easy to decide which of two coupled oscillators influences which. This is even more difficult in the case of more than two oscillators, i.e. where (as for legs 3 and 4) a leg could be influenced by two neighbours. Nevertheless, the results do allow some conclusions. Fig. 3A shows that the return stroke of leg 2 is prolonged when this leg steps so late that the beginning of the power stroke of leg 3 occurs before the return stroke of leg 2 is finished. As leg 2 is the most rostral walking leg, changes in its behaviour must result from the influence of leg 3. This is supported by the observation that with the beginning of the power stroke of leg 3, the speed of the return stroke of leg 2 is decreased (see Fig. 2). This two-fold influence inhibiting the start of a power stroke and decreasing the motor output during the return stroke of leg 2 occurs only during the power stroke of leg 3. In the normally coordinated situation the forward leg continues its power stroke during the time interval between the end of the posterior leg's return stroke and the end of the anterior leg's power stroke. Thus, during this time interval leg 2 is not stimulated sufficiently to start the return stroke. Therefore, we conclude that this forward-directed influence is relatively weak during the first part of the power stroke of the controlling leg. This influence is symbolized in Fig. 8A. The thickness of the bar represents the strength of the ascending influence.

We found qualitatively the same results in looking at the relationship between legs 3 and 4 (Fig. 3B). However, the corresponding conclusions cannot immediately be drawn: leg 3 might also be influenced by leg 2, the phase relationships of which are not shown in the diagram. For example, if leg 3 was experimentally brought out of phase, then both leg 2 and leg 4 might bring leg 3 back into its normal phase relationship. However, in the experiment described in Fig. 3B the experimental manipulation occurred at leg 4. As both leg 2 and leg 3 were in proper phase during the manipulation of leg 4, leg 2 stabilizes the rhythm of leg 3. Similarly, any existing influences from contralateral legs would tend to stabilize the rhythm of leg 3. Thus, influences which, in this experiment, change the phase of leg 3 have to arise from leg 4, not from leg 2. This means that the effect on leg 3 shown in Fig. 3B (i.e. the prolongation of the return stroke) is produced by leg 4. Qualitatively, it is the same effect as that of leg 3 on leg 2 described above. Quantitatively, however, leg 4's influence on leg 3 seems to be weaker, although this may not be the case: other influences on leg 3 which do not act on leg 2 (e.g. those from leg 4) may decrease the relative strength of the effect. Although this cannot be concluded from the results, leg 5 may exert a corresponding influence on leg 4. However, because of other strong influences running from leg 4 to leg 5 (see below), this latter force could rarely become effective. Thus the results show that at least for the first two pairs of legs, there exists a mechanism according to which posterior legs attempt to prolong the return stroke of the next anterior leg as long as the controlling leg performs a power stroke.

Descending influences

Fig. 5 shows another kind of influence on the duration of the return stroke. The return stroke of leg 5 (Figs 2F, 5C) may in some cases be slightly prolonged when it starts quite early relative to leg 4, but for most timing relationships the return stroke duration is shortened. The later the return stroke starts relative to the reference point (the PEP of leg 4), the greater the shortening. The end of the return stroke of leg 5 rarely exceeds the beginning of the return stroke of leg 4 (Fig. 5F). Because leg 5 is the most posterior walking leg, this influence must come from leg 4 as the latter nears the onset of its return stroke. The influence terminates the return stroke of leg 5. However, this influence disappears abruptly about 150–200 ms after the return stroke of leg 4 has begun (Fig. 5C). Thus, the influence acts during the last part of leg 4's power stroke and possibly the first part of its return stroke. The beginning of this influence cannot be determined as exactly as its end. If the return stroke of leg 5 ends before a certain critical value (which probably corresponds to that point in the power stroke of leg 4 at which in normal coordination leg 5 starts its own power stroke), the return stroke may be prolonged; if it ends after that point, it is shortened (see Fig. 5C).

If the slope of the points in the diagram of Fig. 5C had been -1, a definite point in the cycle of leg 4 would have been responsible for stopping the return stroke of leg 5. The much smaller actual slope ($-0{\cdot}51$) shows, however, that the effect increases gradually: stopping the return stroke of leg 5 is apparently more difficult when the leg is in an earlier stage of its return stroke. This effect is illustrated in the sketch below Fig. 5E for the case of leg 4. The duration and increasing strength of this descending effect is symbolized in Fig. 8B by a wedge. The data do not reveal whether this effect reflects a property of leg 5 itself (i.e. greater susceptibility to influence in the last part of its return stroke) or an increase in the strength of the influence from leg 4. However, the latter seems more probable and will be assumed in the model calculation below.

