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Differential Molt-Induced Atrophy in the Dimorphic Claws of Male Fiddler Crabs, *Uca pugnax*

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ABSTRACT Molt-induced atrophy was examined in the closer muscles of the dimorphic claws of the fiddler crab, *Uca pugnax*. In adult males, the major claw, which is about 30 times larger than the minor claw, contains primarily S_1 fibers and some S_2 fibers, while the minor claw contains exclusively S_2 fibers. Ultrastructurally, both S_1 and S_2 fibers resembled slow fibers of other crustacean species: high ratios of thin:thick myofilaments, thickened Z-lines, and myofibrils with long sarcomeres. However, the S_1 fibers had higher thin:thick myofilament ratios (9:1 vs. 6:1), longer sarcomeres (6 μm vs. 4.5–5 μm) and fewer mitochondria than the S_2 fibers. During proecdysis, the muscle of the major claw underwent a greater atrophy than that of the minor claw. Furthermore, atrophy of the major claw muscle was enhanced by the number of walking legs being regenerated. The weight of the major claw muscle in animals regenerating one walking leg decreased about 50%, while that in animals regenerating eight walking legs decreased about 67%. The weight of the minor claw muscle decreased about 25% in animals regenerating either one or eight walking legs. Changes in myofibrillar cross-sectional areas paralleled the reductions in muscle mass. In S_1 fibers, cross-sectional area decreased 43% in animals regenerating one walking leg and 78% in animals regenerating eight walking legs. In S_2 fibers, cross-sectional area decreased 27% and 32%, respectively. Increases in the thick myofilament packing density and decreases in the thin:thick myofilament and actin:myosin heavy chain ratios suggest that this atrophy involves a preferential hydrolysis of thin myofilaments. The ultrastructural changes in S_2 fibers from major and minor claws were similar, suggesting that the preferential atrophy of the major claw muscle results, at least in part, from a differential sensitivity of S_1 and S_2 fibers to factor(s) that trigger protein degradation.

As in other arthropods, decapod crustaceans must periodically shed the old exoskeleton to allow for continued tissue growth (see Hartnoll, '82, for review). At ecdysis, the animal must pull out the large propodus of the claw through a narrow opening at the basi-ischial joint. It is hypothesized that this withdrawal is facilitated by a reduction in the mass of the closer muscle during proecdysis (= premolt) (see Mykles and Skinner, '90a,b, for reviews). The phenomenon of molt-induced atrophy has been most thoroughly studied in the Bermuda land crab, *Gecarcinus lateralis;* the claw closer muscle undergoes a sequential atrophy and restoration during each intermolt cycle (Skinner, '66). Muscle protein decreases 30–60% during proecdysis and is restored following ecdysis. An ultrastructural study has shown that during atrophy there is a fourfold decline in myofibrillar cross-sectional area, which corresponds to a twofold decrease in fiber diameter (Mykles and Skinner, '81). As myofilaments are degraded, intermyofibrillar spaces increase, the myofilament ratio decreases from 9:1 to 6:1, and there is increased degradation of membranous organelles by lysosomes (Mykles and Skinner, '81, '82a). This increased lysosomal activity is correlated with an increase in acid hydrolase activities in claw muscles from proecdysial animals (Yamaoka and Skinner, '75). Myofilament breakdown is apparently mediated by a group of four Ca^{2+}-dependent proteinases, which degrade myofibrillar proteins in vitro (Mykles and Skinner, '82b, '83) and in situ (Mykles, '90) and show increased activity in claw muscles from late proecdysial land crabs (Mykles and Skinner, '82b).

The regulatory mechanism, particularly at the systemic level, is poorly understood. The factor(s) mediating molt-induced protein catabolism in crustacean muscle must be highly specific and tightly regulated. In *G. lateralis,* atrophy occurs in the claws but not the walking legs (Mykles and Skinner, '82a). Presumably, claw muscle atrophy is under hormonal control, since it coincides with elevated ecdysteroid levels in the hemolymph (see

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Abbreviations used: 20-HE, 20-hydroxyecdysone; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

Skinner, '85, for review). The dimorphic claws of male fiddler crabs differ greatly in size, but the diameters of the basi-ischial joint through which the closer muscle must pass are similar between the major and minor claws. Furthermore, the claws differ in slow-fiber-type composition; the S_1 fibers account for most of the fibers in the major claw, while the S_2 fibers comprise the entire mass of the minor claw (Govind et al., '86b; Mykles, '88). The present study quantifies the ultrastructural and biochemical changes that occur during atrophy. The process resembles molt-induced atrophy in *G. lateralis*. However, the closer muscle of the major claw undergoes a greater atrophy than that of the minor claw. Moreover, this differential atropy is enhanced by the number of walking legs being regenerated. Brief summaries of these results have been published elsewhere (Ismail and Mykles, '86, '87).

MATERIALS AND METHODS

Animals and chemicals

Adult male fiddler crabs *(Uca pugnax)* with carapace widths of 16–21 mm were obtained from the western shore of Delaware Bay and maintained at about 24°C with 14 h of daily illumination beginning at 5:00 AM. The aquaria were elevated at one end to give a slope of about 30°. Sand was placed on the bottom of the elevated half, while artificial seawater was placed at the lower half. The crabs were fed TetraMin (a commercial staple food for tropical fish) every other day, water was changed on alternate days, and the sand was cleaned once per week. The crabs were allowed to acclimate in this environment for at least 3 days prior to use in any experiments. Animals were divided into two groups: a control group, in which a third walking leg was autotomized, and an experimental group, in which all eight walking legs were autotomized to induce molting (Skinner and Graham, '70, '72). The removal of a single walking leg in the control group provided a means to assess an individual's molt stage. Autotomy of a single walking leg in *Uca* and other decapods does not markedly affect the duration of the intermolt period (Skinner and Graham, '70, '72; Hopkins, '82). Therefore, crabs missing only one limb were used as sources of anecdysial muscle. However, some individuals missing one walking leg spontaneously entered proecdysis. In these instances, animals were permitted to complete preparations for ecdysis so that the degree of atrophy could be compared with that of animals regenerating eight walking legs.

Four criteria were used to determine the molt stage of an animal: the pigmentation of the carapace, the length of a regenerating limb as measured by the R index (length of limb regenerate $\times 10^2 \times$ carapace width^{-1}; Bliss, '56), the feeding behavior, and the presence or absence of the membranous layer of the exoskeleton (Skinner, '62). Anecdysial animals had dark pigmentation, had no regenerating limb, fed normally, and had a membranous layer. Late proecdysial animals had a yellowish pigmentation, had R indexes between 24 and 31, did not feed, and lacked a membranous layer. Reagents were from the same sources as reported previously (Mykles, '88, '90).

