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Locomotion Behaviour

A.H. JAY BURR[1] AND A. FOREST ROBINSON[2]

[1]Department of Biological Sciences, Simon Fraser University, Burnaby, BC, V5A 1S6, Canada; [2]USDA ARS, Southern Crops Research Laboratory, 2765 F&B Road, College Station, TX 77845, USA

2.1. Introduction

By far the most characteristic traits of nematodes are their extremely narrow streamlined body and undulatory style of locomotion, useful in their common burrowing habit. These traits have enabled them to be successful in an amazingly wide range of free-living and parasitic environments that is without parallel in other meiofauna. This review examines what is known of the mechanism of this locomotion and its adaptations to various environments in the light of their unique body architecture and neuromuscular system.

Nematodes typically move from place to place by an undulatory propulsion that is distinctly different from that utilized by other animals. It has been described and analyzed in considerable detail (Harris and Crofton, 1957; Wallace, 1968, 1969; Croll, 1976; Lee and Atkinson, 1977; Bird and Bird, 1991; Burr and Gans, 1998; Alexander, 2002). Nematode forward undulatory locomotion involves stiff body waves that propagate backward from the anterior end and exert propulsive forces against the environment. This is achieved through dorsoventral bending rather than lateral bending, as in fishes and snakes, and without a backbone. Occasionally, nematodes move backwards by means of forwardly propagated waves. Such reversals usually stop within one body length or so, and the nematode then heads off forward in a new direction, sometimes involving an omega turn (Croll, 1970).

Undulatory propulsion in nematodes is modified or supplemented with other movements in ways that enable nematodes to crawl on or swim through diverse natural surfaces, media, channels and orifices, for example leaf surfaces, intestinal microvilli, plant hairs, water, blood, excreta, rotting plant matter, animal tissues, blood vessels, insect tracheae and stomata. Nematodes can accelerate, turn, stop, reverse, omega-turn, probe, orient, swim, burrow, penetrate, poke, lace, climb, bridge, roll, graze, cruise, nictate, aggregate, swarm, ambush, hitchhike, loop, creep or somersault. They do not fly, but some can leap nine body lengths in the presence of a potential insect host (Campbell and Kaya, 1999a,b) or crawl on to a *Pilobolus* sporangium about to be shot distances up to 2.5 m towards sunlit grass where ungulate hosts feed (Robinson, 1962).

Thus, locomotion in nematodes can be examined in relation to at least six questions:

1. How do nematodes achieve and control the bending of their bodies internally?
2. How does bending of the body gain purchase and provide propulsive forces against external substrates?
3. How is undulatory propulsion controlled by the neuromuscular system?
4. How is bending modified to maximize purchase and propulsion?
5. How have nematodes adapted undulatory propulsion to different environments to achieve specific goals?
6. What are the constraints and advantages of alternative means of locomotion in nematodes that do not involve undulatory propulsion?

2.2. Internal Mechanics

In a recent review on locomotion, Alexander (2002) focused on mathematical and computer models that describe nematode crawling and swimming motion. The present section takes a complementary approach, examining the internal mechanisms behind this motion. Modeling involves assumptions, model formulation and comparing predictions with observations. This approach is useful for conceptualizing complex mechanisms and usually leads to the design of new experiments.

However, this approach has not yet led to new experiments in nematode locomotion. Here we focus on the assumptions, specifically the evidence for a hydrostatic skeleton, the mechanism of bending, how the application of force to objects can propel the worm and the neural control of the body-wall muscles involved. The explanation of hydroskeleton and bending will be mostly intuitive, and the evidence will necessarily be mostly anecdotal because of limited experimental support. We hope that the deficiencies exposed, questions raised and our radical ideas will stimulate new research.

2.2.1. Role of hydrostatic skeleton

Many invertebrate phyla include organisms whose worm-like form is maintained by a hydrostatic skeleton – an elastic body wall surrounding a constant volume of fluid and/or soft tissues under pressure (Chapman, 1958). As for a water-filled balloon, the elastic properties of the body wall determine the resting shape. In a cylindrical balloon, the membrane is stiffer in the circumferential than the axial direction, whereas in a spherical balloon the elastic moduli are isotropic.

In many nematodes, when the body wall is punctured there can be a dramatic effusion of fluid and viscera. Pseudocoelomic pressures from 16 to 225 mmHg were measured in *Ascaris* (Harris and Crofton, 1957). However, note that some nematodes appear not to have such internal pressure (Wright, 1991).

If there were no change in the amount of fluid inside the body cavity, the volume of the body cavity would remain constant, because liquids are incompressible. If more fluid is added to the body cavity, increasing its volume, the internal pressure should increase as the elastic layer is stretched to greater tension. A muscular oesophagus (= pharynx) pumps fluid and food into the intestine against the internal pressure. The energy required to pump in more fluid is stored in the elastic elements of the body wall and would be released by defecation. Defecation causes a short-term decrease in pressure because of the subsequent period of lowered volume (Harris and Crofton, 1957). Over the longer term, nematode internal volume and pressure would be governed by rates of osmoregulation, esophageal pumping, excretion and defecation (Mapes, 1965; Davey, 1995).

The nematode body wall is composed of a cuticle and a single layer of longitudinal muscle cells (Fig. 2.1). These are separated by a thin sheet of hypodermis (= epidermis) that expands medially at intervals around the circumference to form the hypodermal cords. These divide the muscles into four to eight longitudinal bands, depending on species (Fig. 2.1). The body cavity is internal to the basal lamina that covers the muscle and hypodermal cells, and contains pseudocoelomic fluid, intestine and gonads. Since the intestine and gonads have soft, non-muscular walls, their contents are probably at the same pressure as the pseudocoelomic fluid.

If the body wall were a thin membrane, the cylindrical shape should collapse when pressure is released by puncturing the body wall. However, in nematodes the cylindrical shape of the body tube is maintained when punctured and its diameter changes only slightly. Therefore the nematode body wall must have a high circum-

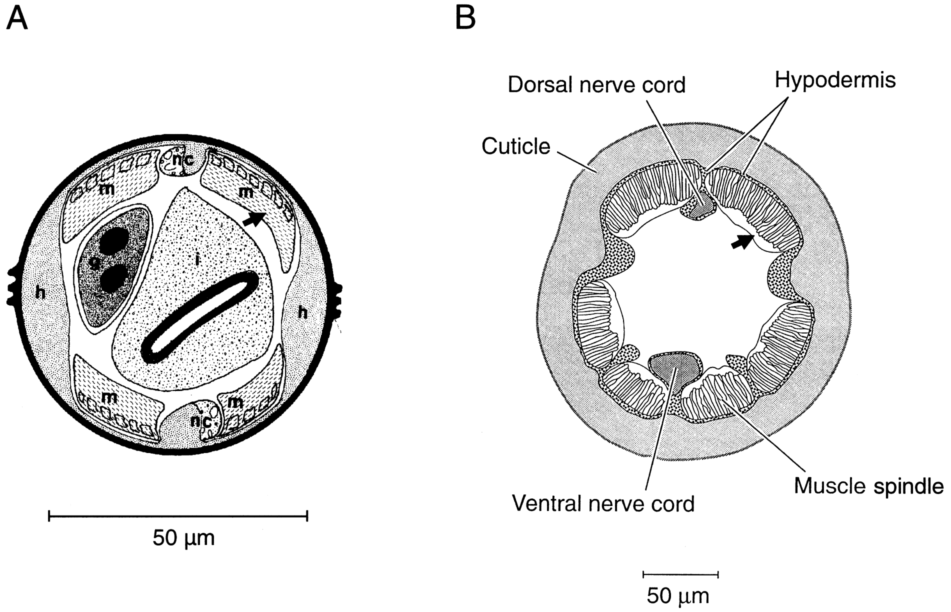

Fig. 2.1. Two examples of body-wall structure in nematodes. A. Hermaphrodite of *C. elegans*. Expansions of the hypodermis (h), called hypodermal cords, contain nerve cords (nc) and separate the muscles (m) into two dorsal and two ventral bands. A thin sheet of hypodermis extends between the muscle spindles (small rectangles) and cuticle. The intestine (i) and gonads (g) lie inside the body cavity. (From Wood, 1988.) B. Female *Mermis nigrescens*. In this much larger nematode (120 mm long) the cuticle and muscle spindles are thicker. The body cavity is usually filled with eggs and a modified gut (trophosome) containing stored food (not shown). Arrow, muscle belly. (From Gans and Burr, 1994.)

ferential stiffness and special properties that maintain the circular cross-section in the absence of internal pressure.

Mechanically, the nematode body wall consists of the multilayered cuticle and the longitudinal strap-like contractile region (spindle) of the muscle cells (Figs 2.1 and 2.2). The sarcomeres consist of a band of thick and thin myofilaments between two cylindrical dense bodies, which are equivalent to the Z lines of vertebrate muscle (see the complete sarcomere in the tangential section of the coelomyarian example, Fig. 2.2). A series of sarcomeres are aligned in columns parallel to the body axis; however, neighbouring columns of sarcomeres are offset to form an obliquely striated pattern in sections perpendicular to the dense bodies (Fig. 2.2).

Mechanically, nematode muscle is unlike any other muscle in that each sarcomere is connected perpendicular to the site of force application, the cuticle. The myofilaments of each sarcomere are attached via the dense bodies to the basal lamina (Fig. 2.2), which in turn is fixed to the cuticle across the sheet-like extension of hypodermal cell via short intermediate filaments and half-desmosomes (Francis and Waterston 1985, 1991; Bartnik *et al.*, 1986; Waterston, 1988; Barstead and Waterston, 1989; Moerman and Fire, 1997). This architecture trans-

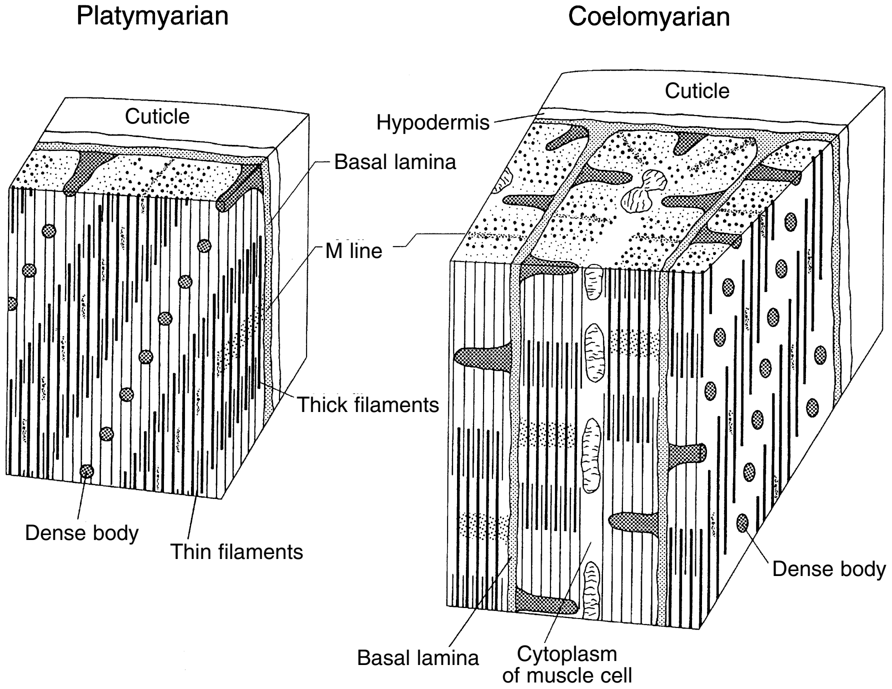

Fig. 2.2. Contractile architecture of the two main types of body-wall muscle: platymyarian (example, *Caenorhabditis elegans*) and coelomyarian (example, *Mermis nigrescens*). The sarcomeres are attached to the basal lamina by dense bodies (Z-line equivalents). In coelomyarian muscle, the basal lamina forms a ribbon between the folded plates of spindle. In both types, the basal lamina is attached to the cuticle across the thin sheet of hypodermis. For clarity, the scale is shortened in the vertical direction and only two thin filaments are shown in the tangential section of *C. elegans* and none are shown in the equivalent radial section of *Mermis*. (From Burr and Gans, 1998.)

mits contractile force laterally to the cuticle rather than serially to the muscle ends, and thus provides for smooth bending and avoids buckling of the body tube (Burr and Gans, 1998). In the coelomyarian muscle type found in large nematodes such as *Ascaris* and *Mermis nigrescens*, the spindle is folded around an inward extension of the basal lamina (Figs 2.2 and 2.3). This ribbon of basal lamina would need to provide a stiff connection between the dense body attachment sites and the part of the basal lamina that is fastened to the cuticle.

