The Journal of Experimental Biology 203, 183–192 (2000)
Printed in Great Britain © The Company of Biologists Limited 2000
JEB2292

ESCAPE BEHAVIOUR IN THE STOMATOPOD CRUSTACEAN *SQUILLA MANTIS*, AND THE EVOLUTION OF THE CARIDOID ESCAPE REACTION

W. J. HEITLER*,‡, K. FRASER‡ AND E. A. FERRERO

B.R.A.I.N. Interdepartmental Center for Neuroscience, Department of Biology, University of Trieste, Via L. Giorgieri 9, I-34127 Trieste, Italy

*e-mail: wjh@st-andrews.ac.uk

‡Present address: The Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife, Scotland KY16 8LB, UK

Accepted 21 October; published on WWW 22 December 1999

Summary

The mantis shrimp *Squilla mantis* shows a graded series of avoidance/escape responses to visual and mechanical (vibration and touch) rostral stimuli. A low-threshold response is mediated by the simultaneous protraction of the thoracic walking legs and abdominal swimmerets and telson, producing a backwards 'lurch' or jump that can displace the animal by up to one-third of its body length, but leaves it facing in the same direction. A stronger response starts with similar limb protraction, but is followed by partial abdominal flexion. The maximal response also consists of limb protraction followed by abdominal flexion, but in this case the abdominal flexion is sufficiently vigorous to pull the animal into a tight vertical loop, which leaves it inverted and facing away from the stimulus. The animal then swims forward (away from the stimulus) and rights itself by executing a half-roll.

A bilaterally paired, large-diameter, rapidly conducting axon in the dorsal region of the ventral nerve excites swimmeret protractor motoneurons in several ganglia and is likely to be the driver neuron for the limb-protraction response. The same neuron also excites unidentified abdominal trunk motoneurons, but less reliably.

The escape response is a key feature of the malacostracan caridoid facies, and we provide the first detailed description of this response in a group that diverged early in malacostracan evolution. We show that the components of the escape response contrast strongly with those of the full caridoid reaction, and we provide physiological and behavioural evidence for the biological plausibility of a limb-before-tail thesis for the evolution of the escape response.

Key words: caridoid, escape response, tail-flip, evolution, *Squilla mantis*, shrimp, giant fibre.

Introduction

The purpose of the present report is to describe the escape system of the stomatopod mantis shrimp *Squilla mantis* and to compare it with the well-known giant-fibre-mediated system found in the decapods. The reason for undertaking the study is that the Hoplocarida, of which *S. mantis* is a member, diverged early in malacostracan evolution, and their escape system may therefore give clues to both the phylogenetic relationships within the class and the evolution of the caridoid escape response itself.

There is considerable controversy surrounding the relationship between the Hoplocarida, the Syncarida and the Eumalacostraca (the main malacostracan sub-class), particularly with regard to the temporal sequencing of the branch points within the group (Schram, 1969; Dahl, 1983; Hessler, 1983; Kunze, 1983). The use of tail flexion as a means of escape is a key eumalacostracan feature; indeed, it has been claimed that this is the only true diagnostic feature of the caridoid facies (Dahl, 1983). The escape tail-flip of eucarids such as the decapod crayfish has been intensively studied, and both the behaviour and the underlying neural circuitry are understood in considerable detail (Wine and Krasne, 1982; Wine, 1984; Edwards et al., 1999). A central feature of the system is the two pairs of dorsally located giant fibres (GFs), the medial (MG) and lateral (LG) giants, which respond to rostral and caudal stimuli respectively, and which command behaviourally distinct types of tail flexion (Wiersma, 1947). These two forms of escape behaviour and their accompanying circuitry must have arisen early in the evolution of the Eumalacostraca, since MG- and LG-type axons, and the accompanying different types of tail flexion, are present in the syncarid *Anaspides tasmaniae* (Silvey and Wilson, 1979; E. Wallis, personal communication), which is 'more primitive that any other caridoids' (Dahl, 1983). In contrast, descriptions of the escape system of mantis shrimps such as *S. mantis* are largely anecdotal and, in part, contradictory (see, for example, the discussion following a paper by Dahl, 1963; Hessler, 1983). Mantis shrimps are certainly capable of abdominal flexion, but it is not clear whether they exhibit the full caridoid escape reaction. Furthermore, although a classic text has stated that a pair of giant fibres is present in the nerve cord of *S.*

mantis (Bullock and Horridge, 1965), to our knowledge no details are available of the anatomy, physiology or function of these fibres. It has been said that the caridoid escape system 'is of the greatest importance for the understanding of the interrelationships of the advanced eumalacostracan superorders' (Dahl, 1983) and, therefore, a fuller description of the hoplocaridan escape system may be useful in indicating the phylogenetic affinities of the group.

In addition to providing information relevant to stomatopod phylogeny, the relatively basal position of the hoplocarids in malacostracan evolution means that their escape system may give clues to the origin of the caridoid escape response itself. The most elaborated known form of the response is that found in the crayfish. In this animal, rapid tail flexion episodes are driven one-to-one by GF spikes, acting through powerful rectifying electrical synapses to drive a specialised fast flexor motoneuron called the motor giant (MoG; Furshpan and Potter, 1959). It was therefore a reasonable early 'best guess' that the rapid GF-mediated tail flexion of the crayfish evolved from a primitive slower tail flexion that was mediated by non-giant pre-flexor interneurons ancestral to the modern GFs (Wine and Krasne, 1982) and that these non-giant neurons simply grew in size as the behaviour pattern evolved. However, a problem with this scheme, and indeed a puzzle regarding the functioning of the entire decapod escape circuit, is that a neuron called the segmental giant (SG), which appears to be derived from a limb protractor motoneuron but has a blind-ending axon, is interposed between the GFs and nearly all the trunk flexor motoneurons (Roberts et al., 1982). Thus, the default view of the evolution of escape requires that this neuron became inserted between the GFs and tail flexor motoneurons after a functioning tail-mediated escape response driven by proto-GFs was already established. Furthermore, in modern crayfish, only the MoG out of a total of 6–10 trunk fast flexor motoneurons in each hemisegment receives significant direct input from the GFs, while the GFs also directly drive some limb protractor motoneurons (Cooke, 1985; Heitler and Fraser, 1989). These facts led to an alternative proposal for the evolution of the escape behaviour that avoids the problem of SG insertion (Heitler and Fraser, 1986). In this view, the interneurons ancestral to the modern GFs drove an escape reaction primarily mediated by a sudden forward flick of the limbs, while tail flexion was originally controlled by separate interneurons, not ancestral to the GFs. Tail flexion was added to the GF-driven limb-mediated escape for reasons of hydrodynamic efficiency, with the tail flexor motoneurons receiving input *via* the ancestral SG motoneuron. One specialised flexor motoneuron eventually bypassed the SG to receive GF input directly and became the MoG. This scheme implies that GF-mediated limb promotion pre-dated GF-mediated tail flexion in the evolution of the escape behaviour.

In comparing the escape system of *S. mantis* with that of the known eumalacostracan systems, two key questions emerge. First, does *S. mantis* show MG- and LG-type escape responses similar to those found in both crayfish and in the most primitive of the uncontested eumalacostracans, the syncarid *Anaspides tasmaniae*? Second, does the response in *S. mantis* provide any support for either the limb-before-tail or the tail-before-limb scheme for the evolutionary origin of the escape response?

