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FUNCTIONAL ORGANISATION OF THE METATHORACIC FEMORAL CHORDOTONAL ORGAN IN THE CRICKET *ACHETA DOMESTICUS*

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Summary

The metathoracic chordotonal organ of the cricket *Acheta domesticus* (Gryllidae) consists of two closely associated scoloparia situated proximally within the femur with their distal ends connected by a pair of ligaments to an apodeme arising from the tibia. The smaller scoloparium is associated with the dorsal ligament, which arises from the dorsal surface of the apodeme 150 μm from its proximal end. The larger scoloparium is attached directly to the proximal end of the apodeme by a larger ventral ligament. Both ligaments are composed of bundles of attachment cells containing densely packed microtubules. Longitudinally orientated, Acid-Fuchsin-staining fibrils are found in an extracellular matrix surrounding the individual attachment cells. Similar fibrils occur in the sheath surrounding each ligament. The fibrils are thickest and most densely packed in the sheath surrounding the ventral ligament. They are thinner and more sparsely distributed in the sheath of the dorsal ligament. The finest fibrils are found in the extracellular matrix surrounding individual attachment cells. Staining with phosphotungstic acid provides the first evidence that they are elastic. The ventral ligament also contains a spring-like cuticular core arising as a proximal extension of the apodeme. As femoro-tibial angle changes, the cuticular core changes in length, shortening with tibial extension and lengthening with flexion. Ventral ligament attachment cells terminate at different levels along the cuticular core. This arrangement provides a new possible mechanism for differential sensitivity of the sensory neurones associated with the attachment cells.

Key words: chordotonal organ, cricket, metathoracic leg, *Acheta domesticus*.

Introduction

The insect metathoracic femoral chordotonal organ (mtFCO) provides sensory input associated with rotatory movements of the tibia with respect to the femur. It is situated some distance from the femoro-tibial joint and is connected to it by ligaments inserted upon a cuticular apodeme arising from the base of the tibia. Recent studies on the mtFCO in *Locusta migratoria* (Field, 1991) and *Schistocerca gregaria* (Shelton *et al.* 1992) have shown that the ligaments of this system have a complex functional anatomy. Although it has previously been described as a single structure (e.g. Usherwood *et al.* 1968), recent work shows that the locust apodeme complex is composed of two closely associated ligaments whose structure and movements during joint rotation have clear functional significance.

To reach a full understanding of the mechanism for encoding changes in joint angle, the functional anatomy and mechanical properties of the ligament system need to be described in full, since they determine the ways in which signals related to joint rotation are transmitted to the proprioceptor. A comparative study of a range of insects is of interest because of the considerable anatomical differences in mtFCO structure from species to species. By adopting this approach, the general properties underlying mtFCO ligaments can be determined and separated from those that are peculiar to particular species or patterns of leg movement such as walking and jumping.

Chordotonal organ ligaments are composed of so-called attachment cells that arise distally from a cuticular insertion and are attached at their proximal ends to the dendrites of the sensory neurones (e.g. Yack and Roots, 1992). In locusts, the mtFCO dorsal ligament arises from the proximal end of the apodeme and consists of a bundle of attachment cells that becomes completely slack when the femoro-tibial angle (FTA) increases above approximately 30–35 ° (M. Walker and P. M. J. Shelton, unpublished observations). The ventral ligament is

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formed from a series of separate bundles of attachment cells with their outer origins at increasingly distal levels on the side of the apodeme. As the tibia is extended, the separate bundles slacken off in a proximo-distal sequence, with only the most distally arising bundle of attachment cells remaining taut when the tibia is fully extended (Field, 1991; Shelton *et al.* 1992). Consequently, the separate bundles of attachment cells in the ventral ligament are always under different degrees of tension. Because there is tension in some parts of the ventral ligament at all joint angles but the dorsal ligament becomes completely slack during extension, the two ligaments move differentially and the dorsal one buckles to form a distinct loop which is very obvious at joint angles greater than 90 °. The arrangement described provides a way of varying the mechanical input to different parts of the mtFCO because, as the tibia undergoes flexion, the number of attachment cells that are under tension increases. The functional role of this sequential recruitment of attachment cells in the ligament system is not yet clear. It has been suggested that it provides a mechanism for range fractionation (Field, 1991; Shelton *et al.* 1992), but there could be additional functional consequences for an arrangement such as this.

Since no similar organisational pattern has been described for femoro-tibial chordotonal organs in other species, we wished to know whether this system is peculiar to locusts or whether there is evidence for sequential recruitment of attachment cells in other orthopterans. In the present study we have examined the mtFCO in a cricket (*Acheta domesticus* L.) which has also developed a system for the differential tensioning of attachment cells and, by implication, subsequent stimulation of the receptor units. However, the mechanism in *A. domesticus* is quite different from that found in locusts. In the cricket, the dorsal and ventral ligaments are anatomically different and the ventral ligament has a substructure that may have a function analogous to that of the loop-forming region of the locust apodeme complex. The fact that locusts and crickets have evolved different mechanical systems for differential tensioning of attachment cells suggests that differential tensioning may be of more widespread occurrence.

There are a number of ways that ligament behaviour could affect sensory output. For example, the viscoelastic properties of the apodeme complex are likely to have an important role in determining rates of sensory adaptation of individual units (Theophilidis, 1986) and may provide an explanation for the observed hysteresis in sensory discharges from the mtFCO (Zill and Jepson-Innes, 1988; Matheson, 1990). The elastic properties of the ligaments will determine the way in which mechanical power is transmitted to the receptor cell dendrites.

Materials and methods

Acheta domesticus L. of either sex were obtained from a laboratory colony maintained at 22 °C. Metathoracic legs were removed from adults anaesthetised with CO_2. To observe the behaviour of the mtFCO in fresh unfixed preparations, legs were attached to a glass slide using a mixture of Plasticine and poster putty (Blu-tak). A 2 mm×5 mm window was first cut through the posterior femur cuticle at the level of the mtFCO. A second similar window was made in the anterior femur so that the chordotonal organ could be seen. Once exposed, the tissues were kept moist with an insect saline solution (NaCl, 140 mmol l^{-1}; KCl, 10 mmol l^{-1}; $NaHCO_3$, 4.0 mmol l^{-1}; $Na_2HPO_4{\cdot}2H_2O$, 6.0 mmol l^{-1}; $CaCl_2{\cdot}6H_2O$, 6 mmol l^{-1}; adjusted to pH 6.8 with 1 mol l^{-1} NaOH). The muscles and connective tissues were carefully removed to expose the organ. For routine microscopy, transmission and scanning electron microscopy, the dissected leg was immobilized flexed (femoro-tibial angle 0–10 °), half extended (80–90 °) or fully extended (160 °), and then fixed for 2–4 h in Karnovsky's (1965) fixative and post-fixed for 1–2 h in 1 % phosphate-buffered (Hale, 1965) osmium tetroxide at pH 7.4. To remove the cellular components from the cuticular core of the chordotonal organ before scanning electron microscopy, the dissected organ was placed into a 10 % KOH solution and gently warmed. It was then fixed in osmium tetroxide as above. Specimens were dehydrated in a graded acetone series. Those prepared for scanning electron microscopy were critical-point-dried, sputter-coated and examined with a Cambridge S-100 microscope. Specimens prepared for light and/or transmission electron microscopy were cleared in propylene oxide after dehydration, infiltrated with Araldite and polymerized. Semi-thin (1 μm) sections were mounted on subbed slides (0.1 % gelatin; 0.01 % chrome alum) and stained with Toluidine Blue (1 % in 1 % borax) or aqueous Acid Fuchsin. Ultrathin sections were stained routinely with aqueous uranyl acetate and lead citrate, and examined using a Jeol 100CX electron microscope (Jeol Ltd, Colindale, London, UK). Light micrographs were taken on Pan F film with either a Zeiss Photomicroscope II or an Olympus PM-10ADS photomicroscope using bright-field, polarized light or Nomarski interference microscopy. Freshly dissected legs (N=20) were used to observe displacements of the mtFCO ligaments and the ventral ligament core during flexion and extension of the tibia. In some, the dissected organs were sprinkled with fine carborundum powder (to establish recognizable markers) and a series of static micrographs was taken of the organ through a Zeiss Photomicroscope as the femoro-tibial angle was changed from 0 to 160 ° in increments of 10 °.

