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Extracellular matrix and muscle fibers in the gills of freshwater bivalves

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Abstract. The supportive tissue in the gills of three species of freshwater bivalves (*Corbicula fluminea, Dreissena polymorpha,* and *Toxolasma texasensis*) is composed of a similar extracellular matrix with interspersed muscle fibers. These matrices consist of a periodic acid Schiff-positive tissue supported by small collagen fibrils as determined by morphological and biochemical examination. The periodicity observed in fibrils and the electrophoretic migration patterns are consistent with other collagens. The ultrastructure of the muscle fibers associated with the connective tissue corresponds with a known type of smooth muscle. The high degree of similarity between the tissues of these species suggests a similar, conserved function.

Additional key words: collagen, smooth muscle, extracellular matrix

The bivalve gill is a highly complex organ composed of regionally specialized epithelial tissue attached to a supportive structure of connective tissue containing blood spaces, nerves, and muscles (LePennec et al. 1988; Kays et al. 1990; Gardiner et al. 1991; Eble & Scro 1996; Gros et al. 1996; Medler & Silverman 1997). LePennec et al. (1988) appropriately emphasized the need for detailed descriptions of the anatomy of littoral bivalve gills. The gill is an important organ involved in nutrition, ion transport, and gas exchange (Dietz 1985; Pearse et al. 1987; LePennec et al. 1988; Kays et al. 1990; Eble & Scro 1996), yet there are gaps in our knowledge of the non-epithelial components of the gill. A number of early investigators noted that muscular movements of the gill were important for integrated gill processes (Setna 1930; Elsey 1935; Atkins 1943; Nelson 1960). We have recently studied the functional organization of the gill muscles and their associated connective tissues (Gardiner et al. 1991; Medler & Silverman 1997), but a detailed understanding of the basic composition of these elements does not exist. This comparative study focuses on the composition of the extracellular matrix and muscle fibers in the freshwater bivalve gill.

The connective tissue of the bivalve gill has historically been termed chitinous (Ridewood 1903; Yonge 1926; Elsey 1935; Atkins 1943), although more contemporary work recognizes the tissues as being composed primarily of collagen and associated muscle fibers (LePennec et al. 1988; Kays et al. 1990; Eble & Scro 1996; Gros et al. 1996; Medler & Silverman 1997). Nevertheless, there are no studies that have explicitly examined the nature of the collagen; the evidence that collagen is the fibrous protein supporting the gill has largely been anecdotal or preliminary (Brown 1952; Ruddall 1955; LePennec et al. 1988; Kays et al.1990; Gros et al. 1996; Medler & Silverman 1997). The intrinsic muscles of the bivalve gill were described many years ago (Setna 1930; Elsey 1935; Atkins 1943) but have received little attention until recently (Gardiner et al. 1991; Medler & Silverman 1997). The ultrastructure of these muscle fibers is poorly known and has been explicitly described for only a single species, *Dreissena polymorpha* (Medler & Silverman 1997). In the present study, we identify the fibrous structural material of the gill and classify the associated muscle fibers according to their comparative ultrastructure.

We studied the gills of the freshwater bivalves *Corbicula fluminea, Dreissena polymorpha,* and *Toxolasma texasensis. Corbicula fluminea* and *D. polymorpha* are members of the subclass Heterodonta (Newell 1965) and share a common evolutionary lineage (Nuttall 1990). *Toxolasma texasensis* is of the subclass Paleoheterodonta (Newell 1965) and is a member of the family Unionidae. Together, these species are representatives from three different bivalve families (Newell 1965) and were selected because they provide a comparative analysis among higher systematic taxa.

Methods

Animals and maintenance

Dreissena polymorpha (PALLAS 1771) were collected from western Lake Erie and the Raisin and Huron

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Rivers in Michigan, and from the Mississippi River near Baton Rouge, Louisiana. *Corbicula fluminea* (MÜLLER 1774) and *Toxolasma texasensis* (I. LEA 1857) were collected from ponds near Baton Rouge, Louisiana. The animals were maintained in artificial pond-water (0.5 mM NaCl, 0.4 mM $CaCl_2$, 0.2 mM $NaHCO_3$, 0.05 mM KCl, and 0.2 mM $MgCl_2$) in aerated aquaria under laboratory conditions of ~22°–25°C.

Preparation of supportive tissues

The supportive elements of the gills were stripped of their epithelia using the method described by Medler & Silverman (1997). Briefly, gills were excised and placed into a calcium-free Ringer's solution to loosen the tissue, and mechanically agitated through a plastic pipette to remove the cells. This procedure was used to prepare tissues for periodic acid Schiff (PAS) staining, scanning electron microscopy (SEM), collagen isolation, and gill homogenization (see below).

Periodic acid-Schiff reactions

The PAS reaction was used to stain the oxidizable carbohydrates commonly found in the proteoglycan matrices that form the ground substance of connective tissues. Supportive tissues were prepared as described above and split with forceps along the interlamellar septae into single lamellae. These lamellae were laid flat on glass slides and air-dried. The whole lamellae were exposed to salivary amylase for 10–20 min and then stained with a standard PAS reaction, following the methods of Troyer (1980). Lamellae were oxidized with 1% periodic acid for 10 min, and the tissues rinsed in running tap water for 5 min. The tissues were then placed in Schiff's reagent (de Tomasi 1936) for 10 min, and the stained lamellae washed in three changes of 0.5% sodium metabisulfite for 2 min each followed by a wash in running water for 5 minutes. Finally, the stained lamellae were dehydrated in a graded ethanol series and mounted with Permount®. The prepared tissues were examined with a Nikon Microphot FXA light microscope using a green filter.