Corresponding effects were found to occur between legs 2 and 3 (Fig. 5A) and legs 3 and 4 (Fig. 5B). For these pairs of legs it is not immediately clear whether the effect is actually produced by the forward leg, since in both cases posterior legs also exist which might be the source of the effect. However, Fig. 3C shows that leg 5 exerts practically no effect on the duration of the return stroke of leg 4. This shows that the results presented in Fig. 5B are produced by the forward leg as well. Since no corresponding argument can be made for the shortening of the return stroke of leg 3 (Fig. 5A), it may be that this results from the influence of leg 4. However, as the results are very similar for all three pairs of legs, we speculate that the same mechanism exists between legs 2 and leg 3. The prolongation occurring when leg 3 starts its return stroke very early may be caused by leg 4.

Thus, the results show that there are at least two different types of coordinating influences. First, as the results in Fig. 5 show, there exists an influence running in a backward direction which acts during the last part of the power stroke and the first part of the return stroke of the controlling leg to start a power stroke by the next backward leg. A second influence can be inferred from the results shown in Fig. 3 to act in a forward direction. This influence acts to bring the next forward leg into return stroke or to maintain it in this stroke as long as the controlling leg is in its own power stroke.

Fig. 5A,D shows an 'empty zone' which is less obvious in Fig. 5B,C,E,F. Although this might be regarded as a 'forbidden zone', that would be misleading. The reason no return strokes start in this zone is not because they are not 'allowed' but because of the following: the strong effect prolonging the return stroke of the forward, reference leg always shifts this leg's AEP and, in consequence, its subsequent PEP in such a way that the PEP of the backward leg cannot approach too near the reference point (which is the PEP of the forward leg). In Fig. 5B,C this time gap is not as clear, indicating that the influences prolonging the return stroke of legs 3 and 4 are not as strong as those acting on leg 2. The empty zones found in Figs 6A and 7A can be explained in the same way as those described above for Fig. 5A.

Combined descending and ascending influences

Fig. 6 shows that the duration of the power stroke can also be influenced. While the power stroke can be prolonged to some extent, except for leg 5 (Fig. 7C), it is generally shortened. As was mentioned in the final paragraph of the Results section, a change in the duration of the power stroke might simply result from a change in the amplitude of the former return stroke, which would not necessarily imply the existence of coordinating influences. Most of the changes in power stroke duration shown in Figs 6B,C and 7A,B (solid circles) can be interpreted in this way. However, this is not immediately possible for either the results shown in Fig. 6A or those marked with open circles in Figs 6B and 7A. These are the cases where the power stroke starts at a normal AEP and ends before it has reached its normal PEP. Examples of this can be seen in Fig. 2A, III; Fig. 2B, III; and Fig. 2D, IV. However, all these results can also be interpreted easily as the consequence of the two influences mentioned – one coming from the backward leg and prolonging the return stroke, and the second coming from the forward leg and inducing the start of a power

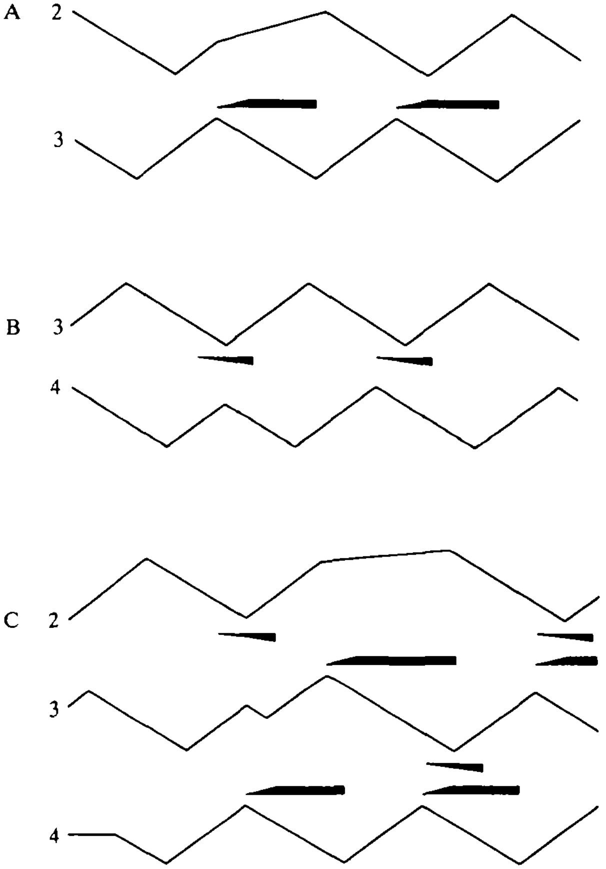

Fig. 8. Schematic drawing summarizing the results. The duration of the hypothesized influences are represented by the length of the horizontal bars, their assumed intensity by the thickness of the bar. In (A) only the ascending influence is shown. This causes the anterior leg to perform a return stroke as long as the posterior leg performs a power stroke. In (B) only the descending influence is shown. This induces the posterior leg to start a power stroke when the anterior leg is near the end of its power stroke. In (C) the effects of both influences on leg 3 are shown, one arising from the anterior, the other from the posterior leg. In some abnormal phase relationships of the three legs both influences combine to produce a short power stroke in leg 3. During normal coordination, as in the final steps of (C), both influences produce qualitatively the same effect and thus form redundant connections.