Results

General anatomy

The general arrangement of the organ and its associated ligaments is summarised in Fig. 1. As in all other insect femoral chordotonal organs studied so far, the sensory cells of the cricket mtFCO are contained in two scoloparia. In this case, they are very closely associated and form a fused structure (Fig. 2A). Their location at the most proximal level of the femur is similar to the location of femoral chordotonal organs in the pro- and mesothoracic legs of the locust (Acrididae) (Bräunig, 1985) and in the pro- meso- and metathoracic legs of *Decticus albifrons* (Tettigoniidae) (Theophilidis, 1986).

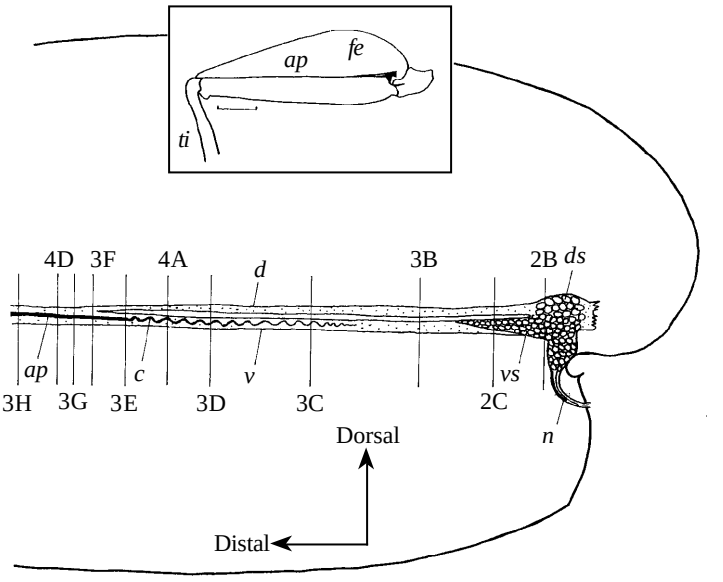

Fig. 1. This semi-schematic diagram shows some of the main anatomical features of the metathoracic femoral chordotonal organ. The dorsal (*ds*) and ventral (*vs*) scoloparia are fused and attached to the anterior femur wall proximally (to the right). The dorsal ligament (*d*) is slightly thinner than the ventral ligament (*v*). The latter contains a tapering cuticular core (*c*) that buckles on tibial extension and is formed by a proximal extension of the apodeme (*ap*). The chordotonal organ nerve (*n*) leaves the organ ventrally. The inset shows the position of the organ within the femur (*fe*) and the long apodeme (*ap*) that connects it to the tibia (*ti*). Labelled vertical bars on the main figure show appoximate levels from which subsequent figures were taken. Main diagram not to scale. Scale bar of inset, 2.0 mm.

Located in this way, the cricket mtFCO differs from that of the locust, where it occupies a much more distal position (Slifer, 1935; Field and Burrows, 1982; Bräunig, 1985). Distally, the scoloparia are connected to the tibia by an apodeme complex that runs the whole length of the adult cricket femur (7.5–9 mm) (Fig. 1).

The apodeme arises from the tibia and is a stiff inextensible structure projecting proximally over most of the length of the femur. It lies just beneath the cuticle of the anterior face of the femur between the main extensor and flexor muscles. At all levels, transverse sections through the apodeme reveal a similar structure consisting of a flattened cuticular tube surrounded by the epidermal cells that secrete it (Fig. 3H). It has a similar cross-sectional area at all levels. At its proximal end, ventral and dorsal ligaments join the apodeme to the two scoloparia (Figs 1, 2A). The ventral ligament arises from the end of the cuticular apodeme, whereas the dorsal ligament arises about 150 μm more distally from the dorsal side of the apodeme (Fig. 3A). The ventral ligament is visibly thicker (28.05±0.54 μm diameter) than the dorsal one (20±0.77 μm diameter) (N=12). A cuticular core (described later; Fig. 3A,C,D) within the ventral ligament makes it stiffer than the dorsal ligament.

The scoloparia

The two scoloparia are fused at their most proximal ends and separate into discrete dorsal and ventral structures distally, where they are attached to the dorsal and ventral ligaments (Figs 1, 2A). From counts of cell bodies in osmium-stained whole mounts, it is estimated that there are at least 100 cells in the fused structure. Proximally, the scoloparia are closely attached to the cuticle of the femur by bands of connective tissue. Although the scoloparia are very closely associated, they can be separated by dissection of lightly fixed material. A series of semi-thin sections through the organ revealed a distinct boundary between a small dorsal scoloparium and a larger ventral one (Fig. 2B). The latter contains many more neurones, some of which form a bulbous ventral projection (Fig. 1). Neurones of the ventral scoloparium extend further distally along its ligament than those of the dorsal scoloparium. The ventral edge of this distally extending group of neurones runs across the ventral ligament at an angle. Consequently, the most dorsal cells of this group are located more distally than the ventral ones and the proximo-distal location of cells in this group depends upon their dorso-ventral position with respect to the ligament (Figs 1, 2A).

The cell bodies of neurones in the two scoloparia are anatomically distinct in sectioned material. Those of the ventral scoloparium are smaller (13–16 μm in diameter), more darkly staining, contain smaller nuclei and are surrounded by a heavy sheathing, which Young (1970) identifies as belonging to Schwann cells. The neuronal cell bodies of the dorsal scoloparium are larger (15–25 μm in diameter), paler staining and have little sheathing around the somata (Fig. 2B).

Neurones often appear to be arranged in pairs in each scoloparium, and distally, at the level where the neurones join the attachment cells, each pair is connected by their two dendrites to a single scolopale unit. In transverse sections, the paired ciliary dendrites can be seen surrounded by single scolopale cells (for terminology, see Young, 1970). The scolopale units of dorsal scoloparium neurones all appear to be situated at the same proximo-distal level of the dorsal ligament (Fig. 2C). However, the scolopale units of the ventral scoloparium are not localised but are distributed over a considerable length of the ventral ligament.

The dorsal and ventral ligaments of the apodeme complex

Just distal to the scoloparia, the two ligaments have a similar structure consisting of attachment cells and associated extracellular components (Figs 3B, 4A). Like the attachment cells described in other chordotonal organs, electron microscopy reveals that they are filled with densely packed

