

## VARIATIONS ON A SEGMENTAL THEME: MUSCLE RECEPTOR ORGANS AND EXTENSOR NEUROMUSCULATURE IN THE SQUAT LOBSTER *MUNIDA QUADRISPINA* (ANOMURA, GALATHEIDAE)

E. J. WALLIS\*, D. H. PAUL†, B. L. ANTONSEN AND R. HOLLENBERG‡

Department of Biology, University of Victoria, Victoria, BC, Canada V8W 2Y2

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### Summary

Extensor neuromusculature and the muscle receptor organs (MROs) associated with them have been conserved during the evolution of malacostracan crustaceans, despite species-specific differences between homologous segments in divergent taxa. Investigations of these differences could provide insight into how sensory and neuromuscular elements are modified to accommodate changing behavioural patterns. The most obvious differences between squat lobsters (galatheid anomurans) and macruran decapods, such as crayfish, are the greater dorso-ventral flattening of the galatheid abdomen and its flexed resting posture. To investigate whether the evolution of this altered posture affected extensor neuromusculature and MRO morphology and physiology, we used Methylene Blue staining, cobalt backfilling and extracellular recording techniques to describe these elements in the caudal thoracic and six abdominal segments of the squat lobster *Munida quadrispina* and compared our results with published descriptions of homologous elements in macrurans. In *M. quadrispina*, there is segmental variation both in the orientation of the MROs along the abdomen

and in their physiological responses to stretch: apparent sensitivity is higher in caudal than rostral MROs. Homologues of three of the four accessory neurones found in crayfish occur, but AN#1 has a major dendrite not present in crayfish. Intersegmental differences in size and morphology of extensor motoneurones occur in *M. quadrispina*, as have been reported in crayfish, but are dissimilar in the two: abdominal ganglion 5 extensor motoneurones are the largest in *M. quadrispina* and the smallest in crayfish; this difference correlates with the difference in relative size of axial muscles along the abdomen reported previously for these species. *M. quadrispina* also differs from macrurans in having a single tonic, and no phasic, MRO on each side of the last abdominal segment. Together, these observations suggest that galatheids have evolved modified or additional neurobehavioural control(s) for the abdomen and tailfan.

Key words: neural evolution, homology, motoneurones, proprioception, squat lobster, *Munida quadrispina*.

### Introduction

Control of abdomen extension in large-tailed decapod crustaceans (macrurans) relies on a complicated neuromusculature, including phasic and tonic extensor muscles, their excitatory and inhibitory motoneurones and a segmental array of proprioceptive muscle receptor organs (MROs). Despite all that is known about the efferent and afferent neurones involved in abdominal extension in crayfish and lobsters (Wine and Hagiwara, 1977; Wine, 1984), the behavioural roles of the MROs are not fully understood in any species. Comparative studies have the potential to provide insights into how neural elements work, as well as how they change through the course of anatomical and behavioural

evolution (Paul, 1990, 1991; Lauder, 1994). In this study, we compare extensor neuromusculature and associated MROs between segments of the same animal (the squat lobster *Munida quadrispina*, an anomuran) and between homologous segments in related animals.

MROs are analogues of mammalian muscle spindles. Investigations into their anatomy, physiology and function have contributed to many areas of neurobiology, such as sensory transduction and coding, efferent control of sensitivity, motor reflexes and intersegmental coordination of reflexes (Wiersma *et al.* 1953; Eyzaguirre and Kuffler, 1955; Edwards and Ottoson, 1958; Fields *et al.* 1967; Jansen *et al.* 1971; Page

\*Present address: Crustacea Department, Museum of Victoria, 71 Victoria Crescent, Abbotsford, Victoria 3067, Australia.

†Author for correspondence.

‡Present address: R.R.1, Site 27, C/1, Kroker Road, Smithers, BC, Canada V0J 2N0.

and Sokolove, 1972). In macrurans, there is typically one pair of MROs associated with the dorsal (extensor) neuromusculature of each abdominal hemisegment. Each MRO consists of a thin receptor muscle into which are embedded the dendrites of a multipolar sensory neurone with its soma lying close to the receptor muscle. The sensory axon projects into the ganglion of the next anterior segment *via* the second nerve (N2) of that ganglion. One MRO in each pair responds phasically to stretch and the other tonically (Wiersma *et al.* 1953), yet the projections of the two sensory axons through the central nervous system are indistinguishable (Bastiani and Mulloney, 1988). Associated with the MROs are motoneurons and accessory neurones, which inhibit the sensory neurones. An excitatory motoneurone is shared between the receptor muscle and the adjacent tonic extensor muscles. Physiological investigations and some morphological descriptions of MROs have generally used a 'representative' pair, usually from the last thoracic segment or from abdominal segment 2 or 3 of macruran species, and ignored intersegmental variation. Exceptions are Alexandrowicz's (1951) original description of the slightly different positions of the MROs along the abdomen in lobsters and several reports of the absence of accessory (inhibitory) neurones for the MRO pairs in the terminal (sixth) abdominal segment (Alexandrowicz, 1951; Wine and Hagiwara, 1977). Thoracic musculature is dissimilar from that of the abdomen, so that the MROs at the thorax–abdomen border are the only ones in which intersegmental variation has been presumed (Macmillan and Field, 1994).

MROs were first described in the six abdominal and last two thoracic segments of crayfish and lobsters by Alexandrowicz (1951), but they are now known to occur in at least five malacostracan orders besides the Decapoda (Pilgrim, 1960, 1974; Alexandrowicz, 1967; Wallis *et al.* 1993), suggesting that this type of proprioceptor may have evolved in early Malacostraca or, perhaps, even pre-date the class. A segmentally homogeneous series of MROs, i.e. with little morphological differentiation between the thoracic and abdominal members, occurs in the taxonomically primitive syncarid malacostracan *Anaspides tasmaniae*, an animal with a segmented and, therefore, flexible thorax and little external differentiation of segments between the head and the tailfan

(Wallis *et al.* 1993). MROs are also present in the anterior thoracic segments of the stomatopod *Squilla mantis* (Alexandrowicz, 1954), a eumalacostracan more distantly related to the decapods than syncarids. In decapods, the thoracic segments are rendered functionally immobile by the carapace, so one might expect the thoracic MROs to be functionally diverse; and, indeed, the MROs of all but the last two thoracic segments are modified and occur as N-cells without associated receptor muscles (Alexandrowicz, 1967; Macmillan and Field, 1994; Wiersma and Pilgrim, 1961). Subtle intersegmental differences between abdominal MROs may exist in macrurans, for there are small differences in segment morphology and some neural elements along the abdomen in crayfish (Mittenthal and Wine, 1978), but this has not been systematically investigated in any species. The differentiation between anterior and posterior abdominal segments is greater in anomurans, so there is greater potential for intersegmental differences in neural elements to be obvious.

