BIOLOGICAL
REVIEWS

Cambridge
Philosophical Society

Biol. Rev. (2014), pp. 000–000.
doi: 10.1111/brv.12073

Locomotion in caterpillars

L. I. van Griethuijsen* and B. A. Trimmer

Department of Biology, School of Arts and Sciences, Tufts University, 200 Boston Avenue, Suite 2600, Medford, MA 02155, U.S.A.

ABSTRACT

Most species of caterpillar move around by inching or crawling. Their ability to navigate in branching three-dimensional structures makes them particularly interesting biomechanical subjects. The mechanism of inching has not been investigated in detail, but crawling is now well understood from studies on caterpillar neural activity, dynamics and structural mechanics. Early papers describe caterpillar crawling as legged peristalsis, but recent work suggests that caterpillars use a tension-based mechanism that helps them to exploit arboreal niches. Caterpillars are not obligate hydrostats but instead use their strong grip to the substrate to transmit forces, in effect using their environment as a skeleton. In addition, the gut which accounts for a substantial part of the caterpillar's weight, moves independently of the body wall during locomotion and may contribute to crawling dynamics. Work-loop analysis of caterpillar muscles shows that they are likely to act both as actuators and energy dissipaters during crawling. Because caterpillar tissues are pseudo-elastic, and locomotion involves large body deformations, moving is energetically inefficient. Possession of a soft body benefits caterpillars by allowing them to grow quickly and to access remote food sources safely.

Key words: caterpillar, locomotion, kinematics, dynamics, soft-bodied, *Manduca sexta*.

CONTENTS

---

* Author for correspondence (Tel: +1 617 627 3924; E-mail: l.vangriethuijsen@outlook.com).

I. INTRODUCTION: THE CONSEQUENCES OF BEING A SOFT-BODIED LARVA

The insect order Lepidoptera contains diverse and widespread species that numerically dominate many forest and field environments. The lepidopteran larval stages (caterpillars) are usually entirely herbivorous, growing rapidly in, on, and around the stems and leaves of their host plants. In addition to providing food, the host plant provides shelter, camouflage and even chemical defences to the caterpillar (Hartmann, 2004). Their reliance on the host plant means that caterpillars must move effectively in branched, complex, three-dimensional structures: in general they cannot afford to fall or fail to reach edible structures. Despite the environmental and agricultural importance of caterpillars, their locomotion has not been studied widely or described systematically. However, recent studies of biomechanics, tissue characterisation, videography and motor patterning have revealed new insights into the mechanisms of movement control in these soft-bodied animals. This review provides a description of these findings and discusses some specialisations of caterpillar locomotion which differ from those of adult insects and other soft-bodied creatures.

Within Lepidoptera, Glossata is by far the largest suborder and encompasses the moths and butterflies in which the adults have a proboscis that can be coiled. Within the Glossata, the Ditrysia includes 98% of all butterflies and moths and almost all caterpillar species that are external plant feeders (Powell, 2009). The species described herein are all members of Ditrysia. Because of its widespread use as a model organism in laboratory studies, locomotion in the tobacco hornworm (*Manduca sexta* L.) (Sphingidae) has been described in the greatest detail. Although the findings described below are expected to be relevant to most surface-crawling caterpillars, some details might not be translatable to other lepidopteran species such as leaf miners and borers that live inside host plant material.

Being a larva has consequences for locomotion. First, reproduction does not take place in the larval stage, so mating and associated behaviours are not direct evolutionary drivers for morphology and locomotion. Second, the size of the adult, which does affect its fitness, is determined by the size of the larva immediately before pupation (Stamp & Casey, 1993). Because large size generally leads to higher adult fitness, it is important for the larva to maximize its size by maximizing feeding opportunities. The distance covered to find food can differ dramatically among different species. *Manduca sexta* caterpillars hatch on their food as the female moth places her eggs on a suitable host plant. These caterpillars tend to stay on the same plant until they climb down the plant to pupate in the soil (Bernays & Woods, 2000). Other caterpillars such as the tent caterpillar (several species in the genus Malacosoma) travel to reach their food sources. During the night these caterpillars reside in silk tents and early in the morning they move to their feeding grounds.

