

DIGGING IN SAND CRABS: COORDINATION OF JOINTS IN INDIVIDUAL LEGS

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Accepted 8 May; published on WWW 25 June 1998

Summary

Sand crabs use their multi-jointed legs to dig into sand. Combined movement and electromyogram (EMG) analyses showed that the pattern of intra-leg coordination in the legs of two sand crabs of different families (*Blepharipoda occidentalis* and *Emerita analoga*) is similar in legs 2 and 3, but very different in leg 4. For example, the sequence of proximal joint movements in legs 2 and 3 is elevation, retraction, depression and protraction (similar to backward walking in most decapods), but the sequence of proximal joint movements in leg 4 is elevation, protraction, retraction and depression (similar to forward walking). The synergies are the same during leg movements in sea water and in sand, suggesting that the same motor programme is used in both situations. At the transition from sea water into sand, however, both the frequency and

amplitude of the EMG potentials increase, and the phasing of the motor output to leg 2 (and presumably leg 3) changes from proportional (both power and return strokes co-vary with period) to return stroke constant (power strokes co-vary much more with period than do return strokes). The motor output to leg 4 remains intermediate between proportional and return stroke constant in sea water and in sand. On the basis of the segmental specialisation of the motor patterns for the legs, we hypothesize that sand crab digging may be an evolutionary mosaic of disparate ancestral locomotor behaviours.

Key words: crustacean, evolution, kinematics, leg, locomotion, digging, swimming, crab, *Blepharipoda occidentalis*, *Emerita analoga*.

Introduction

Many animals use multi-jointed limbs to move through their environment. In theory, the many degrees of freedom allowed by multiple joints should make multi-jointed limbs difficult to control (Whiting, 1984; Turvey *et al.* 1982). How central pattern generators (CPGs) coordinate the movements of multi-jointed limbs is somewhat less well understood than the production of rhythmic motor output to single joints and the coordination among multiple limbs. Nevertheless, the control of multi-jointed limbs will result from an interplay of extrinsic factors, such as sensory cues and reflexes (e.g. Cattaert *et al.* 1993; El Manira *et al.* 1991a,b; Müller and Clarac, 1990), and intrinsic factors, such as centrally generated motor programmes (e.g. Chrachri and Clarac, 1990; Bässler, 1993; Jamon and Clarac, 1997).

Decapod crustacean locomotion is well suited for studying intra-limb coordination, and walking has been investigated extensively. Decapods are relatively large, which facilitates movement analyses; their legs have several joints, which are typically simple hinges (Lochhead, 1961); and decapods have a diverse set of locomotor behaviours, both within and among species. Movements of the two most proximal joints, the thorax-coxa and coxa-basis joints, are important in the kinematics (Ayers and Clarac, 1978; Clarac *et al.* 1987;

Barnes, 1977; Jamon and Clarac, 1997; Macmillan, 1975) and proprioceptive regulation of decapod walking (Head and Bush, 1991, 1992; Sillar *et al.* 1986; Skorupski and Sillar, 1988). Movements of the merus-carpus joint are also important in some cases, notably in sideways walking (Ayers and Clarac, 1978; Barnes, 1977; Clarac *et al.* 1987; Jamon and Clarac, 1997), although their contribution to proprioception is less well investigated. The remaining three leg joints typically make smaller movements than the other joints (Barnes, 1977).

Sand crabs use their multi-jointed legs and 'tail' for digging. Forward-going power strokes by legs 2 and 3 shovel sand from underneath the animal. Leg 4 pushes the rear end of an animal down into the sand, increasing the purchase of the other legs (Faulkes and Paul, 1997c). Rapid movements of the tail liquefy the sand, enabling the animal to descend rapidly into sand (Faulkes and Paul, 1997b). Digging leg movements are similar to (and, we hypothesise, evolutionarily derived from) walking leg movements in other decapods: both are locomotor behaviours using the legs. The intra-limb coordination of sand crab digging interests us for several reasons. First, the legs of the sand crabs are behaviourally specialised, which offers the possibility of studying different motor outputs in serially homologous limbs in a single animal. Second, digging

movements occur under a wide variety of sensory regimes, ranging from swimming in water (Paul, 1981) to digging several body lengths below the surface of sand (Hill, 1979). Changes in sensory input alter coordination between the legs (Faulkes and Paul, 1997c) and between the legs and the tail (Faulkes and Paul, 1997b). Our results, however, suggest that individual legs use a similar, if not the same, motor programme for both swimming and digging (e.g. Fig. 4 in Faulkes and Paul, 1997c). Third, there is the evolutionary question of how a locomotor innovation such as sand crab digging originated. Finally, sand crab digging is somewhat more complex than walking: joints that play relatively minor roles in walking make large movements during digging. In this paper, we analyse the coordination among the joints within individual legs of two sand crab species from different families, with the following aims. First, we wish to determine whether the segmental differences in sand crab leg movements are due to differences in motor output. Second, we wish to determine whether the motor output to a single leg changes

during the transition from sea water to sand in a comparable manner to the changes in interleg coordination (Faulkes and Paul, 1997c). Third, we will further test the hypothesised homology between digging and walking. Abstracts of this work have been published (Faulkes and Paul, 1993, 1995).

Materials and methods

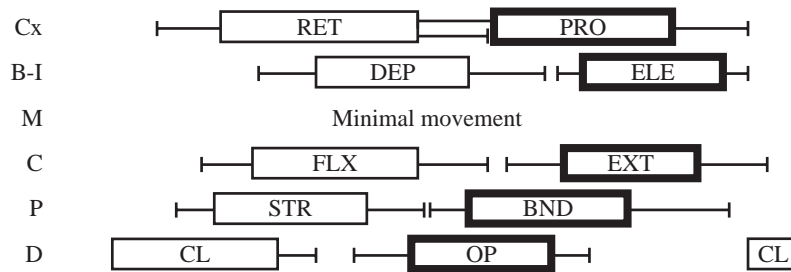
Spiny sand crabs *Blepharipoda occidentalis* Randall and mole sand crabs *Emerita analoga* (Stimpson) were collected and housed as previously described (Faulkes and Paul, 1997c). All experiments were conducted in accordance with Canadian Council of Animal Care guidelines.

Video and electromyogram (EMG) recordings were made using the techniques described in a previous paper (Faulkes and Paul, 1997c). The two recordings were synchronised using a device that stripped a 30Hz signal from the video camera, which was synchronised with the camera's electronic shutter.