Nematodes are unique among worm-like organisms in lacking circumferential muscles; therefore their motions are limited to what can be accomplished by applying longitudinal forces to the body wall. In other worms, contraction of circumferential muscles, thereby decreasing the circumference of one region as in squeezing a water-filled balloon, can displace a large volume from that region and expand the body elsewhere (Chapman, 1958). Thus crawling, as in earthworms, by peristaltic extension-with-thinning and contraction-with-thickening (Quillin, 1999) is not

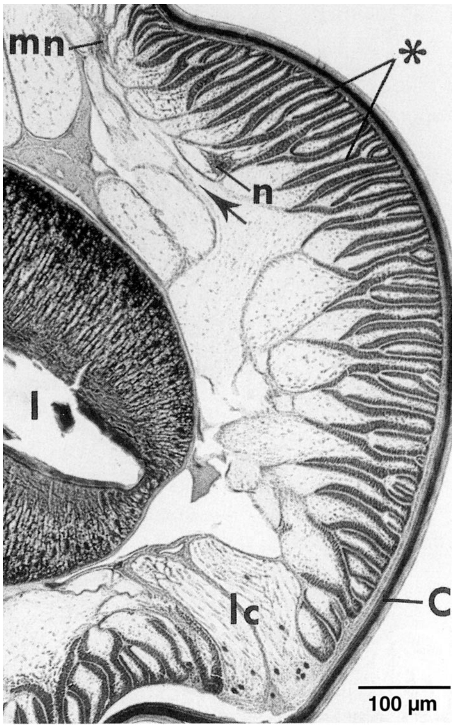

Fig. 2.3. Cross-section through mid-body of a large ascarid, *Toxascaris* sp., showing coelomyarian muscle spindles, muscle bellies and an innervation process (arrow), which fill space in the pseudocoelom not occupied by the intestine (I) and median and lateral cords (mn and lc). C, cuticle, n, nucleus; *, darkly stained myofilaments in folded muscle spindle. (From Wright, 1991.)

possible in nematodes. The peristaltic locomotion of *Criconemoides* is unusual for nematodes and involves relatively small changes in circumferential and axial dimensions (Stauffer, 1924; Streu *et al.*, 1961).

It is commonly assumed that the pressurized body cavity plays an essential role in bending motion, with muscle contraction shortening one side of the body tube while pressure maintains the length of the other. Then, when the muscle relaxes, the internal pressure is thought to straighten the tube, providing the 'restoring force'. These assumptions have been incorporated in several mathematical models of nematode locomotion (Harris and Crofton, 1957; Seymour, 1983; Alexander, 1987, 2002). However, these assumptions are based on only one experimental study, which established that the pseudocoelom is under a pressure that is uniformly distributed throughout the body length (Harris and Crofton, 1957). The role of internal pressure in bending has never been directly investigated in nematodes, nor has the alternative possibility that elastic compression of the cuticle provides all or part of the restoring force (as was assumed in the model developed by Niebur and Erdos (1991)). This lack of experimental investigation of the hydrostatic skeleton and its role in body movements is surprising in view of the keen interest in nematode function spawned by genetic, developmental and molecular studies of *Caenorhabditis elegans*. In contrast, there has been extensive work on hydroskeleton and locomotion in the leech (Miller, 1975; Stern-Tomlinson *et al.*, 1986; Wadepuhl and Beyn, 1989) and the earthworm (Quillin, 1999).

2.2.2. Role of cuticle

If one compares the body motion of a nematode with that of a fly larva or earthworm, the comparative stiffness of the nematode body is very apparent. This could be partly due to the much greater thickness of the cuticle relative to body diameter, the ratio being about 1: 30, but it can be 1: 5 to 1: 3 in *M. nigrescens* (Fig. 2.1B) and 1: 100 in adults of large ascarids (Fig. 2.3; Bird and Bird, 1991; Gans and Burr, 1994). It could be due also to special elastic properties of the cuticle with or without internal hydrostatic pressure. When stripped of muscle, basal lamina and epidermis, the cuticle of *C. elegans* maintains a cylindrical shape in aqueous suspension (Cox *et al.*, 1981a); however, it may require muscle tonus and/or internal pressure to maintain this adequately against external forces.

The nematode cuticle has been described at the electron microscope level in many species and is the subject of many reviews (Wharton, 1986; Wright, 1987, 1991; Bird and Bird, 1991; Kramer, 1997; Baldwin and Perry, 2004). A wide variation in cuticle structure, even in different juvenile stages of a species, probably represents important adaptations to the widely diverse environments in which nematodes are found (Inglis, 1964; Edgar *et al.*, 1982; O'Grady, 1983; Fetterer and Urban, 1988; Baldwin and Perry, 2004). However, the basic structure is a thin (6–40 nm) smooth epicuticle covering three thick layers (Figs 2.4 and 2.5B): the cortex, median zone and basal zone (Wright, 1991). These are constructed of protein secreted by the underlying epidermis during both molting and growth (Bird, 1980; O'Grady, 1983; Wright, 1987). Here, we shall focus on features that may be significant for mechanical function and locomotion.

The cortex generally consists of one or two layers of relatively homogeneous and electron-dense material digestible by elastase (Fujimoto and Kanaya, 1973; Cox *et al.*, 1981a). Together with its outer location, apparent flexibility (Fig. 2.5) and fine structure, this suggests that the cortex provides a tough but flexible contact with the environment.

The median zone is mechanically very compliant (Fig. 2.5B) and may contain a fluid. In the simplest cases it consists of an electron-lucent region crossed by fibrils connecting the cortex and basal zone. In a number of nematode examples the median zone contains radial struts that join the cortex and basal zone through a fluid layer (Figs 2.4 and 2.5B; Inglis, 1964; Lee, 1965; Wright and Hope, 1968; Baldwin and Perry, 2004).

The basal zone is the layer most directly connected to the body-wall muscle and is where the circumferential stiffness of the body wall most probably resides. A loosely organized network of fibers connects the inner surface of the cuticle with a

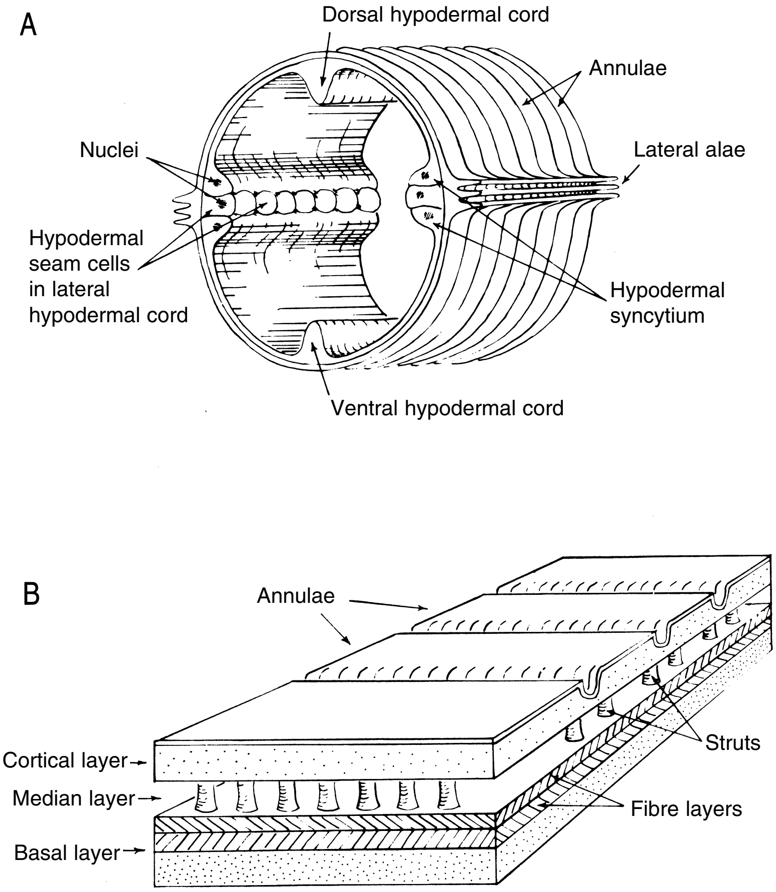

Fig. 2.4. Cuticle and hypodermis in *Caenorhabditis elegans* hermaphrodite. A. General organization of cuticle and hypodermis. B. Structure of cuticle. (From Cox *et al.,* 1981a.)

stiff layer, which occurs in three different forms, depending on species and life stage: (i) two or three layers of helical fibers that cross at characteristic angles, (ii) a layer of electron-dense striated material (Fig. 2.5B); or (iii) a multilayered, nonhelical fibrous material curving in a scalloped pattern.

In addition to the basic three-layered structure covering most of the circumference of the cuticle, lateral longitudinal ridges are found in certain species or life stages. Examples include the external alae of *C. elegans* hermaphrodites or the internal ridges in mermithids (Figs 2.1A,B and 2.4). Unlike in the rest of the cuticle, positive birefringence and electron micrographs indicate that longitudinal fibers are present (Cox *et al.*, 1981a,b; Edgar *et al.*, 1982). These structures are likely to bias bending motions, stiffening the body against lateral bending while allowing dorsoventral bending.