Collagen isolation

Collagen isolation followed a modified version of Murray et al. (1982), designed for the acid extraction of polychaete cuticle collagen. Gills were excised and stripped of their epithelium as described above. The remaining supportive tissues were stirred overnight at 4°C in a neutral salt solution containing protease inhibitors (0.05 M tris-HCl; 1.0 M NaCl; 0.01 M N-ethylmaleimide [NEM]; 0.025 M ethylenediaminetetraacetic acid [EDTA]). The gills were homogenized with a hand-held glass homogenizer in 0.1 M acetic acid at 4°C and then extracted for at least 24 h in 0.1 M acetic acid at 4°C with stirring. Homogenates were centrifuged at 25,000 g for 30 min. The supernatant was collected and dialyzed against 3–4 changes of 20 mM Na_2HPO_4, which caused the collagen to precipitate. Precipitated collagen was collected by low speed centrifugation and freeze-dried. Dried collagen was suspended in deionized water at a concentration of 1 mg/ml and frozen in 50 µl aliquots at −20°C.

Gill homogenization

Whole gill tissues were stripped of their epithelium as described above and homogenized in deionized water at ~25°C using a hand-held glass homogenizer. Suspensions (~2 demibranchs/ml) were applied to grids and negatively stained (see below). This procedure allowed direct examination of collagens present in the gills prior to being isolated, and thus provided a complementary perspective.

Negative staining

Collagen isolates (1 mg/ml) or gill homogenates (~2 demibranchs/ml) were suspended in deionized water. Formvar-coated grids were floated on a drop of the suspension for 10 min. The grids were drained and allowed to air dry. A solution of 3% uranyl acetate in 30% ethanol was dripped across the face of the grids and the grids were drained and allowed to air dry. The prepared grids were examined with a JOEL 100CX transmission electron microscope operating at 80 kV.

Gel electrophoresis

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS PAGE) of collagen proteins was conducted at 20 V for ~12 hours on 8% polyacrylamide gels (acrylamide: methylene bisacrylamide = 37:1) following a modified version of Murray et al. (1982). The running buffer consisted of 0.16 M tris-HCl in 1% sodium dodecylsulfate adjusted to pH 6.6 with acetic acid. The sample buffer consisted of 0.05 M sodium phosphate buffer at pH 7.4 with 20% (w/v) glycerol, 2% mercaptoethanol, 2% SDS, and 0.033% phenol red as tracking dye. Samples (50 µg) were loaded on gels with bovine type I collagen (Sigma C-9879) and high molecular weight markers (Sigma M-3788) as standards. Gels were stained with 0.1% Coomassie Blue R-250 in a solution of 10% acetic acid / 25% isopropanol for 1 h, and destained with a solution of 10% acetic acid/10% isopropanol for several hours.

Preparation of gill sections

Prior to gill excision, hemolymph samples were taken from animals by inserting a 26 gauge needle between the valves and into the pericardial space (Fyhn & Costlow 1975; Dietz et al. 1994). Hemolymph osmolality (~40–60 mOsm) was measured on a Precision Systems freezing point osmometer. A 2% glutaraldehyde solution was adjusted with phosphate buffer to match the hemolymph osmolality. Excised gills were cut along the filaments into strips ~3 mm wide, and quickly fixed by immersion in the glutaraldehyde solution for 1 h. Gills were rinsed twice in phosphate buffer and post-fixed in 1% OsO_4 for 1 h, rinsed twice in phosphate buffer, and dehydrated in a graded ethanol series. Gill strips were embedded in LR White (London Resin Co.) medium grade resin by placing them in a 1:1 mixture of ethanol and resin for 24 h. They were transferred to 100% resin for 12 h, and embedded flat in fresh resin at 60°C for 24–48 h.

Gills were sectioned with a Reichert-Jung ultracut E ultramicrotome at 60–90 nm thickness with glass knives, or at 2 μm and stained with toluidine blue. The gills were sectioned in three planes: (1) in cross section, transverse to gill filaments, (2) in a dorso-ventral plane between adjacent filaments, and (3) in frontal sections (*en face*) parallel to the gill surface. Thin sections (60–90 nm) were stained with 3% uranyl acetate for 8 min followed by Reynolds' (1963) lead citrate for 2–5 min. Thick sections (2 μm) were examined with a Nikon Microphot FXA and thin sections with a JOEL 100CX transmission electron microscope (TEM) operating at 80 kV.

Scanning electron microscopy

The supportive structures of the gill were isolated and fixed with 2% glutaraldehyde and 1% OsO_4 as described above. After osmication, gills were rinsed in phosphate buffer and dehydrated in a graded ethanol series. Gills were wrapped in lens paper, critical-point dried, and mounted on stubs. Specimens were sputter coated with a mixture of gold and palladium (20 nm) and viewed with a Cambridge S-260 SEM.

Microscopic measurements

Measurements were made with digital calipers from photographic prints to the nearest 0.1 nm. The dimensions of the collagen fibrils and fibers were estimated from several measurements within a number of different micrographs. Each estimate is given with its standard error and the number of elements measured. The reported variability represents error in measurement, calculated magnification, and possibly artifacts of fixation.

For the estimation of thick filament diameter, 500 thick filaments were measured from several different muscle fibers for each species. Thin:thick filament ratios were estimated by counting filaments from several regions of different fibers from each species.