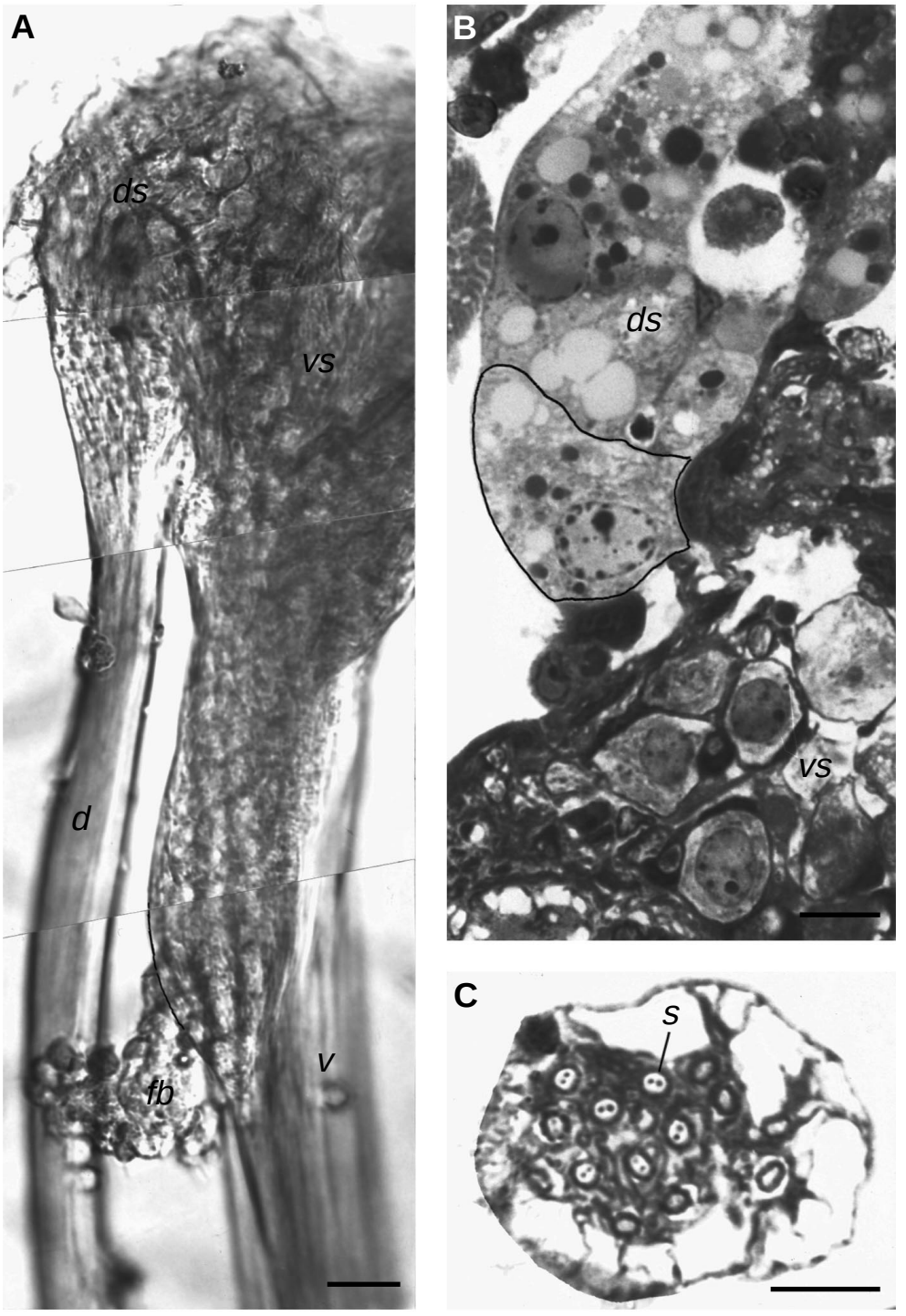

Fig. 2. (A) Nomarski interference micrograph of the scoloparia showing neurones associated with the dorsal (*d*) and ventral (*v*) ligaments. Somata of the dorsal scoloparium (*ds*) are restricted to a region at the proximal end of the ligament, while those of the ventral scoloparium extend further distally (*vs*). A group of fat body cells (*fb*) lies close to the organ but is not part of it. Dorsal towards the left. (B) Semi-thin section through the fused scoloparia showing the large somata of dorsal scoloparium (*ds*) neurones (one outlined) and the smaller somata of those in the ventral scoloparium (*vs*). The different staining properties of the two types of neurone create a distinct boundary between the two scoloparia. (C) A section through the dorsal ligament at the level where pairs of dendrites from the dorsal scoloparium are inserted into scolopale units (*s*) of the dorsal ligament. (B and C dorsal towards top of page). Scale bars, A, 20 μm; B, C, 10 μm.

microtubules (Fig. 4C). Each cell is surrounded by fine fibrils (Fig. 3B) lying in an amorphous matrix (Fig. 4C). Similar extracellular material forms a sheath surrounding each ligament. Both the microtubules and the extracellular fibrils are orientated longitudinally with respect to the attachment cells. The fibrils stain pink with Acid Fuchsin and are variable in size: the thickest (up to 1.2 μm in diameter) are those forming the sheath of the ventral ligament; narrower (0.5–1.0 μm) fibrils surround the dorsal ligament. In both dorsal and ventral ligaments, the fibrils are noticeably thicker in proximal regions than in distal ones (Fig. 3B–D). The finest (0.05–0.3 μm) fibrils surround each individual attachment cell (Fig. 3B–D). There is also a marked difference in the spacing of fibrils in the sheaths surrounding the two ligaments. Those surrounding the dorsal ligament are fairly widely spaced, while those surrounding the ventral ligament are very close together, forming a more or less continuous layer (Fig. 3B–D). In electron micrographs

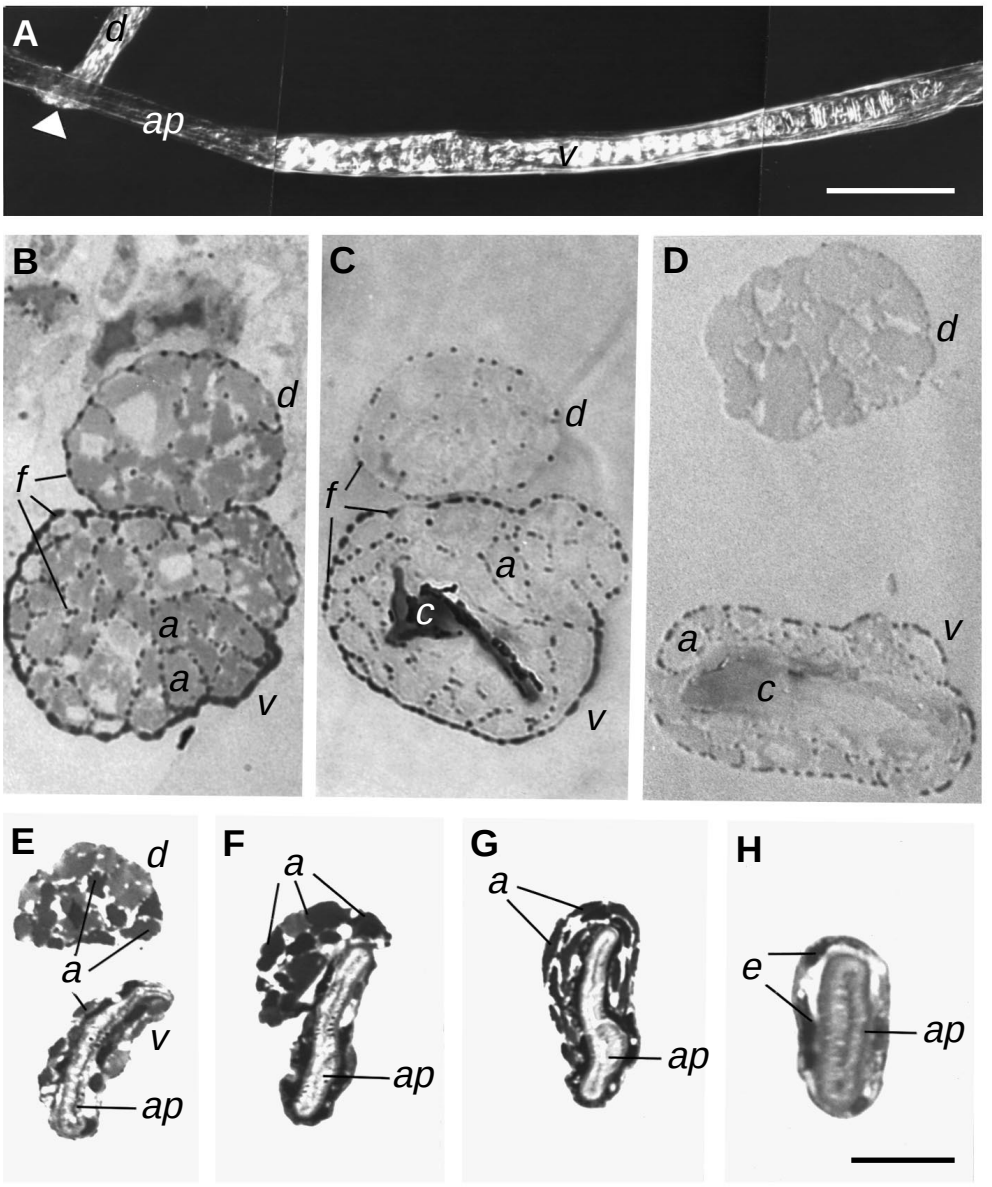

Fig. 3. (A) Nomarski interference micrograph of the apodeme complex with the tibia in the extended position. The apodeme (*ap*) is formed from a stiff rod of cuticle which extends as a pleated strip to form the core of the ventral ligament (*v*). The dorsal ligament (*d*), detached from its scoloparium at its proximal end, is distally attached to a point some distance from the end of the apodeme (arrowhead). Distal is to the left. (B–H) Oil immersion micrographs of transverse semi-thin sections through the apodeme complex. (B–D) Sections at progressively more distal levels stained with Acid Fuchsin to show the extracellular fibrils (*f*) and cuticular core (*c*) surrounded by the attachment cells (*a*). Fibrils in both dorsal (*d*) and ventral (*v*) ligaments are thinner and more widely spaced distally. (E–H) Sections stained with Toluidine Blue to show attachment cells (*a*) and the apodeme (*ap*) at increasingly distal levels. In E, the dorsal ligament (*d*) is still separate from the apodeme, and the attachment cells of the distal ventral ligament (*v*) are closely associated with the cuticle of the apodeme. In F, the dorsal ligament joins the apodeme. In G, the apodeme is surrounded by dorsal ligament attachment cells. H shows the apodeme surrounded by epidermal cells (*e*) distal to both ligaments. Scale bars, A, 100 μm; B–H, 10 μm.