Among the Anomura, squat lobsters (Galatheidae) are the least divergent from macrurans in body form and behaviour (Pike, 1947; Paul *et al.* 1985; Sillar and Heitler, 1985; Wilson and Paul, 1987; Paul, 1991). The most obvious difference is that, at rest, their abdomen is flexed (Fig. 1). But, they locomote like macrurans: they walk around the benthos on three pairs of thoracic legs, chelae extended in front, and swim backwards by rapid extensions and flexions of the abdomen, a behaviour homologous to non-giant tailflipping in crayfish (Sillar and Heitler, 1985; Wilson and Paul, 1987).

Galatheids show neuronal differences from the macruran pattern of flexor (power stroke) motoneurons that are correlated with the loss of the paired medial and lateral giant interneurons, but these neuronal differences are not reflected in changed behaviour, nor are they identical in the two squat lobsters that have been studied: *Galathea strigosa* has homologues of the giant motor neurones (MoG) that resemble unspecialised fast flexor motoneurons (Sillar and Heitler, 1985), whereas *Munida quadrispina* has lost MoG homologues altogether, as well as the anterior cluster of fast flexor motoneurons (Wilson and Paul, 1987), which *G. strigosa* has retained (Sillar and Heitler, 1985). An investigation of the neural components which contribute to abdominal extension in *M. quadrispina* would indicate, by comparison with macruran

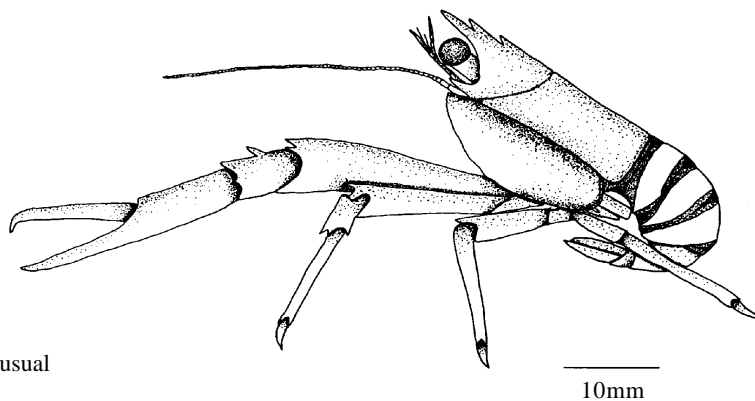


Fig. 1. Side view of the squat lobster *Munida quadrispina* in its usual resting posture.

homologues, whether the loss of the giant interneurons influenced any elements involved in abdominal extension.

Here we describe the anatomy and response properties of all of the MROs and the structures of the efferent neurones involved in abdominal extension in the squat lobster *M. quadrispina*. We discuss the relationship between the intersegmental differences in proprioceptors and neuromusculature, and compare the MROs and extensor neuromusculature in *M. quadrispina* with homologous elements in macrurans.

### Materials and methods

*Munida quadrispina* were collected from Saanich Inlet, Vancouver Island, by trawling and housed in recirculating seawater aquaria. Animals of both sexes, 5–8 cm long, were used in all experiments. Muscle receptor organs (MROs) and extensor musculature were exposed in freshly dissected animals that had been anaesthetised by chilling, then decapitated. Dissections and physiological experiments were performed in saline, composition ( $\text{mmol l}^{-1}$ ): 427 NaCl, 11.8 KCl, 13  $\text{Na}_2\text{SO}_4$ , 12.7  $\text{CaCl}_2$ , 9.29  $\text{MgCl}_2$ , 9.29 Trizma; approximately 4.3 maleic acid was used to adjust pH to 7.5.

#### Identification of receptor muscle position

MROs were located using reduced Methylene Blue staining: 4.5–5 parts of filtered, acidified, 0.5% Methylene Blue (4–5 drops of 24% HCl per 100 ml of Methylene Blue) to 1–1.5 parts of 12% Rongolite (crystals in distilled water), heated until the solution cleared. 5–10 drops of this solution were added to fresh saline or sea water bathing the tissue. MROs were exposed in the abdomen in two ways. In the first, a window was cut in the dorsal exoskeleton and the MROs were stained *in situ*. In the second, the MROs were exposed by ventral dissection; the nerve cord (all segmental nerves cut), the gut and all the flexor and extensor musculature were removed to leave the MROs and their innervation intact. MROs in the thorax were exposed by removal of the carapace and dissection of the dorsal musculature. Exposed nervous tissue was stained at 4°C for 2–18 h.

#### Central projections of sensory neurones; morphology of efferent neurones and accessory neurones

Central projections of the sensory neurones and morphologies of extensor motoneurones and accessory neurones of the MROs were revealed by backfilling second nerves (N2) of the abdominal ganglia and the N2 homologues of ganglia in the thoracic ganglionic mass with  $0.3 \text{ mmol l}^{-1}$   $\text{CoCl}_2$  (at 4°C for 16–24 h). Cobalt was precipitated using ammonium sulphide (Pitman *et al.* 1972), and all fills were subsequently intensified with silver (Altman and Tyrer, 1980). Ganglia were viewed under a Leitz Aristoplan microscope and drawn with the aid of a *camera lucida*. To trace the central projections of the sensory neurones, 17 successful fills were made of abdominal ganglia and 11 of the caudal thoracic ganglia. The descriptions of the efferent neurones are based

on 15 additional backfills of N2 of each of the abdominal ganglia.