Electron microscopy

Animals were anesthetized on ice for about 30 min. Carapace length and width, claw length from the heel of the propodus to the tip of the pollex (Rhodes, '86), and claw width at the widest part of the propodus were measured with a Vernier caliper. The claws were pinned with the dactyl in the fully opened position in a wax-bottomed dish. After 30 min under ice, muscles were injected with a constant flow of fixative, which was composed of 2.5% ultrapure glutaraldehyde, 0.67 M glucose, and 0.10 M sodium cacodylate buffer (pH 7.4) (Mykles, '79). The claws were kept on ice for another hour, during which the epidermis partially separated from the exoskeleton, facilitating removal of the muscle mass (O'Brien et al., '86). Muscles were weighed, postfixed in OsO_4, stained en bloc with uranyl acetate, dehydrated, embedded, and sectioned as described elsewhere (Mykles and Skinner, '81). Sections were stained with Sato's solution, which was composed of 0.5% lead nitrate, 0.5% lead acetate, 0.5% lead citrate, 1% sodium citrate, and 0.36% sodium hydroxide (Sato, '67), and examined with either a Phillips 200 or Phillips 400T transmission electron microscope operating at 80 kV. To enhance visualization of thin myofilaments, some fibers were fixed overnight at 4°C in 2.5% purified glutaraldehyde, 0.67 M glucose, and 0.10 M sodium cacodylate buffer (pH 7.4) containing 8% tannic acid (LaFountain et al., '77; Mykles and Skinner, '81).

An Analyst Kevex 8000 was used to quantify myofibrillar cross-sectional areas in printed electron micrographs by using a size/shape computer feature analysis program. The packing density of thick filaments as well as thin:thick filament ratios were counted in printed electron micrographs of transverse sections of S_1 and S_2 fibers fixed with glutaraldehyde-tannic acid. A minimum of 20 fibers from each animal were analyzed.

Analysis of myofibrillar proteins

Muscle fibers were glycerinated to extract soluble proteins; myofibrillar proteins were dissolved in a sodium dodecyl sulfate (SDS) sample buffer without bromophenol blue as described (Mykles '85b, '88). Protein concentrations were measured by fluorescence emission spectroscopy at 338 nm (excitation = 286 nm); bovine serum albumin was used as the protein standard (Mykles and Skinner, '82b, '83). Samples were mixed with SDS sample buffer containing 0.002% bromophenol blue and heated at 90°C for 3 min before electrophoresis. Proteins were separated with discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (gel thickness 0.75 mm, 10% acrylamide) according to Laemmli ('70); each sample was loaded at 2, 4, 6, and 8 μg protein. Gels were stained with Coomassie brilliant blue R-250 (Mykles, '88). The amounts of myosin heavy chain and actin were quantified by densitometric scanning of gels with a Beckman DU-8 spectrophotometer. The integrated peak area of the actin band was expressed as a function of the integrated peak area of the myosin heavy chain band at each of the four loadings of each sample. Linear regressions were calculated, and the slope of each case was used to quantify the ratio of actin to myosin heavy chain; only samples with correlation coefficients greater than 0.90 were included in the analysis. A minimum of seven fibers of each type were quantified from each animal.

Statistical analysis

The two-tailed Student's test was used to determine the statistical significance of differences between measurements from anecdysial and late proecdysial animals.

RESULTS

Normal muscles: Fibers from anecdysial animals

The claw closer muscles of fiddler crabs contain two types of slow fibers (Govind et al., '86; Mykles, '88). The S_1 and S_2 fibers shared the majority of the protein isoforms, but differed primarily in the isoforms of paramyosin, troponin-I, and troponin-T (Fig. 1). In particular, a large-molecular-weight variant of troponin-T (T_1; M_r = 55,000) was present only in S_2 fibers (Fig. 1, lanes B, C), as has been shown in *Uca* as well as other decapod crustaceans (Mykles, '85a,b, '88). The entire mass of the minor closer muscle was composed of S_2 fibers. S_1 fibers occupied about 90% of the major claw closer muscle, while the remainder of the muscle was composed of S_2 fibers, which occurred as a small cluster in the proximal part of the claw muscle mass. The two fiber types were distinguished easily, since the diameters of the S_2 fibers are much smaller than those of the S_1 fibers (Mykles, '88). The position and orientation of the S_2 fibers in the major claw was the same as that found in the claws of *H. americanus* (Costello and Hummon, '86) and *G. lateralis* (Mykles, '88). The identity of the S_2 fibers in both claws was confirmed by analysis of the myofibrillar protein isoform composition; S_2 fibers, regardless of source, contained the same isoforms of paramyosin, troponin-T, and troponin-I (Fig. 1, compare lanes B and C).

The myofibrils exhibited the features generally found in the striated muscles of both vertebrates and invertebrates, such as A-bands, I-bands, and Z-lines (Figs. 2A, 3A). The sarcomere length of the S_1 fibers was longer (6 μm; Fig. 2A) than that of the S_2 fibers (4.5–5 μm; Fig. 3A). The Z-lines, which mark the limits of each sarcomere, were not in register in the S_1 fibers (Fig. 2A) but were in register in the S_2 fibers (Fig. 3A). In both fiber types, the Z-line bisected the I-band into two equal parts. The width of the I-band, which contained only thin myofilaments, varied according to the extent of contraction of the fiber. In contracted fibers, the I-band width was ~1 μm in S_1 fibers and 0.5 μm in S_2 fibers (Figs. 2A, 3A). The width of the A-band, which contained thick myofilaments and overlapping thin myofilaments, was 5 μm in S_1 fibers (Fig. 2A) and 4 μm in S_2 fibers (Fig. 3A) regardless of the contraction state of the fiber.

Transverse sections showed that the myofibrils of both S_1 and S_2 fibers were irregular in shape and variable in cross-sectional area (Figs. 2B, 3C). Myofibrils were surrounded by sarcoplasmic reticulum, which appeared as a series of vesicles or as flattened cisternae in the intermyofibrillar spaces centered at the level of the A-band (Figs. 2B, 3C). Dyadic contacts between the sarcoplasmic reticulum and transverse tubules were located at the ends of the A-band near the level of the I-band (Figs. 2B, 3C). The cross-sectional areas of the myofibrils of the S_1 and S_2 fibers were 2.17 μm^2 and 0.96 μm^2, respectively (Table 1). At higher magnifications, transverse sections of the A-band showed that both fiber types contained high ratios of thin:thick myofilaments (Fig. 2B, inset); the ratios were 9:1 and 6:1 in S_1 and S_2 fibers, respectively. The packing of myofilaments was irregular in both types, which is characteristic of crustacean slow fibers (Jahromi and Atwood, '69, '71; Crabtree and Sherman, '81; Mykles and Skinner, '81).