stained with uranyl acetate and lead citrate, all of these extracellular fibrils have an amorphous appearance similar to those previously described for the locust mtFCO (Shelton *et al.* 1992) (Fig. 4A–D). In an attempt further to characterize the fibrils, some specimens were fixed in glutaraldehyde and stained with phosphotungstic acid. After such treatment, they appear very electron-dense under the transmission electron microscope (Fig. 4B) and similar to the types of fibril found in insect connective tissues noted for their elasticity (Locke and Huie, 1972).

The attachment cells of both the ventral and dorsal ligaments are fusiform, being broad in the centre (Fig. 3B) and narrow at their distal points of insertion onto the epidermal cells surrounding the cuticular apodeme (Fig. 3E–G). They also taper proximally in the region of the sensory cells where they join the scolopale units (not shown).

While the dorsal ligament is similar along its whole length and is entirely formed from attachment cells and associated fibrils (Figs 3B–D,F, 4A), the more distal region of the ventral ligament also contains a cuticular core (Figs 3C,D, 4D), a modified proximal extension of the apodeme (Fig. 3E–H). The presence of the cuticular core clearly has some functional significance.

The cuticular core of the ventral ligament

The spring-like core surrounded by attachment cells is easily visualised in fresh material using either bright-field or Nomarski interference microscopy. In such preparations, this structure is very obvious in legs where the tibia is extended (see Fig. 5A,B), but only its most proximal tip can be seen when it is flexed (see Fig. 5C,D).

In longitudinal sections of the fixed apodeme, the core is most clearly visible when stained with Acid Fuchsin, which stains the cuticle and the extracellular fibrils associated with

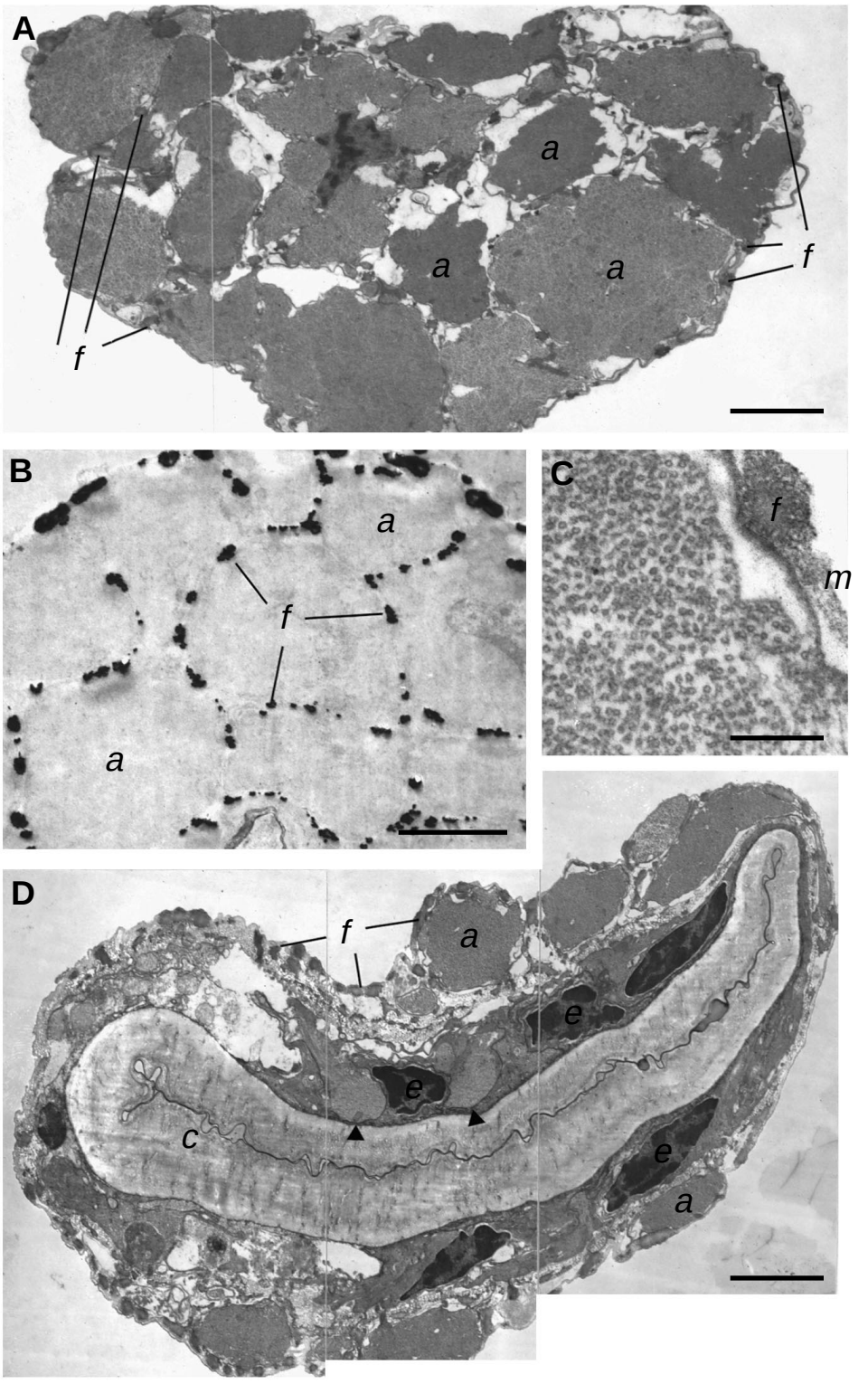

Fig. 4. (A) Transmission electron micrograph (TEM) of a transverse section through the dorsal ligament showing attachment cells (*a*) and extracellular fibrils (*f*). (B) TEM using phosphotungstic acid as a stain for elastic fibrils. All the extracellular fibrils (*f*) stain darkly but the attachment cells (*a*) are unstained. (C) High-magnification TEM showing the attachment cells to be filled with densely packed longitudinally disposed microtubules. An elastic fibril (*f*) is present in the extracellular matrix (*m*). (D) TEM of a transverse section through the distal end of the ventral ligament. The cuticular core (*c*), the epidermal cells (*e*) which secreted it and attachment cells (*a*) are visible. Two attachment cells between adjacent epidermal cells are closely associated with the core (arrowheads). The extracellular sheath contains typical fibrils (*f*). Scale bars: A, B, D, 2 μm; C, 0.2 μm.

attachment cells. Cytoplasm is less heavily stained. Such preparations confirm that the core is cuticular in nature and show that it arises as a proximal continuation of the apodeme. It is thickest at its point of origin where it joins the apodeme proper and it tapers towards the proximal tip, where it is very thin (Fig. 6A).