#### Physiological responses of MROs to stretch

The physiological responses to stretch of the MROs were characterised in isolated MRO preparations. The abdominal MROs were exposed by ventral dissection as described above, then the appropriate N2 was cut close to the ganglion. Sensory branches of N2 innervating the hypodermis were cut to minimise extraneous electrical activity in the recordings. All extensor muscles were removed and the cuticle was trimmed so that a small square remained around the attachments of the MROs. In the thorax, MROs were exposed as described above and N2 homologues were cut close to the ganglionic mass. The dissected tissue was immersed in a bath of fresh saline and anchored on the posterior side to a Sylgard-lined (Dow Corning) dish. The cuticle on the anterior side of the receptor muscles was clamped in forceps attached to a micromanipulator. This arrangement allowed a mechanical stretch stimulus to be applied to the receptor muscle strands. Glass suction electrodes were used to record electrical activity in the N2. The signals were amplified and stored on magnetic tape before being transferred to a computer for analysis (AxiTape, Axon Instruments, CA) and selection of records for Fig. 6. Data were collected from 15 animals, 1–4 MROs per animal, giving 4–9 replicate experiments for the MROs of each segment.

### Results

#### Nomenclature of MROs

Two features of MROs can cause confusion about nomenclature: they span the articulation between two segments and their innervation is through the second nerve (N2) of the ganglion in the next anterior segment, the dorsal myotome in decapods being offset posteriorly with respect to the central nervous system (Alexandrowicz, 1951; Wiersma and Pilgrim, 1961). Here, MROs are labelled according to the segment of their receptor muscle's anterior insertion. Hence, the S6 MRO spans the articulation between the terminal abdominal segment (S6) and the telson, and its innervation is via N2 of abdominal ganglion 5 (A5). Similarly, S2 MROs span the articulation between abdominal segments S2 and S3, and their sensory and motor axons are in N2 of the first abdominal ganglion (A1). In galatheids and other anomurans, shortening of interganglionic connectives has resulted in fusion of A1 and the last five thoracic ganglia into a single ganglionic mass in the posterior thorax (Pike, 1947; Paul *et al.* 1985; Wilson and Paul, 1987). Nevertheless, the individual neuropiles remain discrete and identifiable. Therefore, in order to facilitate future comparisons between different malacostracan taxa, we identify the thoracic nerves by ganglion number according to the primitive plan of eight thoracic neuromeres (Horridge, 1965; Wallis, 1995). The first three thoracic ganglia, T1–T3, are fused with the three ventral 'head ganglia' into the suboesophageal ganglion; the

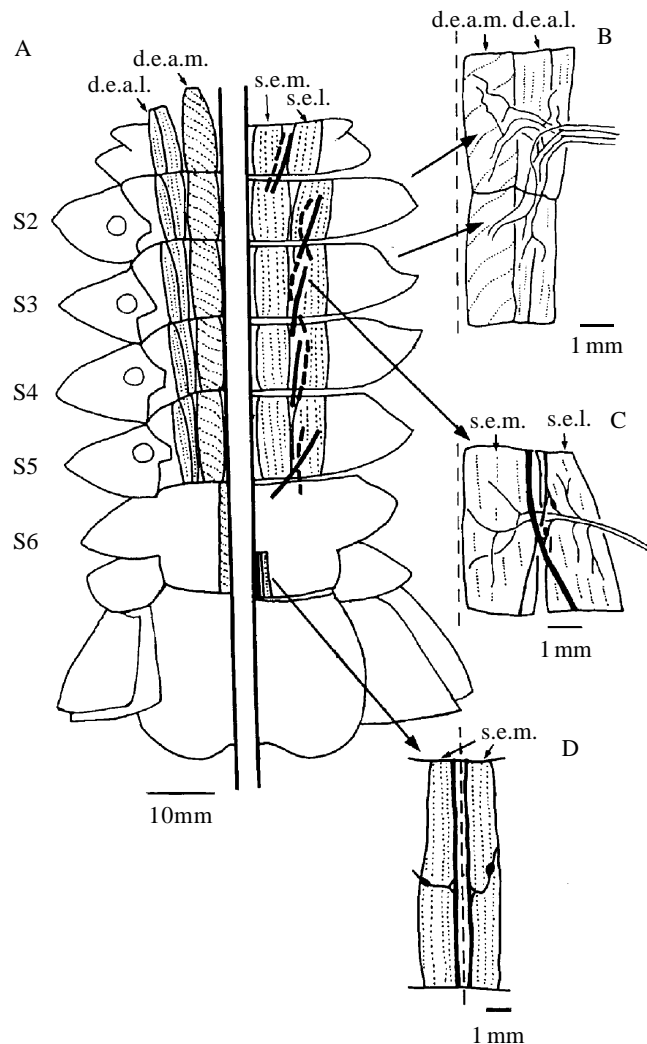
Fig. 2. (A) Diagrammatic views of the dorsal part of the abdomen laid flat and dissected to show the extensor muscles and muscle receptor organs (MROs). Left side, ventral view of deep (phasic) extensor muscles. Note the narrow band of deep extensor muscles in S6. Right side, dorsal view showing the superficial (tonic) extensor muscles and MROs. In all segments except S6, the MROs are paired: dashed lines, the presumably phasic (thicker) receptor muscles; continuous lines, the presumably tonic (thinner) receptor muscles. In S2–S4, the MROs are quite lateral; in S5, the receptor muscles cross at a large angle; in S6, there is only a single MRO on each side. (B) The characteristic branching pattern of phasic extensor motoneurons over the ventral side of the deep extensor muscles, illustrated by a *camera lucida* drawing of the Methylene-Blue-stained A1 N2 axons innervating the deep extensor muscles in S2 and S3. (C) Dorsal view of a typical pair of MROs, stained with Methylene Blue: *camera lucida* drawing of the right pair of S3 MROs. The relative widths of the receptor muscles identify which is phasic (thicker) and which is tonic (thinner). Accessory axons and innervation of the receptor muscles are not shown. (D) *Camera lucida* drawing of a Methylene Blue stain of the bilateral pair of MROs in S6, in the posterior third of the segment. The single receptor muscles (dark lines) are juxtaposed on the midline (dashed line) between the closely apposed underlying superficial extensor muscles, and the sensory somata lie lateral to them, close to the lateral edge of the slow extensor muscles. d.e.a.l., deep extensor muscle, lateral head; d.e.a.m., deep extensor muscle, medial head; s.e.l., superficial extensor muscle, lateral head; s.e.m., superficial extensor muscle, medial head.

remaining five, T4–T8, innervate the posterior thoracic segments, including the pereopods. The thoracic homologues of abdominal N2s from ganglia T7 and T8, along with N2 of A1, innervate the Th-Ab MROs (at the thoracic-abdominal boundary), the S1 MROs, and the S2 MROs, respectively. We describe all of these MROs.