Materials and methods

Specimens of *Squilla mantis* (Linnaeus, 1758) (15–20 cm length) were obtained locally from the Gulf of Trieste, Italy. They were kept in glass aquaria with a closed circulation system of filtered aerated sea water at 18–21 °C.

Behaviour

Free-moving animals were filmed within the aquaria using an S-VHS video camcorder (Hitachi VM-57200E). Behavioural responses were elicited by attempting to tap the animal with a stiff strut on either its rostral or caudal end. The recordings were analysed using stop-frame video playback and a computer video acquisition system (miroVideo DC20). Video frames were de-interlaced (separated into two fields containing odd- and even-numbered scan lines) using Adobe Premiere software, providing a time resolution of 20 ms per image.

Dissection and recording

Prior to dissection, the animals were anaesthetised by placing them on ice for approximately 10 min. The abdomen was then removed, and the ventral nerve cord was dissected free, pinned with its dorsal surface upwards in a Sylgard-lined Petri dish and submerged in saline (Watanabe et al., 1967) of the following composition (in mmol l^{-1}): Na^+, 450; K^+, 15; Ca^{2+}, 10; Mg^{2+}, 20; Cl^-, 525, pH 7.9. Hook electrodes were placed on the anterior and posterior inter-ganglionic connectives for extracellular stimulation and recording. Pin electrodes were used for extracellular recording from the roots exiting the ganglia.

A patch of sheath material was removed from the dorsal surface of the ganglion, and neurones were penetrated with microelectrodes manufactured from thick-walled fibre-filled glass. Microelectrodes were filled with 2 mol l^{-1} potassium acetate and had resistances in the range 30–60 MΩ. Data were photographed from an analogue storage (Tektronix 5113) oscilloscope, using a Polaroid camera.

Preparations remained active for 30–60 min after dissection. However, stimulation-induced output usually showed strong decrement, and there was a progressive loss of recovery from decrement as the preparation aged. This probably indicates deterioration in preparation viability, and the technique would require modification before data could be obtained from long-term *in vitro* recordings. All experiments comparing differences in stimulation-induced output were carried out early in the preparation, with repeated cross-checks being made to ensure that observed differences were not due to deterioration.

Microscopy

The ventral nerve cord was fixed in 4 % glutaraldehyde in

modified 0.1 mol l^{-1} cacodylate buffer (Schönenberger, 1977) and embedded in Epon 812/Araldite mixture. Sections (1 μm thickness) were taken mid-way between ganglia from the interganglionic connectives.

Results

Behaviour

Mechanical threat stimuli applied to the caudal end of *Squilla mantis* did not elicit any recognisable escape response, even when direct contact was made. Most commonly, the animal either turned to face the stimulus or simply walked or swam away, in the latter case using the pleopods (swimmerets) to provide thrust. We conclude, therefore, that *S. mantis* does not exhibit any behaviour resembling the LG-type tail-flip of crayfish.

In contrast, threat stimuli applied to the rostral end of the animal frequently elicited an avoidance or escape response consisting of a rapid, coordinated movement that displaced the animal away from the direction of threat. This response was often initiated before direct contact was made, indicating triggering by visual or water-borne vibration cues. The response was graded in form and could be categorised into a hierarchy according to the vigour of the movement and the distance moved by the animal (Fig. 1). Here, we describe three categories within this hierarchy.

Limb-flick response

The limb-flick response consists of a synchronised protraction of the thoracic and abdominal limbs. Limb-flicks can themselves be graded in strength (Fig. 1A). The minimal response produces a backward 'lurch' to give a displacement of approximately 1–2 cm (Fig. 1Ai). More vigorous limb, and in particular telson, protraction causes a backward 'jump', which is angled slightly upwards, so that the animal loses contact with the substratum and is propelled over a distance of 4–8 cm, before landing again (Fig. 1Aiii). There is little active flexion of the abdomen during the limb-flick, although the last one or two segments may flex slightly when the uropod/telson is strongly depressed (e.g. Fig. 1Aii, 0.12 s). The anterior cephalon may extend slightly dorsal relative to the posterior cephalon, causing rotation about the junction between the fourth and fifth thoracic segments, thus pulling the head back from the stimulus.

In some limb-flick episodes, movement of the uropod/telson structure was first detected within the same video frame/field as movement at the anterior end of the abdomen, indicating a conduction delay of less than 20 ms (Fig. 2). Assuming that the conduction delay in the motor paths is similar at the two ends of the abdomen, this in turn suggests that the information is carried by axons within the central nervous system with a conduction velocity greater than 4 m s^{-1}. It is obviously not possible to determine an upper limit to the conduction velocity without video recordings with a finer time resolution.

Intermediate tail-flip response

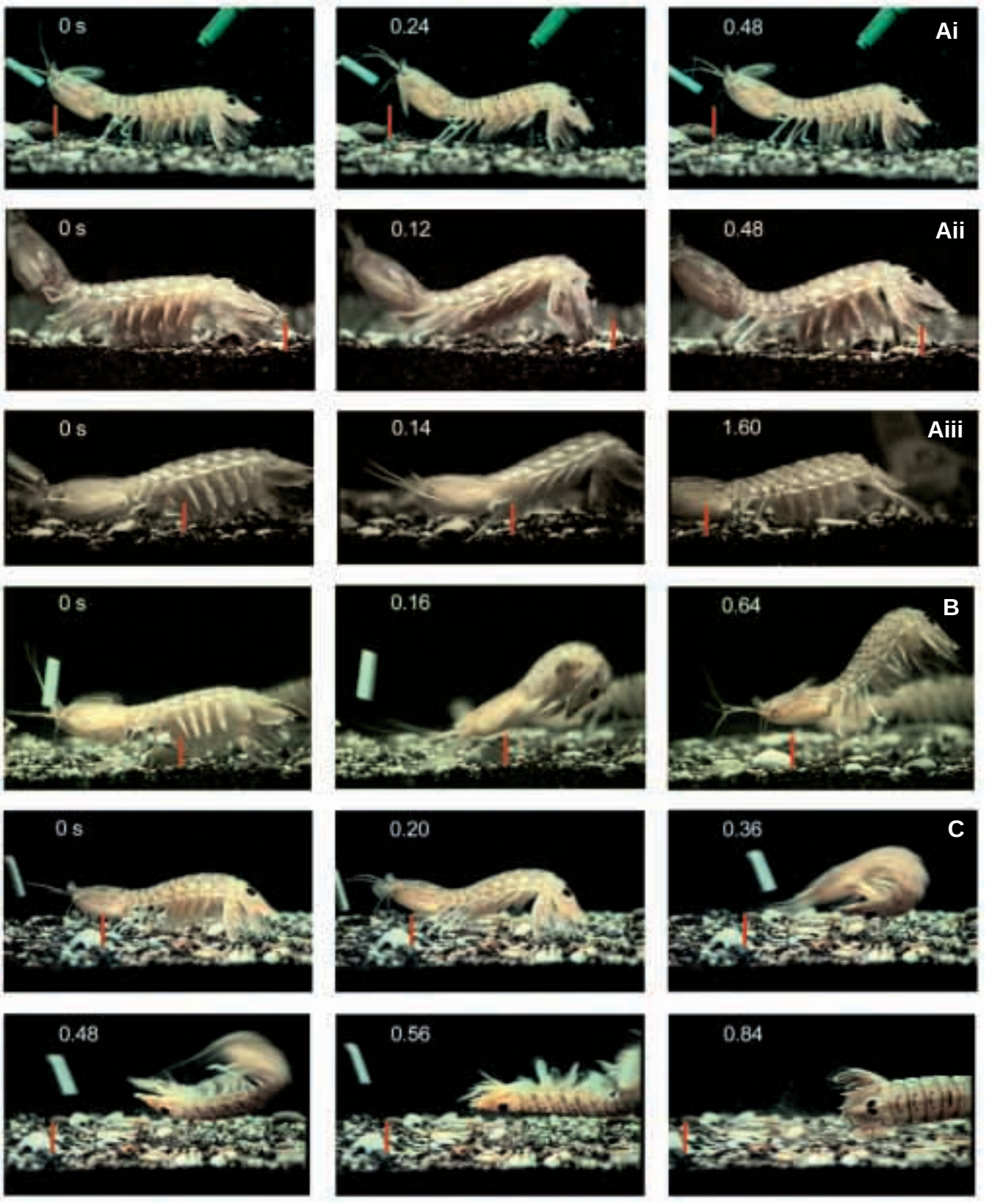
The intermediate response consists of the limb-flick combined with flexion of the abdomen that pulls the animal backwards, but leaves it facing in the same direction. The response invariably starts with limb protraction, like the limb-flick response. However, full limb protraction and telson depression, which is the maximum power phase of the limb-flick response, is followed by a wave of abdominal flexion that progresses anteriorly along the abdomen, to approximately the mid-abdominal position (Fig. 1B, 0.16 s). This pulls the animal directly backwards, causing a displacement of between one-third and half a body length. The form of this response is reminiscent of the MG-type giant-fibre-mediated tail-flip of crayfish, but in crayfish the limb-flick and tail flexion are synchronous, the response is much faster and it produces a greater relative displacement.