How are the different cuticle layers affected during locomotion? The cuticle is compressed on the inside of a bend by the contraction of the sarcomeres and it may

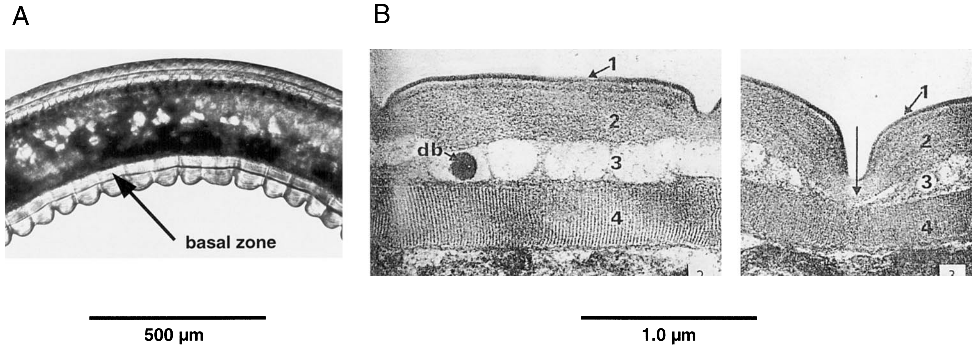

Fig. 2.5. Folding of cortex of compressed cuticle. A. Corrugations form on inside of bend of *Mermis nigrescens* female. The basal zone is thicker on compressed side. Optical section of whole mount, using differential interference contrast. B. Annuli in *Heterodera* juvenile. Right, fold along annule indents the fluid-filled medial layer (arrow). 1, epicuticle; 2, cortex; 3, median zone containing struts and fluid-filled space; 4, striated basal zone; db, dense ball. (From Wisse and Daems, 1968.)

be under tension on the outside. The cortex on the inside of the bend may fold to form large (50–150 µm) corrugations (Fig. 2.5A), or the 0.3–2.0 µm spaced annuli of the cortex (Fig. 2.4) may become indented (Fig. 2.5B). On the other hand, the cortex on the outside of the bend remains smooth and may be relatively stiff against extension (Rosenbluth, 1967; Wright and Newall, 1976; Robinson and Carter, 1986; Gans and Burr, 1994). The basal zone, on the other hand, yields to compression by becoming thicker (Fig. 2.5A; Fig. 1 in Rosenbluth, 1967). The spongy median zone appears to provide the internal compliance necessary for the two layers to react differently to mechanical stress (Fig. 2.5B). The struts or fibers of the median zone would transfer forces across the fluid-filled layer to the cortex and external objects during locomotion. The fluid in the median zone could flow circumferentially to the outside of the bend, increasing flexibility by decreasing cuticular compressive resistance on the inside of the bend. Thus the cuticle appears to be well adapted to yielding to axial compression; however, on extension, the cuticle response may be complex. The compliant basal zone and folding cortex may allow bending in the normal range, but we suspect that the cortex may strongly resist elongation at higher extensions.

The cuticle must protect against potentially harmful mechanical forces. Much as for human skin, a tough outer layer (epicuticle and cortex) would protect against abrasion, while a compliant medial zone may dissipate local stresses. Compliance of the medial zone may protect the inner layers from damage as the body pushes pass unyielding soil particles during burrowing. The stiff basal zone may resist indentation of the body cavity and provide resistance to shear forces. As noted above, its chief importance is probably provision of circumferential stiffness while allowing axial compression during bending.

2.2.3. Body stiffness and the role of muscle tonus

When a nematode stops crawling on a surface or swimming, its body remains in its undulatory bent shape. When *C. elegans* is picked up with a wire pick, its body appears quite stiff against the applied forces. This short-term resistance to bending forces is especially noticeable in *Mermis* because of its 100 mm length. *Mermis* can stiffen its body enough to cantilever more than a third of its body during head-elevation bouts (Fig. 2.6A) and when bridging gaps in vegetation (Fig. 2.7; Gans and Burr, 1994).

The nematode body wall, in addition, transmits forces axially. When nematodes burrow through agar, soil or host tissues, the propulsive forces that are developed in posterior regions (see below) must be transmitted to the anterior where the greatest resistance to motion would be experienced. This is easily observed in *Mermis* as it pokes its head into felt and pushes through fibers in the surface (Fig. 2.6A). More than 10 mm of body can intervene between the tip and the nearest site of force generation, and body stiffness appears to be essential to transmit the force (Gans and Burr, 1994). In all nematode locomotion, propulsion depends on the transmission of forces generated by muscular contraction in one region to an axially distant contact site (see below) and this would be impossible if the body were limp. Furthermore, each body region must be able to resist mild tensile and compressive stresses as it is either pulled or pushed through the medium by other body regions.

How, then, does the body provide axial stiffness while allowing locomotory bending? Dropping *Ascaris* on to a table causes a sudden straightening and stiffening, probably by stimulating muscle contraction equally in the four quadrants. In contrast, when a nematode is anaesthetized, the body becomes limp. These and other anecdotal observations suggest that muscle tonus is involved. The advantage of using muscle tonus to stiffen the body is that it can be adjusted locally on

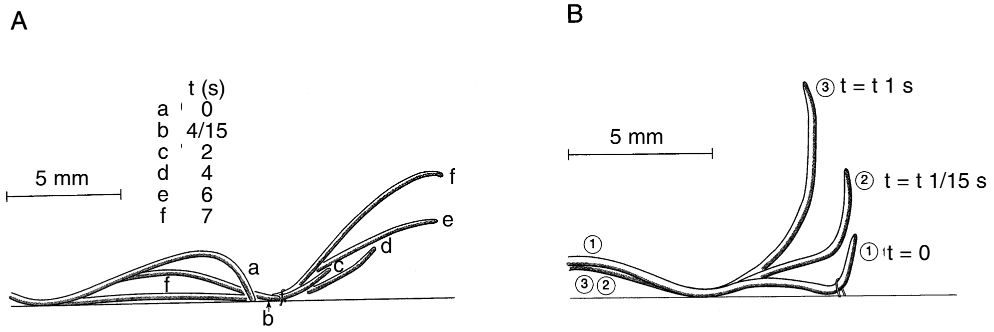

Fig. 2.6. Axial transmission of forces and body stiffness during locomotion of *Mermis nigrescens*. A. Sequence illustrating poking (a) and lacing motion (b–c), followed by application of propulsive force to a fibre loop (e–f) and a head-elevation bout (f) involving cantilevering. B. Tracings from video sequence of a fibre loop breaking due to application of force by the anterior. Position 1/15 second after the break is the result of the force applied to the fibre loop by the anterior and the force on the body transmitted from the posterior (see Fig. 2.12). (From Gans and Burr, 1994.)

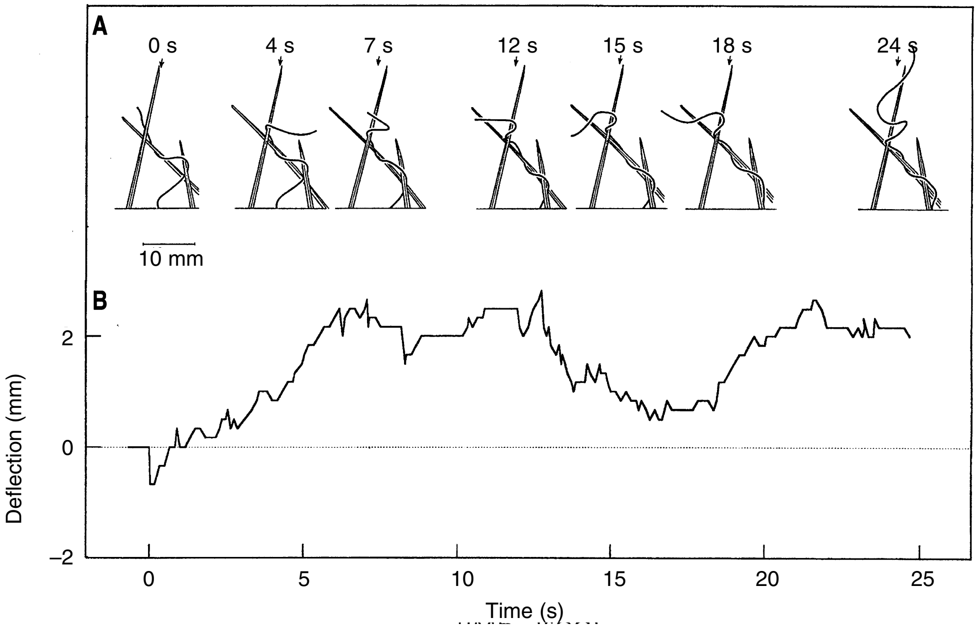

Fig. 2.7. Application of force to a blade of grass by *Mermis nigrescens* female. A. Successive body positions traced from videotaped images. Arrow, original blade position. B. Plot of the deflection of the blade as the body slides past. The deflection would be proportional to the force the blade applies to the nematode – specifically, to the component of this force in the plane of the image and perpendicular to the blade. (From Gans and Burr, 1994.)

command to provide the desired mechanical result within the cuticle's flexible length range. The stiffening would be similar to that caused by increasing the tension in opposing muscles of vertebrate body trunks.

In an attempt to determine the contribution of muscle tonus to stiffness in *Ascaris suum*, one of us (AHJB) measured resistance to elongation before and after injection of γ-aminobenzoic acid (GABA). This inhibitory neurotransmitter is known to relax *Ascaris* muscle (Davis and Stretton, 1996), and, when present, the slope of length/tension curves was much lower (A.H.J. Burr, unpublished results). Since the slope is proportional to stiffness it is clear that the resistance of the body wall to elongation is due primarily to muscle tonus.

The axial force due to tonus would increase internal pressure, and muscle tonus can be modulated to regulate internal pressure. A momentary higher pressure is produced by contraction of the body-wall muscles during the defecation cycle of *C. elegans* (Thomas, 1990). Harris and Crofton (1957) recorded a rhythmic variation in pressure in the posterior half of *Ascaris* that was correlated with rhythmic changes in posterior length.

Normally, muscle tension is opposed by the hydrostatic skeleton, and a decrease in pressure caused by volume reduction would lower this opposing force and cause shortening of the body tube until pressure returns to the level presumably regulated by tonus. Reducing *Ascaris* volume by 24% (1.43 ml) by withdrawing pseudocoelomic fluid caused a 15% body shortening. Under applied tension, the slope of the length/tension curve (stiffness) was the same even though body length was 15% less (A.H.J. Burr, unpublished results). This result seems to support the idea that body-wall stiffness and internal pressure may be regulated by muscle tonus.

A marine *Enoplus* responded to loss of volume in hypersaline solutions by shortening the middle part of the body to the point that the cuticle became corrugated, and they were able to locomote normally while other species could not (Inglis, 1964). Thus, shortening the body may be an important way for nematodes to compensate for fluid loss.

Apparently shortening does not compromise muscle function. The ability for nematode muscle to operate normally over a large range of lengths could be made possible by the unusually long myofilaments found in nematode muscle (Rosenbluth, 1965; Waterston, 1988). Thick and thin filaments are 10 and 6 μm long compared with 1.6 and 1.0 μm in vertebrate cross-striated muscle. Rosenbluth (1967) proposed that the large operating range is due to shearing between the offset columns of sarcomeres found in obliquely striated muscle (Fig. 2.2). However, unlike in obliquely striated smooth muscle of other organisms, where the shearing concept originated, in nematode body-wall muscle every sarcomere is attached by dense bodies to the basal lamina and cuticle, whose elastic properties would prevent shearing. Oblique striation might instead be an adaptation for distributing the force application sites of the sarcomeres more uniformly over the basal lamina and cuticle (Burr and Gans, 1998).

2.2.4. Mechanism of bending

What causes bending during undulatory locomotion and other nematode activities? With constant volume and constant elastic moduli, the shape of a hydrostatic skeleton can be changed only by an asymmetrical application of forces. For example, if the membrane on one side of a water-filled cylindrical balloon is shortened longitudinally by pinching it, this external force on the elastic membrane will bend the tube. Similarly, compression of the nematode body wall by contraction of muscle bands on one side would bend the body tube. In the presence of muscle tonus, bending can equally be caused by relaxation of muscle cells on one side, a possibility that appears not to have been considered previously. In nematodes, muscles of the body wall are controlled by inhibitory as well as excitatory neuromuscular synapses (see Section 2.4 on neural control below) and it is possible that relaxation as well as contraction, on opposite sides of the body, could be responsible for bending.