#### Receptor muscle position

At rest, the abdomen of *M. quadrispina* is curled ventrally and the tailfan is held under the body (Fig. 1). In this posture, there is some degree of flexion between all abdominal segments, except the first and second. When the abdomen is laid flat, with the dorsal surface uppermost, and the MROs stained *in situ* with Methylene Blue, it is evident that the MRO positions and orientations show greater intersegmental variability (Fig. 2A, right side) than in macruran decapods, for example *Homarus vulgaris* (Alexandrowicz, 1951), or even in hermit crabs (Anomura, Paguridae) (Alexandrowicz, 1952). The evolution of segmental differences in MRO orientation with respect to the underlying extensor musculature is attributed to the flexed abdomen at rest – they may have evolved to retain sensitivity to further flexion of the abdomen during postural adjustments (Antonsen and Paul, 1994) as well as to gross movements, such as tailflicking (Sillar and Heitler, 1985; Wilson and Paul, 1987).

Methylene Blue staining of the MROs reveals that, with the exception of those spanning the S6–telson articulation (S6 MRO), MROs in each abdominal hemisegment are paired, there being two receptor muscles and two sensory neurones. Close inspection revealed that one receptor muscle was



invariably slightly thicker than the other; in several other species, such a difference is correlated with physiological differentiation into phasic and tonic receptors (Wiersma *et al.* 1953). The positions of the receptor muscles with respect to the extensor muscles in each segment are shown in Fig. 2A. Extensor muscles are named according to their presumed homologues in crayfishes (Pilgrim and Wiersma, 1963). The receptor muscles of S1 MROs lie directly over the medial superficial extensors (s.e.m.) and are quite close to the midline (Fig. 2A). The receptor muscles of S2 MROs are considerably more lateral: they lie over the lateral deep extensor muscles (d.e.a.l.). The receptor muscles of both S2 MROs and S3 MROs cross (for detail of S3 MROs, see Fig. 2C). The receptor muscles have similar orientations in the S3 and S4 MROs and, in both segments, they are more medial than the receptor muscles of the S2 MROs. The receptor muscles of S5 MROs have the most unusual orientation. The two muscle strands form a broad cross, with widely separated insertions. In segment 6, there is only one receptor muscle and a single sensory neurone (S6 MRO) on each side of the midline, rather than two (Fig. 2A,D). The receptor muscles of the two sides lie next to each other in the midline groove between the

underlying slow extensor muscles of each side in the posterior third of S6; both the slow extensor muscles and receptor muscles are relatively short compared with their anterior segmental homologues.

#### Central projections of the sensory neurones

The stretch-receptive neurones (SR) of the MROs in each segment send their axons through the N2 of the next anterior ganglion in the ventral nerve cord. We traced the SR axons by making cobalt backfills of the N2s of ganglia T7, T8 and A1–A5.

The SR axons of crayfish (Bastiani and Mulloney, 1988) have a characteristic morphology in the ganglion of entry, where they form a T-shaped bifurcation that gives rise to rostral and caudal projections to the brain and A6, respectively. Similarly, in *M. quadrispina*, when axons from both MROs in the same hemisegment are filled, it is clear that they overlie each other in the ganglion and remain closely associated in the connectives of the ventral nerve cord (Figs 3, 4C). Serial backfills of segmental N2s along a single nerve cord show that all of the SR axons bifurcate in the same area of their ganglion

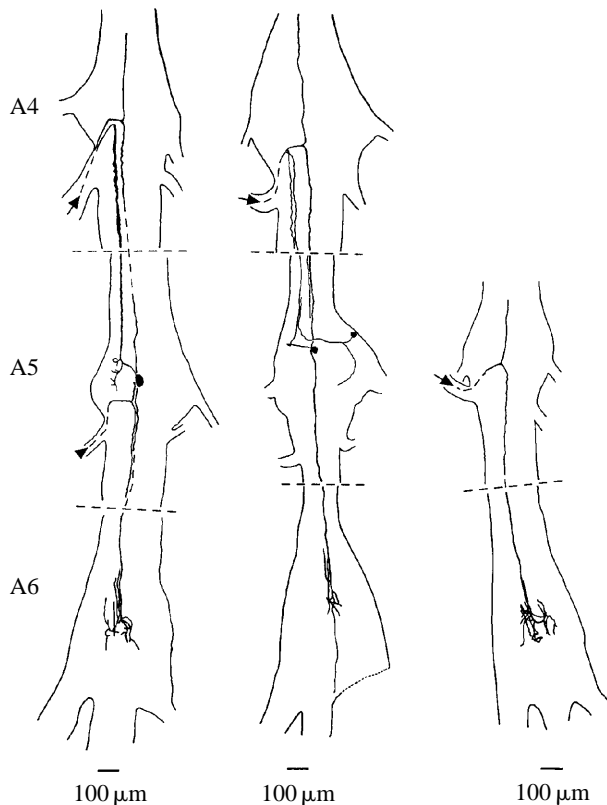


Fig. 3. Each MRO sensory axon bifurcates in its ganglion of entry and the caudal branch terminates in A6. Two examples of the terminations are shown in A6 from S5 MROs (left and middle) and one from an S6 MRO (revealed by unilateral backfills of second nerves, N2, of ganglia A4 and A5, respectively); accessory neurones in A5 from the A4 fills are also shown: *camera lucida* drawings. The N2s from which the cobalt reached A6 are indicated by arrows. Arrowhead (A5, left) indicates a fill which did not reach A6.

of entry and all stay together in the presumed homologue of the dorsal median tract of the crayfish (Skinner, 1985; Liese *et al.* 1987) (Figs 3, 4C).

Backfilling N2 homologues of T7, T8 and A1 revealed T-shaped axonal branching patterns in the thoracic ganglionic mass similar to those characteristic of SR projections in the abdominal ganglia (Fig. 4A). Thus, despite thoracic neuromere fusion, ganglia in the thoracic mass retain discrete neuropilar areas, and it is possible to differentiate fills of each N2 homologue.