Caterpillars may grow several orders of magnitude from hatchling to prepupa. During this period they moult several times, each stage between moults being an instar. Between moults their soft cuticle can stretch extensively. The only hard cuticle is found on the mandibles, spiracles, the thoracic legs, the crochets (hooks) at the tip of the prolegs and the head capsule (Fig. 1A). This overall body softness has important consequences for locomotion. Because muscles are connected to the body wall, their activation can deform the body without leading to locomotory movement. This is a highly energy-inefficient way to move: the cost of transport for gypsy moth caterpillars (*Lymantria dispar* L.) (Erebidae) is 4.5 times greater than expected for an animal of similar size with a rigid skeleton (Casey, 1991). As mentioned previously, lepidopteran larvae grow dramatically. With the exception of approximately 200 species of Lepidoptera that have carnivorous or parasitic larvae (Pierce, 1995), including some that ambush their prey (Montgomery, 1982, 1983), caterpillars are herbivores. Because plants are low in nitrogen, which is essential for growth, caterpillars probably consume more plant material than they need in terms of energy content (Slansky & Feeny, 1977). Therefore, access to calories is unlikely to be limiting and energetic efficiency is unlikely to be a strong evolutionary selective force for caterpillar locomotion.

II. VARIATIONS IN LOCOMOTION IN CATERPILLARS

Caterpillars have a soft cylindrical body, with three pairs of stiff-jointed thoracic legs and 2–5 pairs of soft leg-like structures on the abdomen called prolegs (Fig. 1A). Apart from this overall body plan, caterpillars differ dramatically in body shape and size and hence exhibit different strategies for forward locomotion. For example, inching behaviour in which the posterior legs are pulled forward to grip the substrate just behind the thoracic segments (each ‘step’ is therefore almost one body length and forms a distinctive dorsal body loop) is seen more often in smaller and thinner caterpillars. Anterograde stepping or crawling is generally seen in larger and thicker caterpillars although some species use both or even a combination of these basic modes (Lin, 2011). Thinner caterpillars can be forced to use an anterograde stepping motion by limiting the extent to

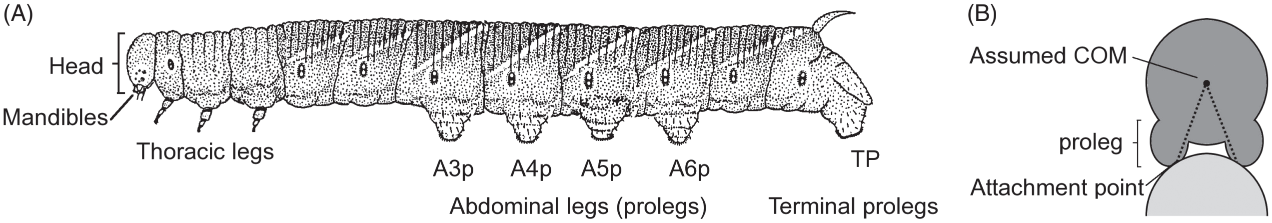

Fig. 1. (A) The anatomy of larval *Manduca sexta* (fifth instar). Caterpillars generally have this body plan but the number of prolegs can vary among species. The body is soft except for the head capsule, mandibles, thoracic legs and the crochets on the tips of the prolegs. (B) Schematic cross section of a caterpillar on a dowel. COM = centre of mass.

which they can lift their bodies from the substrate (Lin, 2011), but this gait is much slower than their normal inching gait.

Several other types of locomotion are used by caterpillars, mainly as escape strategies. When the caterpillar of the mother-of-pearl moth (*Pleuroptya ruralis* Scopoli) (Crambidae) is startled, it can launch itself into the air, form a round shape and roll down an incline like a wheel (Brackenbury, 1997). Other escape strategies include backwards crawling (Brackenbury, 1999), and dropping from a leaf and climbing back up using silk lifelines (Brackenbury, 1996). In this review we will briefly consider these escape behaviours and inching, but then mostly focus on crawling as this mode of caterpillar locomotion has been described most extensively in the literature.

