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REGENERATION OF FIBRE TYPES IN PAIRED ASYMMETRIC CLOSER MUSCLES OF THE SNAPPING SHRIMP, *ALPHEUS HETEROCHELIS*

BY C. K. GOVIND, K. M. MEAROW AND A. WONG

Department of Zoology, Scarborough Campus, University of Toronto, 1265 Military Trail, West Hill, M1C 1A4 Ontario, Canada

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SUMMARY

The fibre composition of the closer muscle in the paired asymmetric (snapper and pincer) chelipeds of the snapping shrimp, *Alpheus heterochelis* (Say), was determined in the pristine and in the regenerated conditions. In the pristine condition, the snapper muscle has exclusively slow fibres in both male and female shrimps. The pincer muscle has a central band of fast muscle sandwiched by slow muscle. This band of fast muscle is proportionately and significantly larger in the female than in the male (36 % compared to 21 %), thus demonstrating a subtle form of sexual asymmetry.

The chelipeds were induced to regenerate following their removal within 24 h of a moult. During the intervening intermoult, limb buds formed which, at the next moult, transformed into newly regenerated chelipeds. In these regenerated chelipeds the snapper muscle was usually entirely composed of slow fibres as in the pristine muscle. In a few animals, however, a scattering of fast fibres occurred in the central region; a condition reminiscent of the pincer muscle. The regenerated pincer muscle resembled its pristine counterpart in having a central band of fast fibres sandwiched by slow fibres. In male shrimps, the proportion of fast fibres in the regenerated muscle was similar to that in the pristine muscle. In females, however, the proportion of fast muscle was significantly smaller in the regenerated than in the pristine condition (21 % compared to 36 %). Since a closer muscle with a central band of fast fibres sandwiched by slow is found during regeneration in pincer and occasionally snapper chelipeds, such a pattern represents an early developmental stage which is being recapitulated during regeneration. There is no evidence in *Alpheus heterochelis* that the pincer muscle initially regenerates exclusively fast fibres as was reported for *A. californiensis*.

INTRODUCTION

In general, crustaceans have impressive powers of regeneration, being able to reform entire parts of their bodies such as antennae and limbs. Such regenerative capacity results in the production of an accurate copy of the original body part within a short period of time. Consequently, regeneration has often been used to model

Key words: regeneration, muscle fibre, shrimp.

developmental processes and such an approach has been strengthened by finding that some developmental events are recapitulated during regeneration. A case in point is the closer muscle in the first pair of thoracic chelipeds in crayfish and snapping shrimps.

The closer muscle in the chelipeds of the newly hatched crayfish, *Orconectes rusticus*, has a central band of fast fibres surrounded by slow fibres (Govind & Pearce, 1985). During subsequent juvenile development, all the fast fibres are transformed to slow ones and the closer muscle assumes its adult condition of 100% slow fibre composition. When a cheliped is lost and a new one regenerates in its place, the closer muscle recapitulates its ontogeny in that the muscle has a central band of fast fibres which over the next few moults transforms into a band of slow fibres. What is even more interesting is that the newly regenerated muscle has a significantly larger proportion of fast muscle than at any stage during development.

Such an enhanced reappearance of fast fibres is taken to the most extreme condition in the closer muscle of the pincer cheliped in the snapping shrimp *Alpheus californiensis* (Stephens & Leferovich, 1984). In pristine chelipeds this muscle has a central band of fast fibres surrounded by slow ones (O'Connor, Stephens & Leferovich, 1982). Yet when newly regenerated, the entire muscle is composed of fast fibres. During the next 2 weeks of this intermoult, the majority of fast fibres in the dorsal and ventral halves are transformed to slow ones, leaving a central band of fast fibres characteristic of the adult pristine condition. The regeneration of exclusively fast fibres in a muscle composed originally of both fast and slow fibres raises the possibility that fast fibres represent a developmentally primitive type. Since this is a possibility of some significance in understanding how muscle fibres differentiate, we were prompted to test the generality of such a finding by examining another species of snapping shrimp, *Alpheus heterochelis*.

We did not find that the closer muscle in the pincer of *A. heterochelis* regenerated exclusively fast fibres, but rather a central band of fast fibres sandwiched by slow ones, in a pattern reminiscent of the pristine condition. Moreover, we report for the first time differences between the sexes, in both the fibre composition of the pincer closer muscle and in its pattern of regeneration.

MATERIALS AND METHODS

Adult snapping shrimp, *Alpheus heterochelis*, of both sexes were purchased from suppliers in Panacea, Florida and Beaufort, North Carolina; males were distinguished from females by a characteristic fringe of hair on the minor cheliped. The animals were held in artificial sea water in 5·5 gallon (= 25 l), glass aquaria which were divided into 12 equal-sized compartments with fibreglass mesh screens. The numbered compartments in individual aquaria allowed us to monitor precisely the moult history of each animal. The aquaria were kept at room temperature, approximately 23 °C, and an underground filter maintained water quality. The animals were fed a daily ration of frozen fish and any uneaten food was removed at the

end of the day. Under these conditions and a 12 h light/dark cycle the animals moulted approximately every 16–20 days.