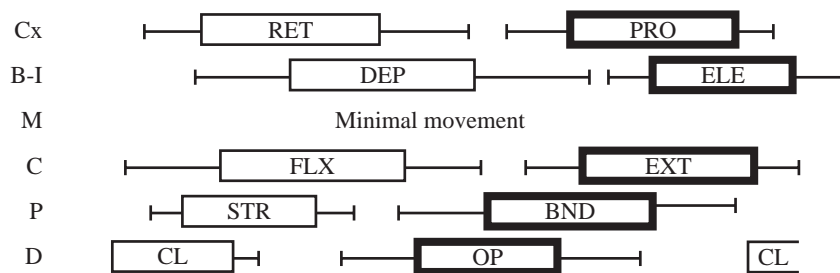


Blepharipoda occidentalis

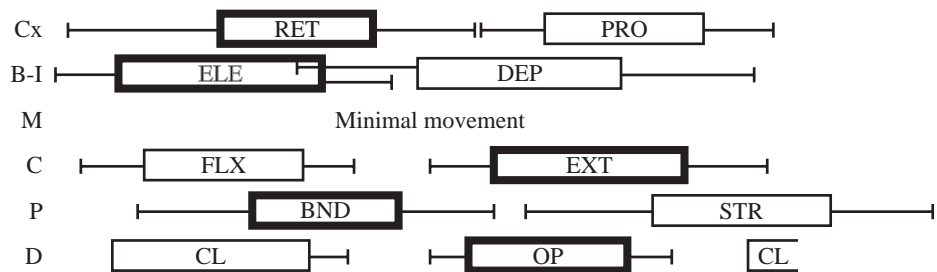
A Leg 2



B Leg 3



C Leg 4



100 ms

Fig. 1. Intra-leg coordination in *Blepharipoda occidentalis* in (A) leg 2, (B) leg 3 and (C) leg 4 analysed from video recordings. Boxes indicate the mean duration of movement of leg segments (abbreviated at left); muscles listed inside boxes are those predicted to be responsible for the movement; bars show the standard deviation of the mean start and stop of joint movements. (A,B) Thin boxes, leg segment movements comprising the return stroke; thick boxes, leg segment movements comprising the power stroke. (C) Thin boxes, movements produced by serially homologous muscles to return stroke muscles in legs 2 and 3; thick boxes, equivalent movements to leg 2 and 3 power stroke. Phases are measured from the onset of closer-generated movement in all legs. This figure does not show normal interleg coordination (see Faulkes and Paul, 1997c). Mean phase and standard deviation are calculated for two strokes each from four individuals. Leg segments (left): Cx, coxa; B-I, basi-ischium; M, merus; C, carpus; P, propus; D, dactyl. Muscles (boxes): PRO, protractor; RET, retractor; ELE, elevator; DEP, depressor; EXT, extensor; FLX, flexor; STR, stretcher; BND, bender; OP, opener; CL, closer (these abbreviations are used in all subsequent figures and in the tables).

A manually activated event marker turned on a light-emitting diode visible in the video recording and superimposed a 1 kHz wave on top of the signal taken from the video camera. The combined signal from the camera and event marker was recorded on one channel of the FM tape, along with up to four channels of EMGs, and the event markers on the video and tape recordings were aligned for the analysis. Some whole leg movements were digitised using the Peak 5 movement analysis system (Peak Performance Technologies, Inc.), and the movements of individual leg segments were analysed using Eshkol–Wachman movement notation (Eshkol and Wachman, 1958; Eshkol, 1980; Golani, 1976, 1992).

The movements of legs 2 and 3 are so similar (Faulkes and Paul, 1997c) that we analysed only leg 2 in detail in *B. occidentalis*. The forward and backward movements of leg 2 define the power stroke and return stroke of the leg, respectively. The movement of leg 4 is not divided into power stroke and return stroke components (Faulkes and Paul, 1997c). The intra-leg coordination of legs in *E. analoga* was examined using EMGs because individuals tend not to make leg movements when held in sea water (Faulkes and Paul, 1997b,c), the telson and carapace conceal several leg joints, and the spatial and temporal resolution of video recordings was inadequate to resolve the rapid movements of individual joints.

The burst durations and periods of EMG activity were measured using Axotape 1.2 (Axon Instruments, Inc.). We measured the EMG parameters for each muscle separately, viewing a single channel at a time. Inspection of the EMGs showed that any measurement with a period greater than 2 s was either the last stroke in a digging sequence or reflected

missing data (e.g. movement artefacts obscuring the EMG signal), so data with periods greater than 2 s were removed from all analyses.

Results

Intra-leg coordination

Video analysis showed that intra-leg coordination is very similar in legs 2 and 3 of *B. occidentalis* (Fig. 1), as predicted by their similar tip trajectories (Faulkes and Paul, 1997c). In legs 2 and 3 of *B. occidentalis*, movements caused by the opener, bender, extensor, protractor and elevator muscles make up the power stroke, and movements caused by the closer, stretcher, flexor, depressor and retractor muscles make up the return stroke (Figs 1, 2). The movements of the merus caused by the reductor muscle are too small to notate effectively, but EMGs show that the reductor functions as a power stroke synergist during digging (Fig. 2A). Two features of the sequence of joint movements (and EMGs) are consistent in both power and return strokes. First, the onset of dactyl movement always precedes that of the other joints. Second, the onset of basi-ischium movement is consistently last (Fig. 2A,C). Thus, the power stroke and return stroke can be divided into three parts: (1) opening or closing of the dactyl; (2) synergistic movements at the thorax–coxa, merus–carpus and carpus–propus joints (protraction, extension and stretching during a power stroke; retraction, flexion and bending during a return stroke); and (3) elevation or depression at the coxa–basis joint.