Maximal tail-flip response

The maximal tail-flip response starts, like the limb-flick and intermediate tail-flip responses, with limb protraction (Fig. 1C, 0.20 s; Fig. 3, frames 0–3). Next, the uropod–telson structure, which acts as a broad paddle, is thrust forward by approximately 2 cm by tight flexion of the posterior abdomen, until it is completely inverted (Fig. 1C, 0.36 s; Fig. 3, frames 4, 5). After this, the telson–uropod remains more-or-less fixed in space, while the flexion spreads anteriorly in a wave up the abdomen and into the posterior, pereiopod-bearing, thoracic region (Fig. 3, frames 5–10). In stomatopods, the segments in this region are unfused (whereas in decapods the pereiopod-bearing thoracic segments are enclosed in a rigid cephalothoracic carapace) and so are capable of considerable flexion. At this point, the abdomen is approximately straight but inverted, with the thorax strongly flexed in the posterior region (Fig. 1C, 0.48 s; Fig. 3, frame 12). Finally, the thoracic-abdominal hinge starts to straighten (Fig. 1C, 0.56 s; Fig. 3, frame 16). The overall effect of the stable inverted caudal end combined with the rostral flexion wave is to pull the animal round in a tight vertical loop, leaving it upside-down and facing in the opposite direction to its initial orientation. It is displaced approximately three-quarters of a body length from its initial location, away from the direction of threat. Finally, the animal swims forwards (away from the threat) using the abdominal pleopods and rights itself by executing a half-roll (Fig. 1C, 0.84 s). The total displacement is highly variable, since it depends on the length of the swimming episode, but it can be several body lengths.

The coupling between the limb-flick and tail-flip components is variable; a vigorous limb-flick does not always lead to the maximal tail-flip (e.g. Fig. 1Aiii), while a maximal-type tail-flip can be preceded by a relatively weak (long-latency) limb-flick (e.g. Fig. 1C).

We never observed repetitive episodes of tail flexion in freely moving *S. mantis*. We did frequently note repetitive movements of the pleopods in swimming episodes, but this is a metachronally coordinated behaviour pattern producing continuous forward motion. We also noted vigorous repetitive cycling of the uropods if the animal was held restrained against the substratum, but this appeared to be a struggling or digging



response and was not observed in unrestrained animals. We therefore conclude that *S. mantis* does not exhibit the category of repetitive non-GF tail-flips known as backwards swimming, which are commonly seen in decapods such as the crayfish and the squat lobsters (Reichert et al., 1981; Sillar and Heitler, 1985b).

Fig. 1. (A–C) Categories of escape response. (A) The limb-flick response involves synchronous protraction of the thoracic and abdominal limbs, which drives the animal backwards. Limb-flicks can be graded in terms of the degree of backward displacement produced (i=weak, ii=intermediate, iii=strong). There is little abdominal flexion in the limb-flick response. (B) The intermediate response involves a limb-flick followed by pronounced abdominal flexion. This drives the animal backwards, but leaves it facing in the original direction. In the final frame illustrated, the animal has reached the maximum backward displacement achieved in the episode, but has not yet 'landed' on the substratum. (C) The maximal response involves a limb-flick followed by powerful abdominal flexion, which completely inverts the animal. The animal then swims forwards (away from the stimulus) and rights itself with a half–roll. The relative time in the sequence is marked on each frame in seconds. In Ai, B and C the manually operated pale blue stimulating strut is visible in some or all frames, anterior to the animal. In Ai, a fixed aquarium aerator (green) is visible above the abdomen. In each sequence, a stationary reference point is marked with a red vertical bar (applied to the video frame, not present in the aquarium) to facilitate visualization of the displacement. In Ai and C, the bar is approximately 2.5 cm long, in B it is approximately 2 cm long, in Aii and Aiii it is approximately 1.5 cm long at the plane of the animal.

Neurobiology

Cord giant fibre

Stimulating the abdominal ventral nerve cord at either the anterior end between ganglia 1 and 2 (g1–2) or the posterior end between ganglia 5 and 6 (g5–6) activates a low-threshold large-amplitude unit that can be recorded at the opposite site (Fig. 4A,B). Because of the large amplitude of its extracellular spike, its low threshold for extracellular stimulation, its conduction velocity of 8–10 m s^{-1} and its ability to follow high-frequency stimulation with fixed latency, we tentatively identify this unit as a through-conducting giant fibre (GF). A neuron was penetrated with a microelectrode in the third abdominal ganglion (g3), mid-way between the midline and the lateral margin of ganglion and near the dorsal surface. Stimulating the anterior connective elicited a spike in this neuron at exactly the same