(1) Inching

Inching is the most common form of locomotion in small slender caterpillars. An inching caterpillar uses the thoracic legs and the posteriormost prolegs as anchors. When the posterior anchor is moved forward, the middle of the body is lifted away from the substrate and the body of the caterpillar resembles an Ω (omega). Next, the caterpillar releases the front grip and extends the body forward forming a cantilever that requires the body to be relatively stiff (Lin & Trimmer, 2010*a*). This is perhaps why inching is observed less often in larger caterpillars; stiffening requires disproportionately large increases in body pressure (Lin *et al.*, 2011). Also inching requires the body to be lifted away from the substrate. This raises the centre of mass (COM) leading to increased rotation around the attachment points and an increased risk of tipping sideways due to small non-axial forces. Compared to crawling, inching therefore requires enhanced grip while simultaneously decreasing the number of contact points. Despite a strong grip, the shifted COM could limit what substrates can be utilized by the caterpillar.

Certain lepidopteran families are known for inching, in particular the Geometridae. This name means ‘earth measuring’ and refers to an inching movement in which the caterpillar seems to measure a certain distance with each step. Caterpillars of the family Noctuidae also display this type of locomotion (Miller, Janzen & Hallwachs, 2006).

(2) Rolling, thread-climbing, backward locomotion and galloping

Caterpillars also use a variety of short-term locomotion strategies as part of their escape responses. At least 13 species are known to use silk threads as lifelines, to allow the caterpillar to climb back up after dropping from a leaf when threatened (Sugiura & Yamazaki, 2006). Thread-climbing represents a very different type of locomotion to the anterograde wave of steps seen in crawling. Only the thoracic legs are used to haul the body back up. At least one species can use an anterograde wave of steps but most caterpillars observed in the field alternate their left and right thoracic legs. The body is swung to the left and right actively to aid in climbing (Brackenbury, 1996).

In two species, the mother-of-pearl moth and the carnation tortrix (*Cacoecimorpha pronubana* Hübner) (Tortricidae), Brackenbury (1999) recorded fast backward locomotion that represented the reverse of forward locomotion. Strong stimulation of the anterior of the caterpillar resulted in reverse galloping, which had a lower duty factor (the proportion of legs attached to the substrate) than crawling, and eventually a rolling behaviour, where the animal curled into a wheel shape and rolled (Brackenbury, 1997, 1999). Both forms of locomotion were much faster than forward crawling, with rolling being up to 40 times faster. Some species, such as the mullein moth (*Cucullia verbasci* L.) (Noctuidae), can increase the speed of forward locomotion by decreasing the duty factor. Effectively these animals move from an anterograde stepping wave to a form of locomotion resembling inching. Although the first proleg lifted from the substrate is still the most posterior one, the body arches the segments without prolegs (A1–A3) and prolegs A3–A4, A5 and A6 are lifted in a posterograde wave (Brackenbury, 1999).

(3) Crawling

In anterograde locomotion, or crawling, a wave of steps is initiated at the posterior of the animal. Both legs of each pair are lifted simultaneously. The prolegs do not shorten much during the swing phase of the step; rather the body lifts the entire segment (Belanger & Trimmer, 2000; Trimmer & Issberner, 2007). Crawling is perhaps the most extensively studied form of locomotion in caterpillars, and will be described in detail below.

III. HISTORICAL MODELS OF CATERPILLAR LOCOMOTION

Despite early attempts to understand caterpillar tonus and neuro-anatomy (Kopec, 1919; Holst, 1934) the first detailed description of caterpillar crawling used purely observational methods (Barth, 1937). Barth combined detailed anatomical descriptions of internal muscle arrangements with observations of the complex body motions of crawling and formulated a model based on peristalsis. In his view, each body segment was activated in turn using antagonistic interactions between dorsal and ventral muscles in adjacent segments. The small tergopleural muscles, which run vertically and are positioned next to the spiracles, were suggested to lift the body segment during the proleg swing phase. This model of caterpillar crawling was used in many insect textbooks (Wigglesworth, 1950; Hughes, 1965; Douglas, 1986; Chapman, 1998). More recent work established that such segment-by-segment waves of muscle activation are not distinct and found no evidence for dorsal-ventral antagonism or tergopleural lifting (Trimmer & Issberner, 2007; Simon *et al.*, 2010*a*).

Our current understanding of caterpillar crawling was first described in a little-known booklet called '*The Caterpillar and the Butterfly*', written in 1961 by R. E. Snodgrass. He was the first to realise that, in contrast to most other terrestrial animals, the prolegs were not used to push the body forward. Instead caterpillars use the prolegs as gripping devices and to support the body weight during stance. He also realised that caterpillar locomotion was not a peristaltic movement like that seen in earthworms, and highlighted the crucial role of muscle activity in pulling segments forward (Snodgrass, 1961). However Snodgrass did not discuss the role of hydrostatics and his insights were not based on quantitative kinematics or measurements of forces.