The intra-leg coordination of leg 4 differs from that of legs 2 and 3 (Figs 1, 2). For example, the extensor is active in phase with the elevator in legs 2 and 3, but these two are out of phase

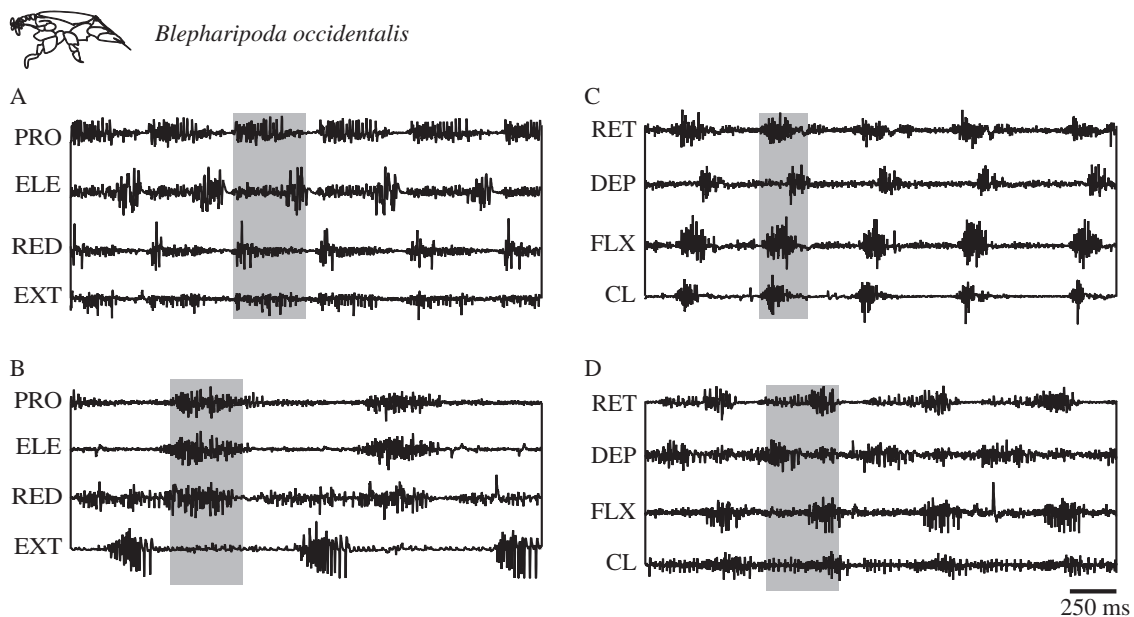


Fig. 2. Leg 2 has a different motor pattern from that of leg 4 in *Blepharipoda occidentalis*. Electromyograms (EMGs), recorded during digging, from (A) power stroke muscles in leg 2, (B) segmentally homologous muscles in leg 4, (C) return stroke muscles in leg 2 and (D) segmentally homologous muscles in leg 4. In this and subsequent figures, shaded boxes highlight a representative sequence of EMGs. RED, reductor. See Fig. 1 for other abbreviations.

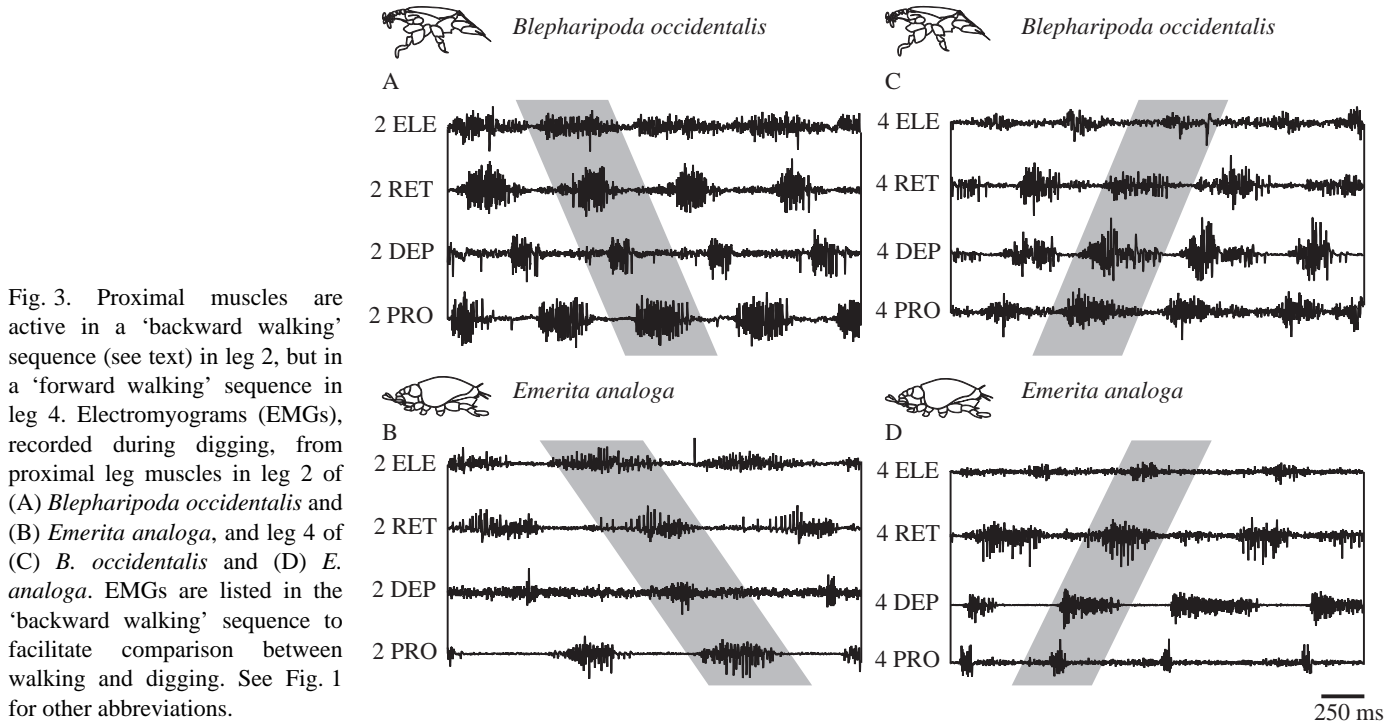


Fig. 3. Proximal muscles are active in a 'backward walking' sequence (see text) in leg 2, but in a 'forward walking' sequence in leg 4. Electromyograms (EMGs), recorded during digging, from proximal leg muscles in leg 2 of (A) *Blepharipoda occidentalis* and (B) *Emerita analoga*, and leg 4 of (C) *B. occidentalis* and (D) *E. analoga*. EMGs are listed in the 'backward walking' sequence to facilitate comparison between walking and digging. See Fig. 1 for other abbreviations.

in leg 4. The muscles of leg 4 are not categorised as power stroke and return stroke synergists, because whole leg movements are not easily separated into power stroke and return stroke components (Faulkes and Paul, 1997c; and see below).