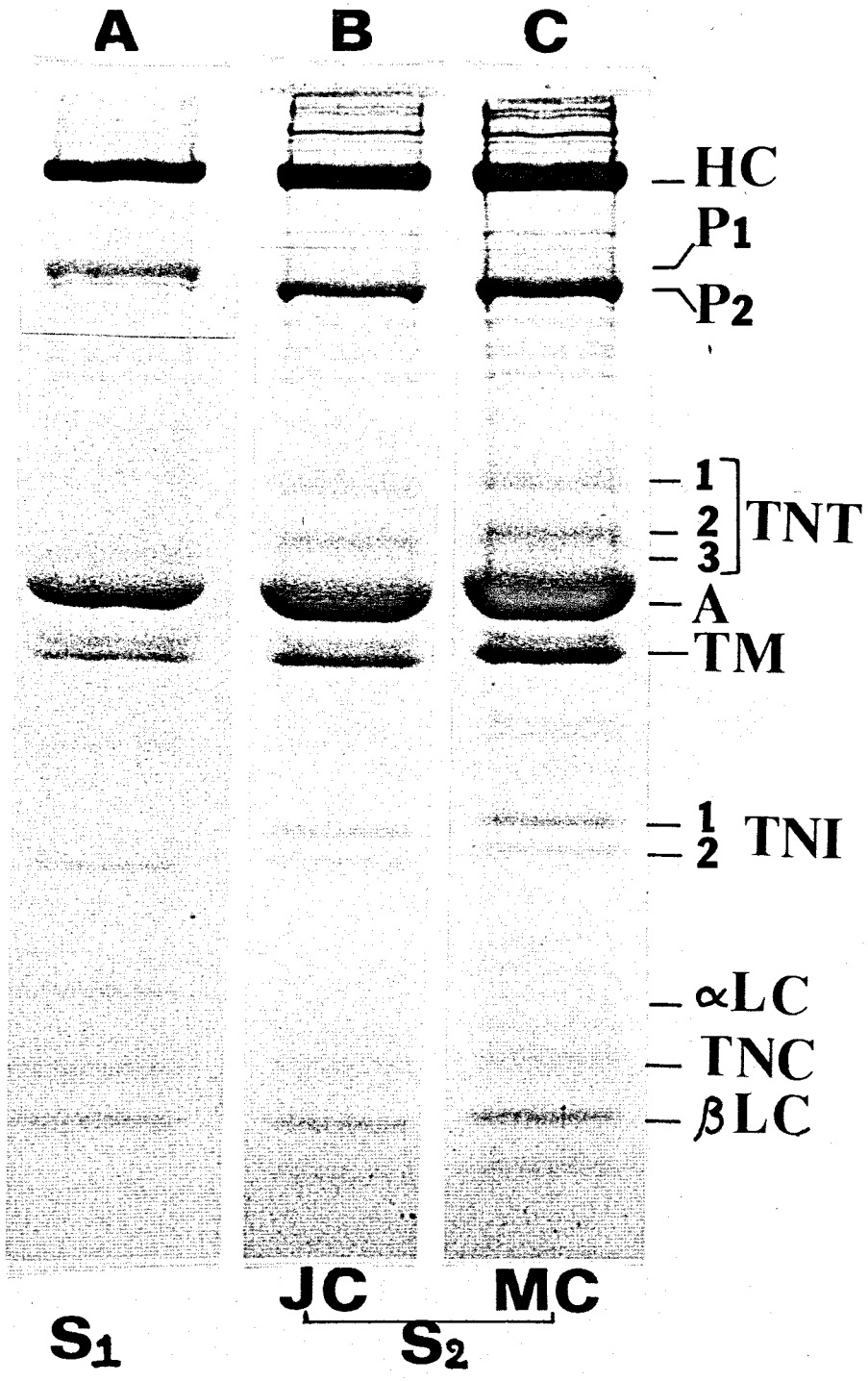

Fig. 1. SDS-polyacrylamide gel (10%) of glycerinated fibers from claw closer muscles of male fiddler crab. Single fibers (S_1 and S_2) from the major claw (JC; **lanes A, B**) and a group of S_2 fibers from the minor claw (MC; **lane C**) were analyzed. The S_2 fibers from both claws had identical myofibrillar protein isoform compositions. S_1 fibers differed from S_2 fibers in the composition of isoforms of paramyosin (P), troponin-I (TNI), and troponin-T (TNT). Protein loaded was 4 μg for lane A, 6 μg for lane B, and 8 μg for lane C. A, actin; HC, myosin heavy chain; LC, α and β-myosin light chains; TM, tropomyosin; TNC, troponin-C.