IV. RECENT FINDINGS IN CATERPILLAR LOCOMOTION

(1) Detailed description of crawling in Manduca sexta

Caterpillar locomotion has been studied most extensively in the tobacco hornworm, which is the larval stage of the tobacco hawkmoth. Tobacco hornworms are bright green, covered with relatively small sensory hairs and grow to a large size, weighing as much as 8 g at the fifth instar. *Manduca sexta* can sequester and secrete nicotine and other alkaloids (Morris, 1983*a*,*b*,*c*, 1984; Murray *et al.*, 1994) and is usually found on species of Solanaceae such as tomato and tobacco that use these compounds to deter herbivores (Self, Guthrie & Hodgson, 1964). *Manduca sexta* can be easily maintained on an artificial diet (Bell & Joachim, 1978) and several laboratories have kept colonies for decades. The caterpillar has been used as a model for endocrine signalling, programmed cell death and nervous system remodelling. The massive changes that accompany metamorphosis include the rewiring of connections from neurons to muscles, so the muscles and their innervations have been described in detail (Taylor & Truman, 1974; Levine & Truman, 1982, 1985; Weeks & Truman, 1984).

A detailed videography study of caterpillar crawling was carried out by Trimmer & Issberner (2007). Both prolegs on a single body segment are lifted simultaneously. Waves of steps start at the terminal segment and end at the thoracic legs with one such wave of steps termed a 'crawl'. Each leg goes through two phases during each crawl, the swing phase (also called a 'step') during which the leg is moved forward, and the stance phase during which the leg is in contact with the ground or substrate. These stepping motions of caterpillars are similar to those seen in vertebrates: the body moves forward during swing phase. During the stance phase the terminal body segment appears to pivot around the attachment points, but the mid-abdominal prolegs do not pivot and simply act as support struts (Trimmer & Issberner, 2007) (Fig. 2).

Within a crawl steps of different prolegs overlap; the proleg on abdominal segment 6 (A6) lifts off before the terminal proleg (TP) has finished its step, hence two or three pairs of legs are lifted simultaneously. This has been observed in both *Manduca sexta* and *Pleuroptya ruralis* (Brackenbury, 1999; van Griethuijsen & Trimmer, 2009). In normal anterograde crawling, speed seems to be limited by the need for stability. Because of the location of the prolegs under the body, and the relatively elevated centre of mass (Fig. 1B), caterpillars run the risk of rolling sideways and hence a high duty factor is desirable. Brackenbury (1999) noted that if the legs and body segments cannot be lifted faster, then decreasing the timing between each anterograde wave of steps becomes the only possible mechanism to increase speed but this will result in increased instability due to a reduced duty factor. Although the timing between crawls can be altered to change the overall average velocity the timing between steps is relatively constant (van Griethuijsen & Trimmer, 2009).

It generally takes *Manduca sexta* a little more than 3 s to cover 1 cm (Trimmer & Issberner, 2007; van Griethuijsen & Trimmer, 2009). This is a low speed compared to other caterpillars: Brackenbury (1999) reported speeds of 0.62–1.02 cm/s for four species of caterpillar. Joos (1992) reported 0.37–2.35 cm/s for the eastern tent caterpillar (*Malacosoma americanum* Fabricius) (Lasiocampidae) depending on temperature. These faster caterpillars also tend to be smaller (about 2.5 cm long) than fifth instar *Manduca sexta* (about 5 cm) which contrasts with the general trend for larger animals to move at higher velocities (Full, 1997).