Transmission electron micrographs through the cuticular core of the ventral ligament reveal attachment cells terminating along its length (Fig. 4D). The attachment cell terminals interdigitate with epidermal cells at the cuticular surface and the cell membranes in these regions are noticeable for their large number of septate desmosomes (not shown). Counts of the number of attachment cell profiles in electron micrographs of transverse sections were made at different levels along the

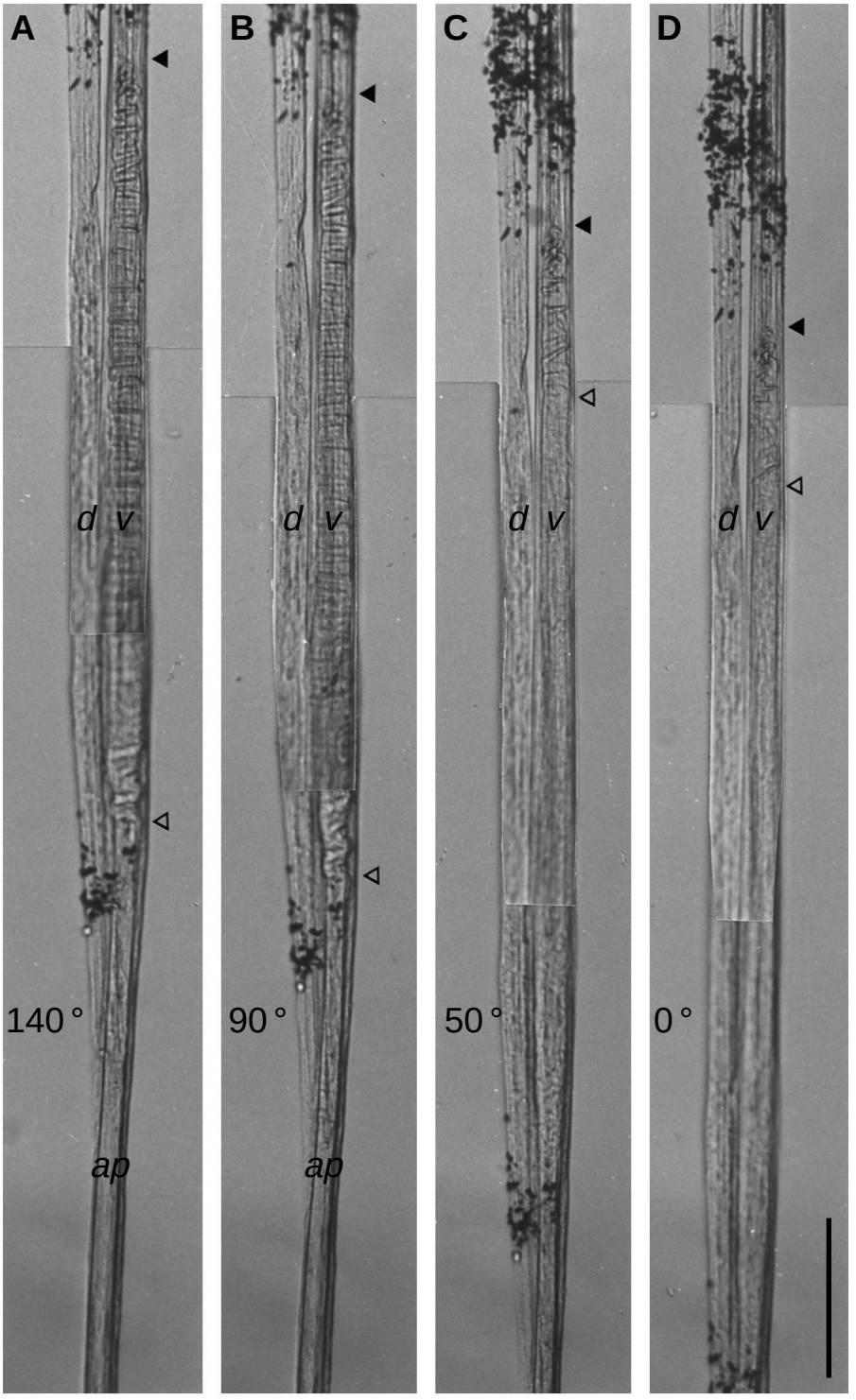

Fig. 5. (A–D) Fresh preparations of the apodeme complex photographed at femorotibial angles of 140 ° (A), 90 ° (B), 50 ° (C) and 0 ° (D) showing increasing stretch with tibial flexion of the dorsal (*d*) and ventral (*v*) ligaments. Also shown is the disappearance of cuticular core pleats of the ventral ligament. In A and B, the core is pleated from its junction (open arrowhead) with the apodeme (*ap*) to its proximal tip (filled arrowhead). As flexing continues, unfolding occurs and the pleats disappear (in C and D). The distal-most point where pleating is visible is marked with open arrowheads. The black grains are carborundum particle markers used for measurements of displacements. (Orientations as in D.) Scale bar, 100 μm.

cuticular core. They show that the number of attachment cells increases proximally (Fig. 7).

The appearance of the fixed cuticular core depends upon the degree of tibial flexion. In preparations preserved with the tibia fully or partially extended, it is buckled along its entire length (Fig. 6A). As flexion of the tibia increases, the buckled core straightens. In fresh preparations, this straightening process can be seen to begin at the most distal level of the core and gradually to spread proximally with increasing flexion. In preparations fixed with the tibia in the fully flexed position, most of the core is straight, although there may still be some folds at the extreme proximal tip (Fig. 6B).

The appearance of the unfixed ligaments and apodeme when viewed using crossed polarising filters also demonstrates that the spring-like core of the ventral ligament unfolds with increasing flexion. The apodeme is strongly birefringent at all