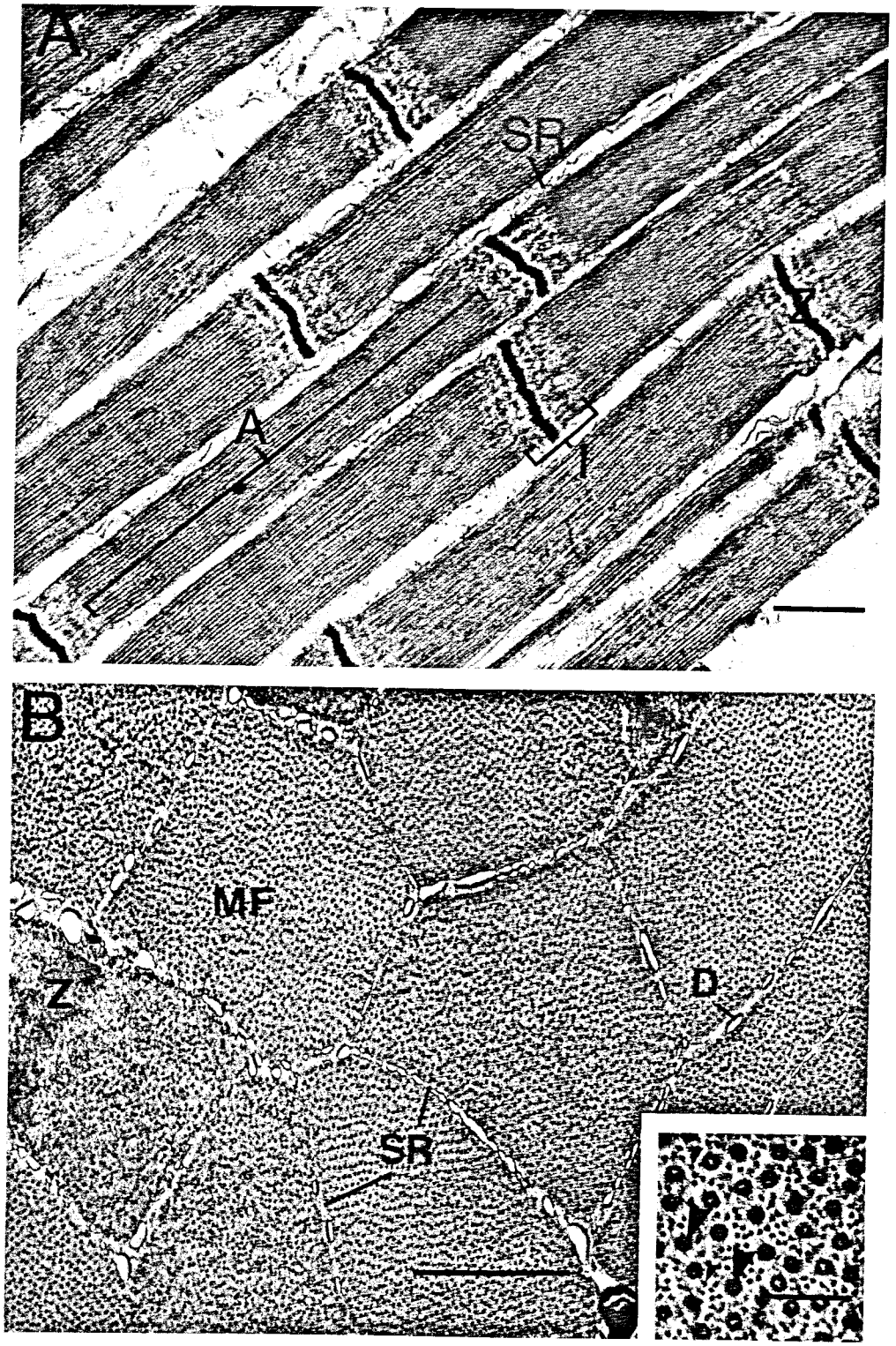

Fig. 2. Electron micrographs of S_1 fibers from the major claw of anecdysial male fiddler crab. **A**: Longitudinal section showing sarcomeres with A-bands (A), I-bands (I), and Z-lines (Z). Sarcoplasmic reticulum (SR) is located in the intermyofibrillar space. Z-lines of adjacent myofibrils are not in register. Bar = 1 μm. **B**: Transverse section showing cross sections of myofibrils (MF), sarcoplasmic reticulum, dyads (D), and transverse section through a Z-line. Bar = 1 μm. **Inset:** Transverse section at the level of the A-band of a fiber fixed with a glutaraldehye fixative containing 8% tannic acid (see Materials and Methods) showing thick myofilaments (large arrowheads) surrounded by orbits of 10–14 thin myofilaments (small arrowheads); the thin:thick myofilament ratio is ~9:1. Bar = 0.3 μm.

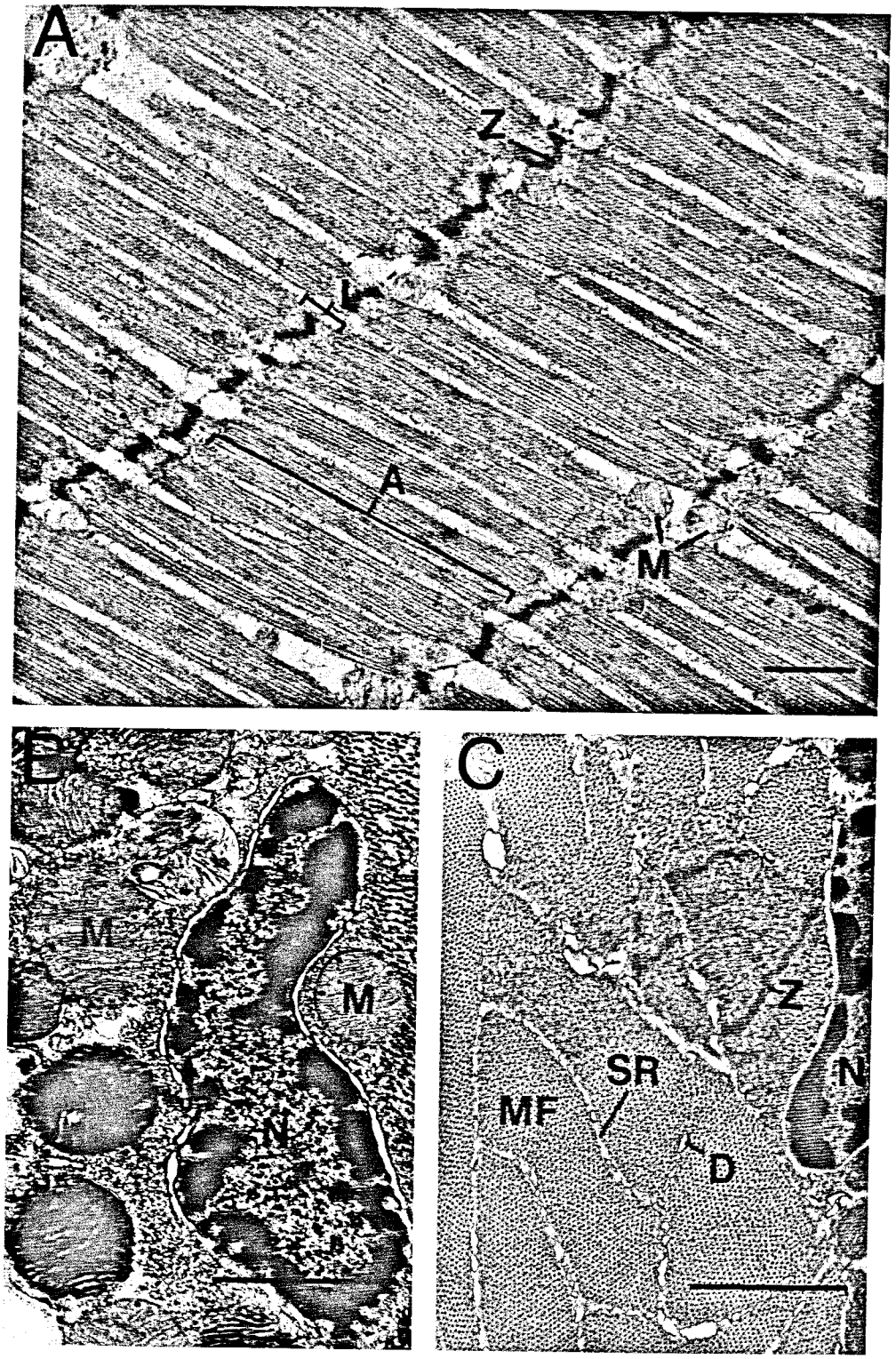

Fig. 3. Electron micrographs of S_2 fibers from the minor claw of anecdysial male fiddler crab. **A:** Longitudinal section showing sarcomeres with A-bands (A), I-bands (I), and Z-lines (Z). Sarcoplasmic reticulum and mitochondria (M) are located in the intermyofibrillar space. Z-lines of adjacent myofibrils are in register. Bar = 1 μm. **B:** Transverse section showing a nucleus (N) and numerous mitochondria (M) in the peripheral sarcoplasm. Bar = 1 μm. **C:** Transverse section showing cross-sections of myofibrils (MF), sarcoplasmic reticulum (SR), dyads (D), nucleus, and transverse section through a Z-line (Z). Bar = 1 μm.