Much is known about the layout of *Manduca sexta* muscles. Each body segment has two layers of longitudinal and oblique muscles; there are no circular muscles. Major muscles of the outer layer consist of four oblique and two longitudinal muscles [dorsal external medial (DEM), dorsal internal oblique (DIO), lateral internal oblique (LIO), ventral internal oblique (VIO), ventral external oblique (VEO) and ventral external medial (VEM)]. The inner layer consists of two

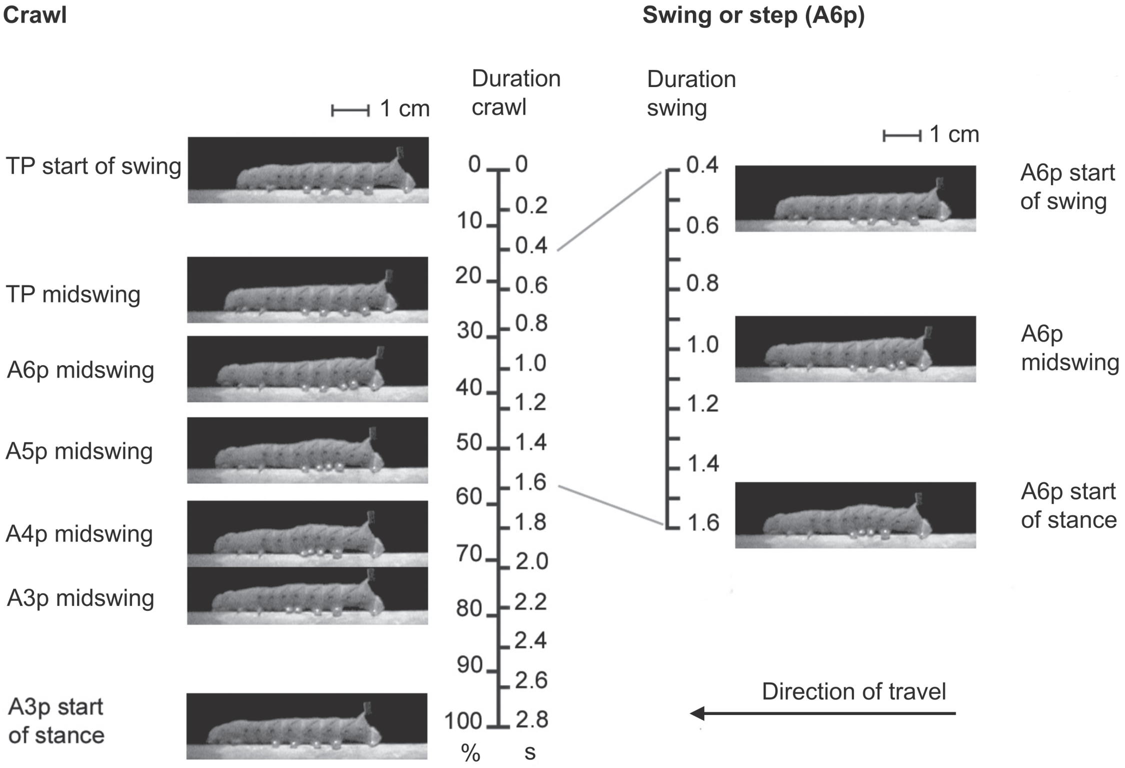

Fig. 2. Sequence of images of a crawling *Manduca sexta* caterpillar. Markers are positioned on the tips of the prolegs to aid in kinematic studies. A full crawl starts with the terminal proleg (TP) lifting away from the substrate (top left). Each proleg is lifted and moved forward. In this individual, a single crawl lasted 2.8 s. On the right the swing phase of A6p is visualized in more detail. The start of the swing phase can be seen on the upper right, followed by the midswing, at which point the velocity of a proleg relative to the substrate is maximal. The swing phase ends with the start of the stance phase. The swing phase of a proleg overlaps with the swing phase of both its predecessor and successor. Adapted from van Griethuijsen & Trimmer (2009).

large longitudinal muscles [dorsal internal medial (DIM) and ventral internal lateral (VIL)] and two small longitudinal muscles [ventral internal medial (VIM) and dorsal internal lateral (DIL)].

The activity of the larger muscles during crawling has been studied using implanted bipolar electromyographic (EMG) wires (Simon *et al.*, 2010*a*) and flexible multi-electrode arrays (Metallo, White & Trimmer, 2011). A wave of muscular activity progresses from the posterior body segments towards the head. In contrast to the model proposed by Barth (1937), the wave of activation is not made up of discrete segment-to-segment motor activity but instead involves substantial overlap of muscle activation between segments. For example, the activation of the VIL in segment A3 begins at the peak of VIL activation in segment A6 (Simon *et al.*, 2010*a*). This means that the VILs (and presumably other major muscles) are coactive in different body segments for much of a crawl, differing only in the phase of activity onset.