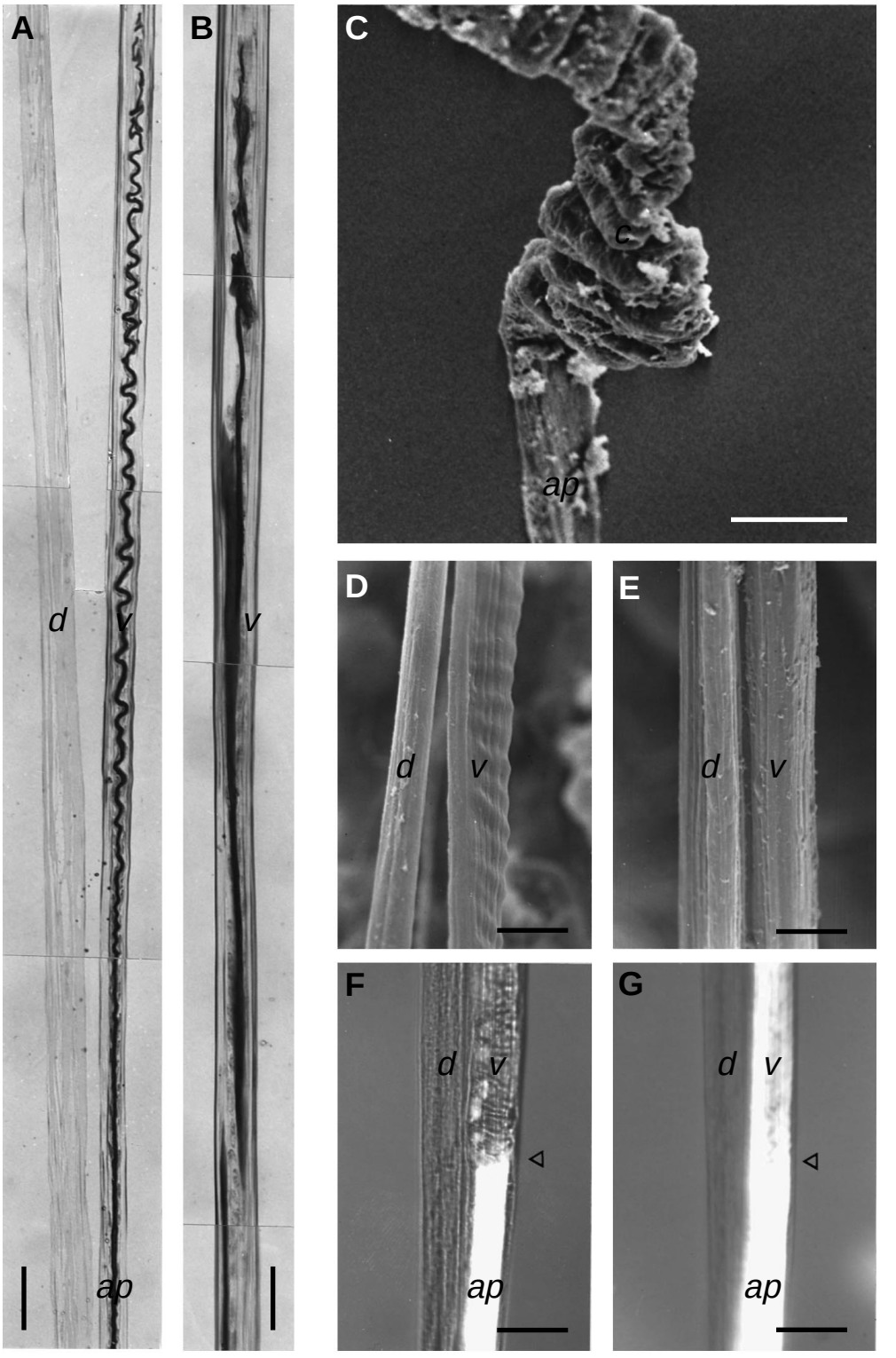

Fig. 6. (A,B) Longitudinal sections through the apodeme complex with the tibia fixed extended (A) and flexed (B), stained with Acid Fuchsin. The straight apodeme (*ap*) continues through the ventral ligament (*v*) as a ribbon of buckled cuticle in A, while in B, the ribbon is straight except at its proximal tip. (The discontinuity in the distal portion is an artefact resulting from sectioning a strip that has a curved face.) Dorsal is to the left. (C–E) Scanning electron micrographs of portions of the apodeme complex. In C, the cellular elements have been dissolved, to reveal the apodeme (*ap*) and its pleated extension, the core (*c*) of the ventral ligament. In D and E, the legs were fixed with the tibia extended (D) or flexed (E). The fibrous sleeves of the dorsal (*d*) and ventral (*v*) ligaments are visible in both cases. (F,G) Fresh preparations of the apodeme complex in legs with the tibia completely extended (F) and fully flexed (G), photographed using crossed polaroids. Arrowheads mark the junction between the birefringent apodeme (*ap*) and the cuticular core (*c*) in the ventral ligament (*v*). (All micrographs orientated as in A.) *d*, dorsal ligament. Scale bars, 25 μm.

femoro-tibial joint angles, but the birefringence of the ventral ligament varies with joint position in a way that is consistent with the anatomical changes of the cuticular core as described above. When the tibia is flexed (femoro-tibial angle 0–10 °), the core-containing region of the ventral ligament is highly birefringent (Fig. 6G); when it is partially or fully extended, it loses its birefringent properties (Fig. 6F). In contrast, the dorsal ligament, which lacks a cuticular core, is much less birefringent in both the flexed and extended conditions.

We examined the apodeme complex using scanning electron microscopy. In whole-mount preparations made with the leg fixed in the extended position, the internal buckled core can be seen to distort the surface of the sleeve of attachment cells surrounding it (Fig. 6D). The distortion disappears when the

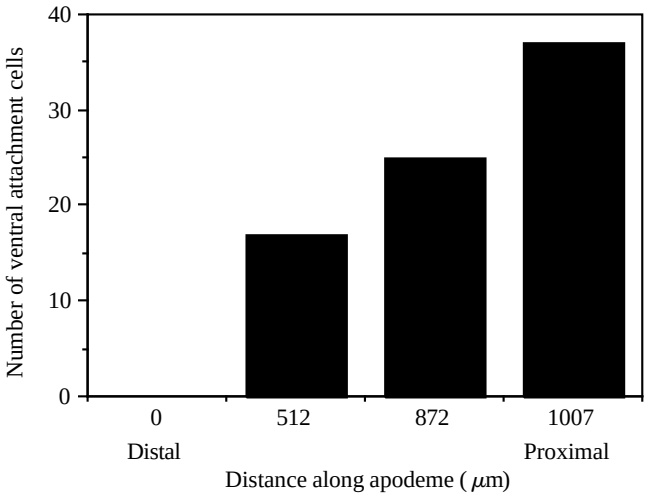

Fig. 7. Bar graph showing the number of attachment cells in the ventral ligament at four levels in the apodeme complex. Data were obtained from electron micrographs of transverse sections though the apodeme and ventral ligament. The first point is the most distal and provides a reference from which the positions of the other more proximal sections were measured. It is from a section through the apodeme just distal to the ventral ligament insertion and here there are no attachment cell profiles. At increasingly proximal levels there are increasing numbers of attachment cells within the ventral ligament so that 1007 μm proximal to the reference level there are 37 cells.

tibia is flexed (Fig. 6E). The outer layer is visibly fibrous in scanning electron micrographs and, even when the internal cuticular structure is buckled, the superficial fibres are still strongly aligned proximo-distally, with no sign that the surface layers of the ligament buckle. This implies that the tissues surrounding the cuticular core are highly elastic and able to shorten as the core buckles. This is consistent with observations on living and fixed material showing that the surface of the ligament remains relatively smooth even though the internal core is buckling (Figs 3A, 6A) and with transmission electron microscopic observations on the attachment cells surrounding the cuticular core (Fig. 8A). In addition, longitudinal sections stained with Acid Fuchsin show that the elastic fibrils within the ventral ligament are straight when the core is buckled (Fig. 8B). In KOH-treated specimens where the soft tissues have been removed, the isolated cuticular core assumes its buckled configuration (Fig. 6C). In contrast, the apodeme itself does not buckle after KOH treatment, and in fresh specimens or those fixed for transmission electron microscopy it is never buckled even when the tibia is fully extended.

The cuticular core of the ventral ligament arises abruptly from the end of the apodeme and is anatomically quite distinct. Scanning electron micrographs show that the core does not have a coil-like organisation but consists of a flattened ribbon of cuticle tightly folded in a pleated manner (Fig. 6C).

The lack of other connections of the mtFCO with muscle, connective tissue or skeletal elements

In the mtFCO of the locust, the scoloparia are not only connected to the tibia by the apodeme complex; there are also two indirect connections. First, there is a ventral attachment connecting the mtFCO to the flexor tibiae muscle (Usherwood *et al.* 1968) and, second, a strand organ closely associated with the mtFCO joins the apodeme of the flexor tibiae muscle at a more distal level (Field and Burrows, 1982). As well as attachments to such moving components, the dorsal ligament of the locust apodeme complex is attached to the femoral exoskeleton by an unloading strand (Field, 1991) also referred to as the guy-rope fibres (Shelton *et al.* 1992). None of these other attachments is present in the cricket mtFCO.

Measurement of displacements in the ligament system

To investigate the role of the cuticular core in the behaviour of the ventral ligament, we measured static displacements at various levels in the system after the tibia had been moved in 10 ˚ increments throughout the possible range of joint movement (femoro-tibial angles of 0–160 ˚) (Fig. 5). Measurement of the displacement from the fully flexed position (0 ˚) of the proximal end of the apodeme during extension shows that up to a femoro-tibial angle of about 100 ˚, the apodeme is displaced proximally with increasing femoro-tibial angle. There is more or less linear displacement with angle over the range 40–100 ˚. However, at angles greater than 100 ˚ the apodeme ceases to move proximally, and at angles greater than 120 ˚ begins to move distally (Fig. 9). This is because of the eccentric attachment (dorsal) of the apodeme relative to the fulcrum of the femoro-tibial joint. In a typical specimen (femur length 8 mm), the proximal end of the apodeme undergoes a maximum proximo-distal excursion of 413 μm over the physiological range of joint rotation.

Changes in the length of the core with femoro-tibial angle were obtained by noting the positions of its proximal and distal extremities as joint angle was changed between 0 ˚ and 160 ˚. Up to 100 ˚, core length decreases, with shortening being approximately linear over the range 40–100 ˚. At larger angles, the core ceases to shorten and begins to lengthen slightly (Fig. 9). From its minimum length to its maximum there is a 32 % increase in length (from 669 to 884 μm) in a typical specimen (femur length 8 mm). These core length changes can be directly correlated with the pattern of apodeme displacement described above. They show that the spring expands and contracts over the whole proximo-distal displacement range of the apodeme.