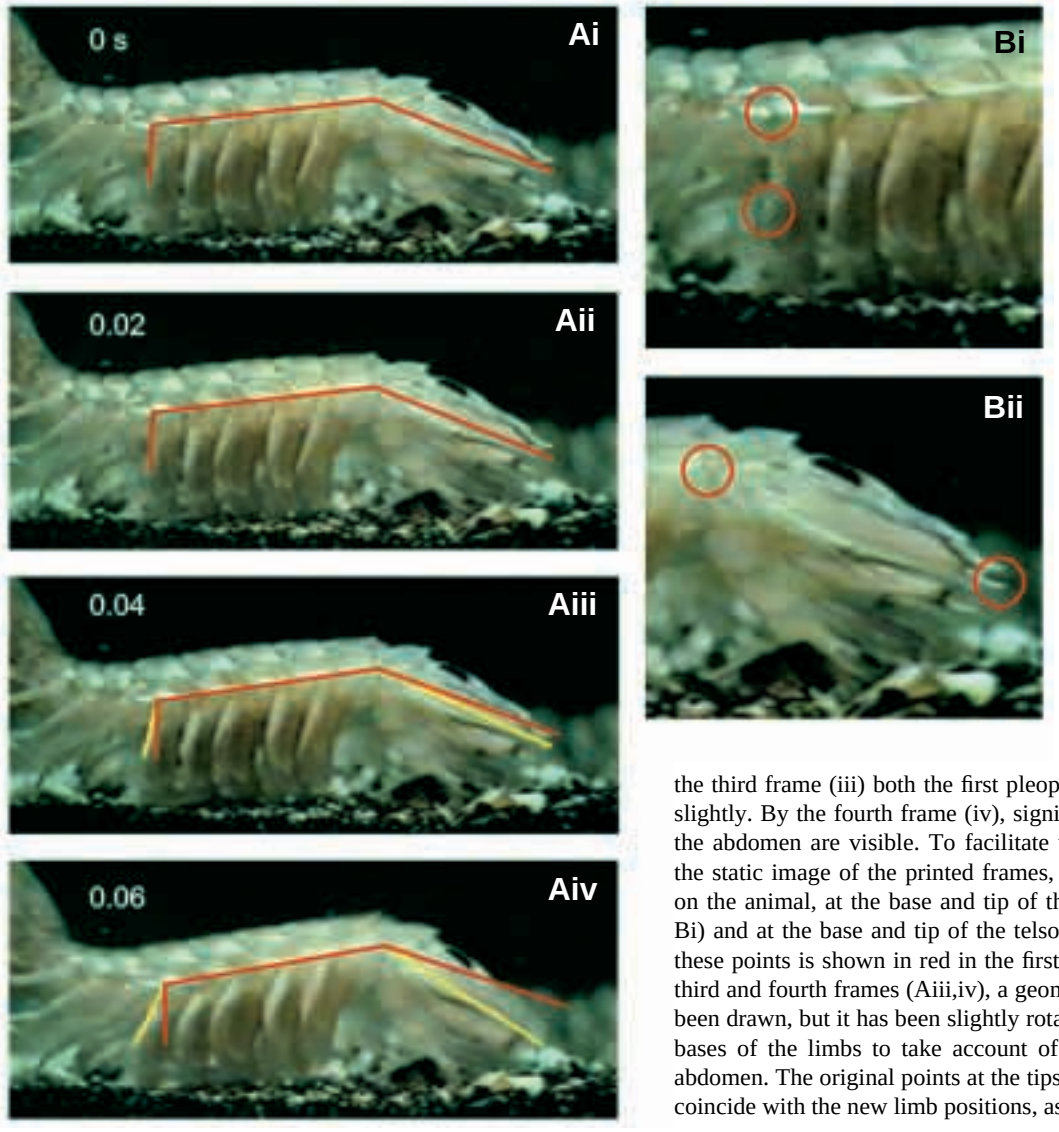


Fig. 2. Near-synchronous movement occurs at opposite ends of the abdomen. (Ai–iv) Consecutive single frames (de-interleaved, frame separation 20 ms) from a video recording of the limb-flick response. The numbers indicate the elapsed time in seconds. There is no observable movement between the first two frames (i,ii), but in the third frame (iii) both the first pleopod and the telson have protracted slightly. By the fourth frame (iv), significant movements at both ends of the abdomen are visible. To facilitate visualization of the movement in the static image of the printed frames, four marker points were selected on the animal, at the base and tip of the first pleopod (shown circled in Bi) and at the base and tip of the telson (Bii). A set of lines connecting these points is shown in red in the first and second frames (Ai,ii). In the third and fourth frames (Aiii,iv), a geometrically identical set of lines has been drawn, but it has been slightly rotated so as to align the points at the bases of the limbs to take account of the slight dorsal rotation of the abdomen. The original points at the tips of the limbs (red lines) no longer coincide with the new limb positions, as indicated by the yellow lines.