The large DIM, VIL and VEO muscles within a body segment are also coactive during crawling with no significant differences in the timing of onset, peak or offset of activity among the three muscles (Simon *et al.*, 2010*a*). Activity in the large longitudinal muscles accounts for the wave of segment shortening during a crawl and it is reasonable to suppose that activity in the oblique muscles helps to bias tension in the body wall so that the body segment lifts away from the substrate. This is analogous to the curvature created in a cylindrical balloon when one side is stiffened with non-extensible tape. It is important to note that muscles can evoke such bending without significantly shortening, they need only stiffen parts of the body wall to redirect forces created by other movements; this might be called a 'dynamic skeleton'.

Given these long-lasting and overlapping waves of intersegmental muscle activation the timing of proleg grip release is critical for normal forward progression. The control of proleg gripping is described in more detail in Section IV.3).

(2) Models of soft-bodied locomotion

(*a*) *Classic hydrostats and muscular hydrostats*

Locomotion in caterpillars has long been compared to that of hydrostats, such as the earthworm, or muscular hydrostats, such as the octopus. Hydrostats are animals without a hard skeleton that have a fluid-filled cavity surrounded by muscle. Because the volume of a fluid does not change when pressurized (at least to the levels seen in physiology), forces produced by muscles can be transmitted to other body regions and to the substrate through the fluid-filled cavity. This can be seen in the clear increases in pressure of a moving earthworm (Quillin, 1998). Constant-volume fluid systems can also produce lever-like mechanics by exchanging forces and displacements in linked cavities of different sizes (the principle employed by hydraulic lifts) (Vogel, 2003). It is tempting to assume that all soft-bodied animals use pressure in this way to extend their body and limbs but caterpillars seem to deviate from this assumption (see Section IV.2*b*). Muscular hydrostats form a subclass of hydrostats: in these the muscles themselves can transmit forces because they consist of fluid-filled cells of constant volume that can be pressurized when the muscle is activated. In caterpillars, however, the body is not packed with muscles so they do not function as a muscular hydrostat.

(*b*) *Use of pressure in Manduca sexta locomotion*

Before discussing the role of pressure in caterpillar locomotion, it is necessary first to describe the properties of the cuticle and how it stretches and folds, as these properties influence the response of the body when a change in pressure occurs. The following descriptions are derived from *Manduca sexta* caterpillars, but the same principles are likely to apply to other species. The cuticle of *Manduca sexta* has several folds running in circles around the body and dividing each body segment into eight annuli or rings. These folds function as wrinkles: the cuticle buckles here longitudinally when it is compressed. At the posterior end of each body segment is a true fold; when the animal is at rest, part of the cuticle is folded inwards. Two large muscles, the dorsal internal medial (DIM) and ventral internal lateral (VIL) attach to this fold and help to pull the cuticle inwards. When the body segment stretches during locomotion or casting (searching) behaviours, this fold disappears (Trimmer & Issberner, 2007). The only folds running longitudinally are the ones above each proleg that allow buckling of the skin when the prolegs are shortened. There are no known behaviours that drastically change the circumference of the animal, although some flattening of the ventral surface occurs during casting behaviour. In addition to the folds and wrinkles described above, the mechanical properties of the cuticle also influence how the tissues respond to pressure. Application of the same stretch to a piece of cuticle produced larger stresses when that piece of cuticle was oriented circumferentially in the caterpillar's body (Lin, Dorfmann & Trimmer, 2009). One possible explanation for this lies in the cuticle's structure: in the Brazilian skipper (*Calpodes ethlius* Stoll) (Hesperiidae), there are microscopic pleats in the cuticle that unfold more easily longitudinally than circumferentially as the caterpillar grows (Carter & Locke, 1993). This difference in response of the cuticle to stress is necessary because, under uniform pressure conditions, hoop stress (circumferential tensile stress) is twice as large as axial stress (longitudinal tensile stress). Without this difference, the caterpillar would not grow uniformly between moults, but would instead mostly increase in circumference (Carter & Locke, 1993).