The difference in the proximal leg muscle coordination between leg 2 and leg 4 is noteworthy. The proximal leg EMG bursts in legs 2 and 3 of *B. occidentalis* and *E. analoga* occur in an 'elevator, retractor, depressor, protractor' sequence (Fig. 3), which is similar to the backward walking sequence in

other decapod crustaceans (Ayers and Davis, 1977; Ayers *et al.* 1994; Clarac, 1984; Evoy and Ayers, 1982; Macmillan, 1975; Sillar *et al.* 1986, 1987). In leg 4 of both species, however, the serially homologous proximal muscles are activated in a 'elevator, protractor, depressor, retractor' sequence, which is similar to that in forward walking (Macmillan, 1975; see also Fig. 3 in Clarac, 1984; Fig. 2 in Chrachri and Clarac, 1990). The mean onset phases of proximal muscle EMG bursts, relative to EMG burst period of a muscle in an adjacent leg segment, are lower in leg 2 than

Table 1. Mean phases of proximal muscles in legs 2 and 4 of *Blepharipoda occidentalis* and *Emerita analoga*

Species	Muscles	Leg 2			Leg 4		
		Mean phase	Phase deviation	<i>N</i>	Mean phase	Phase deviation	<i>N</i>
<i>B. occidentalis</i>	RET in ELE*	0.36	0.10	6	0.86	0.09	5
	DEP in RET	0.05	0.04	3	0.91	0.10	4
	PRO in DEP†	0.19	0.04	2	0.60	0.17	7
	ELE in PRO†	0.39	0.15	4	0.67	0.14	3
<i>E. analoga</i>	RET in ELE	0.06	0.22	5	0.74	0.22	3
	DEP in RET†	0.40	0.17	5	0.92	0.14	3
	PRO in DEP	0.86	0.21	5	0.83	0.17	3
	ELE in PRO	0.97	0.18	5	0.76	0.22	3

Mean phase values of electromyogram (EMG) burst onset in individual muscles controlling one proximal joint relative to the EMG burst onset of a muscle controlling the other proximal joint. The mean phase and phase deviation are equivalent to the mean angle and angular deviation (Batschelet, 1981), respectively, expressed as a value from 0 to 1 instead of in degrees.

An asterisk indicates a significant difference in mean phase between legs 2 and 4 (run test, $h=2$, $P<0.05$; Batschelet, 1981).

Sample sizes for other muscle combinations preclude statistical analysis at the $P<0.05$ level; cases where the distribution of phases in leg 2 does not overlap with that in leg 4 are marked with a dagger.

See Fig. 1 for an explanation of muscle abbreviations.

Table 2. Correlations between the periods of proximal muscles in sea water and in sand

Medium	Leading EMG	Trailing EMG			
		ELE (N)	RET (N)	DEP (N)	PRO (N)
Sea water	ELE	—	0.65 (11)	0.45 (7)	0.61 (10)
	RET	0.66 (11)	—	0.59 (7)	0.59 (10)
	DEP	0.47 (7)	0.55 (7)	—	0.48 (7)
	PRO	0.72 (10)	0.57 (10)	0.42 (7)	—
Sand	ELE	—	0.82 (6)	0.69 (4)	0.54 (4)
	RET	0.84 (6)	—	0.83 (6)	0.88 (3)
	DEP	0.72 (4)	0.77 (6)	—	0.78 (2)
	PRO	0.82 (4)	0.74 (3)	0.76 (2)	—

The duration of electromyogram period in one muscle (leading muscle) was plotted against the period in another muscle (trailing muscle), and the correlation coefficient (r) between them was calculated.

The correlations between periods are generally higher in sand than in sea water, but there is no leading muscle that functions as a substantially better predictor than any other for subsequent periods in trailing muscles in either medium.

See Fig. 1 for an explanation of muscle abbreviations.

the onset phases of the same pair of muscles in leg 4 in both *B. occidentalis* and *E. analoga* (Table 1). The greater variation of the mean phases of *E. analoga* may be partly due to the greater encumbrance of the EMG wires.

Ayers and Davis (1977) suggested that elevator motor

neurons (or cells immediately presynaptic to them) play a central role in organising the walking step in lobsters, partly because the elevator period is the best predictor of subsequent periods in the other walking leg muscles. An analysis of the periods of proximal muscles (measured from EMGs) in leg 2

Table 3. Regression values of electromyogram burst durations in periods in *Blepharipoda occidentalis* and *Emerita analoga*

Species	Muscle	Leg 2			Leg 4		
		r	S.D.	N	r	S.D.	N
<i>B. occidentalis</i>	PRO*	0.81	0.09	4	0.49	0.13	7
	RET	0.18	0.10	10	0.76	0.13	5
	ELE*	0.85	0.09	5	0.68	0.09	4
	DEP	0.35	0.12	6	0.66	0.11	11
	RED*	0.93	0.06	1	0.68	0.11	4
	EXT*	0.77	0.11	4	0.52	0.13	10
	FLX	0.46	0.11	4	0.52	0.07	2
	BND*	0.93	0.08	4	0.64	0.13	4
	CL	0.20	0.07	8	0.57	0.09	11
	OP*	0.73	0.11	4	—	—	—
<i>E. analoga</i>	STR	—	—	—	0.68	0.11	5
	PRO*	0.66	0.20	5	0.49	0.12	2
	RET	0.42	0.19	4	0.66	0.14	2
	ELE*	0.51	0.19	5	0.65	0.12	2
	DEP	0.47	0.17	4	0.61	0.11	2
	RED*	0.53	0.18	2	—	—	—
	EXT*	0.56	0.25	5	—	—	—
	FLX	0.65	0.03	1	—	—	—
	BND*	0.83	0.14	7	0.81	0.21	3
	CL	0.29	0.12	7	0.74	0.13	4
OP*	0.81	0.09	4	—	—	—	
STR	—	—	—	0.94	0.09	6	

Leg 2: calculated regression values are higher for all power stroke (*) muscles than return stroke muscles. The one exception is the extensor and flexor pair in *E. analoga*, for which the flexor burst records were adequate for analysis in only one animal.