Only shrimps with well-differentiated pincer and snapper chelipeds were chosen and these were allowed to moult between six and eight times before they were used. This was done to ensure that the chelipeds were in a pristine conditon (Stephens & Mellon, 1979*a*). After this holding period the chelipeds were removed, usually 7–10 days into an intermoult, to characterize the closer muscle in the pristine state. The regenerated condition was obtained by inducing autotomy of the pristine cheliped within a day after moulting. In the intervening intermoult a limb bud formed which became a complete limb at the next moult. This newly formed limb, which represents the first regenerate, was removed at various times during the intermoult. Removal of the first regenerate limb within 1 day after moulting resulted in the production of a second regenerate limb at the next moult. The closer muscles in the chelipeds of both the first and second regenerates were examined.

The paired chelipeds of snapping shrimps are asymmetrical, consisting of a minor (pincer) and major (snapper) form, either of which appear with equal probability on the right or left side of the body (Wilson, 1903). In order to induce regeneration of the pincer cheliped only the pincer needs to be removed. But to induce snapper regeneration, both chelipeds have to be removed and both will regenerate at their original sites. If the snapper alone is removed, then the existing pincer transforms to a snapper and a new pincer regenerates at the site of the old snapper (Wilson, 1903). We did not examine the transformation of the pincer to snapper but concerned ourselves solely with the regeneration of each type of cheliped.

The closer muscle in *Alpheus heterochelis* is subdivided into a principal and minor component (Mellon & Stephens, 1979) and the present study is concerned only with the principal muscle. The fibre composition of the cheliped closer muscle was assessed by staining for myofibrillar adenosine triphosphatase (ATPase) activity, in frozen sections of the muscle, by histochemical techniques (Ogonowski & Lang, 1979). Since the specific activity of this enzyme is two to three times greater in crustacean fast muscle than slow muscle (Lehman & Szent-Gyorgi, 1975), fast fibres stain more intensely than slow ones. In the present study, serial thick (16 μm) sections of the entire cheliped were obtained, stained for myofibrillar ATPase activity and examined for the presence of fast and slow fibres. Subsequently, selected sections, usually from the middle of the cheliped, were photographed, and suitably enlarged for calculating the percentage of fibre types. This was done either by cutting out the area occupied by each fibre type, weighing it and calculating the percentage (Govind & Pearce, 1985) or by sampling the muscle *via* a grid drawn on an acetate sheet (Josephson & Young, 1981). The percentage of fibre types calculated by these two methods showed little difference (approximately 1 %) in the same samples. Altogether the closer muscle composition was analysed in 53 chelipeds from 30 snapping shrimps.

The newly regenerated pincer and snapper muscle was also viewed with the electron microscope in order to identify fast and slow fibres on the basis of their sarcomere length (SL). Crustacean fast fibres have a short ($<4\,\mu$m) SL while slow

fibres have longer SLs (>6 μm) (Atwood, 1976). Such a scheme has been used extensively to classify fibre types in the cheliped closer muscle in snapping shrimps (e.g. Stephens & Mellon, 1979*a*,*b*; Mellon & Stephens, 1980). The identification of fibre types in the regenerating cheliped muscle of *A. californiensis* was based on SL measured under the light microscope (Stephens & Leferovich, 1984), where the contractile state of the sarcomere cannot be assessed accurately unless A-bands are measured. We opted for the higher magnification possible with electron microscopy which permits an unequivocal measurement of A-band width and SL.

RESULTS

Pincer muscle

Previous studies have established, on the basis of SL (Stephens & Mellon, 1979*a*,*b*), ultrastructural features (Mellon & Stephens, 1980) and myofibrillar ATPase activity (O'Connor *et al.* 1982), that the closer muscle of the pristine pincer is composed of a central band of fast fibres flanked by slow fibres. We confirmed such a fibre composition of the pincer closer muscle in *A. heterochelis* by using myofibrillar ATPase to distinguish between fast and slow fibres. The pincer muscle is made up of a mixture of dark-staining, fast fibres and light-staining, slow fibres (Fig. 1). The fast fibres occur as a central band on either side of the tendon. They originate on the exoskeleton at the proximal end of the muscle and insert distally on the tendon. The fast band continues to the extreme distal end in the medial half of the muscle but not in the lateral half. This asymmetry of the fast band on either side of the tendon is in keeping with the unequal distribution of the muscle mass, with a greater mass occurring on the medial than the lateral half.

The distribution of fast and slow fibres described above was typical of the pristine closer muscle in both sexes (Fig. 1). However, the band of fast muscle appeared to be larger in the female than in the male in all regions of the muscle (Fig. 1). In order to verify this observation, the relative amounts of fast and slow fibres in the closer muscle were calculated for several animals of each sex. For this we used three consecutive cross-sections in the mid-region of the muscle as they provided the largest surface area with the best bilateral symmetry and the largest proportion of fast fibres (cf. Govind & Pearce, 1985). While the muscle in the male cheliped possessed an average of 21 % fast fibres, its counterpart in the female had 36 % (Table 1); an almost twofold difference which was highly significant ($P < 0{\cdot}005$, Student's t-test).