Results

General organization of gill lamellae

The gills of all three species are homorhabdic; the general organization of the gill lamellae forming them is shown in Fig. 1. The gill filaments consist of regionally specialized epithelial cells attached to a fibrous connective-tissue matrix. The connective tissue in the filaments is continuous with an outer connective-tissue sheet at the base of the filaments. A second connective-tissue sheet supporting the internal water channel epithelium is separated from the outer sheet by a hemocoel. The muscles of the gills are restricted to the subfilamentar tissue. They are found in the two connective-tissue sheets of the lamellae and in bands of connective tissue running perpendicular to the bases of the filaments. The bands of muscle and connective tissue are antagonized by connective-tissue cross struts more superficial to the muscle bands and perpendicular to the filaments.

While the general organization of the gills from the three species is similar, some important differences are noteworthy. A detailed description of these differences is beyond the scope of this paper, but preliminary observations include the following. The lamellae of *Toxolasma texasensis,* like those of other unionids, have calcified concretions in the connective-tissue sheets and calcified rods at the base of the filaments (Silverman et al. 1983; Gardiner et al. 1991). These structures are absent in *Corbicula fluminea* and *Dreissena polymorpha.* The gills of *T. texasensis* are also generally thicker than in the other species, thus the water canals connecting to the central water channel are longer (on the order of 100 μm in *T. texasensis* versus ~20 μm in *C. fluminea* and *D. polymorpha*). The filaments in *T. texasensis* alternate with the ostia (as in Fig. 1b), while in *C. fluminea* and *D. polymorpha* the external ostia are effectively divided in half by every other filament (as in Figs. 1c, d). The cross-struts in *C. fluminea* and *D. polymorpha* have an elongate, figure-eight morphology, while those of *T. texasensis* are straight (not shown). The gills of *C. fluminea* are slightly plicate, while the others are flat. Structurally, the gills of *C. fluminea* and *D. polymorpha* more closely resemble one another than those of *T. texasensis.*