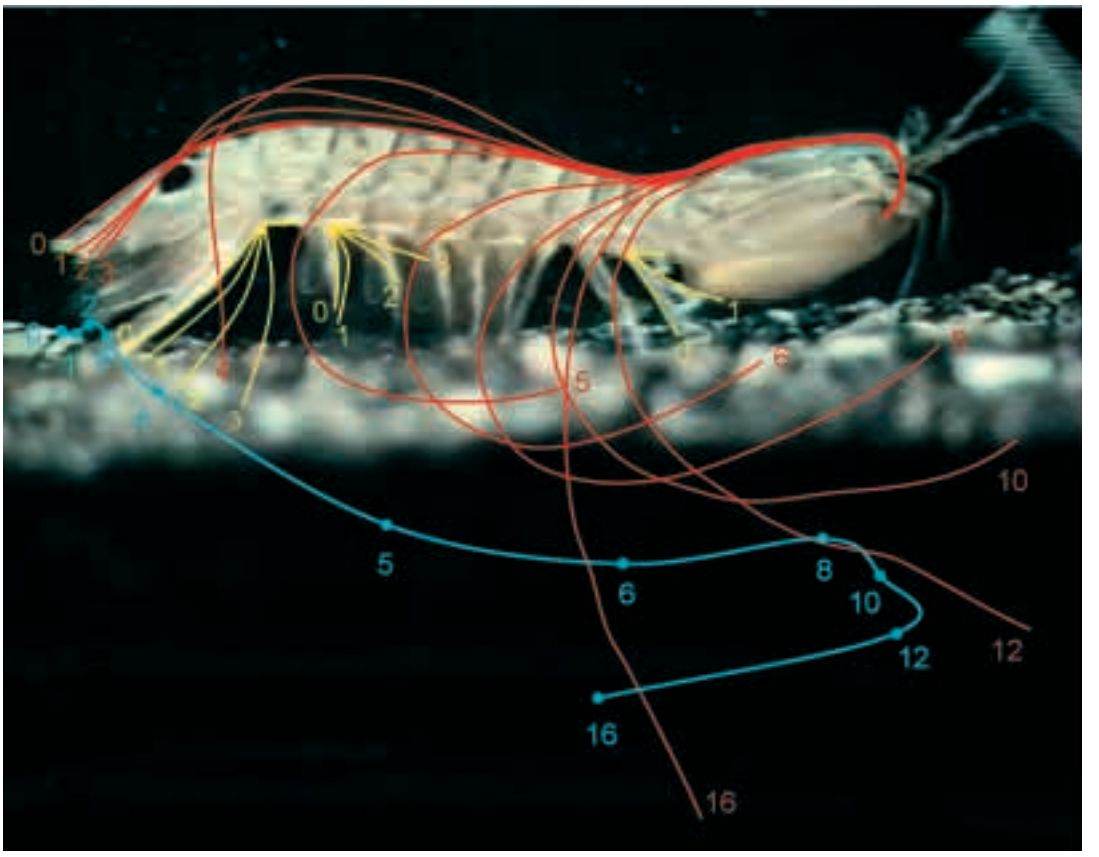


Fig. 3. The relative timing of components of a maximal escape response (different episode from that shown in Fig. 1C). The image shows a video frame just before the start of the response. The dorsal outline of the thorax and abdomen has been traced in red, the leading edge of the uropod, posterior swimmeret and anterior walking leg on the near side have each been traced in yellow, together with the ventral cuticle immediately anterior to each structure, and a fixed point on the substratum has been marked with a blue dot. Similar lines/dots were traced in subsequent video frames showing the escape response (40 ms separation), and superimposed upon the image. The red lines and blue dots were rotated and translated so as to maintain the anterior thorax in a constant relative position. The blue dots were joined by a line (curve fitted by eye) to show the movement of the substratum relative to the anterior thorax. The anterior thorax is enclosed in a rigid carapace, and so is the structure best suited to act as a fixed datum point to show relative movements of the abdomen and substratum. The leg, uropod and swimmeret lines were aligned relative to the ventral cuticle immediately anterior to each structure, not to the anterior thorax, since this best shows their relative movements. The numbers beside each line/dot refer to the video frame from which the datum derives, relative to that shown in the image (frame 0). Full promotion of the walking leg was achieved within one video frame, while full promotion of the uropod and swimmeret was achieved within three video frames.

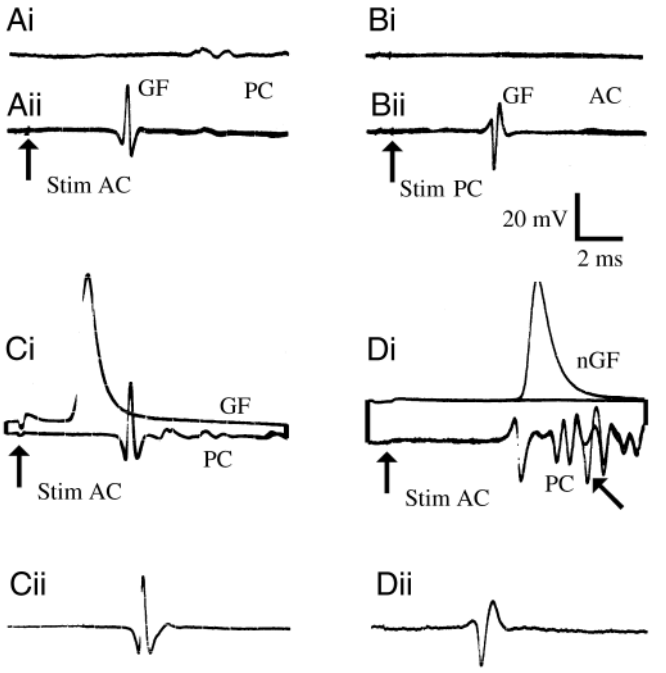


Fig. 4. (A–D) Giant spikes in the abdominal nerve cord. (A) Stimulating the anterior connectives between the first and second ganglia (g1–2; anterior connectives, AC) initiates activity in a low-threshold large unit (GF) in the g5–6 connectives (posterior connectives, PC). (i) Stimulation below the GF threshold (but just above the threshold for some small-amplitude units). (ii) A slight increase in stimulation amplitude compared with i elicits a GF spike. (B) As A, except that the stimulus is applied to the PC while recording at the AC. (C) Physiological identification of the GF. (i) Stimulating the AC while recording intracellularly from the GF (upper trace) in g3 and extracellularly from the PC (lower trace) elicits intracellular and extracellular spikes at the same threshold. (ii) Current injected into the GF (throughout trace) elicits a spike in the PC identical to that caused by AC stimulation. (D) Another unit recorded intracellularly (upper trace) in g3. (i) Two sweeps are superimposed showing AC stimulations, both of which are above GF threshold, but one is above and one below the threshold for the unit recorded intracellularly. Note the change in the PC recording (lower trace, arrow), indicating that the unit has a longer latency than the GF. (ii) Current injected into this unit (throughout trace) elicits a spike in the PC that is similar in amplitude to the GF spike. A and B are from one preparation, C and D are from two other preparations.

threshold as that eliciting the large spike in the posterior connective. When this neuron was depolarised by injecting positive current, it caused a spike in the posterior connective that was very similar in amplitude and shape to those of the large unit elicited by stimulating the anterior connective (Fig. 4C). We therefore think it likely that this neuron is the same unit as that recorded extracellularly. Increasing the intensity of stimulation to the anterior connectives recruits additional units in the posterior connectives, some of which have a similar amplitude in the extracellular recording to the low-threshold large unit, but which have a lower conduction velocity (Fig. 4D).

A cross section of the g2–3 abdominal connectives (Fig. 5) reveals an axon in the dorsal region with a diameter of 50–85 μm, which is significantly larger than other axons in the section. A cross section of the g4–5 abdominal connectives (Fig. 5) reveals a large axon in a similar dorsal region, but this axon is not so obviously different in size from other axons in the section. There is at least one axon located more ventrally that has a similar diameter, and several axons that are only slightly smaller.

These anatomical findings fit both with the physiological results and with our identification of the dorsal axon as the GF. When stimulating the anterior connective, the dorsal axon is likely to be amongst the first recruited, since large-diameter axons have lower thresholds to extracellular stimulation than small-diameter axons. It produces a large spike in the posterior connective, because the amplitude of spikes recorded extracellularly scales with axon diameter. Increasing the amplitude of anterior connective stimulation will recruit smaller-diameter fibres. Some of these presumably either increase in diameter in the more caudal location or make synaptic connections with large-diameter axons, hence accounting for the large extracellular amplitude, but slower conduction velocity, of these units (Fig. 4D).

Root responses to GF stimulation

Extracellular recordings were made from the three roots that exit the fourth abdominal ganglion, while stimulating the anterior connectives above and below GF threshold (Fig. 6). The most reliable response was found in the first root, which innervates the limb protractor muscles (Pilgrim, 1964). At least one unit followed GF stimulation one-to-one in the initial stages of most preparations (11 out of a total of 13 tested). The latency of this unit was almost, but not completely, constant (Fig. 6Aii,D), and it failed if the stimulation frequency was increased above approximately 4 Hz (see Materials and methods). No units in the second root, which innervates limb retractor muscles, were observed to follow GF stimulation (Fig. 6B,D). In the third root, which innervates both flexor and extensor muscles of the abdomen, units were encountered in some preparations (two out of a total of seven tested) which were recruited in response to connective stimulation at the same threshold as the GFs, but these varied both in latency and