When a pincer cheliped is removed early in the intermoult, the snapping shrimp regenerates a new pincer at the next moult. This regenerated pincer is a diminutive replica of the original. Examination of these newly regenerated chelipeds revealed the typical closer muscle consisting of a central band of fast fibres sandwiched by slow ones, in both sexes (Fig. 2). In the male snapping shrimp, the regenerated muscle possessed approximately the same proportion of fast and slow fibres as the pristine muscle, i.e. 21 % fast and 79 % slow fibres (Table 1). This is reflected in the pristine to regenerate ratio of approximately one for both fibre types. But amongst the female snapping shrimps the regenerated muscle contained significantly less ($P < 0{\cdot}005$,

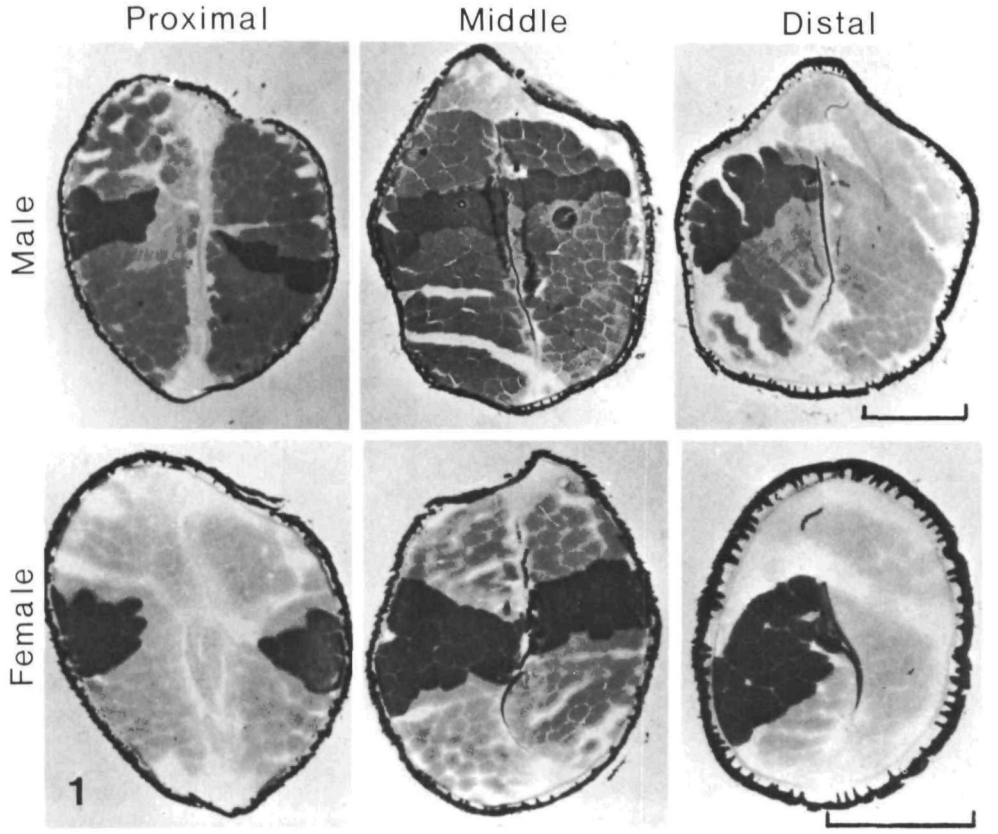

Fig. 1. Representative cross-sections of the pristine closer muscle stained for myofibrillar ATPase in the proximal, middle and distal region of male (upper row) and female (lower row) pincer chelipeds. The distribution of fibre types is similar in both sexes with a central band of dark-staining, fast fibres extending along the entire length of the muscle on both sides of the tendon except in the most distal region. The small dorsal opener muscle is seen in the distal sections. Magnification bar, 1 mm.

Student's *t*-test) fast muscle than its pristine counterpart, i.e. 21 % compared to 36 %. This is reflected in a pristine to regenerate ratio of two for the fast fibres and less than one for the slow fibres.

Table 1 also shows that the fibre composition of the regenerated closer muscle is essentially similar over the entire intermoult period as animals were examined from 1 to 21 days of the intermoult. This contrasts with the finding in *A. californiensis*, by Stephens & Leferovich (1984), that the regenerated closer muscle had all fast fibres 1 day after moulting and after 14 days it had transformed into the pristine pattern. In view of this discrepancy, a second series of experiments was done in which the regenerated pincer closer muscle was examined within 24 h after moulting and on a daily basis throughout the intermoult. In all cases the regenerated closer muscle had a central band of fast fibres surrounded by slow ones.

The finding of 100 % fast fibre composition in the newly regenerated pincer muscle in *A. californiensis* was based not only on myofibrillar ATPase activity but

also on measurement of sarcomere length, which was typically 2–3 μm (Stephens & Leferovich, 1984). Consequently, we examined the fibre composition of a pristine and a newly regenerated pincer closer muscle of *A. heterochelis* with the electron microscope and found them to be essentially similar. Both showed distinct fast and slow populations based on several ultrastructural criteria (Atwood, 1976). First, the fast fibres had characteristically shorter sarcomere lengths than their slow counterparts (Fig. 3). The histogram depicting the distribution pattern of sarcomere length in both pristine and regenerate muscles showed a distinct bimodal shape (Fig. 4). Second, a similar trend was seen for measurements of the A-band length, which provide a more reliable indicator of sarcomere length as they are not subject to length change. Third, the thin to thick filament ratio was characteristic of fast and slow fibres with a thick filament having an orbit of six thin filaments for the fast fibres and 10–12 thin filaments for the slow fibres. Fourth, the Z-line is typically thicker and more wavy for the slow fibre than the fast one (Fig. 3). Fifth, diads are located in the centre of the A-band in fast fibres and towards the ends of the A-band in slow fibres (Fig. 3). Sixth, there is a characteristic M-line in the centre of the A-band in the fast fibres but not in the slow ones. Evidence from all these different ultrastructural