To determine whether the two ligaments move differentially, displacements of carborundum particle markers on the surfaces of both ligaments were observed during extension and flexion of the tibia. Absolute displacements of markers were measured both distal and proximal to the level of the core in the ventral ligament and at equivalent levels in the dorsal ligament. The general pattern of displacement is similar at both levels in each of the ligaments (Fig. 10). However, displacements of proximal markers are much smaller than those that are distal to the core. This is because both scoloparia are attached to the proximal femur and both ligaments are elastic.

At femoro-tibial angles larger than 70 ˚, rotation of the joint

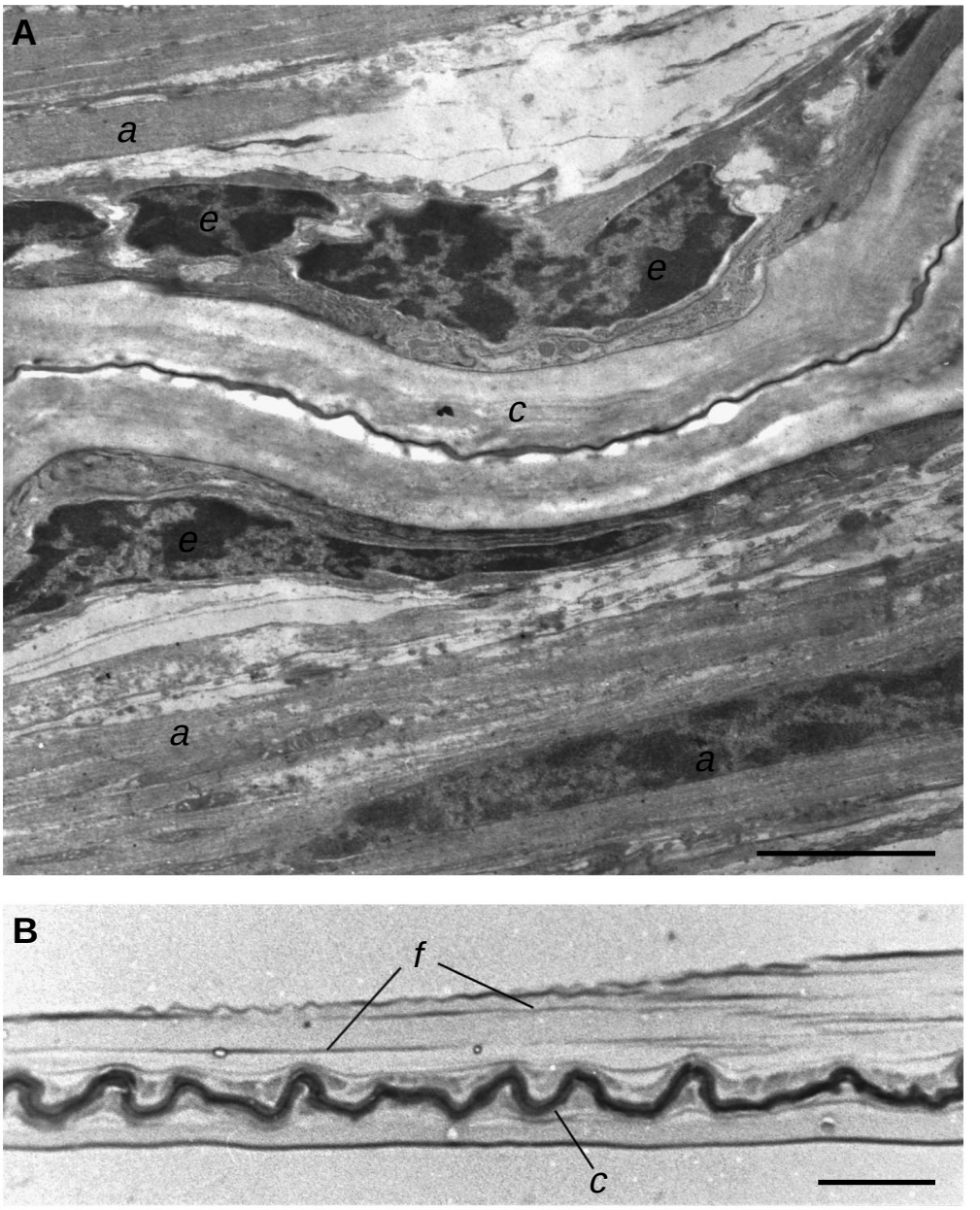

Fig. 8. Transmission electron micrograph (A) and light micrograph (B) of longitudinal sections through the apodeme complex. (A) This section, taken half way along the cuticular core (*c*) of the ventral ligament from a leg in which the tibia was extended shows that the core is folded but that the attachment cells (*a*) are not. Epidermal cells (*e*) are associated with the core and bend with it. (B) This semi-thin section stained with Acid Fuchsin shows the buckled core (*c*) surrounded by unbuckled fibrils (*f*). (Distal is to the left in A and B.) Scale bars, A, 2.0 μm; B, 15.0 μm.

results in displacements of similar magnitude in both ligaments. This is true both proximal and distal to the core. At angles between 0 and 70 ˚, displacements in the ventral-core-containing ligament are larger than in the dorsal ligament (Fig. 10). This shows that over the part of the range where the core is extending to its maximum length, there is demonstrable differential displacement of the two ligaments.

Discussion

The mtFCO in *Acheta domesticus* is composed of two scoloparia. In other femoral chordotonal organs there is a similar situation with a varying degree of fusion between the two organs. In the locust, the pro- and mesothoracic chordotonal organs are separate from each other, but they are fused in the mtFCO (Matheson and Field, 1990). In grasshoppers, the two scoloparia are referred to as proximal and distal because of their relative positions (Slifer, 1935). In the locust mesothoracic chordotonal organ (msFCO), the proximal scoloparium contains many tiny neurones (Burns, 1974) and is sensitive to vibrations. The distal scoloparium contains larger cell bodies and mediates resistance reflexes (Field and Pflüger, 1989). In the mtFCO of the cricket, it is not possible to say with certainty which of the two scoloparia is homologous with the proximal and which is homologous with the distal scoloparium because both are located at the same proximo-distal level within the femur. However, the clear anatomical differences between the two scoloparia may be useful in deciding their homologies. The dorsal organ contains fewer and larger neurones; their scolopales are often paired and are all located at a similar level within the dorsal ligament. The ventral organ is much larger and contains the majority of the

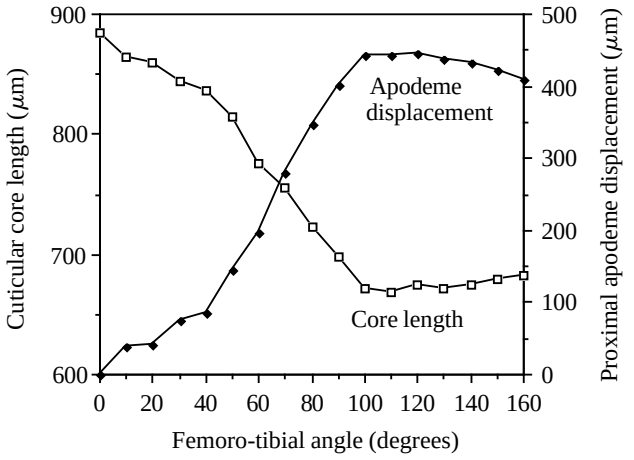

Fig. 9. Graph showing the relationships between femoro-tibial angle (FTA), apodeme displacement (filled symbols) and cuticular core length (open symbols) in a typical preparation. Over the FTA range 0–100 °, extension results in the apodeme moving proximally. At greater angles, the proximal movement ceases and it moves distally. With extension of the tibia over the FTA range 0–100 °, the length of the cuticular core decreases. At greater angles, the core stops shortening and begins to lengthen.