stroke. This is illustrated in Fig. 8C. The influence from the backward leg (leg 4 in Fig. 8C) continues during most of its power stroke, whereas the influence from the forward leg (leg 2 in Fig. 8C) acts only around the end of its power stroke. When both influences occur at the same time, the effect from the forward leg can be stronger, thus overriding the influence of the backward leg and producing a power stroke in the influenced leg (leg 3 in Fig. 8C). However, shortly after the forward leg has started its return stroke the effect of this influence vanishes, allowing that from the backward leg to dominate again. Thus no further influences have to be assumed to explain these results.

The weak prolongation of the power strokes of legs 2, 3 and 4 can also be interpreted as a consequence of the prolongation of their previous return strokes. Thus this effect does not necessarily result directly from coordinating influences. However, the considerable prolongation of the power stroke of leg 5 (Fig. 7C) cannot be explained in this way, as no corresponding prolongation of the previous return stroke occurs and this leg obviously rests at its PEP until a coordinating signal allows it to start the return stroke. Is it necessary to postulate an additional mechanism in order to explain this phenomenon? According to the two mechanisms described, leg 5 receives only influences from leg 4 which can inhibit the start of leg 5's return stroke. In contrast to the other legs, leg 5 receives no coordinating influences which support the performance of a return stroke because there is no leg behind leg 5. Thus the results could be explained without assuming additional coupling mechanisms. Because of this unidirectional influence, the onset of the return stroke of leg 5 is delayed so far that the return stroke occurs in nearly normal phase relative to leg 4. This in consequence means that no obvious effect is found to arise from leg 5 to change the duration of either the return stroke (Fig. 3C) or the power stroke (Fig. 6C) of leg 4. As was mentioned earlier, this does not necessarily mean that a corresponding mechanism does not exist between leg 5 and leg 4. A less obvious but corresponding influence from leg 3 apparently prolongs the power stroke of leg 4 (Fig. 2E, IV).

Considering the results presented in Fig. 2 raises the question why in some cases (Fig. 2B, IV; Fig. 2D, V) no interruption of the return stroke occurs, whereas in others with more or less the same phase such interruptions do appear. This difference might result from different temporal occurrences of both influences, which was tested in the following way. The end of the power stroke of the next forward leg was used as a rough approximation of the backward-directed influence, and that of the return stroke of the next backward leg as an approximation of the forward-directed influence. The probability of producing a short power stroke during the return stroke was indeed significantly ($P < 0{\cdot}001$, $N = 26$) increased when the end of the power stroke of the next forward leg occurred before the end of the return stroke of the next backward leg. Thus each leg can be considered as resembling a sort of bistable flip-flop, the state of which depends upon the strength of the two coordinating influences as well as signals from the leg's own sense organs.

As the experiments were only performed at one walking speed, nothing can be said about how the beginning and end of the coordinating influences are determined;

such a mechanism might depend upon a constant time delay following a definite point in the cycle of the controlling leg, a definite phase value within the cycle of the controlling leg, etc. As the preliminary experiments showed that the time between the end of the return stroke of the backward leg and that of the power stroke of the forward leg is not constant for the periods investigated, the first possibility seems improbable. However, further experiments are needed to answer this question.

A model calculation

To test whether the two influences described above are sufficient to account for the observed behaviour, a model calculation – based on an earlier one (Cruse, 1983) – was performed. Two coupling mechanisms were incorporated, using the properties just discussed, but these will not be explained here in detail. The influence acting in a backward direction is assumed to increase in strength when the leg approaches its PEP. This calculation provides a good description of the behavioural results. In particular, it should be mentioned that the model predicts the prolongation of the power stroke of leg 5 resulting from the pause at the end of the stroke. Thus, the two mechanisms discovered here are sufficient to describe our experimental results. Nevertheless, further 'redundant' or non-redundant, but weak, coupling mechanisms may exist. Furthermore, as the two influences demonstrated here differ qualitatively from each other, they are not only sufficient but necessary. In addition, the model calculation supports our assumptions that, in our experiments at least, contralateral coupling plays only a small role compared to ipsilateral coupling and that ipsilateral influences between non-neighbouring legs can be neglected for a first approximation.

Both the experimental results and the model calculation show that within the limits of measurement, coordination is normally restored after one step. This occurs mainly through direct influences on the duration of the return stroke; the change in the following power stroke is only an indirect consequence. However, direct influences may also shorten or prolong the duration of the power stroke.