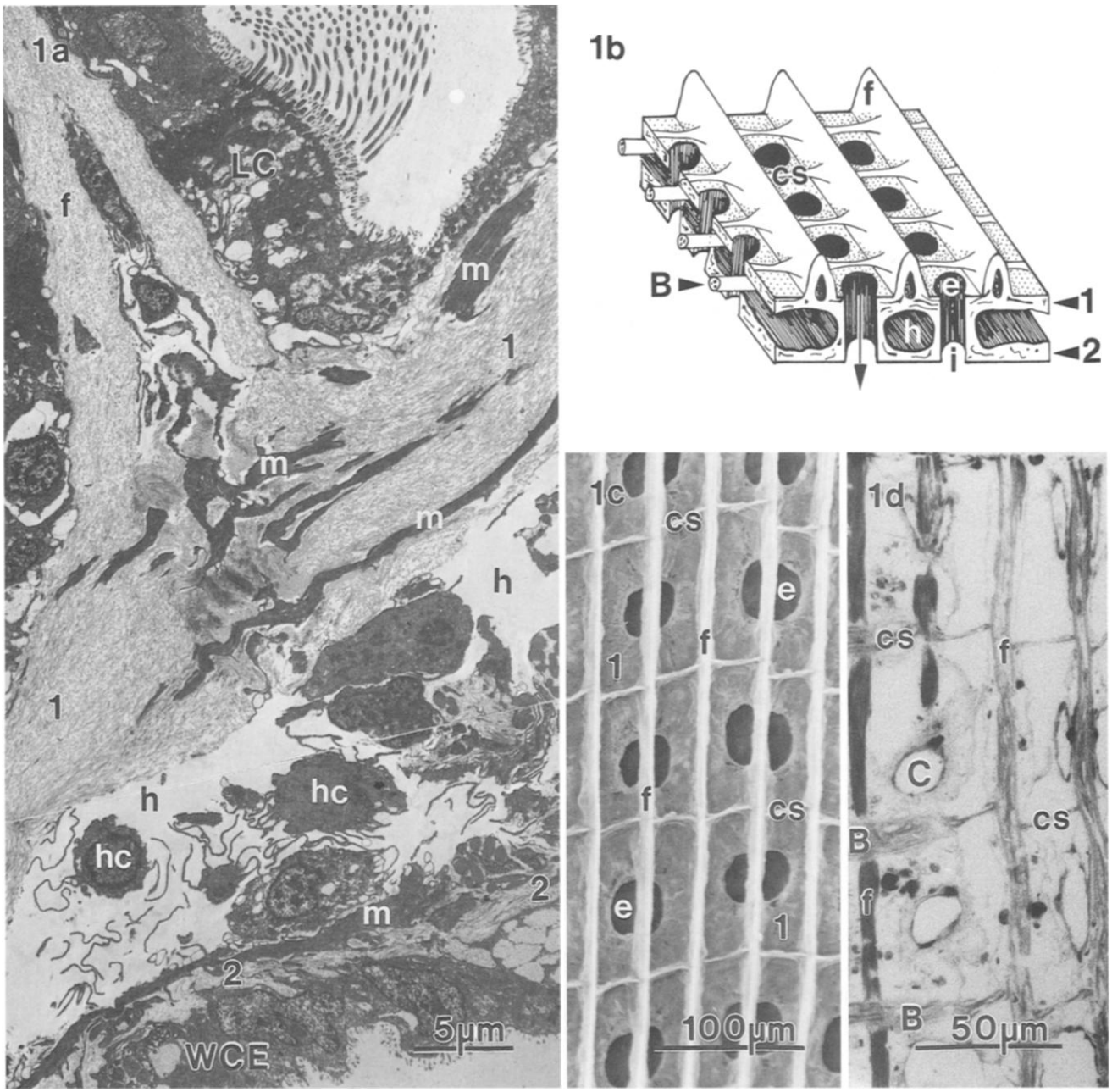

Fig. 1. General organization of connective tissue and muscle in the gills of the bivalves studied. **(a)** Transverse section through a single lamella of *C. fluminea.* The connective-tissue fibers of the filament (f) merge into the connective tissue of the outer connective-tissue sheet (1). This connective-tissue sheet envelops muscle fibers (m), as does the inner connective-tissue sheet (2). Between the two connective-tissue sheets is a hemocoel (h) with various hemocytes (hc). Epithelial tissue, including a lateral ciliated cell (LC), covers the outside of the gill and water channel epithelium (WCE) lines the central water channel. TEM. **(b)** Diagrammatic composite of the common supporting structure of the gills studied (not to scale). Dorso-ventral filaments are supported by a fibrous connective-tissue structure (f) that merges into a flat connective-tissue sheet (1). These filament supports are held apart by connective-tissue cross struts (cs) that are superficial to bands of connective tissue and muscle fibers (B). A second connective-tissue sheet (2) supports the water-channel epithelium. Both sheets of connective tissue are interspersed by muscle fibers (wavy black lines). The space between the two connective-tissue sheets comprises the hemocoel (h). Water moves through the gill by entering external ostia (e) and into water canals (arrow) which empty into the central water channel via internal ostia (i). **(c)** External view of the supportive structure in the gill of *D. polymorpha.* The filament supports (f), cross struts (cs), outer connective-tissue sheet (1), and external ostia (e) are shown. SEM. **(d)** 2 μm frontal section through a gill lamella of *D. polymorpha,* showing the internal structure of Fig. 1c. Filament supports of varying density (f), cross struts (cs), muscle/connective-tissue bands (B), and water canals (C) are visible. Light micrograph.

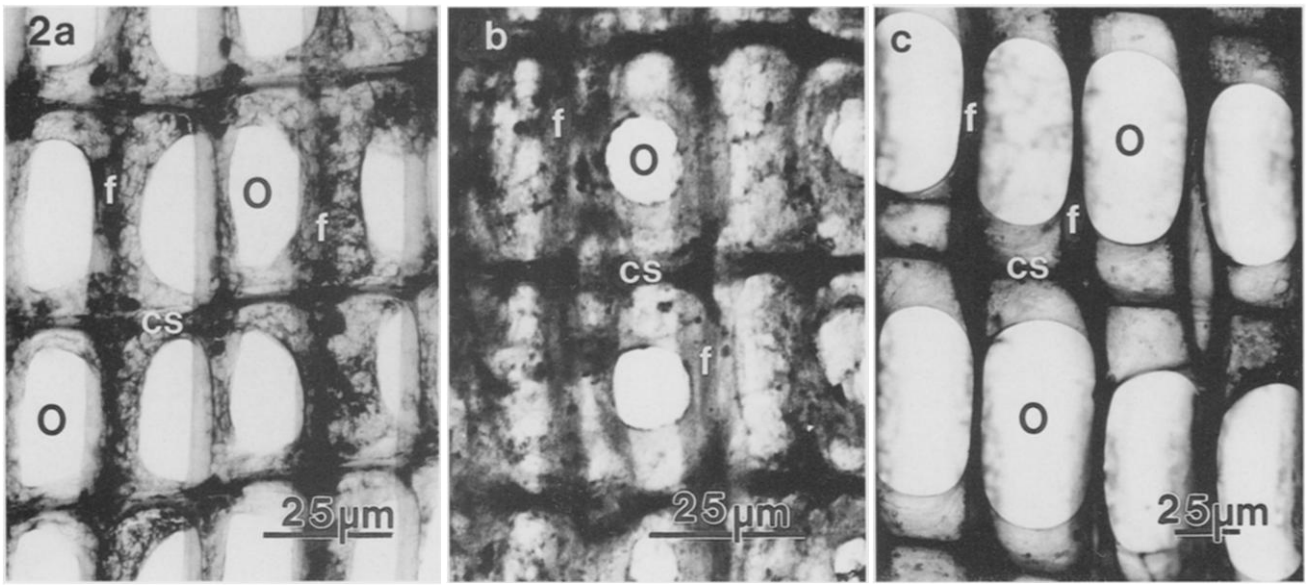

Fig. 2. PAS-stained connective-tissue elements in the gills of **(a)** *D. polymorpha,* **(b)** *C. fluminea,* and **(c)** *T. texasensis.* All of the major connective-tissue regions show a positive PAS reactivity including the filament supports (f), cross-struts (cs), and the connective-tissue sheets that delineate ostia (O). Light micrograph.

Connective tissue

The supportive tissue of the gills (filament supports, cross-struts, and connective-tissue sheets) was found to be PAS-positive in each of the three species (Fig. 2). Sections of the gills revealed that the connective tissue is composed of small fibrils 18 ± 1 nm (n=30) in diameter, having an axial periodicity of 8 ± 1 nm (n=10) (Figs. 3a, c, d). In some regions, interfibrillar banding was observed with an axial periodicity of 59 ± 3 nm (n=5) (Figs. 3c, d). In some dense regions, such as the main support of the filaments, the banding pattern was obscured by a ground substance (not shown).