In crayfish, the axon of each SR terminates bilaterally in A6 with a characteristic J-shaped morphology, the ramifications being of about equal density on both sides of the midline (Bastiani and Mulloney, 1988). In *M. quadrispina*, the morphology of the SR terminations in A6 is quite variable,

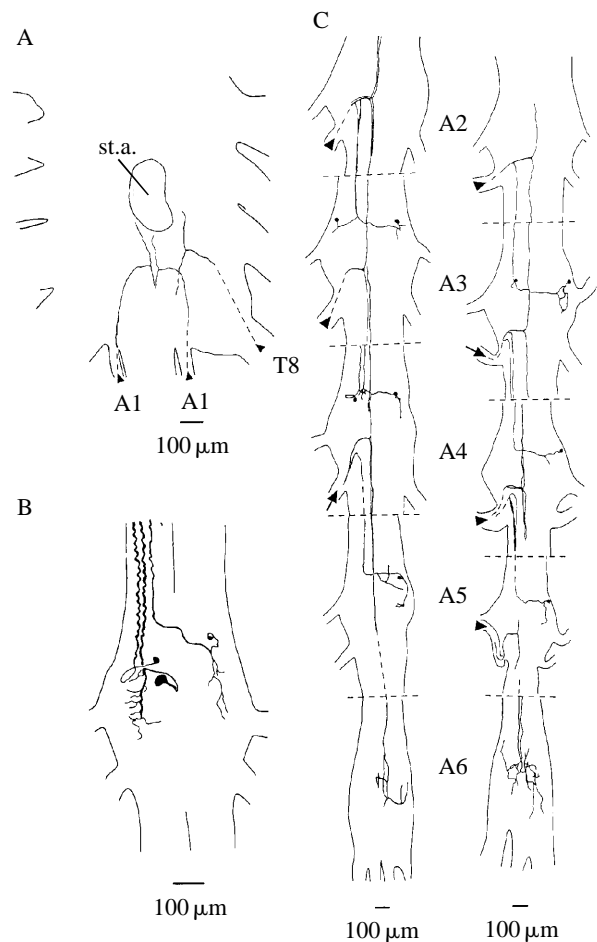


Fig. 4. Morphology of MRO sensory neurone axons and accessory neurones revealed by backfilling with cobalt the second nerves (N2) of different ganglia: *camera lucida* drawings. (A) Backfills of N2 homologues of T8 and A1 (bilateral fill) in the thoracic ganglionic mass. (B) Morphology of accessory neurones in A4. AN#1 has the largest soma; the soma of AN#2 is contralateral; the soma of AN#3 may be medial (as shown) or lateral to its axon. (C) Backfills of N2s of A2–A5 in two individuals. The N2s from which the cobalt reached A6 are indicated by arrows; those that were filled but the cobalt did not reach A6 are indicated by arrowheads. st.a., sternal artery.

although most of the backfills showed at least a few contralateral projections (Figs 3, 4C), and several fills displayed a hook-like termination (for example, see Fig. 4C, left). This morphological heterogeneity of A6 terminations in *M. quadrispina* may be explained in part by the finding of Bastiani and Mulloney (1988) that A6 axonal terminations were more variable in adult crayfish than in juveniles, presumably due to postembryonic growth. Only adult *M. quadrispina* were used in this study.

#### Morphology of efferent neurones

Other axons in N2 besides the SR axons include the motoneurons innervating the receptor muscles and the fast and slow extensor muscles (Wine and Hagiwara, 1977) and the accessory neurones, inhibitors of the sensory neurones (Wiersma *et al.* 1953; Jansen *et al.* 1971).

Our backfills revealed three groups of motoneurons in abdominal ganglia A2–A5 (Fig. 5C–G); we tentatively identify them as phasic and tonic exciters and peripheral inhibitors of the extensor muscles by their positional and morphological resemblance to these well-studied motoneurons in crayfish (Wine and Hagiwara, 1977). These motoneurons have been similarly identified in another squat lobster, *G. strigosa* (Sillar and Heitler, 1985). Thus, the anterior cluster of small ipsilateral (to the axons exiting N2) somata are presumed to be the tonic extensor motoneurons, the posterior ipsilateral group of large somata to be the phasic extensor motoneurons, and the two contralateral somata to be the phasic and tonic peripheral inhibitors (Fig. 5C–G). In T8 and A1, the large and small ipsilateral somata are interspersed, rather than forming two groups (Fig. 5A,B). As in crayfish (Wine and Hagiwara, 1977), one or two of the motoneurons in the caudal group (in A2–A5) are sometimes much smaller than the others, so that to distinguish between the smaller phasic and larger tonic motoneurons on the basis of soma size and position is particularly problematic. There are fewer exciters than in *G. strigosa* which, in turn, has fewer than crayfish (*Procambarus clarkii*, Table 1; Sillar and Heitler, 1985). These number differences correlate with the size difference between the three animals, but otherwise the extensor motoneurons and muscles are qualitatively very similar in all except the last of their abdominal segments (Table 1; Fig. 5).

The phasic extensor musculature from the thorax to S5 in *M. quadrispina*, as in macrurans (Pilgrim and Wiersma, 1963), comprises a medial, spiralled group of muscles (deep extensor muscles, median head: d.e.a.m.), with the segmental units joined end-to-end, flanked by two lateral muscles (deep extensor muscles, lateral heads: d.e.a.l.). The lateral muscles lie deep with respect to the much thinner sheets of superficial, tonic extensor muscles (superficial extensor muscle, medial and lateral heads: s.e.m., s.e.l.) (right side of Fig. 2A), which are hidden in the ventral view (left side of Fig. 2A). The S6 extensors in crayfish have been largely ignored, apart from reports by Daniel (1931) and Alexandrowicz (1951), who indicated the position of the superficial extensors in *Homarus vulgaris*. In *M. quadrispina*, the extensor muscles in S6 are