TABLE 1. Comparison of myofibrillar cross-sectional area, thick myofilament packing density, and ratio of actin to myosin heavy chain of S_1 and S_2 fibers before and after muscle atrophy. Measurements of S_2 fibers from both major and minor claws were combined; data are presented as mean ± 1 standard deviation (number of animals)

	Myofibrillar cross-sectional area (μm^2)		Thick myofilament packing density[a]		Actin:myosin heavy chain ratio	
Molt stage	S_1	S_2	S_1	S_2	S_1	S_2
Anecdysial animals	2.17 ± 0.21 (11)	0.96 ± 0.13 (7)	350 ± 11 (17)	300 ± 15 (14)	1.30 ± 0.29 (13)	0.80 ± 0.35 (10)
Late pro-ecdysial regenerating one leg	1.23 ± 0.11 (11)	0.70 ± 0.20 (7)	457 ± 12 (17)**	330 ± 18 (14)**	0.72 ± 0.31 (15)	0.65 ± 0.25 (10)
Late pro-ecdysial regenerating eight legs	0.48 ± 0.12 (11)*	0.65 ± 0.10 (7)	529 ± 11 (17)**	340 ± 13 (14)**	0.47 ± 0.28 (12)	0.60 ± 0.22 (10)

[a]Number of thick myofilaments per μm^2
*Significantly different from anecdysial animals at $P < .01$.
**Significantly different from anecdysial animals at $P < .001$.

Nuclei were located in the peripheral sarcoplasm adjacent to the cell membrane (Figs. 3B, 5B). Mitochondria, containing large numbers of densely packed cristae, were found in both fiber types. However, they were more numerous in S_2 fibers, occurring in the cortical zone of the sarcoplasm beneath the cell membrane in the vicinity of the nuclei (Fig. 3B,C) and in the intermyofibrillar spaces (Fig. 3A). In contrast, mitochondria in the S_1 fibers were largely restricted to the peripheral sarcoplasm and were rarely observed in the intermyofibrillar spaces (Figs. 2A, 5A). Lysosomes occurred infrequently in fibers from anecdysial animals.

Atrophied muscles: Fibers from late proecdysial animals

Since the mass of the closer muscle is related to the size of the claw, it was necessary to establish a meaningful reference point for making comparisons between animals of different sizes (Skinner, '66). The total muscle weight was expressed as a function of the product of the width and length of the propodus; this relationship was linear over the claw sizes used in this study with correlation coefficients >0.90 (Fig. 4). The weight of the major claw muscle decreased ~50% by the end of proecdysis in animals regenerating one walking leg and ~67% in animals regenerating eight walking legs (Fig. 4, left). In contrast, the weight of the minor claw muscle decreased ~25%, whether animals were regenerating one or eight walking legs (Fig. 4, right).

Changes in the myofibrillar cross-sectional area paralleled the changes in muscle mass during proecdysis. The myofibrillar cross-sectional area of the S_1 fibers decreased 78%, from 2.17 to 0.48 μm^2, in animals regenerating eight walking legs (Table 1; compare Figs. 2B and 5C), but only 43%, from 2.17 to 1.23 μm^2, in animals regenerating one walking leg (Table 1). The myofibrillar cross-sectional area of the S_2 fibers decreased 27%, from 0.96 to 0.70 μm^2, in animals regenerating one walking leg (Table 1). The number of limbs being regenerated had little effect on the S_2 fibers; the cross-sectional area decreased 32%, from 0.96 to 0.65 μm^2, in animals regenerating eight walking legs (Table 1; compare Figs. 3C and 6B). Since the ultrastructural changes were similar between S_2 fibers from the major and minor claws, data from S_2 fibers in both claws were combined and presented in Table 1.

The general structure of the myofibrils, nuclei, mitochondria, and sarcoplasmic reticulum was retained in fibers from late proecdysial animals (Figs. 5A,B, 6A). The dimensions of the remaining thin and thick myofilaments were unchanged. Secondary lysosomes, many with myelin figures, were observed only in atrophic fibers (data not shown). Atrophic fibers were also characterized by enlarged intermyofibrillar spaces and the presence of gaps in the myofilament lattice, described previously as areas of erosion (Fig. 5C; Mykles and Skinner, '81). These features were more common in S_1 fibers than in S_2 fibers. In animals regenerating eight walking legs, atrophy of S_1 fibers resulted in a reduction in the ratio of thin:thick myofilaments from ~9:1 to ~6:1 (compare Fig. 5C, inset, with Fig. 2B, inset), which was correlated with a 51% increase in packing density of the thick myofilaments (Table 1). Accurate myofilament ratios could not be obtained in S_2 fibers from late proecdysial animals due to poor resolution of the

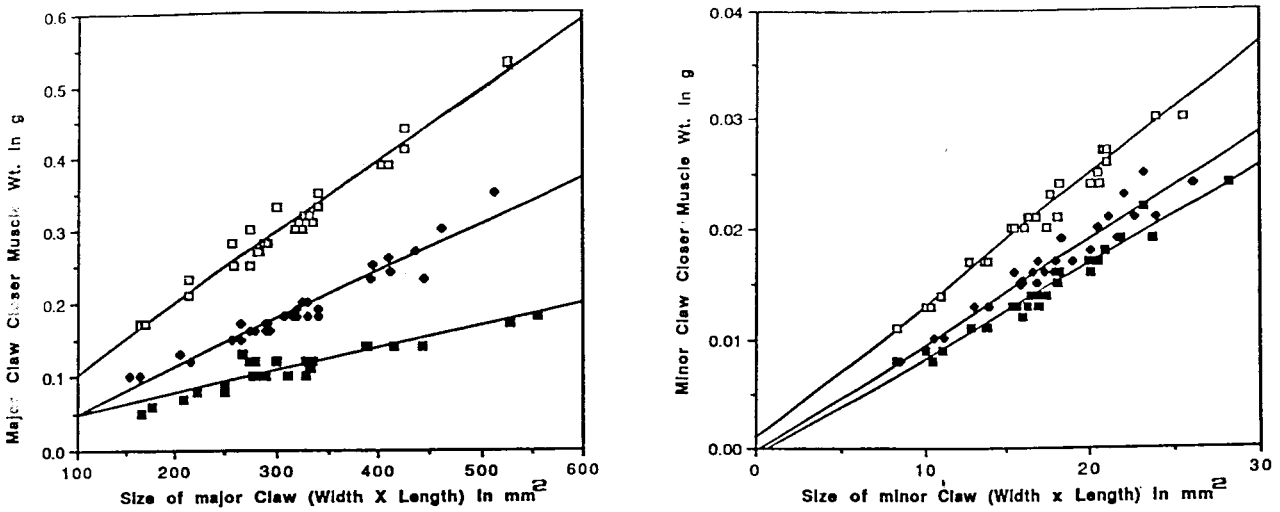

Fig. 4. Relationship between closer muscle weight vs. size of major (**left**) and minor (**right**) claws of male fiddler crabs before and after atrophy. Data from anecdysial animals (□) are compared with data from late proecdysial animals regenerating one walking leg (◆) or eight walking legs (■).

thin myofilaments, even in fibers fixed with the glutaraldehyde/tannic acid solution. However, the thick myofilament packing in atrophic S_2 fibers was only 10–13% greater than that of S_2 fibers from anecdysial animals (Table 1).