The anecdotes described in the preceding section illustrate that there may be an equilibrium between the force exerted by body-wall muscles and an opposing force due to the pressurized hydroskeleton. If muscles in all four quadrants contract, as during defecation, muscle tension would exceed the opposing force and the body tube would shorten, thus increasing pressure until the forces rebalance. Activating muscles in all four quadrants in a short segment of the body tube would compress the body tube locally, and the increase in local pressure would be transmitted through fluid inside the body cavity to other parts of the body tube. There, the hydroskeletal force would exceed muscle force and would cause elongation until opposing elastic forces build up there. When the local muscles relax, the process would reverse, the net force (the 'restoring force') stretching the local body wall, decreasing local pressure and moving fluid back from other parts of the body. During the process, some internal fluid would be translocated from one region to another. Transfer of gut contents can often be observed (Seymour, 1973; Thomas, 1990).

In the case of bending, the net forces due to increased muscle tension would shorten only one side of the local body tube, while the length on the other side would be maintained by the equilibrium of forces there. The increase in local pressure and the axial volume transfer could be relatively small and may even be negligible if the local muscles on the opposite side relax at the same time. When the muscle tension is released, a restoring force would straighten the body tube.

Until this point, our development of this idea has assumed that the restoring force arises from the hydrostatic skeleton under pressure, as when the force pinching the water-filled balloon is released. In nematodes the elastic membrane is commonly assumed to lie in the cuticle and the pressurized internal fluid to be the pseudocoelomic fluid plus the fluid contents of the soft internal organs of the body cavity. However, there are two other possible origins of restoring force that have not been investigated: (i) pressurized cytoplasm of the muscle bellies, and (ii) the compressed cuticle. Note that the proportion of the body cross-section taken up by muscle bellies and cuticle thickness varies strikingly among nematode examples (Figs 2.1 and 2.3) and with the body region of a nematode, and therefore it is likely that the relative contribution of the three possible sources of restoring force could vary.

The necessity of internal pseudocoelomic pressure to bending was investigated in *Ascaris* (A.H.J. Burr and R. Davis, unpublished results). Only the anterior third of this species contributes to locomotion; the posterior regions filled with gonads appear to be dragged along by the anterior. Pseudocoelomic volume in the anterior is small, as evident in cross-sections such as Fig. 2.3 and also shown by injecting 0.1 ml dye solution into the head of intact *Ascaris* – the solution passed right through the active zone. Transecting the worm posterior to this active region or inserting a cannula into the pseudocoelom just posterior to the region did not prevent normal locomotion. The cannula evidently had successfully equalized the pressure between the pseudocoelom and the exterior, because *Ascaris* injected with Ringer's solution into the head passed through the active region and immediately exited via the cannula. Thus, a pressurized pseudocoelom is not necessary to provide a force opposing muscle tension during locomotory bending, although the results do not rule out that it may contribute if present.

This surprising result indicates that there may be some other restoring force opposing muscle tension in *Ascaris*. It would most probably arise by compression of the cytoplasm of the large muscle belly when the contractile region of the same cell shortens. Surrounded by an elastic basal lamina, the pressurized muscle bellies could serve as an intracellular hydroskeleton that could provide the necessary restoring force. Nematode basal lamina contain cross-linked type IV collagen fibers (Kramer, 1997). In the esophagus, a basal lamina provides the elastic restoring force for the pumping action (Roggen, 1973; Saunders and Burr, 1978). Muscle bellies occupy a large proportion of the cross-section internal to the muscle spindles in the active zone of *Ascaris* (Rosenbluth, 1965) and in *Toxascaris* (Fig. 2.3) and *Nippostrongylus* (Wright, 1991). When the muscle contracts, the muscle bellies become tightly pressed together and expanded medially (Fig. 1 in Rosenbluth, 1967) and extension of muscle spindles and bellies on the outside of the bend would provide space for this locally. The pressurized cytoplasm would not be translocated out of the local region; however, the expanded muscle bellies could displace some pseudocoelomic fluid or gut contents.

Seymour (1973) suggested that the thickening of the muscle layer caused by local contraction in all four quadrants creates radial forces that can displace gut contents in small nematodes such as *Aphelenchoides blastophthorus*. When some marine species such as *Oncholaimus oxyuris* and two monhysterid species are transected, there is no explosive release of fluid and locomotion continues (Coomans *et al.*, 1988; Van de Velde and Coomans, 1989). Large vacuoles in the hypodermal cords of these species are suggested to be a local hydrostatic compartment.

The third possible elastic restoring force, provided by the compressed cuticle, was proposed by Wisse and Daems (1968), and it deserves investigation. This system would be like a bent section of thick-walled latex tubing, which tends to straighten even in the absence of internal pressure. We have noted above that the basal zone is compressed on the inside of a bend, becoming thicker (Fig. 2.5A), and that fluid in the intermediate zone could translocate to the outside of the bend, expanding the cuticle there.

2.2.5. Model of crossed helical fibers

The elastic properties of the nematode body wall – high circumferential stiffness with longitudinal flexibility – occur regardless of which type of basal zone is present in the cuticle. However, the interesting geometrical structure of the crossed-helical fiber layers in one type has attracted attention. Harris and Crofton (1957) proposed a mechanical model and provided a mathematical formula that governs the geometrical relationship between fibre crossing angle and body length, diameter, volume and pressure. They did not directly measure the relationships to crossing angle, however. By measuring the length and volume changes in the normal physiological range and applying the model to these data, they predicted a fibre crossing angle of 73°. As this turned out to be very close to that measured in sections of fixed worms, they proposed that the crossed fiber array is the prime determinant of body dimensions in *Ascaris.* The model predicts that for geometrical reasons alone, and in the absence of any elastic stiffness in the cuticle, an increase in pressure would lengthen the body tube rather than increase its circumference.

Harris and Crofton (1957) recognized that their model was an oversimplification because it considers an insignificant any elastic component that may be contributed by other components of the cuticle, including the matrix in which the fibers are embedded. In addition, the model assumes that the collagen fibers are rigid, contrary to findings in other organisms (Shadwick and Gosline, 1985; Wainwright, 1988). Further experiment on the elastic properties of nematode cuticle is required along the lines of investigation of the mechanical properties of blood vessels, in which stress/strain functions are measured at different inflation pressures (Shadwick and Gosline, 1985). Since the fiber crossing angles in *Ascaris* can be measured *in situ* with a microscope, changes in fiber crossing angles can be measured at the same time. Until such measurements are done, the Harris and Crofton model should be considered speculative.

Alexander (1987, 2002) extended the crossed-helical fiber model to include bending; however, there is no experimental evidence. Indeed, the measurement of length and volume changes that predicted a crossing angle were done by Harris and Crofton (1957) on the posterior third of *Ascaris*, which does not contribute to locomotion and where bending motion is slight. Their observations of pressure fluctuations with a 30 second periodicity cannot be related to locomotory bending, which is faster, and the phenomenon which produces this periodicity is unexplained.

Wainright (1988) suggests several other possible functions for helical fibre structures in animals: preventing bulging under pressure, providing resistance to buckling under external axial loads (such as when pushing through soil or tissue) and allowing smooth bending without kinking. These functions would be important in all nematodes, and the other two types of basal zone structure must somehow provide these as well.

2.3. Transfer of Forces and Propulsion

Locomotion in nematodes, as in snakes and spermatozoa, is usually accomplished by travelling waves of bending that apply forces to external objects or fluids as the body passes by. Force transmission from sarcomere to contact point at the surface of the cuticle traverses many structural components of the body wall. The forces would be transmitted across the compliant medial zone of the cuticle via fibers or struts. Smoothness of the epicuticle and lubrication provided by the surface coat are probably important in reducing friction. The hydrophilic surface coat must also set up the aqueous surface tension that causes adherence to a moist substrate important in transmitting propulsive forces to the substrate in the case of nematodes crawling on thin moisture films (Wallace, 1959, 1968).

It is evident that understanding undulatory propulsion has two levels: the neuromuscular mechanisms that generate the waves of forces within the body, and the relationship of these and the body form to the external forces that drive the nematode forward (Gray, 1953). The first level will be treated in the next section. The

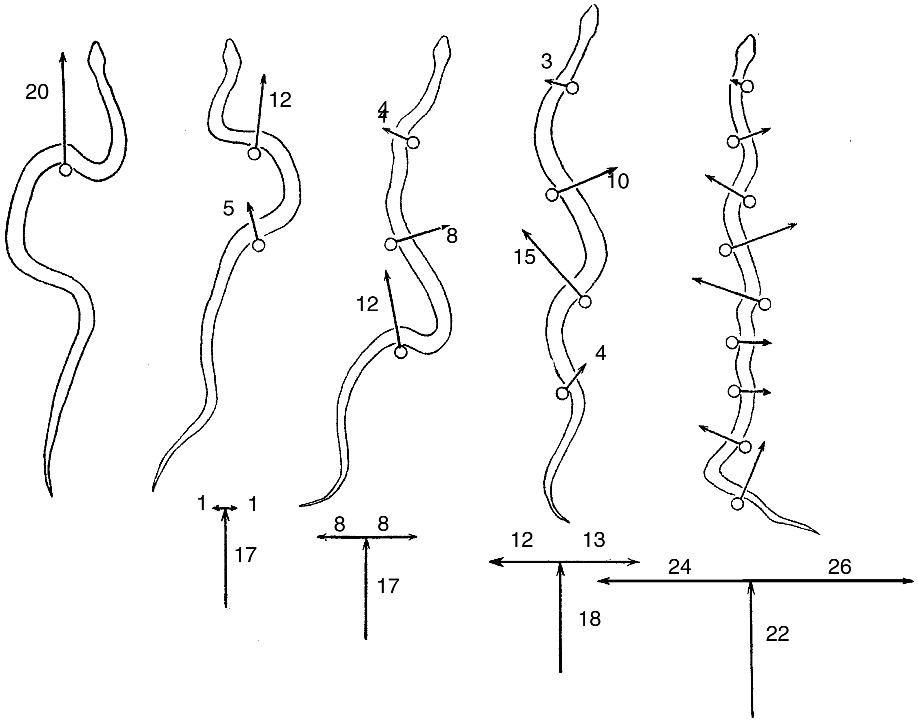

Fig. 2.8. Reactive forces exerted by a series of objects as a result of forces applied by a grass snake (arrows). The vectors beneath each record show the sums of the forward or lateral components of the forces. (From Gray and Lissman, 1950.)

current section will connect muscle tension to the forces that propel the nematode during undulatory locomotion. The more complete treatments by Gray (1953) and Wallace (1968) would be required reading for anyone interested in investigating the topic further.

Measurement of the forces against external objects would be difficult to determine with most nematodes, though conceivable with the larger species. However, the pattern of forces would be similar to that measured on snakes. Figure 2.8 shows the reactive forces exerted by objects on to a grass snake that are equal and opposite to the forces applied by the body during undulatory propulsion. Each of the vectors can be resolved into one component in the forward direction, the propulsive thrust, and one in a lateral direction, the restraining force, and these can be summed to give the overall effect on the center of mass (Fig. 2.8). Note that the lateral components cancel and there is a net forward propulsive force exerted on the snake. If the net propulsive force equals the net frictional force (not shown), the speed will be constant.