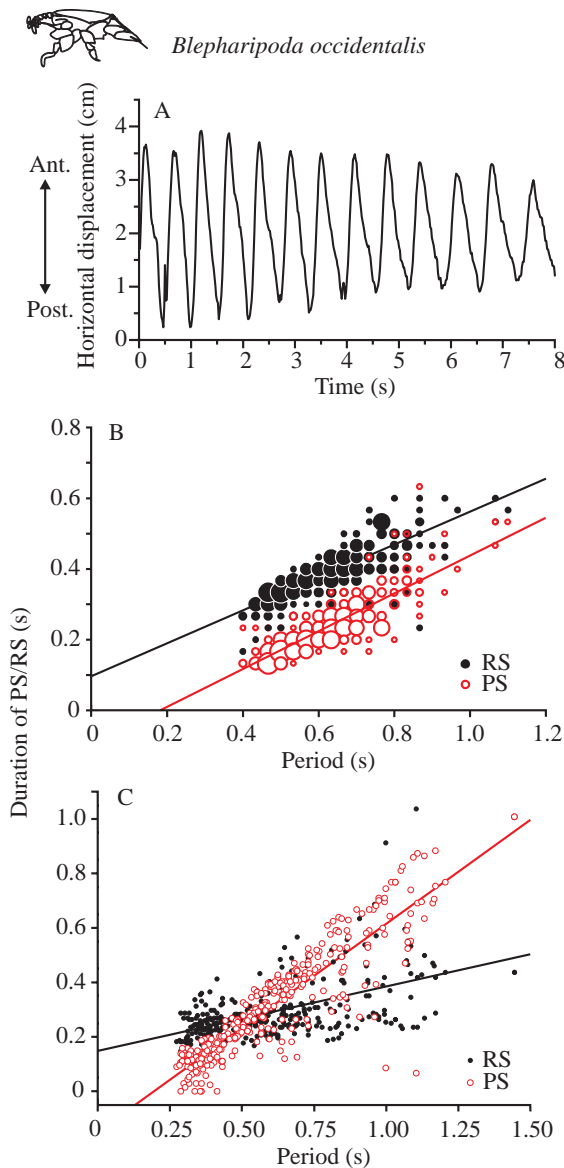
Leg 4: in *B. occidentalis*, the r values for leg 4 are intermediate to those calculated for leg 2 muscles except for the protractor and retractor. The reductor muscle has no antagonist and functions as a power stroke synergist (Fig. 2A). Although the stretcher and opener bursts are identical, their movements are not, and so the electromyogram activity is involved in both the power and return stroke movements.

See Fig. 1 for an explanation of muscle abbreviations.

of *B. occidentalis* revealed no equivalent evidence for the elevator motor output acting as a common pacemaker, either in sea water or in sand (Table 2). The correlations between the leading and trailing EMG periods are higher in sand than in sea water, probably because of the influence of loading by sand and systematic slowing as the animals dig (Faulkes and Paul, 1997c).

Changes in motor output when switching from swimming to digging

B. occidentalis swims by rowing legs 2 and 3 while tail-flipping (Paul, 1981) and, despite the changes caused by the load of sand on the legs, apparently makes similar leg movements when digging (Faulkes and Paul, 1997c). We investigated whether there were any changes in intra-leg coordination comparable to the sudden changes in interleg (Faulkes and Paul, 1997c) and leg/tail coordination (Faulkes and Paul, 1997b) that occur at the onset of digging.



When *B. occidentalis* make digging movements above sand, the amplitude and speed of forward and backward leg movements are proportional. The power stroke and return stroke make up approximately 35% and approximately 65% of the period, respectively, regardless of period (Fig. 4). The increase in the durations of the forward and backward movements of the whole leg as the frequency drops could be due to the lengthening of EMG bursts in individual muscles, to the movements of different joints becoming less synchronous, or to a combination of these two factors. The first explanation best fits the data, at least for the closer EMG bursts, because EMG burst durations in the closer (a return stroke muscle) vary with period when an animal makes digging movements above sand ($r=0.66$; $N=4$), but are not correlated with period when the animal is actually digging (Table 3). We have no indication that the activation of other return stroke muscles is different.

During the transition from rowing with the legs in sea water to digging into sand, the motor output to leg 2 ceases to be proportional. The duration of the power stroke continues to covary with period, but the duration of the return stroke (estimated by measuring from the start of the closer burst to the end of the depressor burst) changes much less with period (Fig. 4). A similar pattern is evident in the correlations between the burst durations in the individual muscles and period. The EMG burst duration increases with period in leg 2 power stroke muscles ($r>0.7$ in *B. occidentalis*; Table 3), whereas the burst duration changes little with period in return stroke muscles ($r<0.5$ in *B. occidentalis*; Table 3). We refer to this motor output as 'return stroke constant', because variations in return stroke burst duration are poorly correlated with variations in period.

There are few opportunities for comparing the motor output

Fig. 4. Changes in leg 2 motor output in *Blepharipoda occidentalis* swimming in sea water and digging in sand. (A) Movement of leg 2 during a long bout of swimming in sea water above sand. The most vigorous movements occurred at the start of the sequence, and the amplitude and frequency of leg movement decreased together. Leg movement digitised from video recordings using Peak 5. Ant., anterior (direction of power stroke movement); Post., posterior (direction of return stroke). (B) In sea water, the duration of both the power stroke (PS) and return stroke (RS) lengthen with period. Leg movements measured from video recordings. The size of a circle corresponds to the number of observations occupying the same point on the graph. PS regression line: $y=-0.096+0.53x$; $P<0.01$; $r^2=0.70$. RS regression line: $y=0.096+0.47x$; $P<0.01$; $r^2=0.64$. (C) In sand, the power stroke lengthens with period but the return stroke changes much less with period. The return stroke is estimated from electromyograms (EMGs) by measuring from the onset of the closer burst to the end of the depressor burst (see Fig. 2); the power stroke is estimated as the period minus the return stroke duration. Initial analyses of simultaneous video and EMG recordings indicated that these two methods of estimating power and return strokes corresponded reasonably well. PS regression line: $y=-0.15+0.76x$; $P<0.01$; $r^2=0.81$. RS regression line: $y=0.15+0.24x$; $P<0.01$; $r^2=0.29$. See Fig. 1 for other abbreviations.

of leg 4 in both media, because leg 4 tends to be still when an animal is above sand (Faulkes and Paul, 1997c). When digging, the EMGs from leg 4 do not show the straightforward relationships between burst duration and period seen in leg 2: almost all *r* values are intermediate to those calculated for the muscles in leg 2 (Table 3). This supports our interpretation of the movement analysis, which revealed no straightforward division of the leg 4 movement into power and return stroke components (Faulkes and Paul, 1997c).