Table 1. *Percentage distribution of fast and slow fibres in the closer muscle of the pincer cheliped in adult snapping shrimp,* Alpheus heterochelis

Identity	Sex	Pristine (P) % fibre types Fast	Slow	Regenerate (R) % fibre types Fast	Slow	P/R ratio Fast
DJ 5	M	19	81	20	80 (2)	0·95
DJ 6	M	25	75	26	74 (4)	0·96
PM 7	M	24	76	17	83 (6)	1·41
PM 8	M	21	79	20	80 (6)	1·05
HI 9	M	25	75	23	77 (6)	1·09
EMP 1	M	16	84	16	84 (7)	1·00
AL 1	M	20	80	24	76 (21)	0·83
PM 12	M	20	80	—	—	—
JP 1	M	22	78	—	—	—
$\bar{X} \pm$ S.D.		21 ± 3	79 ± 3	21 ± 3·7	79 ± 3·7	1·04 ± 0·18
DJ 10	F	32	68	19	81 (1)	1·68
DJ 3	F	41	59	17	83 (3)	2·41
PM 6	F	40	60	24	76 (6)	1·48
DJ 1	F	40	60	23	77 (7)	1·74
LVW 6	F	42	58	14	86 (7)	3·00
RY 2	F	40	60	27	73 (10)	1·48
PM 1	F	33	67	—	—	—
DJW 6	F	36	64	—	—	—
DJW 7	F	34	66	—	—	—
RY 3	F	22	78	—	—	—
$\bar{X} \pm$ S.D.		36 ± 6·1	64 ± 6·1	21 ± 4·8	79 ± 4·8	2·00 ± 0·59

The number in brackets denotes the number of days after moulting that the muscle was sampled.

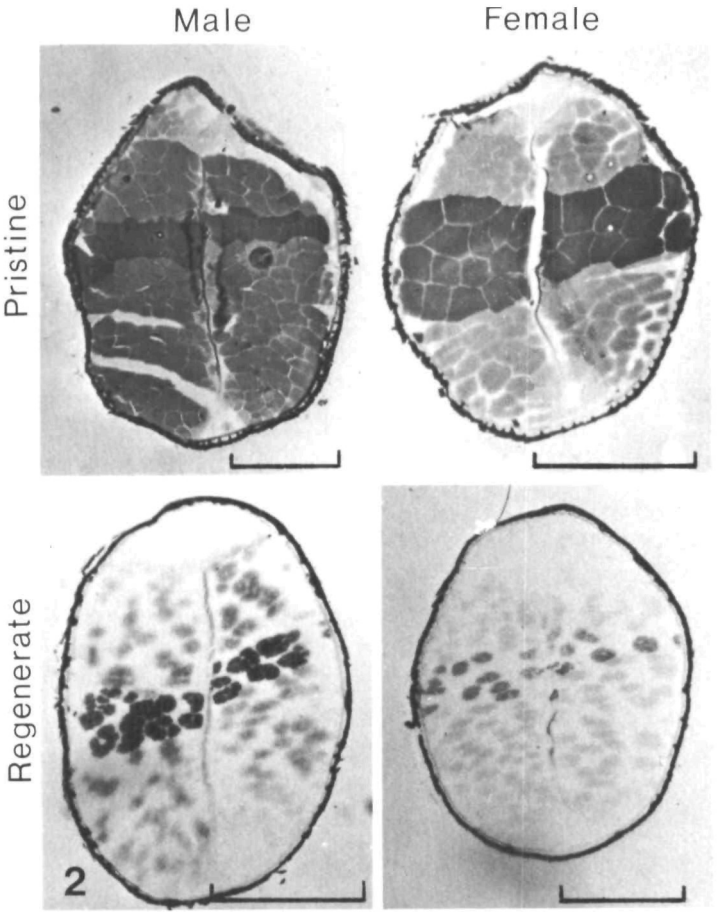

Fig. 2. Representative cross-sections from the middle region of the pristine (upper row) and regenerate (lower row) pincer closer muscle stained for myofibrillar ATPase activity in male and female chelipeds. All have a central band of fast fibres sandwiched by slow, although the proportions vary between sexes and between pristine and regenerate conditions. Magnification bar, 1 mm.

criteria show unequivocally the presence of fast and slow fibres in the newly regenerated pincer muscle.

Snapper muscle

In both male and female adults, the snapper muscle of the pristine cheliped is composed of a homogeneous population of slow fibres (Fig. 5). This confirms earlier reports of a similar fibre composition of the snapper muscle in other species of snapping shrimp (Stephens & Mellon, 1979*a*,*b*; O'Connor *et al.* 1982). During regeneration of this cheliped, the closer muscle usually reappears with exclusively slow fibres except in a few cases where we detected a scattering of fast fibres in the central area of the muscle (Fig. 5). This phenomenon was examined further in first and second regenerates of the snapper in several female shrimps (Table 2). In both

the first and second regenerate conditions, only two out of six snapper muscles showed some fast fibres. Consequently, the appearance of fast fibres in the snapper muscle bears no correlation with the frequency of regeneration nor with the time of the intermoult, as it was found as early as 2 days into the intermoult or as late as 15 days. Altogether four regenerates out of 12 showed fast fibres and amongst these the fast muscle constituted less than 10%. Thus, typically the snapper muscle regenerates its original fibre composition though occasionally a few fast fibres are regenerated in the central area.