Collagen isolates were found to contain fibrils 24 ± 1 nm (n=10) in diameter with an axial periodicity of 26 ± 2 nm (n=10) (Figs. 3e–g). Many of these fibrils consisted of 2 or more individual fibrils, so the dimensions of individual fibrils were difficult to determine. The gill homogenates contained large fibers of up to ~150 nm in diameter with axial periodicities of 57 ± 1 nm (n=30) (Figs. 3h–j). These fibers display the "hole" and "overlap" zones seen in negatively stained vertebrate collagen fibers. The "hole" regions in the large fibers correspond to accumulated stain in the gaps between sequentially packed molecules, while the "overlap" regions are where stain is excluded by the overlapping molecules (Figs. 3h–j) (Linsenmayer 1981; Wolfe 1985). The large connective-tissue fibers observed when the gills are homogenized in deionized water (Figs. 3h–j) have never been observed *in situ* and may be formed by reorganization of the smaller fibrils.

Collagen samples on SDS PAGE gels demonstrated protein bands that are consistent with the banding patterns seen in vertebrate collagens. Figure 4 shows an isolate from *Dreissena polymorpha* next to bovine type I collagen. This banding pattern was also observed in other gels of *Corbicula fluminea* and *Toxolasma texasensis* isolates (not shown). The most prominent bands in Fig. 4 correspond to individual alpha chains of the collagen molecules. Collagen alpha chains have a molecular weight of ~95 kDa, but migrate more slowly than globular proteins of similar size (Miller & Gay 1982). Bands corresponding to alpha chain dimers (beta chains, Fig. 4) were also observed in our gels but less consistently, since fully reduced gamma and beta chains dissociate into alpha chains. Trimers of alpha chains (gamma chains) are too large to be seen in these gels, but another band was consistently observed in the range of 66 kDa (Fig. 4) and probably corresponds to fragments of individual alpha chains. The resolution of our gels was insufficient to make inferences about the alpha chain composition in these samples.

Muscle fibers

The muscle fibers in the gill are similar in each of the species (Fig. 5). They are smooth muscle fibers with a thin:thick contractile filament ratio estimated to be 13 ± 1 (n=15). The electron-dense bodies are generally peripherally placed, and occasionally observed in the internal part of the fiber with no apparent organization (Figs. 5a–c). The cytoplasmic organelles