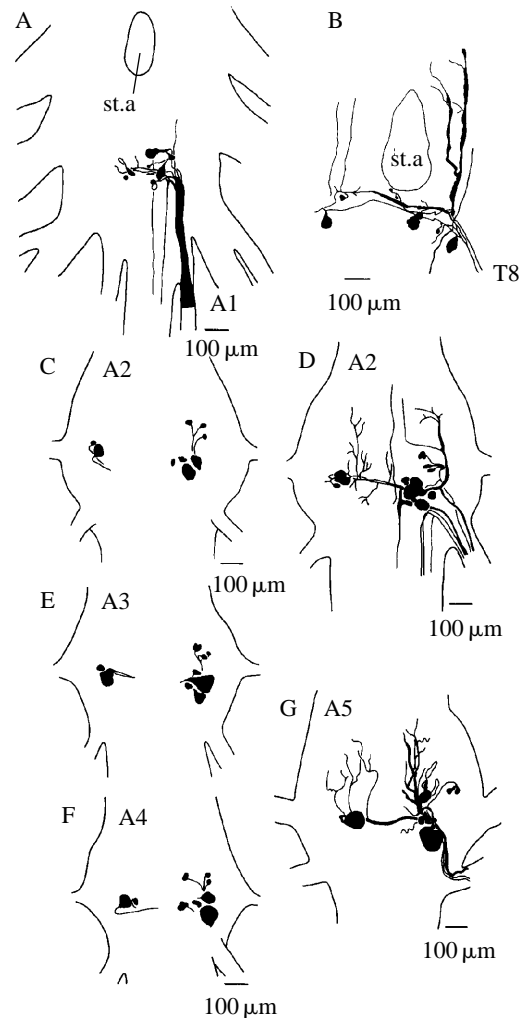


Fig. 5. Extensor motoneurons in the last thoracic ganglion, T8, and abdominal ganglia A1–A5 stained by backfilling one N2 (or N2 homologue from the thoracic ganglionic mass) of each ganglion: (A) A1; (B) T8; (C) A2, somata only shown; (D) A2, details of dendritic branching. The bilateral neurites of the large contralateral cell (the putative fast extensor inhibitor) shown in A2 are typical of this cell in all segments. Bilateral ramifications of the putative exciters are not shown, because they would have obscured the distinctive morphology of the peripheral inhibitor. (E) A3, somata only shown; (F) A4, somata only shown; (G) A5, including details of dendritic branching. Note the very large size of the soma and ipsilateral dendritic tree of the largest excitor in A5. st.a., splanchnic artery.

very narrow bundles along the mid-dorsum, the phasic muscles (d.e.a.m. continuation) spanning the whole segment and the tonic muscles (s.e.m. continuation) arising two-thirds of the way back, along the midline (Fig. 2A). The posterior insertions of the extensor muscles of S6 are on the midline of the anterior telson, with the tonic (superficial) bundles lying dorsal to the phasic (deep) bundles. The pair of largest phasic extensor motoneurons in the animal occurs in A5 (Fig. 5G), which was unexpected, because of the small size of the phasic extensor muscles in S6 compared with their homologues in anterior segments (Fig. 2A, left). The large

Table 1. Efferent neurones in segmental nerve 2 of abdominal ganglia

Ganglion	<i>Procambarus clarkii</i> <sup>a</sup>			<i>Galathea strigosa</i> <sup>b</sup>			<i>Munida quadrispina</i>		
	E	I	AN	E	I	AN	E	I	AN
A1	10	2 (3)	4	7	2	?	5	2	?
A2	10	2 (3)	4	8	2	?	7 (8)	2	3
A3	10	2	4	8 (9)	2	?	7 (8)	2	3
A4	10	2	4	8	2	?	7	2	3
A5	6	2	4	6	2	?	5	1	3

<sup>a</sup>From Wine and Hagiwara (1977); <sup>b</sup>from Sillar and Heitler (1985); these authors labelled A1 TAG (true abdominal ganglion) and numbered the free abdominal ganglia 1–5 instead of 2–6.

E, excitor (phasic and tonic); I, inhibitor; AN, accessory neurone; bracketed numbers indicate this value was observed in a minority of experiments.

size of these neurones may result from the absence of competition for postsynaptic sites on the muscle fibres from branches of the next anterior N2: in the anterior segments, fast extensors are innervated by two adjacent ganglia (see below).

Methylene Blue staining revealed that four phasic motor axons per N2 ramify over the ventral surface of the deep extensor muscles in adjacent pairs of hemisegments (the hemisegment that the N2 enters and the next caudal hemisegment; Fig. 2B) in a pattern virtually identical to that described for four of their counterparts in crayfish and lobsters (Parnas and Atwood, 1966). Using the numbering scheme of Parnas and Atwood (1966), *M. quadrispina* has #1, which innervates the medial spiral muscles in the segment of nerve entry and the next caudal segment; #2, which innervates the medial spiral and the lateral muscle bundles in the segment of nerve entry only; #3, which innervates the lateral muscle bundles in both the segment of nerve entry and the next posterior segment; and #5, which innervates both spiral and lateral muscle bundles in both segments (except, perhaps, the lateral part of the lateral muscle in the segment posterior to nerve entry). Axon #5 is presumed, from its wide distribution, to be the peripheral inhibitor. *M. quadrispina* apparently lacks a homologue of the macruran #4 phasic, excitatory, extensor motoneurone. We observed this pattern of segmental overlap for the deep branch of N2 of all ganglia from T8 (which innervates deep extensors of the last thoracic segment and abdominal segment 1) to A3 (which innervates S4 and S5 deep extensors). Fast extensor motoneurones in N2 of A4 innervate only S5 muscles and do not branch into the next segment (S6) which, as suggested above, may account for the large size of A5 extensor motoneurones. Thus, only in S6 are the extensor muscles innervated by a single ganglion (A5). It is unknown whether this is a peculiarity of *M. quadrispina*, of all galatheids, or whether it also occurs in macrurans. This single-ganglion innervation of S6 extensor muscles could, presumably, facilitate the control of telson extensions independently of the abdomen, movements which are allowed by the greater flexibility of the articulation and exoskeleton of the tailfan in galatheids than in macrurans (Paul *et al.* 1985).

#### Morphology of accessory neurones

The somata of accessory neurones (AN), inhibitors of the SRs, are located in the ganglion caudal to the N2 through which their axons run (Jansen *et al.* 1971; Wine and Hagiwara, 1977; Skinner, 1985; Liese *et al.* 1987). The accessory neurones of the crayfish are distinguished by soma position and by the shape and location of their dendritic trees (Wine and Hagiwara, 1977); morphologically very similar neurones occur in *M. quadrispina*.