Electron microscopic examination of a pristine and a newly regenerated snapper muscle showed a single population of fibres, with features characteristic of slow

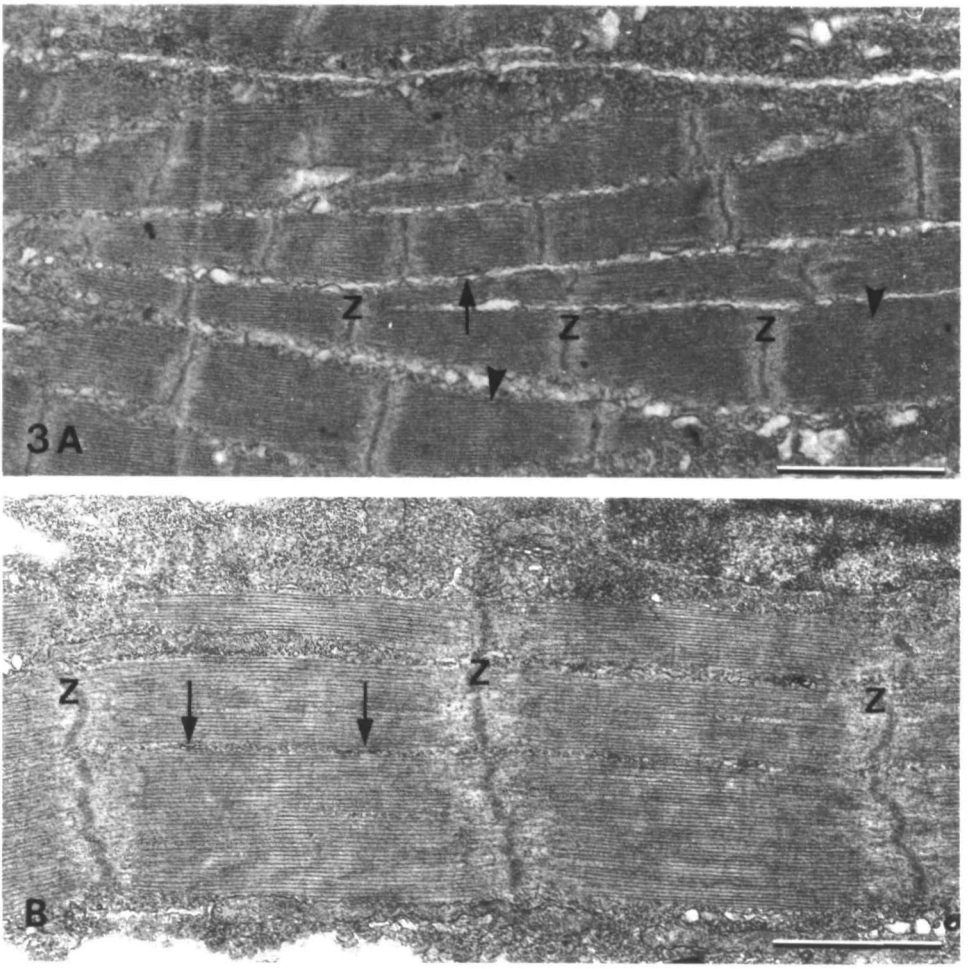

Fig. 3. Electron micrographs of a newly regenerated pincer closer muscle from a female cheliped, showing the fine structure of typically fast (A) and slow (B) myofibrils (see text for full description). Sarcomeres are demarcated by Z-lines; diads by arrows and M-lines by arrowheads. Magnification bar, 2 μm.