Comparison with other arthropods

Few experiments on the mechanisms coordinating the walking legs of crayfish have been reported in the literature. Evoy & Fourtner (1973) showed for *Procambarus* that leg 5 is less strongly coupled to leg 4 than is the case for the other neighbouring pairs. This is in agreement with our observations. After a leg has been disturbed it will normally return to the proper phase with its neighbouring leg by the next step. However, leg 5 sometimes deviated from this rule, taking several steps to reach the proper phase value. We found this to be the case as well for contralateral coupling for each segmental leg pair (Müller, 1985). Another experiment was performed by Barnes & Kidd (cited in Clarac & Barnes, 1985) with *Astacus*, in which they tied up leg 4 by means of a thread so that it could not reach the ground. This leg was then normally coordinated with leg 5 but not with leg 3. This demonstrates the existence of a coordinating influence acting in a forward direction which may well be the same influence as the one we have described.

More extensive data are available for lobsters than for crayfish. Our results support the suggestion by Clarac (1982), Clarac & Chasserat (1983) and Chasserat & Clarac (1983) that for the rock lobster *Jasus*, the duration of both the return stroke and the power stroke are changed to obtain a proper phase-relationship between neighbouring legs.

In a detailed analysis, Chasserat & Clarac (1983) found changes in the period length of leg 4 and inferred the existence of a reciprocal influence between legs 4 and 5 that was both 'excitatory' and 'inhibitory' in nature. When these two terms are taken to refer to the excitation or inhibition of a return stroke, their results can be interpreted to mean that: (a) the return stroke of leg 5 inhibits the start of the return stroke of leg 4, thereby extending the duration of the power stroke of leg 4 (as occurs in insects, see below); and (b) the end of the return stroke of leg 5 excites the start of a return stroke by leg 4, thereby shortening the power stroke duration of leg 4. This influence would have the effect of preventing the overlap of return strokes by neighbouring legs. This does not seem to fit our results obtained using crayfish: such overlapping return strokes were observed quite often (see Fig. 2A, II; Fig. 2B, II; Fig. 2C, IV; and Fig. 2E, V). However, the results from the rock lobster are also open to other interpretations. For crayfish, we were able to show that in some cases the shortening of the power stroke can be explained by the forward-directed influence, which 'excites' the start of a return stroke (see Fig. 2A, II; Fig. 2B, III) during the power stroke of the controlling leg. This effect, together with backward-directed influences from leg 3, could also explain the behaviour of the rock lobster.

An exciting study of coupling influences between the legs of a rock lobster was performed by Clarac & Chasserat using the technique of autotomy (1979). After autotomizing successive legs and measuring the muscle excitation in the remaining leg stumps, these authors demonstrated the existence of two coordinating influences. One acts in a forward direction and influences the stump in front of an intact leg to perform movements in antiphase compared to the latter leg (Clarac, 1982). The experiment did not determine whether this influence is active during only one part of the cyclic movement of the controlling leg (e.g. power stroke or return stroke) or during the whole cycle. Thus this result remains open to different interpretations. It could reflect either an inhibition of the start of the return stroke of the forward leg during the return stroke of the controlling leg; or an inhibition of the start of the power stroke of the forward leg during the power stroke of the controlling leg (as described here for the crayfish); or possibly a combination of both. Clarac & Chasserat (1979) have also described a second influence acting in a backward direction whereby the cyclic excitation of the leg stump muscles follows the cycle of the forward intact leg nearly synchronously. However, no quantitative comparison is possible with the backward-directed influence described for the crayfish. As was described above, the latter influence is assumed to act at about the end of the power stroke of the controlling leg and to elicit a power stroke in the backward leg. It is quite possible that both effects result from the same influence. So far, however, a final decision on the matter cannot be made.

Thus, although there are a number of experimental results for the lobster, because of the different methods of measurement and evaluation it is not yet possible to determine whether or not the coupling influences in the lobster and the crayfish are the same.

In insects only one coordinating mechanism has to date been described clearly enough to allow comparison with our results. Several authors have demonstrated the existence of a forward-directed influence which inhibits the start of the return stroke of the forward leg as long as the controlling leg performs a return stroke (Graham, 1978; Dean & Wendler, 1982; Cruse & Epstein, 1982; Foth & Graham, 1983). As was mentioned above, corresponding mechanisms were not found in the crayfish although the experimental results of Chasserat & Clarac (1983) seem to suggest that they do exist in the rock lobster. Backward-directed influences have also been found in insects (Bässler & Wegner, 1983). However, comparison is difficult because no information is available concerning the phase dependency of this mechanism. In sum, although the available data are scanty, they show at the very least that the coupling mechanisms between the walking legs of insects and of crustaceans are not always the same.

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