We could identify at least three of the four ANs described in crayfish ganglia T7–A5 (Wine and Hagiwara, 1977) in A2–A5 of *M. quadrispina* (Table 1; Figs 3, 4B,C). The soma of AN#1 lies on or close to the midline in the anterior part of the ganglion and its axon, unlike its homologue in crayfish (Wine and Hagiwara, 1977), gives off a major ipsilateral caudal projection on its way to the connective (Fig. 4B). The morphologies of AN#2 and AN#3 are similar in *M. quadrispina* and crayfish. The soma of AN#4 lies contralateral to the axon, as does its major caudally projecting axonal branch, which, as in crayfish, ramifies extensively over the branching neurites of extensor motoneurones in the same hemiganglion. AN#3 appears to be entirely unilateral, its soma variably medial or lateral to its axon (in crayfish, its soma is usually quite close to the lateral edge of the ganglion); its axon extends into the connective without branches in the soma ganglion (Fig. 4B). A fourth, very small, caudal contralateral soma stained in a few backfills of N2 of A2–A5, but its axon was never discernible. It might be the homologue of AN#4 of crayfish (Wine and Hagiwara, 1977), which has not been stained in its entirety and is not included in Table 1.

#### Physiological responses of MROs to stretch

The responses to stretch of the MROs at the thoracic–abdominal boundary and in all abdominal segments in *M. quadrispina* are illustrated in Fig. 6. It was not possible to obtain reliable recordings from N2 homologues more anterior than T7, so these have been excluded. In general, all segments have phasic and tonic MROs, except S6, which lacks a phasic unit. There is variation, however, between the segments in rate of tonic firing during the stretch stimulus. A more detailed description of the responses of the MROs in each of the

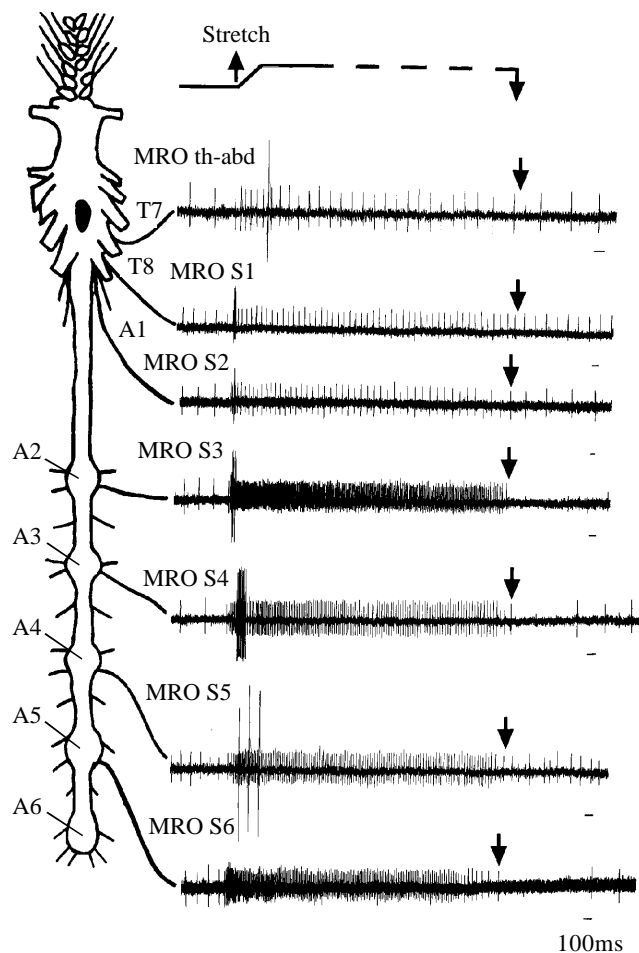


Fig. 6. Responses to stretch of MROs at the thoracic-abdominal (th-abd) articulation and in each segment along the length of the abdomen were recorded extracellularly. All records have the same amplification. MROs were set with a resting tension that gave a basal firing rate in the tonic unit of  $3\text{--}5\text{ spikes s}^{-1}$ . A standard stretch stimulus was applied and held for 2–3 s by means of a micromanipulator, after which the stretch was released and the discharge of the MRO was allowed to equilibrate for 5 min before the stimulus was repeated. The records shown are from different animals as it was not possible to obtain recordings from all segments in a single animal. The segmental differences were consistent in different animals: recordings were made from each segment in 4–9 different animals (15 animals in total).

segments, highlighting the differences between the tonic units, follows. All descriptions refer to Fig. 6.

Th-Abd MROs (SR axons in N2, T7) span the thoracic-abdominal boundary, which is loosely articulated in galatheids. When a rapid stretch stimulus is applied, the tonic unit increases its firing rate, followed, always after the onset of the stimulus, by a single large spike of the phasic unit. Increasing stimulus amplitude or acceleration usually resulted in breaking the preparation.

S1 and S2 MROs (SR axons in N2 of T8 and A1, respectively) respond very similarly to stretch and will be treated together. The phasic unit in both fires briefly,

immediately after the onset of a stretch stimulus. The tonic units show a three- to fourfold increase in firing rate following the onset of the standard stretch stimulus. There is no cessation of tonic activity following release from stretch.

S3, S4, and S5 MROs (SR axons in N2 of A2, A3 and A4, respectively) respond similarly. All have a large phasic unit which fires immediately after the onset of stretch and adapts more slowly than the phasic units in the more anterior segments. The tonic units respond to stretch with an approximately 10- to 15-fold increase in firing rate. This is greater than that observed in the more anterior segments. In all three segments, although only shown for S3 and S4 MROs in Fig. 6, a pause in tonic activity is common upon release of the stretch stimulus. Tonic firing resumes spontaneously within 10 s of the stimulus release.

In segment 6, there is neither anatomical (Figs 2, 3) nor physiological evidence of a phasic MRO. The single S6 MRO (SR axon in N2 of A5) responds with an approximate 15-fold increase in firing rate at the onset of the stretch, as do the tonic units of the MROs of S3, S4 and S5. Some adaptation to the stimulus occurs, and firing ceases completely for a period after release from stretch (Fig. 6).