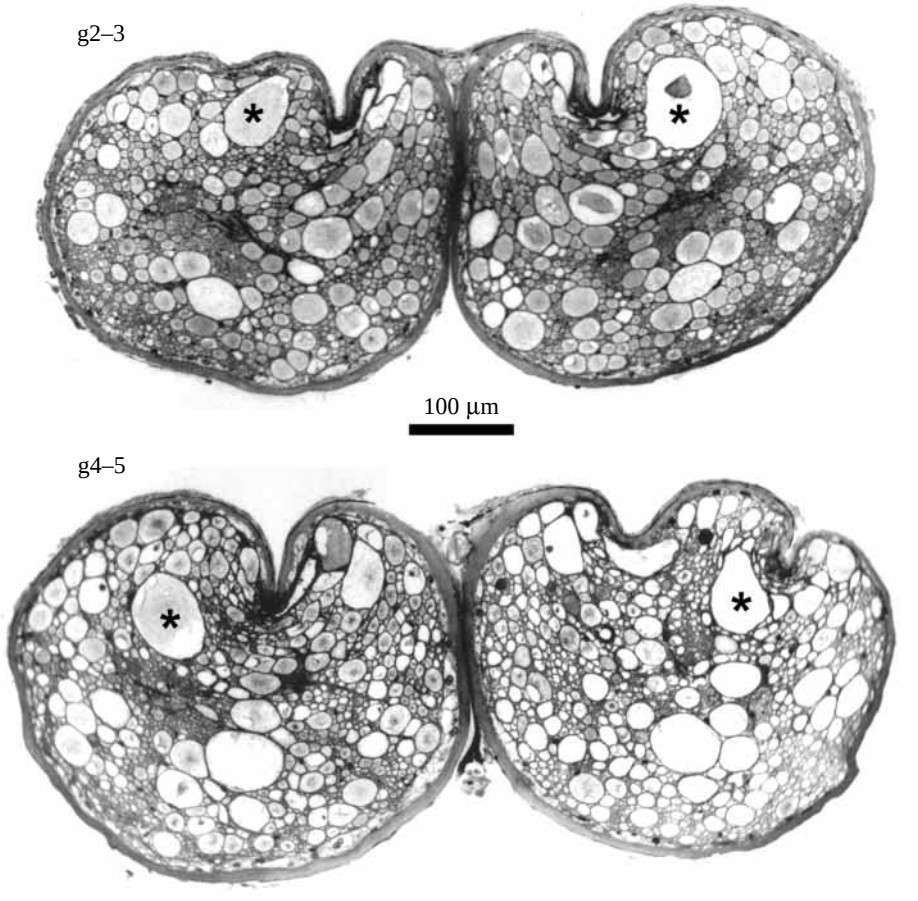


Fig. 5. Cross sections of the interganglionic connectives reveal a large-diameter axon (asterisk) in the dorsal region of each hemi-connective. In the g2–3 connectives, this axon is larger than all others, but in the g4–5 connectives there is another more ventrally situated axon of similar size.

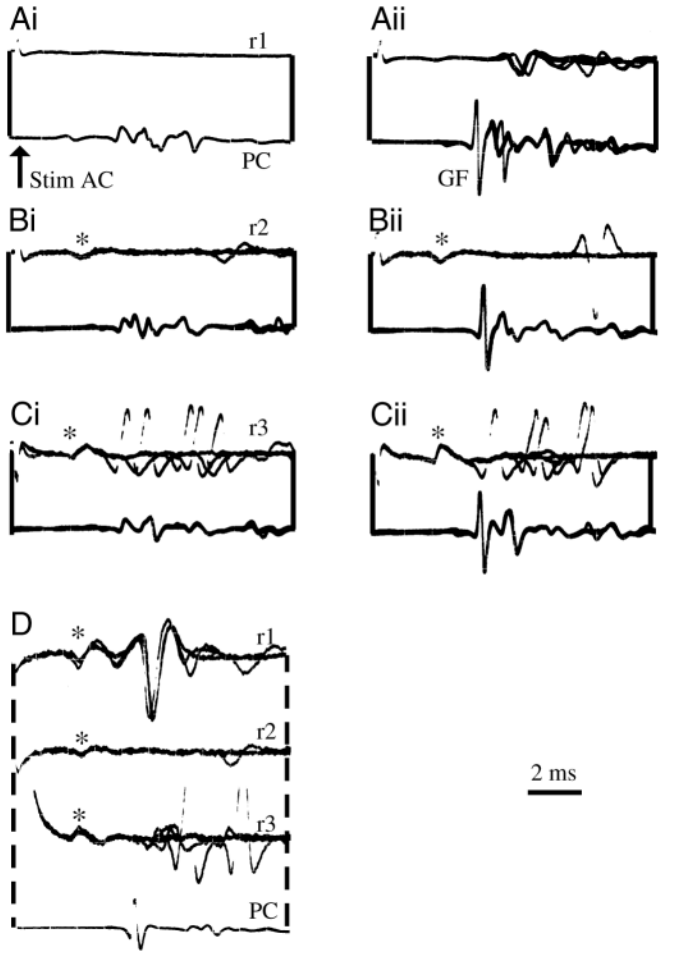


Fig. 6. (A–D). Root responses to giant fibre (GF) stimulation. (A–C) Responses from the first root (r1; A), second root (r2; B) and third root (r3; C) to stimulation of the anterior connective (AC) below (i) and above (ii) GF threshold. Only the first root (A) shows a response that depends upon the stimulation. In C, the third root activity was tonic and unrelated to the stimulus either above or below the GF threshold. (D) Another preparation, showing a consistent response to GF stimulation in the first root, no response in the second root and an inconsistent response in the third root. The record is a montage constructed from separate recordings, which have been aligned to show their relative timing. Each trace shows superimposed sweeps from five stimulation episodes applied at 1 Hz to a preparation that had been rested for at least 30 s prior to stimulation. The early response (*) seen in several root recordings is pick-up from cord units. PC, posterior connective.

amplitude, and were less reliable in occurrence than the first root unit (Fig. 6D).

Current injected through a microelectrode into a third ganglion GF, identified by the criteria described above, elicited spikes in the first roots of the third and fourth ganglia (Fig. 7). These spikes were similar in shape and amplitude to the spikes recorded in the first root in response to stimulating the anterior connectives at GF threshold, which confirms that it was indeed the GF itself that was eliciting the root response.

The anterior connectives were stimulated in a preparation in

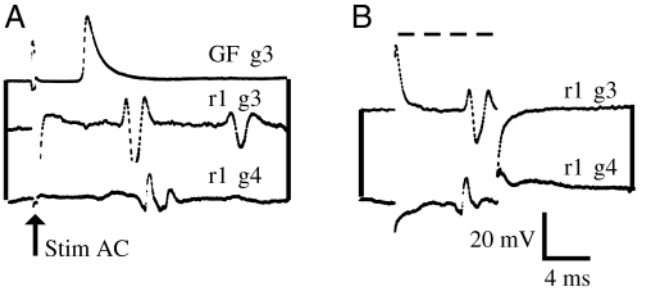


Fig. 7. (A,B) The giant fibre (GF) activates a swimmeret protractor motoneuron. (A) Stimulating the anterior connective (AC) at the threshold of the GF (recorded intracellularly in g3, upper trace) elicited spikes in the first roots of ganglion 3 (g3; middle trace) and ganglion 4 (g4; lower trace). (B) Injecting depolarising current into the GF (dashed line) elicited similar spikes in the first roots to AC stimulation. The gain of the g3 first root recording is higher in A than in B.

which the nerve cord was left *in situ* in an isolated abdomen. Protraction twitches were observed in the swimmerets innervated by the fourth and fifth ganglia in response to a single stimulus pulse at a threshold that recruited a GF spike in the posterior connectives. We attempted to elicit a stronger response by increasing the frequency of stimulation, but the response did not persist for more than four or five stimulus pulses and did not show full recovery even after a period of rest (see Materials and methods). We did not observe any contraction of tail flexor musculature in response to connective stimulation.