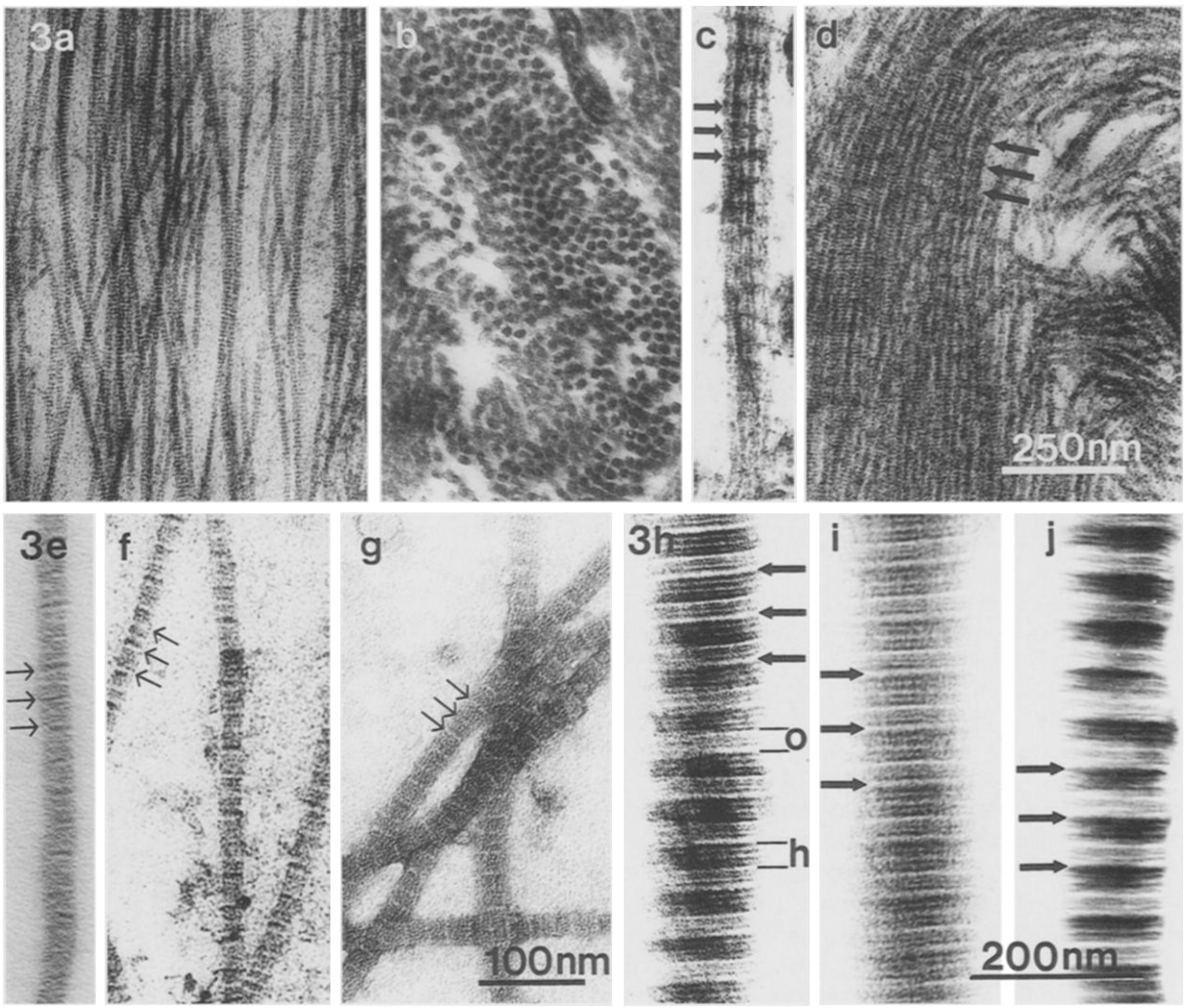

Fig. 3. Collagen fibrils (a–g) and fibers (h–j) from the gill tissue. TEM. **(a–d)** Fibrils observed in sections of gill tissues of (a) *D. polymorpha,* (b, c)*C. fluminea,* and (d)*T. texasensis.* Axial banding patterns of ~8 nm are visible for each species. A very regular and tightly packed arrangement of fibrils is demonstrated in the section from *C. fluminea* (b). An axial periodicity of ~59 nm (arrows) is visible between fibrils in sections from *C. fluminea* (c) and *T. texasensis* (d). (250 nm scale bar is for a–d) **(e–g)** Negatively stained collagen fibrils isolated from (e)*D. polymorpha,* (f) *C. fluminea,* and (g)*T. texasensis.* Each of these samples contains fibrils that show a definite axial periodicity of ~26 nm (arrows) and are ~24 nm in diameter. (100 nm scale bar is for e–g) **(h–j)** Collagen fibers from homogenized gill tissues in (h)*D. polymorpha,* (i)*C. fluminea,* and (j) *T. texasensis.* These fibers are large (up to 150 nm diameter) and show an axial periodicity of ~57 nm (arrows). Alternating ''hole'' zones (h) and ''overlap'' zones (o), characteristic of collagen, are visible in each case. The ''hole'' regions in the large fibers correspond to accumulated stain in the gaps between sequentially packed molecules, while the ''overlap'' regions are where stain is excluded by the overlapping molecules. (200 nm scale bar is for h–j)

and scanty sarcoplasmic reticulum are peripherally placed (Figs. 5a, c, d, e), while microtubules are scattered throughout along the long axis of the muscle fibers (Figs. 5a–c, inset in c). In cross-section, the muscle fibers vary from ovate to elongate and are only a few microns in diameter. The thick contractile filament diameter in cross section is variable, with the three species having overlapping distributions (Fig. 6). The maximum diameter is ~40 nm in *Corbicula fluminea* and ~50 nm in *Dreissena polymorpha* and *Toxolasma texasensis.* The minimum diameter is ~11 nm in each of the species. These dimensions are consistent with thick filaments 40–50 nm in the middle, tapering to ~11 nm at the ends for each of the three species (Fig. 6).

Discussion

The supportive skeleton of these freshwater bivalve gills is composed mainly of an extracellular matrix of

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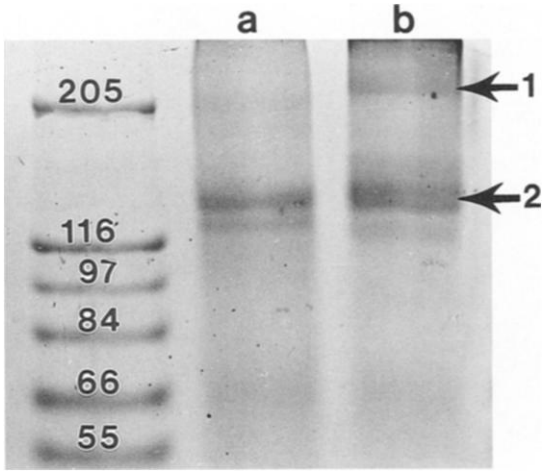

Fig. 4 SDS PAGE of vertebrate type I collagen **(a)** and that isolated from *D. polymorpha,* **(b).** The left lane shows the molecular weight of standards in kDa. The arrows on the right mark the beta (1) and alpha (2) chains inferred from the migration pattern of vertebrate type I collagen. Faint bands can also be seen in the range of 66 kDa and are presumably fragments of alpha chains.

collagen fibrils and the associated ground substance. The reactive carbohydrates identified with the PAS stain probably represent both the glycosylated components of the collagen, a glycoprotein exhibiting varying degrees of glycosylation (Linsenmayer 1981), and the ground substance in which the collagen is embedded. An abundance of smooth muscle fibers is present throughout this matrix, along with a variety of other cell types not described here. Overall, the composition and organization of these supportive structures are reminiscent of many molluscan organs where muscular systems are encased and embedded by connective-tissue layers (Bairati 1985). A few examples of such organs include the bivalve mantle (Morrison 1996), palps (Morse & Zardus 1997), and neural sheath (De Biasi et al. 1985).

The collagen fibrils[1] *in situ* are ~18 nm in diameter with an apparent axial periodicity of ~8 nm that may correspond to part of a larger, unresolved pattern. The fibrils are similar in structure to other collagens reported for a number of different molluscan species (Bairati 1985). This similarity is not surprising given that collagens in general are widely distributed among animal phyla and have an evolutionary history that is both long and conserved (Baccetti 1985; Mathews 1985). Negatively stained collagen samples and gill homogenates also demonstrate axial banding patterns similar to one another and to those of collagens in general, but these are not directly comparable to the fibrils *in situ.* Positive- and negative-staining patterns are determined by different molecular mechanisms and do not produce the same results (Katayama & Nonomura 1979; Chapman 1985). While an axial periodicity of 64 nm (Gosline & Shadwick 1983; Kelley et al 1984) or 67 nm (Galloway 1985; Wolfe 1985) is often considered to be characteristic of collagen, the period is variable and dependent on tissue source, preparation, and tensile strength (Trelstad & Silver 1981). The periodicity in this study of ~57 nm is reasonably close to that of other collagens, as is the banding pattern. As individual collagen molecules associate into larger fibers they are aligned end to end, with regular gaps left between sequential molecules. The molecules of adjacent rows are staggered and show regions of regular overlap where no gaps exist (Linsenmayer 1981; Kelley et al. 1984 ; Wolfe 1985). This arrangement produces the banding patterns observed in the large collagen fibers (Figs. 3h–j). While these large fibers have not been observed in the gill, it is interesting that the interfibrillar banding patterns *in situ* have a correspondent periodicity of ~59 nm (Fig. 3c, d).