### Discussion

Neuronal elements are generally conserved during evolution (Katz, 1991; Arbas *et al.* 1991), which led us to expect that there would be a strong resemblance between neural elements associated with the MROs and the extensor musculature of *M. quadrispina* and those previously described in crayfish. This prediction was generally supported. The MROs in *M. quadrispina* are morphologically similar to crayfish MROs and, except in the terminal segment, occur in pairs, two receptor muscles and associated sensory neurones per hemisegment. Each sensory neurone's axon bifurcates in the ganglion of entry, projecting one branch anteriorly and one posteriorly; the latter terminates bilaterally in A6. Homologues of at least three of the four accessory neurones identified in crayfish are present. There are, however, fewer extensor motoneurones. Other studies have also reported smaller pools of identified motoneurones in anomurans compared with their counterparts in macrurans (Bent and Chapple, 1977; Chapple and Hearney, 1976; Paul *et al.* 1985). That anomurans are generally smaller than macrurans does not in itself explain the difference, since homologous neuronal pools can have identical numbers of neurones in species of very different body size (Chapple, 1977; Thompson and Page, 1982; Paul, 1981). There are also subtle morphological differences between some homologous neurones, which are likely to be functionally significant.

The physiological responses of MROs to stretch show pronounced intersegmental variation in *M. quadrispina*, a phenomenon not detailed in crayfish. Although our method of recording electrical activity makes it impossible to relate the discharge frequencies of the sensory neurones to normal postures in intact animals, the segmental differences we found



are unlikely to be artefactual, because each receptor was held at the same, low resting firing rate and standard stretch stimuli were used; furthermore, the results (segmental differences between responses) were repeatable in different animals (which would inevitably have been dissected and mounted somewhat differently). The apparently greater sensitivity of the caudal (S3–S6) MROs than of the anterior MROs, implied by the much higher discharge frequencies in response to the standard stretch stimulus, may be an adaptation to the flexed resting posture of squat lobsters: angular excursions at intersegmental articulations are smaller in the posterior than in the anterior abdomen, a situation opposite to that in macrurans. This conjecture could be tested by comparing the sensitivities of the MROs along the abdomen of a crayfish or lobster.

More prominent morphological, as well as physiological, differentiation of serially homologous elements along the abdomen in *M. quadrispina* than in crayfish was previously described in a comparative study of the flexor neuromusculature of these animals: the size ratio of extensor to flexor muscle (cross-sectional areas measured in mid-segment) is larger in S5 than in anterior segments in *M. quadrispina*, whereas this ratio is approximately the same in S5 as in the anterior segments in crayfish (Wilson and Paul, 1987). It is probable that the species difference in extensor: flexor ratios (*M. quadrispina* > crayfish) is even larger in S6 than in S5, although the ratio has not been determined directly in either animal. This is because the extensor muscles in S6 appear to be somewhat larger relative to the size of the segment in squat lobsters than in crayfish, although in both they are much smaller than in S5. Furthermore, squat lobsters lack a homologue of the principal axial flexor muscle in S6 of crayfish (the ventral telson flexor muscle; Paul *et al.* 1985). The relatively greater development of the extensor system in the caudal than in the rostral abdominal segments is also reflected in the posterior-to-anterior decrease in size of the fast extensor motoneurons, which appears to be characteristic of squat lobsters, since Sillar and Heitler (1985) reported a similar gradient in size of extensor motoneurons in *G. strigosa*. These segmental differences in the relative sizes of the axial neuromusculatures correlate with the difference in resting posture (caudal abdomen flexed in galatheid anomurans and extended in macrurans). The different postures presumably reflect different neutral points for the skeletal system: extension is relatively more work for squat lobsters, starting with the abdomen flexed, than for macrurans, starting with a partially extended abdomen.

There are also some differences in morphology of the fast extensor motoneurons in galatheids and crayfishes. In *M. quadrispina*, as in *G. strigosa* (Sillar and Heitler, 1985), the bilateral dendrites of the fast extensor motoneurons contrast with the predominantly ipsilateral dendrites of the fast extensor motoneurons in crayfish (Wine and Hagiwara, 1977). This arrangement may be a correlate of the loosely articulated segments in squat lobsters and suggests that the reflex control of abdominal movements in squat lobsters could be different from that in crayfish. This idea is supported by the

morphological difference of one of the accessory neurones: AN#1 in *M. quadrispina* has a prominent ipsilateral projection in the soma's ganglion (Fig. 4B), in contrast to the crayfish AN#1, which ramifies only in the anterior ganglion (Wine and Hagiwara, 1977).

The differentiation of posterior from anterior segments in *M. quadrispina* is accentuated in S6, with its single MRO per hemisegment. In crayfish, the pairs of MROs in S6 resemble those of anterior segments (except for the absence of accessory neurones), whereas S6 in *M. quadrispina* (and, presumably, other galatheids) lacks phasic MROs, and the bilateral pair of single tonic MROs are juxtaposed on the dorsal midline. Given the lateral flexibility of the S6–telson articulation in galatheid anomurans, a flexibility that does not exist in macrurans (Paul *et al.* 1985), the midline may be the best position from which proprioceptors can unambiguously distinguish flexion from twisting of the telson. Squat lobsters are continually adjusting the position of their tailfan and they hold it in characteristic poses during agonistic behaviours (Antonsen and Paul, 1994); their S6 MROs are likely to be intimately involved in these behaviours. Squat lobsters are also lively swimmers, using non-giant tailflipping (Sillar and Heitler, 1985; Wilson and Paul, 1987). Adaptations in proprioceptive circuits involving the MROs during this behaviour (not well understood even in crayfish) are likely to have occurred during galatheid evolution, but they remain to be explored.

Fossil evidence suggests that anomurans and macrurans had diverged by the Cretaceous period (approximately 100–65 million years ago) (Schram, 1977). Freshwater crayfish and marine macrurans had, apparently, already separated in the Triassic period (some 220 million years ago) (Hasiotis and Mitchell, 1993). The similarity in morphology of the MROs, extensor motoneurons and accessory neurones in *M. quadrispina* and macrurans, despite their different external appearances and postures, presumably reflects conservation of developmental programmes (Thomas *et al.* 1984). What differences there are between the extensor neuromusculature of crayfish and squat lobsters appear to be related to specific differences in body form, such as size and dorsal–ventral compression of the body, which, in turn, would influence proprioception. This conservation in the extensor neuromusculature supports the conclusion of Wilson and Paul (1987) that the differences in the flexor neuromusculature of *M. quadrispina* compared with the flexor systems of both *G. strigosa* and macrurans are unrelated to functional and behavioural differences.

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