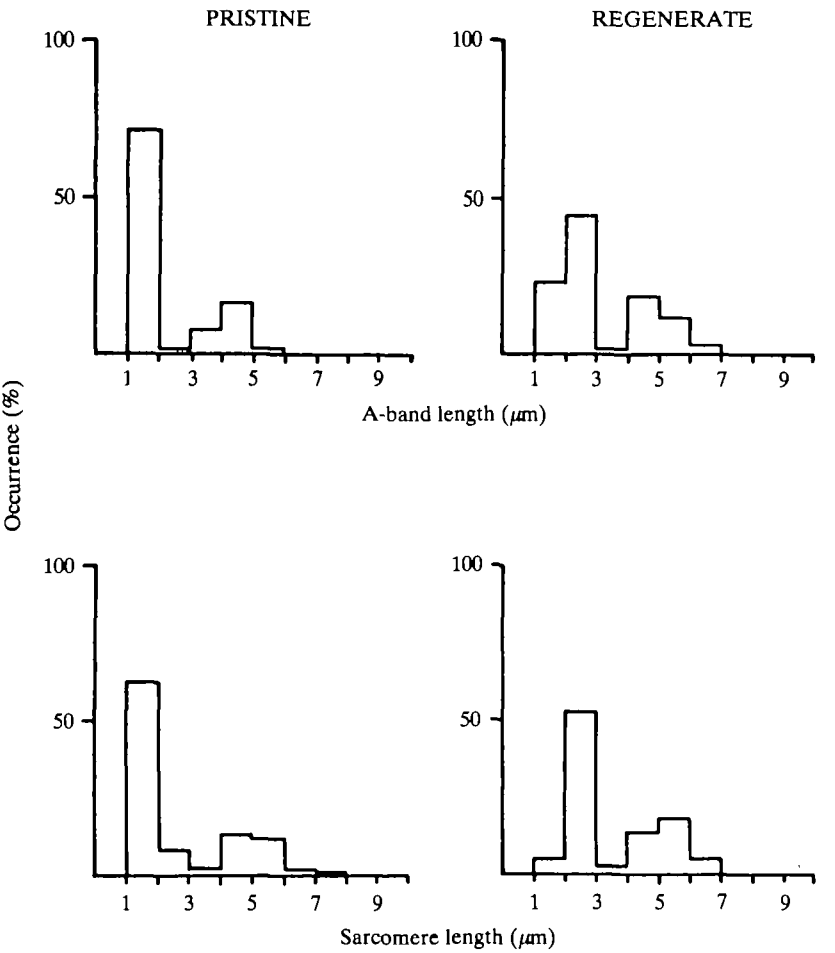

Fig. 4. Frequency histogram of A-band and sarcomere length measured from electron micrographs of pristine and newly regenerated pincer closer muscle in female snapping shrimps. Both muscles have a distinct bimodal distribution of fibre types denoting fast and slow fibres. $N = 268$ for pristine and 350 for regenerate muscles.

muscle. Thus A-band and SL were typically long (Fig. 6), the Z-lines were thick and wavy, and a single thick filament had an orbit of 12 thin filaments. Other features characteristic of slow fibres were the location of diads towards the ends of the A-band and the absence of an M-line. Our measurements of SL at 5–10 μm are slightly shorter than the 11 μm reported for *A. armillatus* using electron microscopy (Mellon & Stephens, 1980) but within the range of 6–13 μm reported for *A. armillatus* and *A. heterochelis* using light microscopy (Stephens & Mellon, 1979*b*). It was not within the scope of our study to make a detailed analysis of the variability in SL amongst slow fibres other than to employ SL as a guide to fibre typing. This electron microscopic examination did not reveal evidence for the few fast fibres occasionally seen with histochemical examination of regenerate snapper muscles.

DISCUSSION

A primary objective of the present study was to confirm, in another species of snapping shrimp, the observation made by Stephens & Leferovich (1984) that the pincer closer muscle in *Alpheus californiensis* regenerates exclusively fast fibres. We failed to confirm this in *A. heterochelis*, where the regenerated closer muscles possessed a central band of fast muscle sandwiched by slow, in a pattern typical of the pristine muscle. Indeed, in an earlier study of *A. heterochelis* and *A. armillatus* (Stephens & Mellon, 1979*b*), a regenerated 6-day-old pincer closer muscle had fibres with SLs ranging from 2 to 6 μm, denoting putative fast and slow types. Earlier than 6 days, the authors were unable to measure SL of newly regenerated closer muscles with light microscopy. However, using electron microscopy, we have shown that the newly regenerated pincer muscle fibres in *A. heterochelis* are already differentiated into fast and slow types on the basis of SL. That this is not the case in *A. californiensis* (Stephens & Leferovich, 1984) may be a species-specific difference, even though the fibre composition of the pristine muscle is similar in the two species.

On the other hand, it is possible that the techniques for identifying fast and slow fibres varied sufficiently in the two studies to yield different results. In both studies, fast and slow fibres were characterized on the basis of their enzymatic and structural