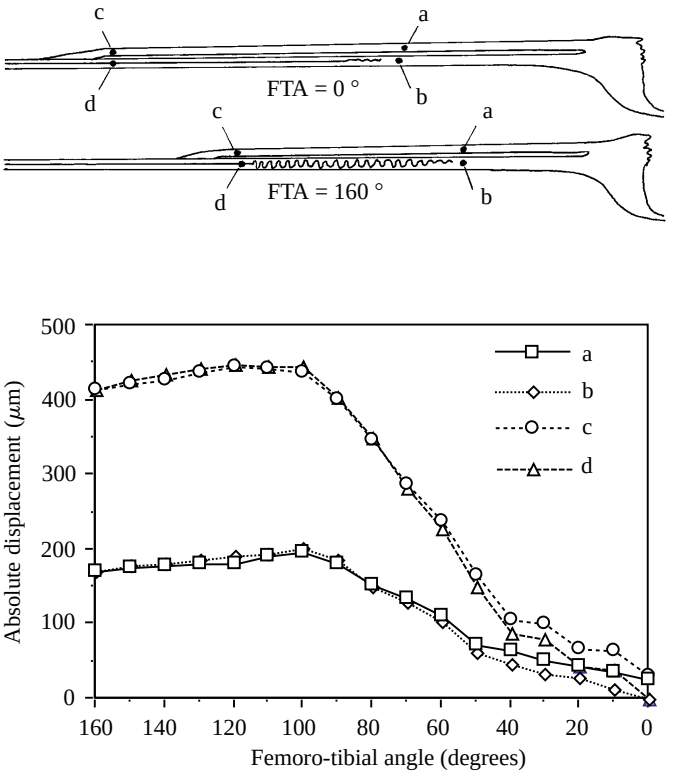

Fig. 10. Graph showing displacements of four marker carborundum particles on the surface of the ligaments as femoro-tibial angle changes. In this preparation, particles b and d were, respectively, located just proximal and distal to the cuticular core of the ventral ligament. Particles a and c were at similar levels in the dorsal ligament. Diagrams above show the positions of the markers at full flexion (FTA=0 °) and full extension (FTA=160 °). (Dorsal up; distal to the left.) The graph shows differential movement between the ligaments over the FTA range 0–70 °, with larger displacements in the ventral ligament both proximal and distal to the core.

sensory cell bodies. The scolopale units of the ventral scolopidium are not restricted to one particular proximo-distal level within the ventral ligament.

Our main finding is that the cricket mtFCO has a mechanism for differential tensioning of attachment cells in the ventral ligament that is significantly different from that in the locust. There does not appear to be a comparable mechanism in the dorsal ligament. In the cricket, the selective tensioning of ventral ligament cells is dependent upon the order in which they are attached to the cuticular core. During flexion, the cuticular core begins to unfold at the thicker distal end first and the unfolding spreads proximally as flexion increases. With extension of the tibia, the core buckles as it shortens. The mechanism for this process is not completely elucidated by the present study. It is not known to what extent shortening depends upon spring-like properties intrinsic to the core. In preparations where the cellular components have been removed, the core assumes its buckled state. This implies that it is naturally in its short configuration unless stretched. However, the buckling of the core during tibial extension could be dependent upon the properties of the tissues surrounding the core. The contributions of the core and the surrounding cellular components to the buckling process must depend on their relative compliances. The present study provides clear evidence that the attachment cells surrounding the core are themselves elastic because, even when the core is buckled, the attachment cells remain unbuckled. Similarly, the extracellular sheath surrounding the ligament also shortens without buckling. This elasticity is almost certainly dependent upon the extracellular fibrils associated with both the ligament sheaths and the individual attachment cells. The fibrils stain with phosphotungstic acid in a way similar to fibrils found in highly elastic insect tissues (Locke and Huie, 1972). A significant feature of the ligament system is that proximally the fibrils are much thicker and they are present in larger numbers than they are distally. Such an arrangement means that, as the ventral ligament is stretched, the distal region with the fewest and thinnest fibrils will be more compliant than more proximal regions. If the core is much more compliant than the surrounding attachment cells with their fibrils, the tapering nature of the fibrils could easily explain why unfolding of the core advances from the distal end.

The progressive nature of the buckling and unbuckling process provides a mechanism for differential tensioning of attachment cells in the ventral ligament. Attachment cells that arise from the most distal levels of the core will develop higher tensions than those associated with the more proximal levels. This means that different neurones in the ventral scoloparium will receive different mechanical inputs, and it is likely that their response properties will vary accordingly. This system has considerable parallels with the locust mtFCO system,

where attachment cells in the ventral ligament also tighten differentially (Field, 1991; Shelton *et al.* 1992). In that case, the attachment cells associated with the more distal parts of the cuticular apodeme also tighten first. The physiological consequences of such a pattern of organisation are unknown, but it is known that some sensory cells in the mtFCO of the locust fire over different ranges of femoro-tibial joint angle range (Matheson, 1990).

There is evidence that the sort of structure we have described for the cricket mtFCO also occurs in another orthopteran, the tettigoniid *Decticus albifrons* (Theophilidis, 1986). In that case, a cuticular core in one of the ligaments has been noted in both the prothoracic and metathoracic femoral chordotonal organs. The present study provides the first detailed description of the structure and behaviour of this structure.

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References

BRÄUNIG, P. (1985). Strand receptors associated with the femoral chordotonal organ of locust legs. *J. exp. Biol.* **116**, 331–341.

BURNS, M. D. (1974). Structure and physiology of the locust femoral chordotonal organ. *J. Insect Physiol.* **20**, 1319–1339.

FIELD, L. H. (1991). Mechanism for range fractionation in chordotonal organs of *Locusta migratoria* (L) and *Valanga* sp. (Orthoptera: Acrididae). *Int. J. Insect Morph. Embryol.* **30**, 25–39.

FIELD, L. AND BURROWS, M. J. (1982). Reflex effects of the femoral chordotonal organ upon leg motor neurones of the locust. *J. exp. Biol.* **101**, 265–285.

FIELD, L. H. AND PFLÜGER, H.-J. (1989). The femoral chordotonal organ: a bifunctional orthopteran (*Locusta migratoria*) sense organ? *Comp. Biochem. Physiol.* **93**A, 729–743.

HALE, L. J. (1965). *Biological Laboratory Data*. London: Methuen.

KARNOVSKY, M. J. (1965). A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**, 137A–138A.

LOCKE, M. AND HUIE, P. (1972). The fiber components of insect connective tissue. *Tissue & Cell* **4**, 601–612.

MATHESON, T. (1990). Responses and locations of neurones in the locust metathoracic femoral chordotonal organ. *J. comp. Physiol.* **166**A, 915–927.

MATHESON, T. AND FIELD, L. H. (1990). Innervation of the metathoracic femoral chordotonal organ of *Locusta migratoria*. *Cell Tissue Res.* **259**, 551–560.

SHELTON, P. M. J., STEPHEN, R. O., SCOTT, J. J. A. AND TINDALL, A. R. (1992). The apodeme complex of the femoral chordotonal organ in the metathoracic leg of the locust *Schistocerca gregaria*. *J. exp. Biol.* **163**, 345–358.

SLIFER, E. H. (1935). Morphology and development of the femoral chordotonal organs of *Melanoplus differentialis* (Orthoptera, Acrididae). *J. Morph.* **58**, 615–637.

THEOPHILIDIS, G. (1986). The femoral chordotonal organs of *Decticus albifrons* (Orthoptera: Tettigoniidae). I. Structure. *Comp. Biochem. Physiol.* **84**A, 529–536.

USHERWOOD, P. N. R., RUNION, H. I. AND CAMPBELL, J. I. (1968). Structure and physiology of a chordotonal organ in the locust leg. *J. exp. Biol.* **48**, 305–323.

YACK, J. E. AND ROOTS, B. I. (1992). The metathoracic wing-hinge chordotonal organ of an atympanate moth, *Actias luna* (Lepidoptera, Saturniidae): a light- and electron-microscope study. *Cell Tissue Res.* **267**, 455–471.

YOUNG, D. (1970). The structure and function of a connective chordotonal organ in the cockroach leg. *Phil. Trans. R. Soc. Lond. B* **256**, 401–428.

ZILL, S. N. AND JEPSON-INNES, K. (1988). Evolutionary adaptation of a reflex system: sensory hysteresis counters muscle ‘catch’ tension. *J. comp. Physiol.* A **164**, 43–48.