Quantification of the amount of actin and myosin heavy chain, the major proteins of the thin and thick myofilaments, respectively, by scanning densitometry of SDS-polyacrylamide gels confirmed the reductions in the thin:thick myofilament ratio observed with the electron microscope. S_1 fibers showed a greater decrease in actin relative to myosin heavy chain than S_2 fibers; the actin:myosin heavy chain ratio decreased 45% and 64% in late proecdysial animals regenerating one and eight walking legs, respectively (Table 1). In contrast, the decrease in the ratio in S_2 fibers was 13% and 25% in the same animals (Table 1).

DISCUSSION

Crustacean muscle fibers are divided into the major categories of fast and slow types based on morphological and contractile properties (see Chapple, '82, and Govind and Atwood, '82, for reviews). The ultrastructure of the claw closer muscle fibers from both the major and minor claws resembles that of slow muscle fibers from other decapod crustaceans (Jahromi and Atwood, '69, '71; Sherman and Atwood, '71; Crabtree and Sherman, '81; Mykles and Skinner, '81); *U. pugnax* claw muscle fibers have long sarcomeres, high ratios of thin:thick myofilaments, thickened Z-lines, and large numbers of mitochondria (Figs. 2, 3). The histochemical properties are consistent with this classification; the fibers in both claws possess low myofibrillar adenosine triphosphatase (ATPase) and high nicotinamide-adenine dinucleotide (NADH) diaphorase activities (Govind et al., '86), which reflect the slow-contractile properties and high oxidative capacities, respectively, of crustacean slow fibers (see Silverman et al., '87, for review).

There are two subtypes of slow muscle fibers in decapod crustaceans that differ in electrophysiological, histochemical, and biochemical properties (Silverman et al., '87; Mykles, '88). In *U. pugnax,* the S_1 fibers are found exclusively in the major claw, whereas the S_2 fibers are found in both claws. Morphologically, the S_1 fibers have longer sarcomere lengths, larger myofibrillar cross-sectional areas, and fewer mitochondria than the S_2 fibers (Table 1, compare Figs. 2 and 3). The difference in myofibrillar cross-sectional area is correlated with the greater diameter of the S_1 fibers (Mykles and Skinner, '81; Mykles, '88). In addition, the S_1 fibers lack the regular alignment of the Z-lines found in the S_2 fibers. These same slow fiber types occur in *H. americanus* and *G. lateralis,* as well as in *U. pugnax* (Mykles, '85a,b, '88; Govind et al., '86). Histochemically, the S_2 fibers have generally lower myofibrillar ATPase and higher NADH diaphorase activities than the S_1 fibers (Govind et al., '86; Mykles, '88). The greater NADH diaphorase activities are consistent with the greater number of mitochondria within the S_2 fibers (Fig. 3). Biochemical analysis has shown that the superficial abdominal muscles of *H. americanus* are also composed of S_2 fibers

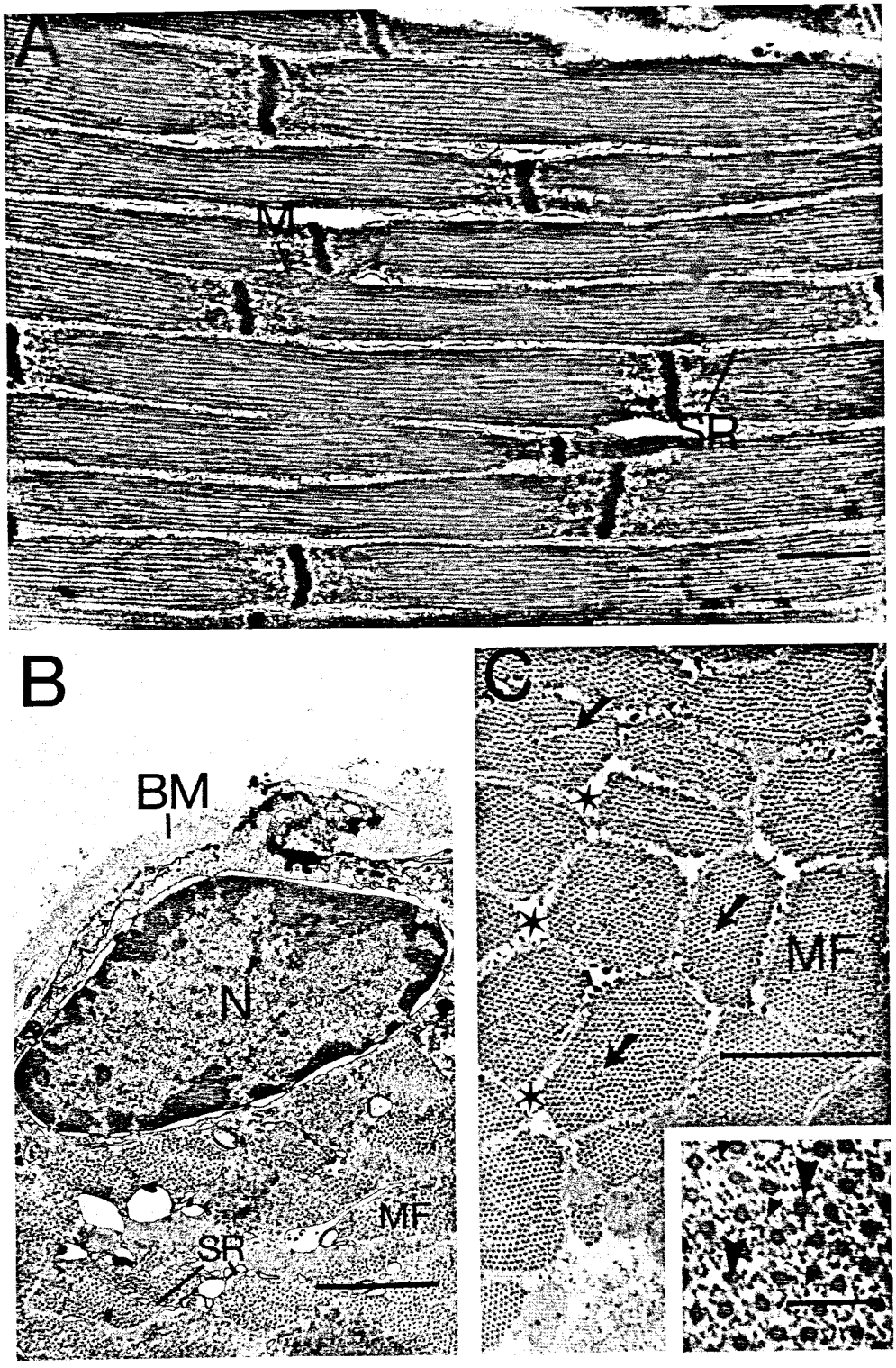

Fig. 5. Electron micrographs of S_1 fibers from the major claw of late proecdysial male fiddler crabs regenerating eight walking legs. **A:** Longitudinal section showing sarcoplasmic reticulum (SR) and an occasional mitochondrion (M) in the intermyofibrillar spaces. Bar = 1 μm. **B:** Transverse section through peripheral sarcoplasm showing basement membrane (BM), nucleus (N), cross-sections of myofibrils (MF), and sarcoplasmic reticulum. Bar = 1 μm. **C:** Transverse section showing myofibrils with areas of erosion (arrows) and enlarged intermyofibrillar spaces (asterisks). Sarcoplasmic reticulum and mitochondria are located in the intermyofibrillar space. Bar = 1 μm. **Inset:** Transverse section at the level of the A-band of a fiber fixed with a glutaraldehyde fixative containing 8% tannic acid (see Materials and Methods) showing thick myofilaments (large arrowheads) surrounded by orbits of 6–10 thin myofilaments (small arrowheads); the thin:thick myofilament ratio is ~6:1 Bar = 0.3 μm.