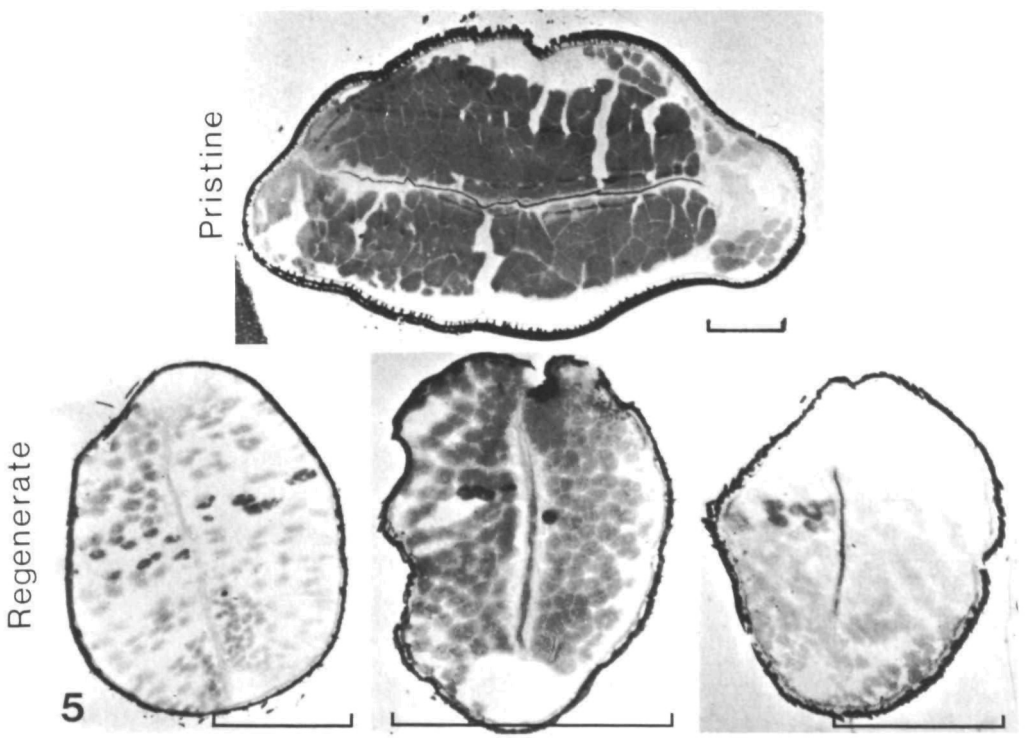

Fig. 5. Representative cross-sections of the snapper closer muscle stained for myofibrillar ATPase in one pristine (upper) and three regenerate (lower row) female chelipeds. The latter show a few darkly-staining, fast fibres in the central region of the muscle. Magnification bar, 1 mm.

Table 2. *Percentage fast and slow fibres in regenerating closer muscles in the snapper cheliped of adult female snapping shrimps,* Alpheus heterochelis

Identity	Number of regenerations	Days into intermoult	% fibre types Fast	Slow
AL 4	1	0 (<24 h)	0	100
AL 5	1	2	0	100
WR 6	1	3	0	100
DJ 6	1	4	10	90
DJ 2	1	4–5	0	100
DJ 12	1	4–5	6	94
AL 9	2	0 (<24 h)	0	100
AL 4	2	0 (<24 h)	0	100
DJ 2B	2	1	7	93
AL 5B	2	2	0	100
AL 12	2	5	0	100
DJ 8	2	15	2	98

properties. The histochemical techniques for detecting myofibrillar ATPase activity are fairly routine and indeed gave comparable results between the two species for the pristine cheliped as well as for the regenerated cheliped which was well into its intermoult. It was only in the 1-day-old regenerate muscle that the fibre composition differed between the two species. In *A. californiensis* the closer muscle stained intensely and uniformly over its entire surface, thereby suggesting that there are only fast fibres (Stephens & Leferovich, 1984); whereas in *A. heterochelis*, two staining intensities were present, suggesting that fast and slow fibres occur in a constant ratio throughout the intermoult period.

The other means for identifying fast and slow fibres is by their fine structure and, in particular, their characteristic sarcomere length (Atwood, 1976). Thus in a 1-day-old regenerate we found, using electron microscopy, these two distinct fibre types based on a number of criteria including the sarcomere and A-band length, the thin to thick filament ratio and the thickness of the Z-band. The earlier study using *A. californiensis* relied on only one of these structural features, i.e. the sarcomere length, to characterize fibre types. Moreover, these measurements were made with the light microscope where, without measuring A-band lengths, it is difficult to judge whether the sarcomere is unduly shortened or lengthened. Such problems are easily resolved with the higher magnification obtained with the electron microscope. Consequently, on the basis of both electron microscopy and enzyme histochemistry, we are reasonably convinced that the newly regenerated pincer muscle in *A. heterochelis* has a mixture of fast and slow fibres, distributed in a manner similar to that of its pristine counterpart.

The many previous studies on the fibre composition of the paired closer muscles have dealt either with *A. armillatus* (Stephens & Mellon, 1979*a*,*b*; Mellon & Stephens, 1980) or *A. californiensis* (O'Connor *et al.* 1982; Leferovich & Stephens, 1982; Stephens, O'Connor & Leferovich, 1983; Stephens & Leferovich, 1984). The species *A. heterochelis* has been used by Mellon and his collaborators to study other aspects of the asymmetric chelipeds (Mellon & Stephens, 1978, 1979; Mellon,

Wilson & Phillips, 1980; Wilson & Mellon, 1982; Phillips, Wilson & Mellon, 1982). In all of these studies, however, the animals were not identified as to their sex and it has been assumed that the closer muscle composition is essentially similar between the sexes. Although this is true for the snapper closer muscle, which is composed entirely of slow fibres, we find a distinct difference between the sexes in the composition of the pincer muscle in *A. heterochelis*. The proportion of fast fibres in the closer muscle of the female is almost double that of the male. Sexual dimorphism in the paired chelipeds is not uncommon in crustaceans, with the female chelipeds being smaller and less elaborate than their male counterparts; e.g. in *A. dentipes* the female snapper and pincer chelipeds are much shorter than their male homologues (Dawes, 1934). Heterochely, when present, is superimposed on this customary sexual dimorphism and may produce considerable divergence between the sexes; for