As the body slides past an object, each region follows the same sinuous path as that preceding it, and curvature of the region and the force applied by it to the object changes to that formerly applied by the region immediately ahead of it. This

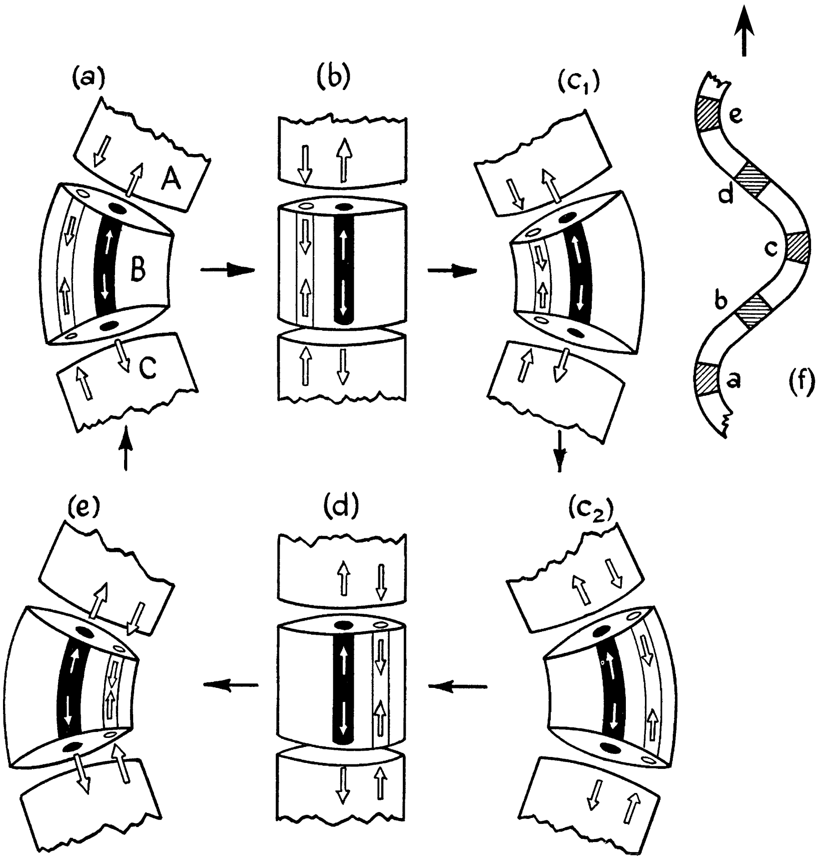

Fig. 2.9. Changes in form of an actively bending region (B) and the forces exerted on its neighbours A and C. The complete bending cycle a–e is marked along the undulatory wave in panel f. Arrow, direction of locomotion. Panels a–c_1 illustrate the bending sequence and bending couple when tension is applied on the left, and panels c_2–e illustrate the effect when applied on the right. (From Gray, 1953.)

should result in a continuous application of force to each object as the body glides by, and a continuous propulsive force applied to the body. The three-dimensional movement of an *M. nigrescens* female climbing among grass blades is more complex; however the effect of force on one of the grass blades could be observed and is seen to be continuous, though variable, as the body glides by (Fig. 2.7B). The net effect of such forces from many such contacts is the continuous upward locomotion indicated by the sequential body positions (Fig. 2.7A).

Figure 2.9 illustrates how muscle tension on one side of a local body region (thick arrows within segment) and a restoring force supplied by the hydrostatic skeleton (thin arrows in segment) act together as a ‘bending couple’ to change the curvature. While the muscle tension tends to shorten the cuticle on one side, the restoring force resists shortening of the center of the tube. During forward locomotion (arrow in Fig. 2.9f), body region *a* acquires the curvature of region *b* and then *c*. For these changes in curvature to occur, the muscle tension in each region must be on the left side, as illustrated in Figs. 2.9a, b, and c_1. Thus, the regions along this half wave can be regarded as a single propulsive unit. Figure 2.9 c_2, d and e illustrate the bending sequence in segments of the second half wave that is generated by applying muscle tension to the right side. Note by comparison with Fig. 2.9f, that muscle tension is always on the forward side of each half wave. The same effect is achieved if the medial restoring force due to a hydrostatic skeleton is replaced by a lateral restoring force applied by a compressed cuticle.

How such internally generated local bending couples can apply forces to external objects is illustrated in Fig. 2.10. Muscle tension (shading) is on the forward side of each half wave, as in Fig. 2.9. In order to think about a half wave as an iso-

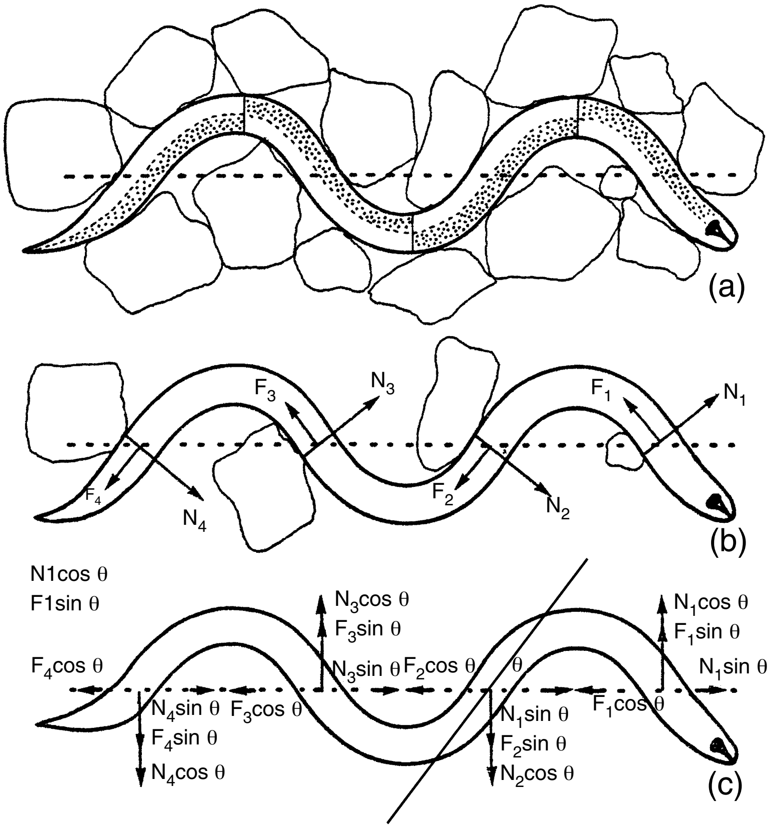

Fig. 2.10. Propulsive forces in the presence of external restraints. Each half wave marked in (a) can be regarded as a locomotory unit. Shaded areas indicate on which side muscle tension is developed. (b) A further simplification, isolating one restraining object in each half wave. Muscle tension in each half wave exerts a backward pressure on each restraint, and the normal reactive forces N_i and tangential frictional forces F_i are shown. (c) Vectors shown are the components of the N_i and F_i along axis of progression and axes perpendicular to it. (From Wallace, 1968, and based on Gray, 1953.)

lated propulsive unit, however, it must be recognized that lateral movement of both ends of the half wave is restrained by transmission of forces from preceding and following half waves. In the presence of these restraints and the external objects at the mid-point of the half waves (Fig. 2.10b), the result of shortening the forward side of each half wave causes the body to press against the objects. Note that, as in Fig. 2.8, the reactive force, N_i, exerted by each object on to the body is normal to the body surface and has a component in the forward direction. As the body slides by each object, a frictional force, F_i, develops tangential to the body. In Fig. 2.10c the forces applied by the four objects are resolved into components along the axis of progression and components transverse to it. The sum of the transverse components, $\Sigma N_i \cos\theta + \Sigma F_i \sin\theta$, is zero and forward propulsive forces $N_i \sin\theta$, are observed to arise from the bending couples in each half wave.

When the body is moving at a constant speed, the sum of propulsive forces acting on the nematode, $\Sigma N_i \sin\theta$, is equal and opposite to the sum of frictional forces, $\Sigma F_i \cos\theta$. From this equality an important relationship can be derived between total frictional force, F, and total propulsive force, N, at constant speed: $F = N \tan\theta$. This can explain changes in wave-form that are observed when friction increases, as when the surface of agar dries (Fig. 2.11). According to the equation, increases in friction can be overcome by increasing either the amount of muscle tension in order to increase N, or by increasing θ, the angle the body makes at mid-wave with the axis of progression (Fig. 2.10c). The latter can be accomplished by decreasing wavelength and/or increasing amplitude; note that *Heterodera schachtii* decreases wavelength on dry agar.

Thus Fig. 2.10 shows how the forces against external objects can develop from the bending couples of Fig. 2.9 during undulatory locomotion. Except on agar, which provides a uniform field of external constraints, wave-forms are not likely to be symmetrical as in Fig. 2.10. External constraints vary in position and different parts of the body contribute unequally to the propulsive force. Note also that the two examples on the left of Fig. 2.8, where propulsive forces arise from one or two objects, are not typical sinusoidal undulatory locomotion. Interestingly, the body patterns resemble what develops when *M. nigrescens* obtains propulsive force from a single fiber (Fig. 2.6). In this case, tension generated by dorsal muscles would apply forces to three restraining points (Fig. 2.12): the fiber loop, the substrate and an arbitrary point on the body through which force is transmitted from posterior regions. The change in body curvature 1/15 second after the fiber breaks (Fig. 2.6B) reflects these forces. How these restraining forces can sum to provide that propulsive force is diagrammed in Fig. 2.12.

2.4. Neuromuscular Control

Figure 2.10a is a snapshot of the body wave at one instant in time. For undulatory locomotion to occur the motor nervous system must accomplish the following:

1. Create a pattern of tension and relaxation somewhat like that in Fig. 2.10a by

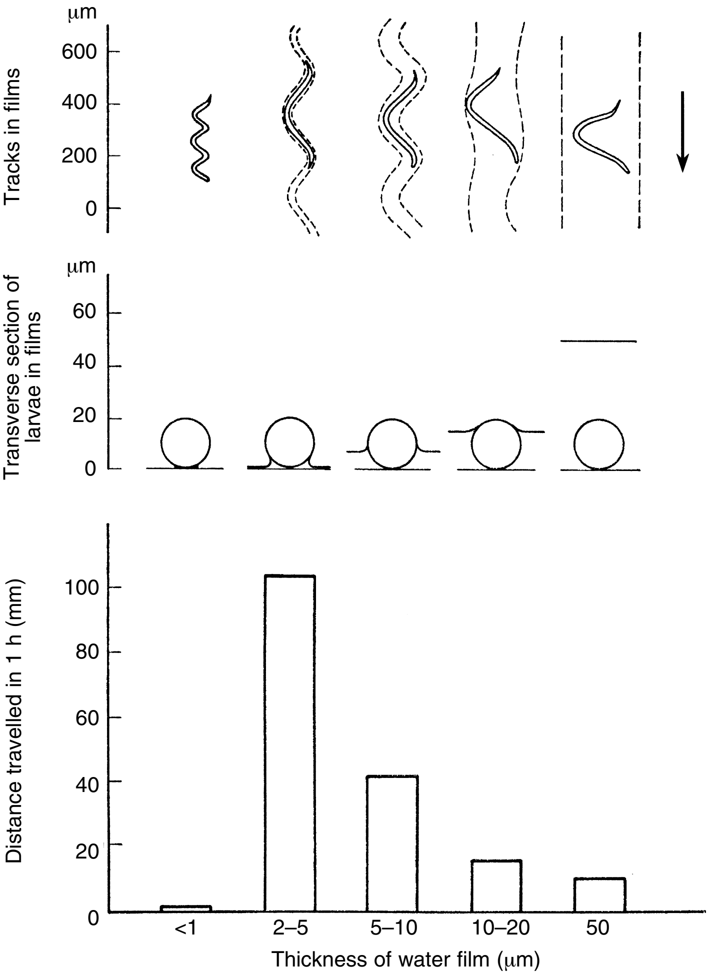

Fig. 2.11. The relationship between thickness of water films on agar, wave-form and speed in *Heterodera schachtii*. For thicknesses greater than 2 µm, speed is inversely related to slippage; however, for thicknesses below 1 µm, friction slows locomotion and wavelength decreases. (From Wallace, 1958.)

exciting dorsal or ventral muscle bands on the anterior edge of the body waves and inhibiting those on the opposite side.