The opener and stretcher muscles share excitation but generate separate movements

In other reptantian decapods, only a single, shared excitatory neuron (OE≡SE) innervates the opener and stretcher muscles (Wiersma and Ripley, 1952; Wiens, 1989). The anatomy of sand crab leg motor neurons is consistent with them having the same innervation scheme (Faulkes and Paul, 1997a). In both sand crab species, the stretcher and opener EMG potentials often match potential for potential (Fig. 5), with a small lag

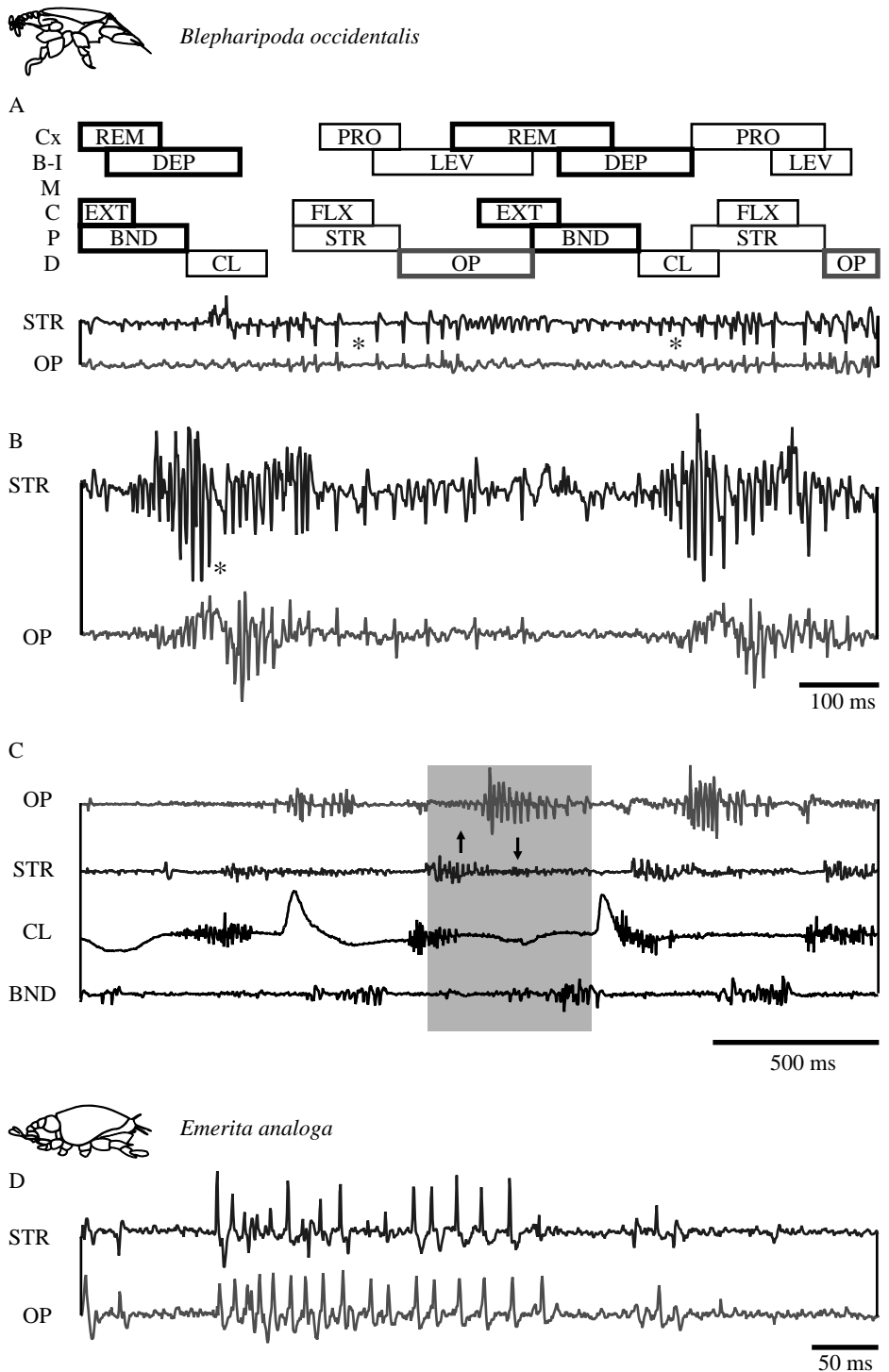


Fig. 5. (A) Analysis of a video sequence and electromyograms (EMGs) recorded simultaneously from the opener and stretcher muscles in leg 2 of *Blepharipoda occidentalis* during swimming. (B) EMGs from the same individual during digging in sand. Same scale in A and B. Asterisks in A and B indicate the drop in potential frequency at the end of the stretcher-generated movement, as the opener-generated movement begins. (C) EMGs from OP, STR, CL, BND during digging movements in sea water. If antagonistic muscle activity alone explained why opener- and stretcher-generated movements do not occur simultaneously, the bender should be co-active with the opener and the closer co-active with the stretcher muscle. The shaded box is aligned with the opener burst. Arrows indicate attenuation of opener and stretcher potentials that presumably reflects peripheral inhibition. (D) Potential-for-potential correspondence in the opener and stretcher EMGs in leg 2 of *Emerita analoga*. See Fig. 1 for other abbreviations.

between stretcher and opener potentials (approximately 1.5 ms in *B. occidentalis*, approximately 1 ms in *E. analoga*) that presumably results from conduction delays. Such close correspondence in EMG potentials has been seen in other decapods (Atwood and Walcott, 1965; Clarac *et al.* 1987) and provides physiological evidence for shared innervation between the muscles.