The electrophoretic migration pattern of the collagen is consistent with the patterns shown by vertebrate collagens (Miller & Rhodes 1982). Collagens are formed from three intertwined alpha helices (alpha chains), with different collagens made of unique combinations of alpha chains (Miller & Gay 1982; Gosline & Shadwick 1983; Wolfe 1985). Bands representing dimers (beta chains) of alpha chains were sometimes resolved in our gels, but not as prominently as the alpha chains. Our resolution of individual bands was too low to make any interpretation about the number or types of alpha chains, and further work is required to understand this level of molecular detail for these collagens.

Tapered thick filaments are characteristic of many invertebrate smooth muscles (Sobieszek 1973; Castellani et al. 1983; Paniagua et al. 1996) and tapered filaments of similar dimension were observed in each of the species studied. Although we have previously described the integral gill muscles in bivalves as obliquely striated (Kays et al. 1990; Gardiner et al. 1991; Medler & Silverman 1997), the differences between obliquely striated and invertebrate smooth muscles often follow a continuum (Morrison & Odense 1974;

[1] The terms *fibril* and *fiber* refer to fibrous collagen molecules of different size. Individual collagen molecules are organized into *fibrils* which may in turn form larger *fibers.* We use the term *fibril* to denote collagen with a diameter on the order of ~10–50 nm and the term *fiber* for any collagen larger than this.