Discussion

The baseline descriptor for the rapid escape synapomorphy shared by the eucarid crayfish and the syncarid *Anaspides tasmaniae* is a unitary episode of tail flexion driven one-to-one by spikes in one of two pairs of giant fibres (GFs). Medial giants (MGs) respond to rostral environmental stimuli and mediate a uniform abdominal flexion resulting in backward displacement of the animal, while lateral giants (LGs) respond to caudal stimuli and mediate a jack-knife abdominal flexion resulting in upward and forward displacement. The aim of this report has been to describe the escape behaviour of the hoplocarid mantis shrimp *Squilla mantis*, which diverged early in malacostracan evolution, and to compare it with this descriptor.

Comparative escape behaviour

In our observations, mechanical stimulation applied to the caudal end of *S. mantis* never elicited abdominal flexion, even when the stimulus consisted of a sharp tap on the telson delivered to a naïve animal. We conclude that *S. mantis* does not exhibit LG-type escape reactions. *S. mantis* does, however, exhibit an escape response to rostral stimulation that has some formal similarities to the MG-type tail-flips of crayfish, in that both mediate backward displacement and can involve abdominal flexion. However, in *S. mantis*, the escape response is very much slower than that mediated by the MG in crayfish. Furthermore, in *S. mantis*, the response is graded, with two

sequential components; limb-flick and tail flexion. The limb-flick can occur in isolation, mediating a weaker response, while tail flexion only occurs following a limb-flick and mediates a more vigorous response. In crayfish, the MG-mediated response also has limb-flick and tail-flexion components, but the two are inextricably linked, since the motor paths of both are directly driven through powerful electrical synapses by the MG neuron itself (Furshpan and Potter, 1959; Heitler and Fraser, 1989).

There is a bilateral pair of large-diameter dorsally located axons within the abdominal nerve cord of *S. mantis*, and these have the highest conduction velocity of all the cord units. In the anterior connective, the axons are obviously distinguishable from other axons in terms of size, which justifies their designation as giant fibres, although in the posterior cord the size differential is not so marked. There is clear physiological evidence that the dorsal GFs mediate limb protraction. First root units (which innervate swimmeret protractor muscles) are driven one-to-one by GF spikes elicited by positive current injected directly into a GF, and extracellular stimulation of the nerve cord at GF threshold elicits visible protraction movements of the swimmerets in the posterior abdomen. The dorsal GFs are therefore a candidate for mediating the limb-flick response to threat. An apparently plausible counter-argument is that the limb-flick is too slow to be GF-mediated. In crayfish, the entire GF-mediated tail-flip response occurs within 100 ms, while in *S. mantis* the maximal protraction of the limb-flick alone can take more than 200 ms. However, GFs only determine the speed of conduction within the nerve cord and, hence, the synchronisation of activity along the animal; they do not determine the latency to the peak of the behaviour. The latter variable is highly dependent upon the neuromuscular properties of the system. In crayfish, the tail-flexion motor output is mediated by very specialised motoneurons (the MoG neurons), which produce a massive contraction of the entire abdominal flexor musculature in response to a single motor spike, driven by a single GF spike. The crayfish limb promotor motoneurons that receive input directly from the MG have similar neuromuscular properties to the MoG (Heitler and Fraser, 1989), although their peripheral terminal distribution is unknown. There is no similar specialised trunk flexor neuromuscular system in *S. mantis* (Dijust, 1981), and single-impulse cord stimulation only produces weak limb protraction twitches. In the absence of the highly specialised motor pathway found in crayfish, multiple motor and pre-motor spikes would be required to elicit the full limb-flick in *S. mantis*. This is consistent with the graded nature of the limb-flick demonstrated in the behaviour pattern, and would clearly result in a much longer latency to peak response than the one-shot activation and all-or-none behavioural response found in crayfish.

We therefore consider that the dorsal GFs are very likely to be pre-motor drivers for the limb-flick component of the escape response in *S. mantis*. Are they also drivers for the tail flexion? There is some physiological evidence for recruitment of third root units by the GFs, but this excitation is much less consistent than that of the first root units (and the identity of the third root units is unknown; in *S. mantis*, the root contains extensor motoneurons as well as flexors). The fact that abdominal flexion progresses as a rostral wave argues against the GFs being drivers for abdominal flexion, although since a similar, albeit faster, rostral progression in abdominal flexion occurs in MG-mediated tail-flips in *Nephrops norvegicus* (Newland and Neil, 1990) and in crayfish (J. J. Wine, personal communication quoted in Newland and Neil, 1990), this cannot be regarded as conclusive. However, the delay between limb protraction and abdominal flexion, coupled with the fact that a strong limb-flick can be followed by a relatively weak tail flexion, and *vice versa*, strongly suggests that the two systems are not controlled by a single common command element. While we cannot discount the possibility that the GFs make some contribution to tail flexion, it is clear that it is not of the powerful, one-to-one form found in crayfish. Tail flexion in the escape response probably involves the recruitment of additional non-giant pre-motor drivers.

Squilla mantis *escape behaviour and evolutionary considerations*

Our data clearly show that adult *S. mantis* do not exhibit the two types of GF-mediated tail flexion which, had they existed, would have placed the hoplocarids in the same group as the syncarids and eucarids with regard to the escape system. However, the significance of the absence of this feature depends upon whether it represents the primary condition or a secondary loss. It is true that the escape system has shown considerable evolutionary plasticity involving such loss within the decapods (see, for example, hermit crabs, Chapple, 1966; squat lobsters, Sillar and Heitler, 1985a; Paul, 1989; and the true crabs), but where there has been total or partial loss of the escape system, this has usually been associated with massive morphological change, such as the abdominal reduction and permanent reflexion of the true crabs, or the use of discarded molluscan shells for abdominal protection in hermit crabs. In contrast, *S. mantis* has not only retained a fully extended abdomen, but appears to have substituted (or retained) another, more primitive, escape behaviour. This fact, taken in combination with other evidence such as the apparently primitive absence of transverse abdominal flexor musculature (Kunze, 1983), makes it seem likely that the absence of a clear GF-mediated tail-flexion escape in *S. mantis* is a primary condition. This reinforces the phylogenetic distinction between the hoplocarids and the fully caridoid syncarid/eucarid groups.

If the escape system of *S. mantis* is primary, why have the stomatopods ‘stuck’ with a primitive escape system, rather than fully integrating tail flexion in the escape response? The explanation may lie in the most striking (so to speak) feature of the Hoplocarida, which is, of course, their powerful raptorial second thoracic limbs. These limbs equip the mantis shrimp rather well to follow a ‘fight’ rather than ‘flight’ strategy in response to danger (particularly if the danger were to approach head-on), which in crayfish is the remit of the MG-mediated response. Furthermore, in many mantis shrimps, the telson is a specialised, heavily armoured ‘shield’ that can be held in front

of the animal by tonic full ventral flexion as a defence against the raptorial limbs of an opponent during agonistic encounters (Caldwell and Dingle, 1975). This necessarily means that the telson must be immune to the sorts of sudden mechanical stimuli that initiate the LG-mediated response in crayfish.