Although the EMGs from these muscles are synchronous, the movements they generate are emphatically not (Figs 1, 5): the stretcher-generated movement is part of the return stroke, whereas the opener-generated movement is an important component of the power stroke. Such temporal separation could result from sequential activation of specific inhibitory

motor neurons for the stretcher and opener muscles (OI and SI; Atwood, 1977; Spirito, 1970; Spirito *et al.* 1972; Wiens, 1989; Faulkes and Paul, 1997a) or from co-activation of the antagonistic bender and closer muscles (e.g. Barnes, 1977). Two pieces of evidence indicate that antagonistic muscle activity is not the full explanation for the temporal discrepancy between EMGs and movements of the distal leg segments. First, bender and closer movements do not always overlap with opener and stretcher movements (e.g. Fig. 5C). Second, the amplitudes of EMG potentials in the stretcher are often larger during the first half of the burst (i.e. when stretcher-generated movement is occurring), while the potentials in the opener muscle tend to be larger in the second half of the burst (Fig. 5B,C). Antagonistic muscle activity should not alter the size of EMG potentials, but peripheral inhibition could.

The frequency of the EMG potentials often drops momentarily at the end of the stretcher-generated movement and as the opener-generated movement begins (Fig. 5A,B). This is evident at low frequencies (e.g. during digging movements in sea water), when each EMG potential is presumably elicited by a single spike from the shared OE=SE motor neuron, and suggests that this pause reflects a transient decrease in the firing of OE=SE midway through its burst.

Discussion

Two major goals of this study were to find patterns in sand crab digging behaviour that might suggest how their nervous system generates this behaviour and to find evidence that would suggest how sand crab digging originated and evolved. The similarity of intra-leg coordination in *B. occidentalis* and *E. analoga* is further evidence that digging in these two families is homologous, despite familial differences in interleg coordination (Faulkes and Paul, 1997c) and leg and tail coordination (Faulkes and Paul, 1997b). Because the leg tip trajectories of pearly sand crabs *Lepidopa californica* are similar to those of *B. occidentalis* and *E. analoga* (Faulkes and Paul, 1997c), the patterns of intra-leg coordination described here may be typical of all sand crab species.

Every muscle in legs 2, 3 and 4 of sand crabs is involved in making digging movements, and the joints move in a distinct sequence during the power and return strokes. The legs are clearly not acting as rigid struts or oars. Further, the legs are functionally specialised, with similar patterns of intra-leg coordination in legs 2 and 3, and a different pattern in leg 4. The different motor pattern in leg 4 from that in legs 2 and 3 implies that the neural circuits controlling these legs differ.

Our EMG data provide physiological evidence that sand crabs, like other reptantian decapods, have a shared excitor innervating the opener and stretcher muscles (for a review, see Wiens, 1989), as expected from the similar morphology of distal leg motor neurons in sand crabs and walking species (Faulkes and Paul, 1997a). To our knowledge, sand crab digging is the first known case where the opener and stretcher muscles generate temporally distinct large-amplitude movements at their joints during rhythmic behaviour. This appears to be the result

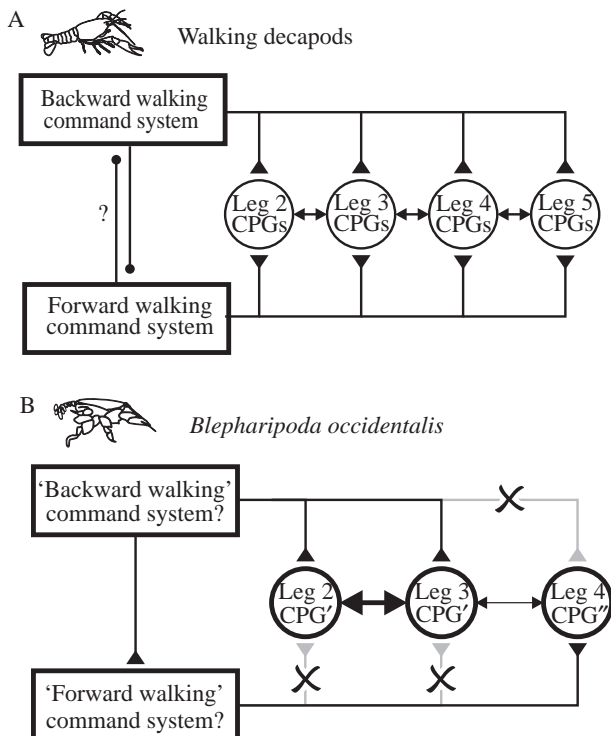


Fig. 6. Models of neural control systems for walking and digging. (A) Decapod walking consists of command networks (boxes), coordinating neurons (arrows) and central pattern generators (CPGs, circles). There is no evidence for how command networks interact, although mutual inhibition is plausible. The CPGs produce many different motor programmes. (B) In sand crab digging, legs 2 and 3 function as a single locomotor module. These legs are tightly coupled, suggesting that their CPGs have a common command system and/or strong coordinating signals connecting them. Leg 4 acts as a separate locomotor module, whose motor output is conditional on strong activation of legs 2 and 3 and/or sensory input. Coordination between leg 4 and the anterior legs is weak (Faulkes and Paul, 1997b). The patterns of intra-leg coordination suggest that the motor programmes of legs 2 and 3 (CPG') are modifications of the same ancestral motor programme, possibly for backward walking, whereas that for leg 4 (CPG'') originated from a different ancestral motor programme, possibly for forward walking, so that sand crab digging appears to be an evolutionary mosaic. The sand crab digging CPGs produce a much more limited range of motor output than those in walking species.

of peripheral inhibition, with antagonistic muscle activation perhaps playing a secondary role.

Predictions about digging pattern generators

Animals maintain leg muscle synergies as they switch from swimming to digging, while the motor output increases (indicated by the greater amplitude and frequency of EMG potentials) and changes smoothly from proportional to return stroke constant. These features suggest that the rhythmic movements of individual legs during swimming in sea water above sand and digging are controlled by a single motor programme, modulated by sensory input. All of the same muscles, active in the same synergies, are involved in making leg movements in both media. The changes in motor output that occur during the transition from swimming to digging appear to be the result of one motor programme operating under different sensory regimes. When leg 2 (or 3) is unloaded, the motor output is proportional, which is similar to waving (Pasztor and Clarac, 1983), to swimmeret beating (Davis, 1969, 1971, 1973), and to the swimming movements of leg 5 in portunid crabs (Spirito, 1972). Conversely, when the legs are loaded (during digging), the motor output is return stroke constant, which is similar to walking in other crustaceans (Ayers and Davis, 1977) and to swimming movements of legs 2–4 in portunid crabs (Spirito, 1972). Similar switches from proportional to return stroke constant motor output have been demonstrated for the uropods of *E. analoga* (Paul, 1976) and the limbs of chicks (Johnston and Bekoff, 1996). In both cases, these switches result from proprioceptive feedback.