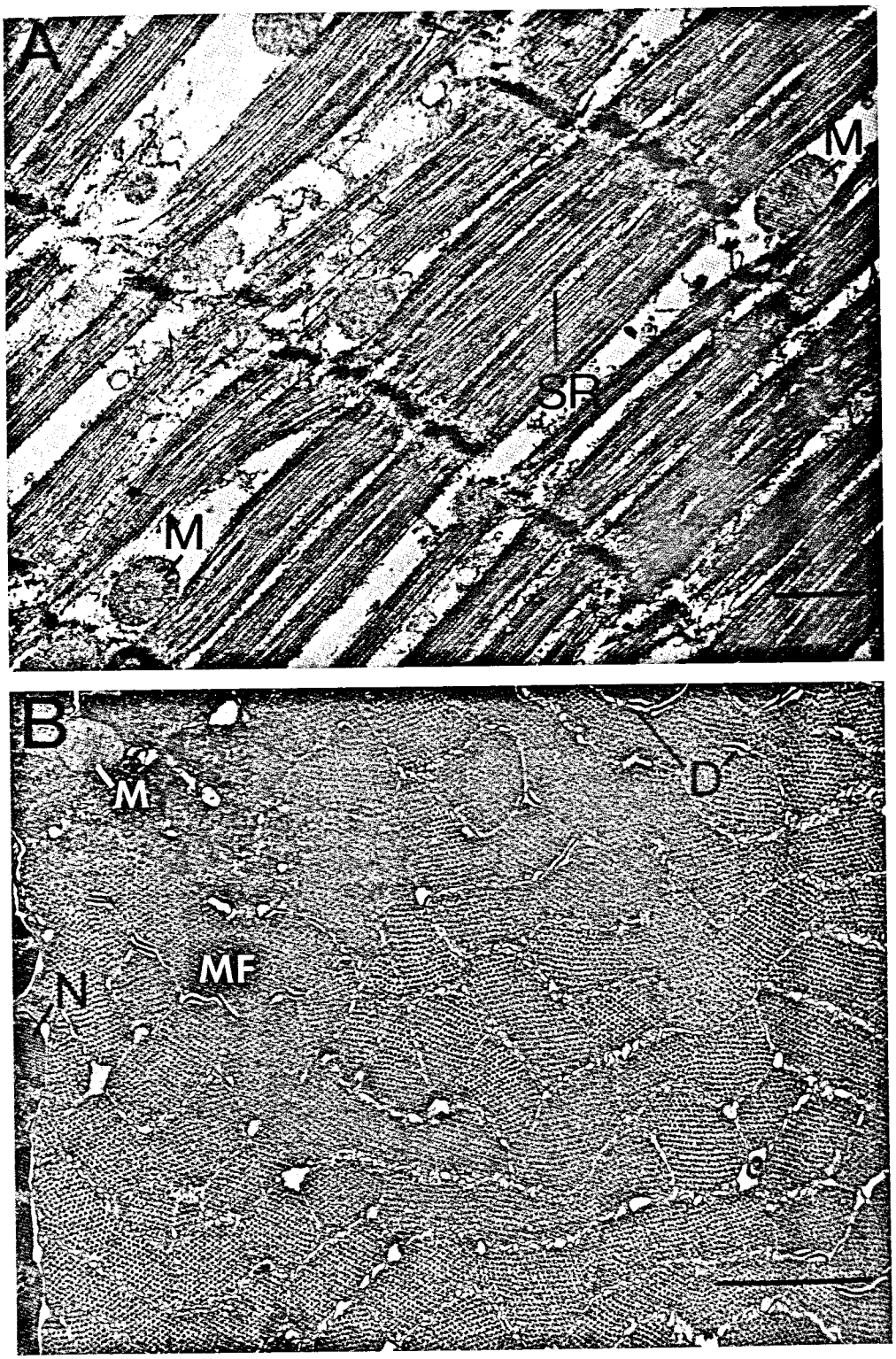

Fig. 6. Electron micrographs of S_2 fibers from the minor claw of late proecdysial male fiddler crabs regenerating eight walking legs. **A:** Longitudinal section showing sarcoplasmic reticulum (SR) and numerous mitochondria (M) in intermyofibrillar spaces. Z-lines of adjacent myofibrils remain in register. Bar = 1 μm. **B:** Transverse section showing myofibrils (MF) surrounded by sarcoplasmic reticulum. D, dyads; M, mitochondrion; N, nucleus. Bar = 1 μm.

(Mykles, '85a, '88). These fibers also contain large concentrations of mitochondria, particularly in the peripheral sarcoplasm (Jahromi and Atwood, '69).

The S_1 and S_2 fibers in decapod crustacean muscles are also distinguished by unique isoforms of myosin heavy chain and troponin-T (Govind et al., '86; Mykles, '88). The distinct myosin heavy chain variants may account for the difference in myofibrillar ATPase, since myosins from fast and slow muscles of *H. americanus* differ in their heavy chains but have identical light chains (Li and Mykles, '90). The presence of troponin-T_1 in S_2 fibers may contribute to the differential sensitivity to stimulation by the nervous system. In *U. pugnax,* the motor axons to the minor claw trigger muscle contraction at lower firing frequencies than their homologs in the major claw, even though the excitatory axons are both of the slow type (Govind et al., '86).

The claw closer muscles of the male *U. pugnax* undergo a sequential atrophy and restoration during each molting cycle. All measurements indicate that this atrophy is differential between the dimorphic claws. The reductions in muscle weight, myofibrillar cross-sectional area, and actin:myosin heavy chain ratio and the increase in the thick myofilament packing show that the muscle of the major claw atrophies more than that of the minor claw (Table 1; Fig. 4). This explains how the muscle in major claw, which is approximately five times wider, six times longer, and 15 times heavier than the muscle in minor claw, can be pulled out through the narrow exit of the basi-ischial segments. Since the diameters of the basi-ischial segments of each claw are similar, there would be a greater mechanical problem associated with extricating the major claw from the old exoskeleton.