The presence in *S. mantis*, which diverged early in malacostracan evolution, of a limb-mediated escape response driven in a graded manner by proto-GFs fits rather well with the proposed ancestral form of the caridoid escape response in the 'limb-before-tail' scheme of evolution. In modern crayfish, non-giant categories of tail-flip are delayed relative to the GF-mediated reaction (Kramer and Krasne, 1984), and so the fact that when tail flexion occurs in *S. mantis* it invariably follows the limb-flick, rather than occurring synchronously with it, also fits with the idea that the tail flexion is mainly driven by non-giant fibres. Our data do not provide evidence that the dorsal GF in *S. mantis* is actually homologous to the similarly located MG neuron in crayfish, although this is a testable hypothesis. If the neurons are homologous, it would provide strong support for the proposed evolutionary path of the caridoid escape behaviour. However, even if the two systems are not homologous but have evolved independently, the escape system in *S. mantis* clearly demonstrates the biological plausibility of the ancestral form implicit in the limb-before-tail evolutionary path proposed for the decapod escape behaviour.

This work was supported by a grant from the BBSCR (formerly SERC) of the UK to W.J.H. and a grant from the Italian MURST (formerly MPI) to E.A.F. We thank the anonymous referees whose comments refined our thinking on the subject.

References

Bullock, T. H. and Horridge, G. A. (1965). *Structure and Function in the Nervous System of Invertebrates*. San Francisco, London: W. H. Freeman, pp. 867–868.

Caldwell, R. L. and Dingle, H. (1975). Ecology and evolution of agonistic behavior in stomatopods. *Naturwissenschaften* **62**, 214–222.

Chapple, W. D. (1966). Asymmetry of the motor system in the hermit crab *Pagurus ganosimanus* Stimpson. *J. Exp. Biol.* **45**, 65–81.

Cooke, I. R. C. (1985). Further studies of crayfish escape behaviour. II. Giant axon-mediated neural activity in the appendages. *J. Exp. Biol.* **118**, 367–377.

Dahl, E. (1963). Main evolutionary lines among recent Crustacea. In *Phylogeny and Evolution of Crustacea* (ed. H. B. Whittington and W. D. I. Rolfe), p. 18. Cambridge, Mass: Mus. Comp. Zool.

Dahl, E. (1983). Malacostracan phylogeny and evolution. In *Crustacean Phylogeny* (ed. F. R. Schram), pp. 189–212. Rotterdam: A. A. Balkema.

Dijust, R. (1981). Citoarchitettonica dei gangli nervosi addominali di *Squilla mantis* (Crustacea: Stomatopoda), evidenziata mediante iontoforesi assonale di $CoCl_2$. Tesi di Laurea in Scienze Biologiche, Università di Trieste.

Edwards, D. H., Heitler, W. J. and Krasne, F. B. (1999). Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends Neurosci.* **22**, 153–161.

Furshpan, E. J. and Potter, D. D. (1959). Transmission at the giant motor synapses of the crayfish. *J. Physiol., Lond.* **145**, 289–325.

Heitler, W. J. and Fraser, K. (1986). The segmental giant neurone of the hermit crab, *Eupagurus bernhardus*. *J. Exp. Biol.* **125**, 245–269.

Heitler, W. J. and Fraser, K. (1989). Thoracic output of crayfish giant fibres. I. Pereiopod promotor motoneurone. *J. Comp. Physiol.* A **166**, 117–124.

Hessler, R. R. (1983). A defense of the caridoid facies; wherein the early evolution of the Eumalacostraca is discussed. In *Crustacean Phylogeny* (ed. F. R. Schram), pp. 145–164. Rotterdam: A. A. Balkema.

Kramer, A. P. and Krasne, F. B. (1984). Crayfish escape behavior: production of tailflips without giant fibre activity. *J. Neurophysiol.* **52**, 189–211.

Kunze, J. C. (1983). Stomatopoda and the evolution of the Hoplocarida. In *Crustacean Phylogeny* (ed. F. R. Schram), pp. 165–188. Rotterdam: A. A. Balkema.

Newland, P. L. and Neil, D. M. (1990). The tail flip of the Norway lobster, *Nephrops norvegicus*. I. Giant fibre activation in relation to swimming trajectories. *J. Comp. Physiol.* A **166**, 517–527.

Paul, D. H. (1989). A neurophylogenist's view of decapod Crustacea. *Bull. Mar. Sci.* **45**, 487–504.

Pilgrim, R. (1964). Observations on the anatomy of *Squilla mantis* Latr. (Crustacea, Stomatopoda). *Pubbl. Staz. Zool. Napoli* **34**, 9–42.

Reichert, H., Wine, J. J. and Hagiwara, G. (1981). Crayfish escape behavior: neurobehavioral analysis of phasic extension reveals dual system for motor control. *J. Comp. Physiol.* A **142**, 281–294.

Roberts, A., Krasne, F. B., Hagiwara, G., Wine, J. J. and Kramer, A. P. (1982). Segmental giant: evidence for a driver neuron interposed between command and motor neurons in the crayfish escape system. *J. Neurophysiol.* **47**, 761–781.

Schram, F. R. (1969). Polyphyly in the Eumalacostraca? *Crustaceana* **16**, 243–250.

Schönenberger, N. (1977). The fine structure of the compound eye of *Squilla mantis* (Crustacea: Stomatopoda). *Cell Tissue Res.* **176**, 205–233.

Sillar, K. T. and Heitler, W. J. (1985a). The neural basis of escape swimming behaviour in the squat lobster *Galathea strigosa*. I. Absence of cord giant axons and anatomy of motor neurones involved in swimming. *J. Exp. Biol.* **117**, 257–269.

Sillar, K. T. and Heitler, W. J. (1985b). The neural basis of escape swimming behaviour in the squat lobster *Galathea strigosa*. II. The motor programme and sensory feeback interactions. *J. Exp. Biol.* **117**, 271–289.

Silvey, G. E. and Wilson, I. S. (1979). Structure and function of the lateral giant neurone of the primitive crustacean *Anaspides tasmaniae*. *J. Exp. Biol.* **78**, 121–136.

Watanabe, A., Obara, S. and Akiyama, T. (1967). Pacemaker potentials for the periodic burst discharge in the heart ganglion of a stomatopod, *Squilla oratoria*. *J. Gen. Physiol.* **50**, 839–862.

Wiersma, C. A. G. (1947). Giant nerve fibre system of the crayfish. A contribution to comparative physiology of synapses. *J. Neurophysiol.* **10**, 23–38.

Wine, J. J. (1984). The structural basis of an innate behavioural pattern. *J. Exp. Biol.* **112**, 283–319.

Wine, J. J. and Krasne, F. B. (1982). The cellular organization of the crayfish escape behaviour. In *The Biology of Crustacea*, vol. III, *Neural Integration* (ed. H. Atwood and D. Sandeman), chapter 15. New York: Academic Press.