The variability of the movement of leg 4 above sand suggests that sensory input is more important in regulating its movements than it is for legs 2 and 3. Sensory input during retraction of the leg may influence motor output to leg 4, since the retractor muscle shows a tighter correlation between EMG burst duration and period than do the other muscles. The thoracic-coxal muscle receptor organ, which signals leg retraction in other species (Ripley *et al.* 1968; Skorupski *et al.* 1992), is a good candidate to shape the motor output of leg 4. It strongly influences rhythmic motor output in crayfish (Sillar *et al.* 1986, 1987) and generates a suite of reflexes across multiple joints in crayfish and brachyuran crabs (e.g. Head and Bush, 1991, 1992; Skorupski and Bush, 1992; Skorupski *et al.* 1992).

Some features of intra-leg coordination, but not all, could be explained by central synaptic connections between the leg motor neurons. Antagonistic muscles generally alternate as sand crabs dig, and reciprocal inhibition between the motor neurons themselves could generate such oscillatory activity (Chrachri and Clarac, 1989; Pearlstein *et al.* 1995; Skorupski and Sillar, 1988).

Some aspects of intra-leg coordination in sand crabs are not explainable by central connections between motor neurons; for example, the dactyl movement in legs 2 and 3 precedes other synergistic joint movements. Synergies of muscles working across multiple joints in sand crabs probably arise from common input to, rather than from mutual excitation between, motor neurons controlling separate joints. In crayfish, no

electrical synapses have been found between motor neurons controlling separate leg joints (Chrachri and Clarac, 1989; Pearlstein *et al.* 1995; Skorupski and Sillar, 1988), and stimulation of a local interneuron evokes a motor pattern that closely resembles the intra-leg coordination of whole animals (Pearlstein *et al.* 1995). There is no obvious candidate for a 'pacemaker' muscle (Ayers and Davis, 1977) or a 'leader joint' (Jamon and Clarac, 1997) in sand crab legs.

Is sand crab digging an evolutionary mosaic?

The search for the physiological causes underlying the different motor pattern in legs 2 and 3 from that in leg 4 can be guided by evolutionary hypotheses (e.g. Paul, 1990, 1991; Paul and Wilson, 1994). Walking is the most plausible homologue of digging (Faulkes and Paul, 1997c), but two lines of evidence indicate that digging leg movements are not simply a modified form of forward or backward walking. First, the sequence of proximal joint movements in legs 2 and 3 resembles backward walking, whereas that in leg 4 resembles forward walking. Second, two command networks appear to be involved in the initiation of rhythmic movements: one for legs 2 and 3, and the other for leg 4 (Faulkes and Paul, 1997b,c).

We suggest that digging is an evolutionary mosaic (Fig. 6). This hypothesis is based on the assumption that the neural substrates of walking and other rhythmic limb movements in decapods have three main elements: command networks, coordinating neurons and CPGs (Ayers *et al.* 1994). Command networks initiate walking in a particular direction by turning on the appropriate CPGs (Bowerman and Larimer, 1974; Pearlstein *et al.* 1995), while coordinating neurons connect CPGs and ensure appropriate ipsilateral and bilateral leg coordination (Stein, 1978; Paul and Mulloney, 1986). Multi-functional CPGs generate a variety of detailed motor programmes for a single limb (Chrachri and Clarac, 1990). In our model of sand crab digging, the command network that starts the CPGs for legs 2 and 3 has little to no input to the leg 4 CPG. Furthermore, coordinating signals between leg 4 and the anterior legs have been weakened, but not lost (Faulkes and Paul, 1997b). A weakening of coordinating signals between CPGs (i.e. parcellation; Wagner, 1996) would allow natural selection to modify individual CPGs without dramatically affecting the others (Gatesy and Dial, 1996). The wide array of walking motor programmes would provide many behaviours for selection to act on, with specialisation resulting from paring the repertoire down to a small number of motor programmes or even to a single motor programme. In this way, the motor programmes for legs 2 and 3 may have become modified from the same ancestral motor programme, possibly that for backward walking (suggested by proximal joint coordination), whereas the motor programme for leg 4 may have had its origins elsewhere, perhaps in the forward walking motor programme. The modular organisation of crustacean nervous systems is compatible with this hypothesis (Liese, 1990, 1991; Mulloney *et al.* 1996; Murchison *et al.* 1996).

Modular neural elements, such as CPGs, can become more tightly associated and form locomotor modules: highly integrated portions of the musculoskeletal and nervous system

that act as functional units during locomotion (Faulkes and Paul, 1995; Gatesy and Dial, 1996). Decapod walking may be seen as a single locomotor module consisting of all the legs (except claws). In sand crab digging, two locomotor modules have been carved from one ancestral locomotor module by parcellation and integration: one for legs 2 and 3, the other for leg 4. These have been paired with a modified version of a separate and originally incompatible ancestral locomotor module for swimming with the tail (Paul, 1981). Re-linking these modified locomotor modules into new configurations (Faulkes and Paul, 1997b; Gatesy and Dial, 1996) could explain how the different sand crab families evolved differences in bilateral coordination (Faulkes and Paul, 1997c) and in leg/tail coordination (Faulkes and Paul, 1997b) while retaining similar intra-leg coordination.

This mosaic hypothesis generates several predictions. If, for example, interneurons are found that are active in forward walking but not in backward walking, the mosaic hypothesis predicts that, during digging, their homologues in sand crabs will be active in the ganglion innervating leg 4, but not in the ganglia innervating legs 2 and 3. Testing this hypothesis will require a better understanding than presently exists of the neuronal control of walking in other decapods, particularly with respect to switching between forward and backward walking motor patterns.

We thank Joseph L. Ayers (Marine Science Center, Northeastern University) and two reviewers for making valuable comments on earlier versions of the manuscript and Sergio M. Pellis and Vivien C. Pellis (Department of Psychology, University of Lethbridge) for teaching movement notation to Z.F. The video synchronisation device was designed by Roberto Racca (Vivitro Video, Victoria) and built by Patrick A. H. Kerfoot (Department of Biology, University of Victoria). This project was financially supported by grants from the Natural Sciences and Engineering Research Council of Canada (OGP08183) to D.H.P. and from the Animal Behavior Society to Z.F.

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