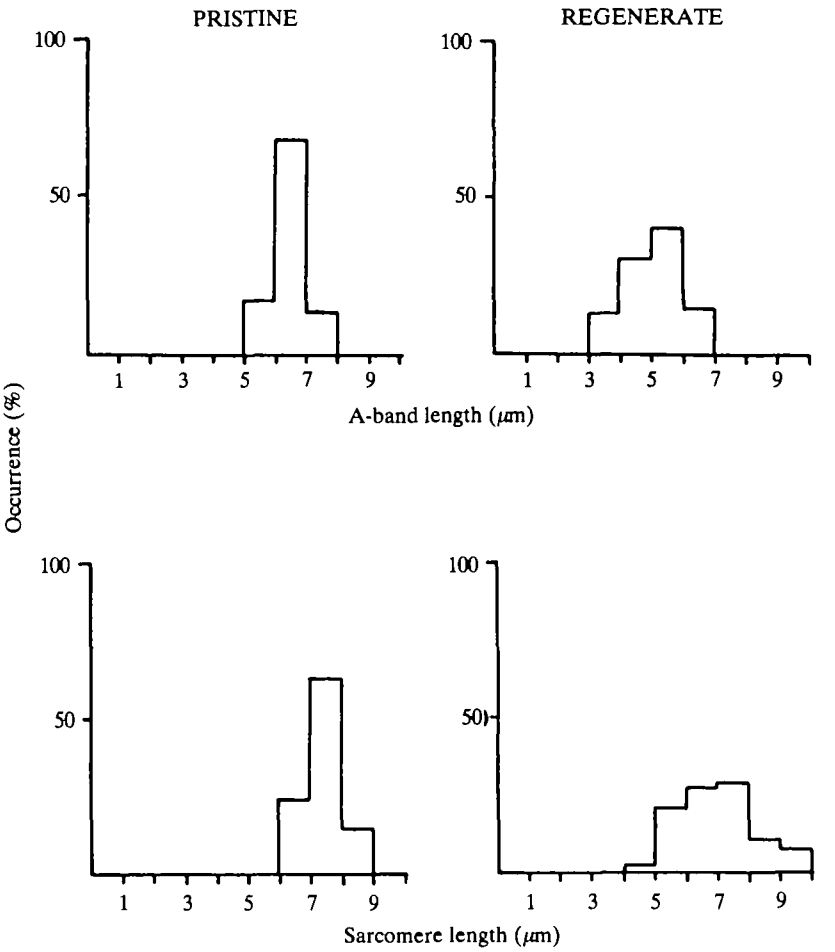

Fig. 6. Frequency histogram of A-band and sarcomere length measured from electron micrographs of pristine and newly-regenerated snapper closer muscle in female snapping shrimps. Both muscles have a unimodal distribution of fibre types denoting slow fibres $N = 80$ for pristine and 125 for regenerate muscles.

example, in fiddler crabs only the male has asymmetric (major and minor) chelipeds and the female has paired minor chelipeds (Crane, 1975). In snapping shrimps, where both sexes are heterochelous, this divergence is more subtle, being expressed only in the composition of the pincer closer muscle and resulting in male and female pincer muscles differing in their fast fibre content.

The regeneration of the closer muscles yielded some interesting results. First, the female pincer cheliped regenerates its closer muscle with a lower proportion of fast fibres than found in its pristine counterpart. During the succeeding moults the fast fibre population increases to a value typical of the pristine condition. A similar process occurs during development of the lobster cutter (minor) closer muscle in which the fast fibre population is increased by transforming the existing slow fibres (Govind & Lang, 1978; Govind & Kent, 1982).

Second, the male pincer cheliped regenerates its closer muscle with the same proportion of fast and slow fibres found in the pristine muscle and consequently needs no further differentiation in the succeeding moults. In this respect, regeneration of the male pincer closer muscle is more straightforward than that of the female where the regenerate does not fully resemble the pristine condition and further refinement is required.

Third, the snapper cheliped of both sexes regenerates its closer muscle usually into its fully differentiated condition of exclusively slow fibres. Occasionally, however, the newly regenerated snapper muscle has a few fast fibres, centrally located on either side of the tendon and resembling the central band of fast fibres of the pincer muscle. Since the pristine snapper has only slow muscle, these few fast fibres either degenerate or are transformed to the slow type in subsequent moult cycles. Which of these two processes prevails is not known. We do know that during transformation of a pincer to a snapper, the existing fast fibres in the pincer degenerate (Mearow & Govind, 1985) rather than transform as had been previously shown (Stephens & Mellon, 1979*a*). Clearly, the exceptional pattern in snapper muscle regeneration, i.e. with centrally located fast fibres, not only resembles the pincer to snapper transformation but is presumably a throwback to an early developmental stage. This latter view is strengthened by the fact that both male and female pincer closer muscles also show this pattern during regeneration. Under these circumstances, regeneration of the cheliped closer muscle in snapping shrimps recapitulates its ontogeny. A similar conclusion was made in the crayfish *Orconectes rusticus* (Govind & Pearce, 1985).

Finally, it is worth mentioning that the typical fibre composition of the pincer closer muscle, with a central fast band sandwiched by slow, is found in several other crustaceans. In the lobster, *Homarus americanus*, this pattern is found in the larval and early juvenile stages when the paired chelipeds are symmetrical and have not differentiated into major and minor forms (Govind & Lang, 1978; Ogonowski, Lang & Govind, 1980). The same pattern is seen in the cheliped closer muscle of the crayfish, *Orconectes rusticus*, during development and regeneration (Govind & Pearce, 1985), and it may persist in the pristine chelipeds of *Procambarus clarkii* (unpublished observations). The adult closer muscle of the minor cheliped of the

hermit crab, *Pagurus pollicaris*, also shows this pattern (Stephens, Lofton & Klainer, 1984). Such a widespread distribution of this pattern amongst Crustacea and its occurrence during development implies that it is of phylogenetically primitive origin.

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