2. Propagate this pattern along the body posteriorly for forward locomotion and anteriorly for backward locomotion.

3. Control the switching from forward to backward locomotion based on sensory input.

4. Provide a rhythmic pattern generator to cyclically initiate the waves.

5. Regulate wave frequency, amplitude, wavelength and rate of propagation according to environmental requirements.

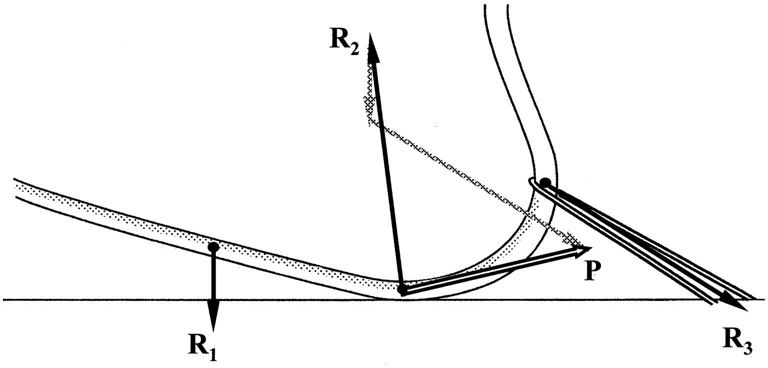

Fig. 2.12. Force diagram illustrating how a propulsive force can be generated by application of force by *Mermis nigrescens* to a loop of fibre as the body slides past, as in Fig. 2.6. Shaded area, regions of muscle tension that apply forces to the loop, substrate and posterior body. The propulsive force P is the resultant of hypothetical restraining forces R_1, R_2 and R_3. Grey vectors show how the three vectors sum. (From A.H.J. Burr, unpublished results.)

6. Utilize sensory feedback to adjust the wave-form according to the position of objects in the environment.

Of the above listed required activities of the neuromuscular system, 1–3 are better understood; the others are more a matter of speculation. See Perry and Maule (Chapter 8, this volume) and previous reviews for details (Johnson and Stretton, 1980; Stretton *et al.*, 1985; Walrond *et al.*, 1985; Chalfie and White, 1988; Wicks and Rankin, 1995; Davis and Stretton, 1996, 2001; Driscoll and Kaplan, 1997; Rand and Nonet, 1997; Thomas and Lockery, 1999; Martin *et al.*, 2002). The neuromuscular anatomy is known in *C. elegans* and *Ascaris* in detail due largely to the work of White *et al.* (1976; 1986) and Stretton *et al.* (Stretton *et al.*, 1978; Angstadt *et al.*, 1989, 2001; Davis and Stretton, 1989). The number, morphology and location of the motor neurons are strikingly similar in these 1 and 300 mm long nematodes. The motor system of all nematodes may be fundamentally similar to these well-studied examples, but note that other nematodes appear to have more complex behaviour and the 120 mm long *M. nigrescens* has many more motor neurons (Table 2.1).

Anyone who has observed nematode behaviour notices a distinction between the exploring, probing and steering motions of the anterior (‘head’ and ‘neck’) of the worm and the undulatory locomotory motion of the remainder of the body which involves only dorsoventral bending. This is a reflection of the innervation of the four to eight rows of muscles that travel through both regions (Fig. 2.1). The anteriormost muscle cells in each row, in the head and neck region, are independently innervated by motor neurons in the nerve ring, providing for complex patterns of movements in both lateral and dorsoventral directions. However, in the region behind the neck, in both *C. elegans* and *Ascaris*, the muscle cells from the two subdorsal quadrants send processes to the dorsal nerve cord, and those from the two

Table 2.1. Number of motor neurons, commissures and muscle cells. The number of muscle cells per mm are equivalent across the three species; however, the number of commissures per muscle cell (and presumably motor neurons per muscle cell) vary dramatically. *Mermis*, with the most versatile locomotion, has the most commissures per mm.

Species	Length (mm)	Motor neurons	Commissures	Commissures per mm	Muscle cells	Muscle cells per mm	Commissures per muscle cell
C. elegans[a]	1	57	36	4	79	79	0.5
Ascaris[b]	300	59	36	0.12	50,000	167	0.0007
Mermis[c]	120	> 1000	1000	12	10,000	83	0.1

[a] Chalfie and White (1988).
[b] Stretton (1976); Stretton *et al.* (1978).
[c] Gans and Burr (1994).

subventral quadrants send processes to the ventral nerve cord (Fig. 2.3). In addition to making chemical synapses with motor neurons in the nerve cords, the processes are tied electrically to each other by gap junctions. This causes the dorsal and ventral pair of muscle bands to act together and only dorsoventral bending is possible. Reciprocal inhibition between dorsal and ventral motor neurons ensures that dorsal muscles relax when ventral muscles contract and vice versa.

What would cause the sinusoidal bending pattern to propagate along the body? Driscoll and Kaplan (1997) review several possibilities. The most interesting is the possibility that distal processes of the motor neurons may act as stretch receptors. The long processes extend well beyond the regions that form neuromuscular junctions and have no other apparent function. When stretched at the outside of a bend, a posterior process could increase the depolarization of the neuron, which would then more strongly excite muscles anterior to the bend. A stronger tension anterior to the outside of a bend and lower tension anterior to the inside of a bend would be appropriate for driving forward locomotion (Figs 2.9 and 2.10).

While this simple model based on the neuroanatomy of *C. elegans* and *Ascaris* would satisfactorily explain locomotion on a smooth agar surface where physical properties of the substrate are uniform, nematodes seldom encounter such conditions outside the laboratory. Changes in wave-form on different substrates are generally consistent across taxa and appear to optimize purchase and reduce slip and yawing. What causes the amplitude to increase as slippage increases (Fig. 2.11)? Why are frequency, amplitude and body form different when a nematode swims? Could these changes be due simply to the smaller lateral restraining forces, or is there a physiological adjustment involved? During locomotion of *Mermis*, it is clear that feedback from touch receptors along the body is needed in order for *Mermis* to maintain the force against a fiber or grass blade as the body glides past (Figs 2.6 and 2.7). Soil-inhabiting nematodes may need a similar mechanism in order to adjust to different particle sizes (Fig. 2.13). While lateral and ventral touch receptors in *C.*

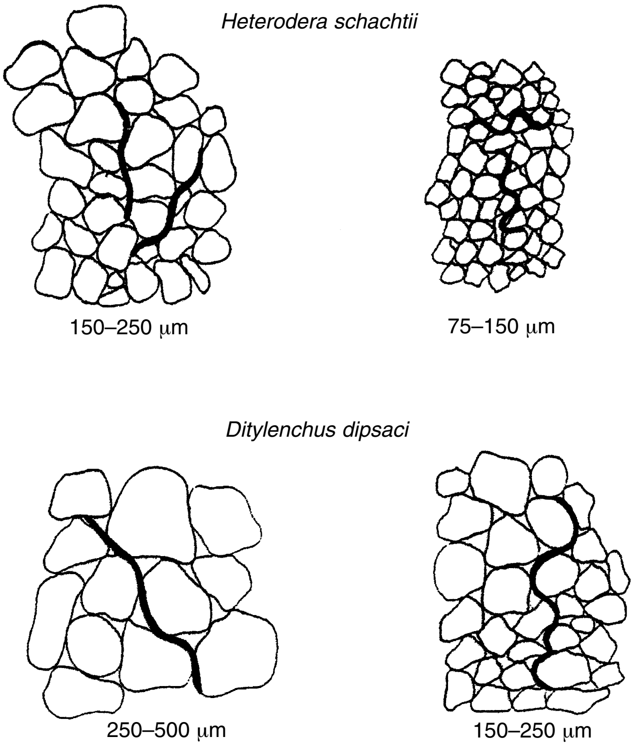

Fig. 2.13. Effect of particle size on wave-form of *Heterodera schachtii* and *Ditylenchus dipsaci*. (From Wallace, 1958.)

elegans have been shown to initiate a reversal reflex, it is not known if they can modulate muscle tension during locomotion. Could some commissures be touch-sensitive? They lie adjacent to the cuticle as they pass from nerve cord to nerve cord and there are many more of them per mm in *Mermis* than in *C. elegans* or *Ascaris* (Table 2.1).

These considerations of locomotion do not cover mechanisms of steering which are initiated by the head and neck. Marine nematodes crawling through filamentous algae display movements that suggest a highly complex sensing and steering capability. Nematodes constrained to crawling on their sides on agar cannot fully display this (Fig. 2.14). Scanning movements of the anterior end can be seen when nematodes forage for bacteria, probe roots or otherwise sample their environment for thermal, chemical or tactile cues, suggesting they thereby gather information that directs their steering. Experiments with *Mermis* demonstrate that, in order for the female to steer towards light, proprioceptive signals of the vertical and sideways head bending must be compared with the periodic signal from the photoreceptor caused by shading by the hemoglobin pigmentation in the eye (Burr and Babinszki, 1990). The innervation of head and neck muscles of *C. elegans* is known to be complex, with motor neurons controlled by interneurons, which integrate

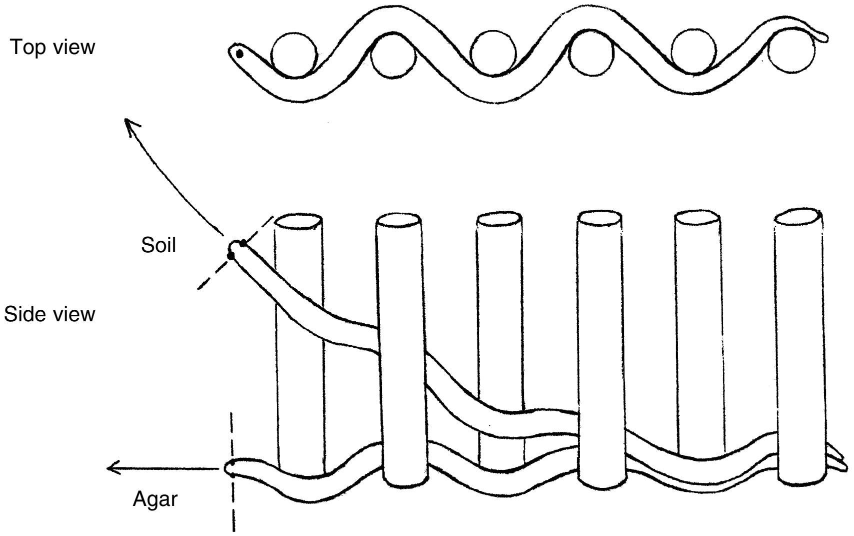

Fig. 2.14. Lateral sampling and steering is prevented when surface tension confines a nematode to a wet planar surface (side-view, lower worm, and top view), because its dorsoventral flexures force it to crawl on its side. However, this is not prevented in three-dimensional media such as a fibre network or soil (upper worm in side-view). Dots represent laterally located sensilla such as amphids. (From A.F. Robinson, unpublished results.)

sensory information in the nerve ring. However, unlike the control of the dorsoventral undulatory motion, the behaviour and physiology of steering are largely unstudied.