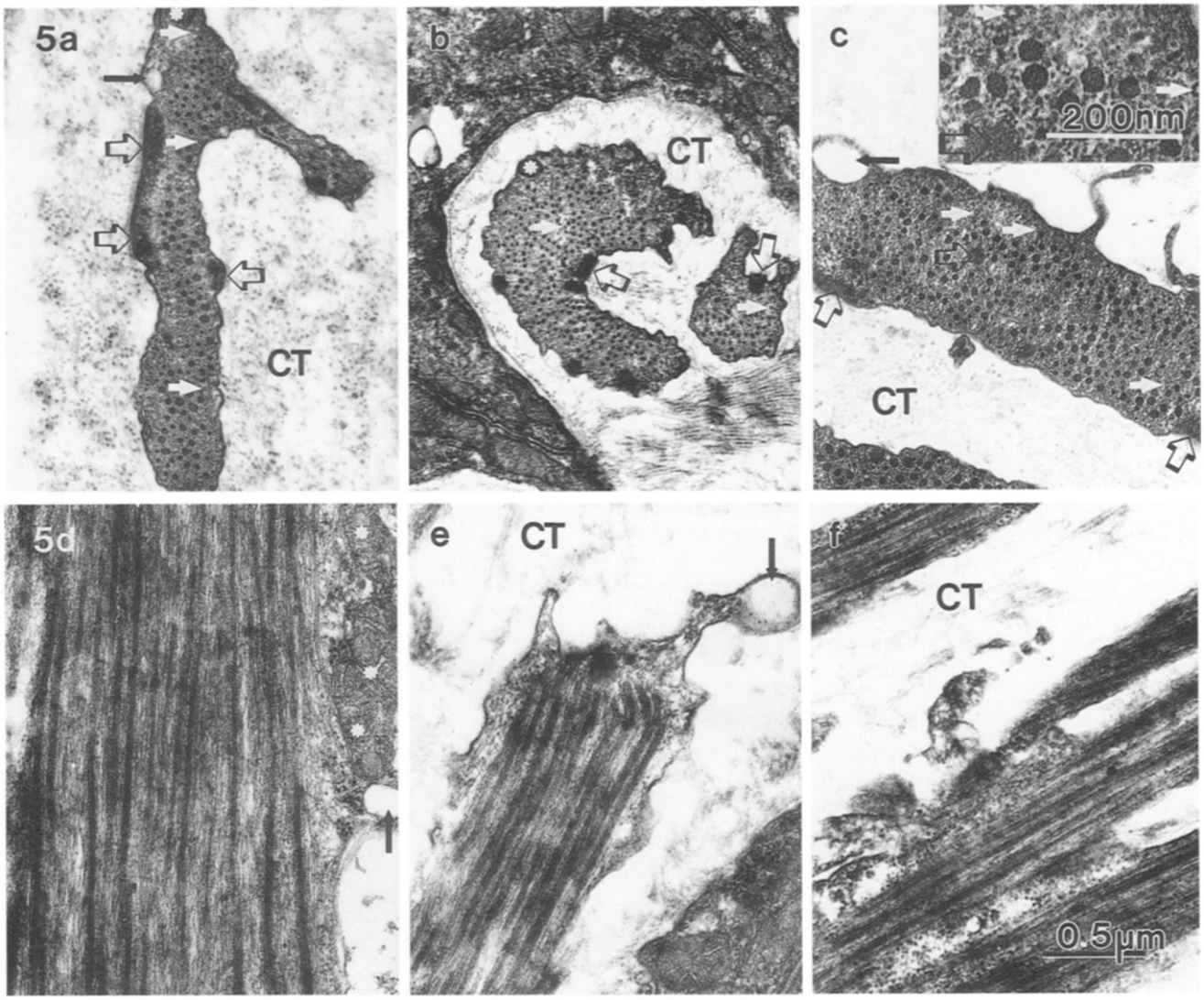

Fig. 5 Sections of muscle fibers from (a, d) *D. polymorpha,* (b,e) *C. fluminea,* and (c,f) *T. texasensis.* TEM. **(a–c)** Cross sections of muscle fibers from the three species. The muscle fibers show densely packed thick and thin filaments with dense bodies (open arrows), peripheral mitochondria (asterisks), scanty sarcoplasmic reticulum (black arrows), and microtubules (white arrows). The inset in (c) is an enlargement of the fiber to show the structure of microtubules. **(d-f)** Longitudinal sections of muscle fibers from the three species. Muscle fibers are always surrounded by a connective-tissue matrix (CT). (0.5 μm scale bar is for a–f).

Paniagua et al. 1996). We conclude here that the fibers in all three species are not obliquely striated, but are more typical of molluscan smooth muscles, based on the arrangement of the electron-dense bodies and on the appearance of the fibers in cross section. Obliquely striated muscles have sarcomeres aligned at a small angle with respect to the fiber long axis, as may be indicated by electron-dense body arrangement (Rosenbluth 1972; Paniagua et al. 1996). Cross sections of the fibers often contain distinct regions representing H bands, I bands, and A bands simultaneously (Rosenbluth 1972; Paniagua et al. 1996). The muscle fibers of the species studied here show no apparent organization between sarcomeres, as the electron-dense bodies are peripheral or occasionally inside the muscle, with no clear organization. Cross sections of the muscles always contain both thick and thin filaments together and do not show organized sarcomeric zones.

These muscle fibers correspond to Matsuno's (1987) invertebrate smooth muscle type B: peripherally-placed cytoplasmic organelles, thick filaments ~40 nm in diameter, disordered electron-dense bodies, and scanty sarcoplasmic reticulum. This type of muscle cell is found in other molluscan species, as well as in echinoderms, coelenterates, and oligochaetes (Matsuno 1987; Paniagua et al. 1996). According to Matsuno

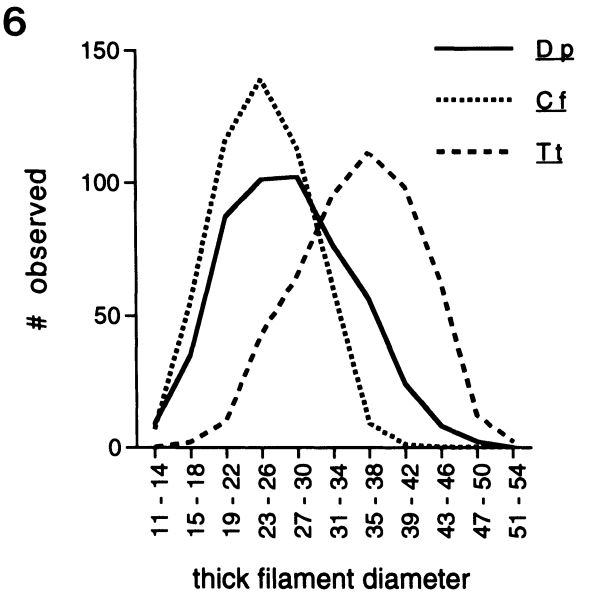

Fig. 6 Distribution of thick filament diameter in 4 nm groupings (n = 500). The thickest part of the filament is just over 50 nm in both *D. polymorpha* and *T. texasensis,* but is ~40 nm in *C. fluminea.* The minimum diameter is ~11 nm in each of the species, and the mean diameters are 28 nm in *D. polymorpha,* 25 nm in *C. fluminea,* and 35 nm in *T. texasensis.* These dimensions are consistent with thick filaments 40–50 nm in the middle, tapering to ~11 nm at the ends, for each of the three species.

(1987), these are the most prevalent muscles in the motile organs of molluscs and echinoderms, where they provide body support and movement. The similarity in muscle type among the three bivalve species we studied is interesting, given the significant differences in invertebrate muscle structure within species (Morrison & Odense 1974; Matsuno 1987; Matsuno 1988; Matsuno & Kuga 1989; Matsuno et al. 1993; Royuela et al. 1995) and between species (Morrison & Odense 1974; Matsuno 1987; Paniagua et al. 1996).

The gross organization of the gills follows a general pattern with some differences among species. Overall, more anatomical similarity exists between the most closely related animals, *Corbicula fluminea* and *Dreissena polymorpha.* Nevertheless, the gills of all three species are constructed from very similar collagenous material and smooth muscle, suggesting a conserved function. Early authors proposed that the integral gill muscles work in a coordinated fashion with the ciliated cells of the bivalve gill (Yonge 1926; Elsey 1935; Atkins 1943; Nelson 1960). We have recently demonstrated that the active gill musculature in unionid and dreissenid bivalves responds to neurotransmitters and is capable of altering the dimensions of the water passageways in excised gills (Gardiner et al. 1991; Medler & Silverman 1997). Similar movements have also been observed in the gills of *C. fluminea* and *T. texasensis* (Medler, unpubl. obs.). The biological significance of the muscles and associated connective tissue may lay in the regulation of water flow through the gill (Gardiner et al. 1991; Tankersley 1996; Medler & Silverman 1997), but further study is needed to unravel all of their functions.

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