The degree of atrophy is correlated with the fiber type composition of the closer muscles of the major and minor claws. The average myofibrillar cross-sectional area of the S_1 fibers from animals regenerating eight walking legs decreased about 4.5-fold, while that of the S_2 fibers decreased only about 1.5-fold (Table 1). It is important to emphasize that this differential sensitivity does not appear to be determined by claw type, since S_2 fibers in both claws underwent similar amounts of atrophy as measured by the reduction in myofibrillar cross-sectional area. This phenomenon also occurs in molt-induced atrophy of the claw closer muscles of *G. lateralis*. Mykles and Skinner ('81) originally showed that large-diameter fibers atrophied to a much greater extent than small-diameter fibers; these were later identified as the S_1 and S_2 fibers, respectively (Mykles, '88). Mammalian skeletal muscles also show a differential atrophy that is correlated with the fiber type composition. For example, the soleus muscle, which contains primarily slow-twitch (type I) fibers, undergoes a greater atrophy than the plantaris, which contains primarily fast-twitch (type II) fibers (see Thomason and Booth, '90, for review). Histochemical analyses have shown that the reduction in diameter is greater for the type I fibers than the type II fibers in the soleus muscles of rats subjected to space flight or whole-body or hindlimb suspension (Musacchia et al., '90; Riley et al., '90a). These studies suggest that the response of a muscle to an atrophy stimulus is determined, at least in part, by the types of fibers expressed.

Claw muscle atrophy is enhanced by the simultaneous regeneration of seven or eight walking legs. This has previously been shown in *G. lateralis,* in which myofibrillar cross-sectional area decreases 52% in animals regenerating one walking leg and 78% in animals regenerating seven walking legs (Mykles and Skinner, '81, '82a). In *U. pugnax,* the effect of limb regeneration is most pronounced in the major claw, while the minor claw appears to be protected (Fig. 4; Table 1). The weight and myofibrillar cross-sectional area of the major claw muscle decreased ~50% and ~43%, respectively, in animals regenerating one walking leg and ~67% and ~78%, respectively, in those animals regenerating eight walking legs. Comparable changes in the thick myofilament packing and the actin:myosin heavy chain ratio are consistent with an enhanced proteolysis in the major claw induced by limb regeneration (Table 1). It is apparent that limb regeneration puts severe metabolic demands on these animals; multiple limb autotomy in *Uca* reduces organismal growth (see Hopkins, '85, for review). Even though these animals feed during most of the proecdysial period, it is possible that dietary intake of amino acids may not be sufficient to meet the increased synthetic demands of a large regenerative load (Mykles and Skinner, '90a,b). The large mass of the major claw muscle would serve, in effect, as a reservoir for amino acids that could be mobilized to other tissues. However, there is no direct evidence showing that amino acids produced by hydrolysis of muscle proteins are used by tissues, such as limb regenerates or integumentary tissue, in which protein synthesis is stimulated.

Crustacean muscle atrophy is induced by a variety of physiological stimuli, such as tenotomy, prolonged denervation, disuse, and molting (Skinner, '66; Boone and Bittner, '74; Moffett, '87). Ultrastruc-

tural studies have shown that, regardless of the stimulus, muscle atrophy shares certain morphological features, which suggests that there is a common biochemical mechanism. Enlarged intermyofibrillar spaces and erosion areas are characteristic of crustacean muscles undergoing atrophy (Mykles and Skinner, '81, '90a; Schmiege and Moffett, '85). Similar features are found in vertebrate striated muscles, such as the rat soleus muscle induced to atrophy by hindlimb unloading (Riley et al., '90b). It is hypothesized that these are areas where myofilaments are removed from the contractile apparatus and proteolyzed, resulting in the reduction in myofibrillar cross-sectional area (Mykles and Skinner, '81).

As in *G. lateralis,* atrophy in *U. pugnax* involves a preferential hydrolysis of the thin myofilaments. In both species, the thin:thick myofilament ratio of the S_1 fibers is reduced from ~9:1 to ~6:1, which is correlated with an increase in the packing density of the thick myofilaments and a decrease in the ratio of actin to myosin heavy chain (Table 1; Mykles and Skinner, '81,'82b). Using the data for myofibrillar cross-sectional areas and thick myofilament packing densities in Table 1, the numbers of thin and thick myofilaments that must be hydrolyzed to obtain this reduction in the myofilament ratio can be calculated. Myofibrils in anecdysial fibers contain an average of 760 thick and 6,840 thin myofilaments. In late proecdysial animals regenerating eight legs, the numbers of thick and thin myofilaments are reduced to 254 and 1,524, respectively, in each myofibril. This means that muscle atrophy involves a preferential degradation of about 11 thin myofilaments for each thick myofilament, which is in remarkable agreement with the 11:1 hydrolytic stoichiometry calculated for *G. lateralis* also induced to molt by multiple limb autotomy (Mykles and Skinner, '81). The occurrence of the same preferential hydrolysis in two different decapod species suggests that the conservation of the thick myofilaments has an important function. Earlier we hypothesized that the thick myofilaments help retain myofibrillar structure and facilitate incorporation of myofilaments as muscles are restored during metecdysis (Mykles and Skinner, '81). In other words, the relative abundance of the thin myofilaments may make these more expendable, so their preferential hydrolysis would help ameliorate the adverse effects of atrophy on muscle performance as well as promote the recovery of the muscle after exuviation.

Lysosomes appear to have a specialized role in muscle atrophy. Activities of lysosomal enzymes, such as cathepsin D and acid phosphatase, are three-and two-fold greater, respectively, in claw muscles from proecdysial *G. lateralis* than in muscles from anecdysial animals (Yamaoka and Skinner, '75). One would expect that, as fiber volume decreases, there would be a concomitant reduction in mitochondria and sarcoplasmic reticulum to maintain a relatively constant density of these organelles. In *G. lateralis,* the surface density of the sarcoplasmic reticulum remains unchanged during atrophy (Mykles and Skinner, '81). Secondary lysosomes, many with myelin figures, are associated with mitochondria and sarcoplasmic reticulum, which suggests they are involved in the turnover of these structures (Mykles and Skinner, '81, '82a). We have made similar observations in *U. pugnax* (data not shown).

Claw muscle atrophy in crustaceans is associated with the secretion of the molting hormone 20-hydroxyecdysone (20-HE), which accumulates in the hemolymph during proecdysis and reaches a maximum concentration immediately before ecdysis (see Skinner, '85, for review). Thus it is possible that protein catabolism is triggered by 20-HE or other endocrine factors. Whatever the regulatory mechanism, it must account for the response of a specific muscle to the molting stimulus. Muscle differentiation and net protein accumulation are stimulated in regenerating limbs at the same time that atrophy and net protein loss proceed in claws, but not the walking legs, of *G. lateralis* (Mykles and Skinner, '82a). In *U. pugnax,* muscle atrophy is even more exquisitely controlled. It may be the number or the mode of action of hormone receptors in the target organs that causes tissues to respond differently to the same stimulus. Alternatively, this may result from the difference in the neural supply to the major and the minor claws. Neuronal regulation appears less likely, however, since the S_2 fibers undergo a similar degree of atrophy regardless of whether they are located in the major or minor claws. This suggests that the claw muscle's mass is determined by differential sensitivities of differentiated fiber types to factor(s) that regulate protein metabolism during the intermolt cycle.

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