2.5. Environmental Challenges and Adaptations

Since most nematodes are microscopic and occupy opaque habitats, their movements are seldom observed directly in nature, although there have been some observations of nematode locomotion on leaves, stems, skin, hair and excised sections of gut. Most observations have been on agar. On rigid surfaces most nematodes require some free moisture for movement. In at least some cases, progression on a moist rigid surface with the appropriate topology can be significantly faster than on agar. There are significant differences in the extent to which nematodes depend on free moisture for movement, and nematodes with tough integuments, such as the ensheathed infective juveniles of entomopathogenic nematodes, appear able to move on virtually dry surfaces.

2.5.1. Locomotion on agar

On the surface of agar wave-form depends on slippage in the surface water film (Fig. 2.11). When placed on water agar weaker than 0.5%, many nematodes will penetrate the surface and move through the agar in a fashion similar to that on the surface, but more slowly. Specific aspects of locomotion on agar can differ greatly, depending on species and goals. Bacteria-feeding rhabditids such as *C. elegans*, for example, typically move slowly on agar that supports a bacterial lawn, frequently pausing to graze with characteristic swinging motions of the head. Conversely, on food-free agar, *C. elegans* cruises for 1–2 min before reversing direction spontaneously for one or more undulations, and then heads off in a new direction. Many infective stages of parasites of vertebrates, foliage or insects move forward on agar without interruption for extended periods, stopping only when the walls of the container are encountered. Nematode tracks on agar often exhibit broad arcs or slew caused by inherent dorsoventral bias in movement, or drag from ventrally bent tails or projected male copulatory organs (Croll, 1972; Robinson, 2004).

2.5.2. Swimming

Some nematodes can swim effectively, especially aquatic species. Among other species, swimming efficiency varies greatly. In general, active nematodes abruptly become more active when transferred from agar to water, and decreasing the viscosity or agar concentration increases wave frequency, amplitude and wavelength. Bacteria-feeding rhabditids, which swim poorly, undulate in water with waves of higher frequency and greater amplitude but otherwise similar in form to those on agar (Fig. 2.11). On the other hand, the vinegar eelworm, *Turbatrix aceti*, which swims more efficiently, generates waves with amplitude increasing by a factor as great as four as they travel posteriorly, and this suppresses yawing (Fig. 2.15; Gray and Lissmann, 1964). In striking contrast, infective juveniles of *Ditylenchus* species, which progress on agar via smooth waves like other nematodes, in free water typically exhibit rapid and repeated bending and unbending in the middle with no progression of the wave along the body. The 120 mm *M. nigrescens* does not swim at all, whereas 300 mm *Ascaris* can swim in intestinal contents. It is worth noting that *Ascaris* can also crawl through a narrow tube by pressing against opposite walls and rolling its body along the opposite surfaces. With forwardly propagated waves they thus accomplish an unusual forward locomotion (Reinitz *et al.*, 2000; A.H.J. Burr, unpublished results).

2.5.3. Movement through soil

Much of our knowledge of factors governing movement of nematodes through soil comes from the classic experiments of Wallace (1968) on nematodes moving in a thin layer of soil (Wallace, 1958). Soil is a mixture of mineral particles that can be placed into three size categories: sand, silt and clay. The relative contents of these particles determine the gross soil texture. This, however, is an oversimplification because the precise distribution of sizes and the surface chemistry of soil particles vary greatly within these particle size classes and among soils. Small particles often

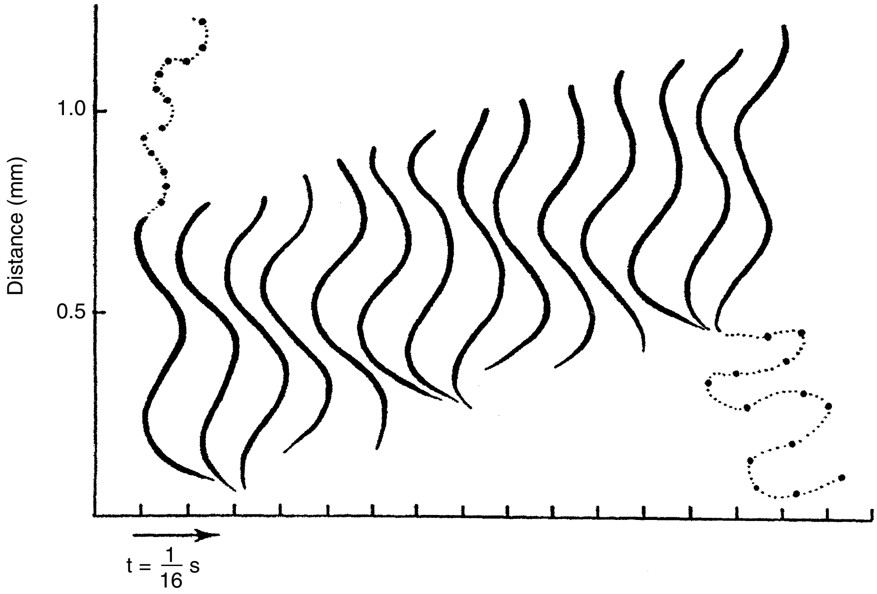

Fig. 2.15. Successive positions at 1/16 second intervals of swimming *Turbatrix aceti*. Note slippage, and amplitude of tail about four times that of head. (From Gray and Lissman, 1964.)

form aggregates or soil crumbs, and the sizes of crumbs present within the soil can be the primary determinant of the suitability of conditions for nematode movement.

The surface tension of a water film draws nematodes towards a flat surface with a force greatly exceeding the force exerted on them by gravity. In soil, a similar effect would be expected but additional forces resulting from the powerful affinity of water for electrically charged soil particle surfaces (primarily clay micelles) must also be considered. These effects are taken into account by expressing the water status of soil in terms of the sum of matric potential (Gibbs' free energy attributable to adhesive forces), osmotic potential (free energy resulting from dissolved solutes) and gravitational potential. Osmotic potential and gravity have little effect on the shape of water films. Instead, the water films in soil for the most part are shaped by surface tension and matric forces, two factors whose relative contributions vary greatly with soil clay content. As a consequence, soils with high clay content have thinner films at the same level of physiological dryness. Wallace's experiments showed that matric potential is the most reliable predictor of the suitability of soil moisture content for nematode movement across a wide range of soil types (Wallace, 1968). A mathematical model has been developed that simulates movement through soil under different controlling factors (Hunt *et al.*, 2001).

As soil dries, solutes become more concentrated. However, osmotic pressure increases little compared with the changes that occur in matric potential, and the resulting differences in osmotic pressure in most soils appear to have essentially no effect on nematodes over the range of moisture contents where surface tension permits nematode movement. This relationship was elegantly demonstrated by an experiment by Blake (1961) in which soil was wetted with a urea solution and subjected to various matric potentials. Movement was similar with water or urea and fastest as the pores of the sand began to drain (Fig. 2.16). These and other results showed that, as soil moisture is removed, reducing thickness of the water films surrounding the soil particles prevents nematode movement long before they are affected physiologically by osmotic pressure (Blake, 1961).

Fastest nematode movement has been shown to occur in soil when it is at field capacity, i.e. the point when soil pores have just partially drained (Wallace, 1968). At field capacity, moisture and gas transport are generally optimum for plant

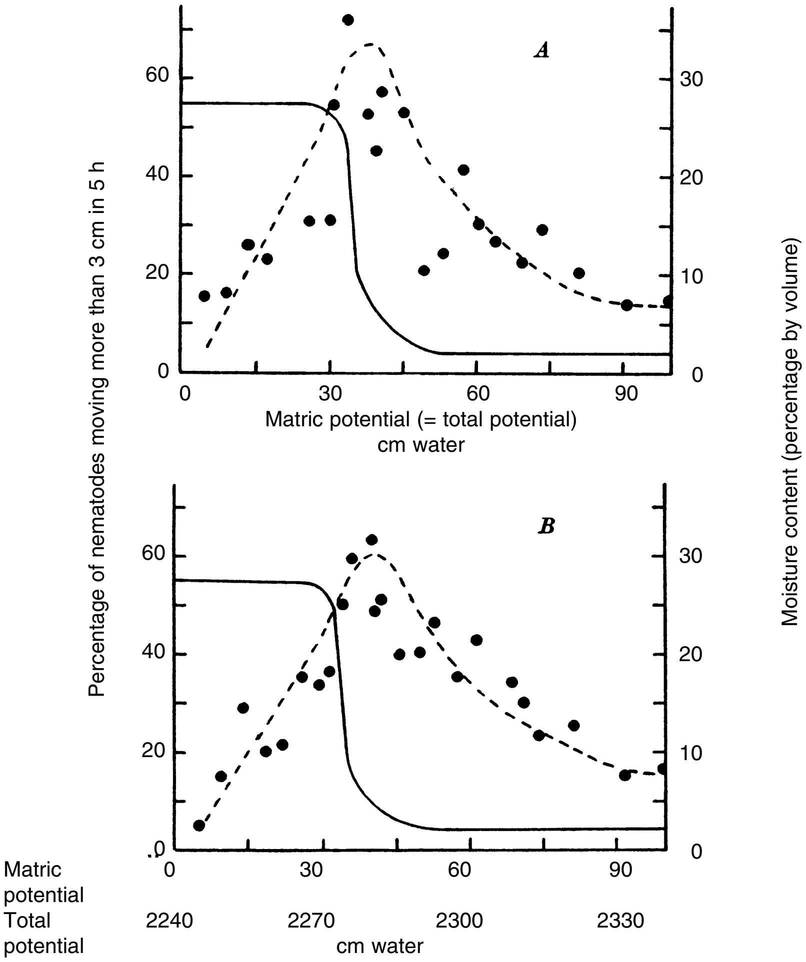

Fig. 2.16. Effect of matric potential on movement of *Ditylenchus dipsaci*. Moisture was removed from soil wetted with water (*A*) or 0.1 M urea (*B*) before stepwise drying. Solid line, moisture content (right-hand scale); filled circles and dashed line, nematode movement (left-hand scale). (From Blake, 1961.)

growth, and the gas phase is continuous, allowing free exchange of respiratory gases as well as the establishment of long-distance gradients within soil channels of volatiles that can serve as attractants and repellents for animals in the soil.

Even though surface tension draws nematodes in a thin film with a force usually exceeding gravity many-fold, a buoyancy differential between head and tail can orient microscopic nematodes in soil if soil pores are sufficiently large. Direct observations of *Heterodera rostochiensis* (Wallace, 1960) and *Rotylenchulus reniformis* (Robinson and Heald, 1993) when suspended in water showed both to point head down, and, when in soil at field capacity, live (but not dead) nematodes moved downward regardless of moisture gradient direction if soil particle sizes exceeded 325 μm.

2.5.4. Adaptations of size and shape

Stages typically found in soil samples appear to fall into two broad size classes with mean lengths of about 400 and 1000 μm. Those in the former may be well suited for moving through soil interstices while the others may utilize channels provided by roots and insects. Observations of juveniles of the sugar beet cyst nematode in a monolayer of soil showed them to move fastest with longest wavelength when the particle size was about 0.3 times the body length, regardless of moisture content (Wallace, 1958). Infective stages of numerous entomopathogenic and vertebrate parasites occurring in soil are similar in overall length and diameter to the bulk of other soil-inhabiting nematodes, suggesting that the soil pore size and tortuosity have selected for optimum length and diameter for movement through soil in diverse trophic groups. Conversely, the retention of a soil stage by most terrestrial vertebrate and arthropod parasites may impose a general constraint on shape that contributes significantly to the highly conserved morphology of nematodes across so many taxa and niches.

The sizes and shapes of nematodes appear attuned to requirements for movement and reproduction not just in soil but in other matrices as well. Thus, the microfilariae of filariids are among the smallest of all nematodes (*c.* 30–40 μm in length), facilitating movement into peripheral dermal capillaries and through the vector's proboscis (Casiraghi *et al.*, 2001). The saccate shape of adult females in the root-parasitic heteroderids reflects a complete trade-off between motility and reproduction. Partial compromises are apparent throughout the phylum. The sacrifice of motility for fecundity by *Ascaris*, which can lay 200,000 eggs per day, is an example. Infective juveniles of *Anguina*, *Ditylenchus*, *Aphelenchoides* and mermithids, which move on to foliage as part of the life cycle are typically similar in diameter to nematodes in soil but are two to five times longer. This probably facilitates movement on foliage, where length-dependent bridging between leaf hairs, leaf folds and isolated water droplets may be critical. *Mermis nigrescens* females, with their unusual 120 mm length and 0.4 mm diameter, bridge large gaps between grass blades (Fig. 2.7A).

2.5.5. Adaptations of speed and activation

The energy cost of movement for nematodes can be considered negligible (Alexander, 2002) and so, not surprisingly, their speed often appears well tuned to ecological needs. The slowest of all nematodes are those that parasitize plant roots and fungi in the soil, i.e. hosts that grow slowly in a highly buffered environment. In comparison, the transient bacterial blooms available to bacteria feeders in and on the soil can require rapid migration to escape desiccation, hitchhike on passing animals and locate new food sources. Nematodes that crawl from soil on to the surfaces of foliage risk a harsh environment where temperature and moisture film availability change abruptly compared with soil. Nematodes that crawl on foliage, be they parasites of plants, insects or vertebrates, typically move in water much faster than soil-inhabiting nematodes. Intestinal parasites must constantly overcome the expulsive effects of peristalsis, and swallowed *Trichinella* juveniles that find themselves in the small intestine following exsheathment in the stomach enter into frenzied activity, which drives them into the intestinal wall and saves them from expulsion (Sukhdeo *et al.*, 2002).

Spontaneity of movement is also typically attuned in relation to ecological needs. For example, cessation or activation of locomotion in response to environmental cues can both result in accumulation within strategic zones that favor survival and maximize the likelihood of host encounters or phoresy. Infective stages of root and foliar parasites typically are spontaneously active and continue to move at a speed linearly related to ambient temperature (Croll, 1975; Robinson *et al.*, 1981; Robinson, 1989). Many nematodes, however, remain lethargic or entirely motionless until activated by triggering stimuli. Examples include activation of *Agamermis* infective juveniles by light (Robinson *et al.*, 1990), of infective juveniles of *Trichinella spiralis* and hundreds of other intestinal parasites on exposure to bile (Sukhdeo *et al.*, 2002), and of *Steinernema* by vibration, air movement and volatile host cues (Gaugler *et al.*, 1980; Campbell and Kaya, 1999a,b).

The wide array of plant, insect and vertebrate parasites that move in large numbers from soil on to foliage sometimes crawl up foliage more than a metre above the soil surface. Such migrations allow nematodes to distribute their eggs or themselves on foliage where their eggs may be eaten by insects or ungulates, or they may gain access to foliar terminals, leaf folds or stomata where they invade plant tissue, induce foliar or floral galls and reproduce. Careful studies of the environmental factors favoring such migrations (Rees, 1950; Adamo *et al.*, 1976; Stromberg, 1997; Robinson, 2000) have shown that rainfall, humidity, foliar pubescence and sometimes sunlight play key roles in activating nematodes when there are suitable physical conditions for locomotion. In the case of *Agamermis*, which infect orthopterans on the Australian tablelands (Baker and Poinar, 1995), orientation precisely perpendicular to light (transverse phototaxis) directs nematodes vertically at sunrise when the moisture films needed for movement on grass blades are present (Robinson *et al.*, 1990). The larger adult female *M. nigrescens* is guided by a positive phototaxis to the upper levels of vegetation, but only under wet conditions (Burr and Babinszki, 1990; Burr *et al.*, 1990; Gans and Burr, 1994).

2.5.6. Other locomotory motions

Undulatory propulsion in three dimensions (Gray, 1953) has been observed for *Hemicycliophora* and *Meloidogyne javanica* during root penetration and for juveniles of *Nippostrongylus* climbing hairs of the animal host (Wallace and Doncaster, 1964). In the case of *Nippostrongylus*, ultrastructural examination revealed a 360° twist of the body for each 360° revolution around the axis of the helix traversed (Lee and Biggs, 1990).

Many different kinds of nematodes move rhythmically and synchronously *en masse* when drawn together by surface tension within a film of water on the surface of agar, glass or plastic Petri dish lids (Gray and Lissmann, 1964; Croll, 1970). Such movement by large numbers of nematodes is called swarming and is commonly observed in crowded populations of *C. elegans*, other bacteria-feeding *Rhabditis* on mushroom beds, *Ditylenchus myceliophagus* and *Aphelenchoides composticola* migrating from foul compost, dauer juveniles of *Pelodera coarctata*, diplogasterids on nutrient plates and entomopathogenic nematodes (Croll, 1970). During swarming, large numbers of nematodes climb up each other, forming a writhing structure above the substrate.

During an additional locomotory subroutine, called nictation, many nematodes lift their anterior or even more of their body off a moist substrate and wave it in the air (Croll and Matthews, 1977). Examples include dauer juveniles of *C. elegans* (Riddle, 1988) and other bacteria feeders (Croll and Mathews, 1977), infective stages of certain hookworms (Lee, 1972; Granzer and Haas, 1991; Muller, 2002) and several entomopathogenic nematodes (Ishibashi, 2002). Nictating can be promoted by adding glass beads or sand grains to agar (Baird, 1999; J. Campbell, personal communication) and by other substrate irregularities (Ishibashi and Kondo, 1990). Nictating at the extremities of objects may improve the chance of phoresy or host encounter. In the presence of its isopod host, dauers of *Caenorhabditis remanei* nictates and crawls onto the host (Baird, 1999). Many rhabditid species associate with isopods, insects or molluscs for transport to new microenvironments, including a potential cadaver (Baird *et al.*, 1994; Baird, 1999; Kiontke *et al.*, 2002). Nictating by the dog hookworm *Ancylostoma caninum* is stimulated by carbon dioxide, warmth and humidity (Granzer and Haas, 1991). During the migration of gravid *M. nigrescens* on vegetation, nictation increases the chance of discovering new surfaces to climb on (Gans and Burr, 1994).

Steinernematid nematodes can jump – for example, the infective juveniles of *Steinernema carpocapsae*. When the nictating anterior does not contact another surface projection, it bends over into a loop, contacting a moist point on the posteriad body. The force generated by the subsequent opposing body wave abruptly breaks the anterior end free of surface tension at the point of contact. This can fling the anterior end upward with sufficient momentum to break the surface tension holding the posterior end to the substrate and jettison the entire body through the air (Reed and Wallace, 1965; Campbell and Kaya, 1999a,b). The entomopathogenic steinernematids use nictating, swarming and jumping to transfer to insects, along with a refinement that allows some species to jump seven or more nematode body lengths above the substrate towards insects. Factors influencing nictating, swarming and jumping in steinernematids have received extensive investigation because these species are important in biological control programmes. Both nictation and jumping are stimulated by carbon dioxide.

Criconemoides curvatum can propel itself forward with no dorsoventral undulatory bending by means of anteriorly propagated peristaltic contractions. Posteriorly angled cuticular ridges provide traction. Cinematography showed worms to progress very slowly (20 μm per contraction) when crawling in water on a glass slide (Streu *et al.*, 1961). A somewhat different pattern, involving non-peristaltic contraction and relaxation of the entire body, was described of *Criconemoides rusticus* as it crawled through soil particles (Stauffer, 1924). *Shaerolaimus gracilis* utilizes adhesive secretions from a caudal gland system and an anteriorly positioned renette cell to release and refasten posterior and anterior ends alternately to the substrate at sequential points in a controlled fashion within water currents (Turpenniemi and Hyvärinen, 1996).

2.6. Summary and Future Directions

Understanding the mechanism of locomotion requires knowledge of cuticle (ultrastructure and elastic properties), hydrostatic skeleton (internal pressure and elastic properties of body wall), muscle (structure, lateral connection to cuticle, tonus and development of bending couples), transmission of forces (along stiff body tube and across cuticle), motor nervous system (connectivity, function of synapses, neurotransmitters and neuromodulators, propagation of waves, mechanism of orientation and lateral steering) and the locomotory role of sensory feedback (of relative location of bends and external objects along the body tube, and of other physical factors, including frictional resistance). Separately these individual areas of investigation have little apparent meaning for locomotion. We have attempted to integrate these separate topics and explain them clearly in the context of their locomotory roles. At the same time, we have highlighted areas that desperately need further study before the basic mechanisms are satisfactorily understood.

Of the special areas listed above, those most in need of further work are: (i) cuticle – elastic properties and transfer of forces across it, including the possible mechanical role of lateral ridges and alae; (ii) muscle – tonus and tension causing stiffness and bending; (iii) proprioception – stretch, pressure and touch reception and all aspects; (iv) hydrostatic skeleton; (v) transmission of forces; and (vi) role of sensory feedback. Especially needed is an investigation of the mechanical properties and putative functions of the hydrostatic skeleton, relating different inflation pressures, changes in dimensions, crossing angle, elasticity, cuticle ultrastructure and bending.

There is current intensive study in other areas: the genetics, molecular composition and development of cuticle and muscle in *C. elegans*, and the effects of neuromodulators on the *Ascaris* neuromuscular system. While important and interesting for other reasons, none of these recent efforts are aimed at attaining the whole picture of locomotion. There is a strong need for more integrative investigations of nematode locomotion.

Knowledge of the mechanism in a few species, however, is not enough. Only by comparative study of the diversity of structure, function and behaviour in the context of adaptation to a varied environment can nematode locomotion be fully understood. We have ended this review by describing the results of some of these excellent studies, hoping to inspire more.

There is an amazing diversity in nematode structure and for taxonomic purposes this has been recorded at the light microscopic scale for more than a century. There is anecdotal evidence for a diversity of nematode behaviour too, and it should also be documented. Technology is now available for archiving locomotion and other behaviour as video clips. These can accompany a new method for archiving morphological information, video capture and editing (VCE) microscopy (De Ley and Bert, 2002), which provides micrographic images of nematodes as a multifocal series (see http://faculty.ucr.edu/%7epdeley/vce.html). These records will soon be provided on a website together with DNA sequences, and behavioural clips can readily be added.

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