Caterpillars have a fluid-filled cavity (the haemocoel) in which the muscles are located together with the fat body, gut and trachea (breathing tubes). The air-filled trachea are relatively stiff and corrugated to protect them from collapse during normal movements. In addition, the trachea are in direct contact with the air outside the animal *via* the spiracular openings. Spiracles are located on each side of most body segments and can be opened or closed to regulate gas exchange and to minimize water loss. Because air is compressible and can be lost from the spiracles the caterpillar haemocoel does not have a constant volume. This can easily be demonstrated by exercising caterpillars underwater. After a short while air bubbles form at the spiracles and these expand and contract with body movements (H. T. Lin, unpublished observation). The relatively large volume of air in *Manduca sexta* hence makes it difficult to control movements by the use of pressure alone. This problem is complicated by the decreasing effectiveness of pressure-stiffening as animals grow larger; to be able to stiffen its body a constant amount, a growing caterpillar must either increase its internal pressure or dramatically increase its diameter:length ratio. *Manduca sexta* does neither (Lin *et al*., 2011). Finally, the internal fluids of *Manduca sexta* are in constant motion (Simon *et al*., 2010*b*) and despite the relatively open haemocoel, the body is not iso-barometric (Mezoff *et al*., 2004). These considerations make it unlikely that caterpillars use a hydrostatic skeleton in the same way as earthworms although pressure and hydraulics may still be important mechanical components in caterpillar locomotion.

(3) **Kinematics and dynamics studies**

(*a*) *A surprising finding: movement of the gut*

Although the body wall and muscles are the most obvious mechanical components of caterpillar locomotion, most of the body mass consists of the gut and other internal tissues. Movements of these tissues might affect locomotion but are difficult to visualize in intact animals. One approach is to image soft tissues using high-energy X-rays. By maintaining a crawling caterpillar on a treadmill in a synchrotron-sourced X-ray beam it has been possible to visualize the movements of trachea attached to the gut. Surprisingly the midgut was found to move an entire body segment forward before the surrounding body wall began to move. It is not clear if the gut is being pushed forward by the terminal segments or being pulled forward by the head and thorax (Simon *et al*., 2010*b*). This phenomenon was seen not only in fifth instar *Manduca sexta* caterpillars, which are relatively large, but also in hatchlings (Simon *et al*., 2010*b*). Movements of the gut during locomotion have also been observed through the translucent cuticle of the Brazilian skipper (B. A. Trimmer, unpublished observation). This suggests that this 'visceral-locomotory pistoning' is a widespread phenomenon in caterpillars that could change the centre of mass during the stance phase. The movement of the gut might therefore be an integrative part of locomotion or could represent a strategy to prevent the anterograde movements of locomotion from interfering with the posterograde movement of the gut contents (Chapman, 1998; Simon *et al.*, 2010*b*).

(*b*) *Effects of body orientation*

Caterpillars crawling on a horizontal surface exhibit similar movements to climbing caterpillars even though gravity acts in different directions in these two situations. Detailed kinematic studies confirmed that the movements are indeed very similar. In fact, none of the parameters tested could be used to differentiate horizontal from vertical crawling (van Griethuijsen & Trimmer, 2009). Statistically significant differences were found in the distance covered by a step and timing between crawls (together resulting in a lower crawling speed). The actual differences were very small compared to the variability within the data, and not believed to be biologically relevant to the caterpillar as caterpillars generally do not rely on speed of locomotion to escape predators or find food sources. The similarities between vertical and horizontal crawling might be due to the strong grip of the prolegs, which is used regardless of the orientation of the body. Locomotion is relatively costly for small animals when expressed as mass moved per unit distance, which makes the extra energy required to lift the body during vertical locomotion relatively small (Taylor, Caldwell & Rowntree, 1972). Caterpillars are particularly inefficient energetically because of their soft body (Casey, 1991). From oxygen use by crawling gypsy moth caterpillars Casey (1991) calculated that their minimum cost of transport was 4.5 times higher than expected for a similarly sized animal with a jointed skeleton. This means that, for a climbing caterpillar, the extra energy required to lift the body is a negligible component of the total.

(*c*) *Movement of the thoracic legs*

Caterpillars have three pairs of thoracic legs and several pairs of prolegs. Most studies on caterpillar crawling have focused on the prolegs because they have a stronger grip, the crawl initiates posteriorly and most of the crawl cycle occurs in the abdomen. If the thoracic legs are removed or their movement constrained, locomotion is unaffected (Kopec, 1919). The thoracic legs are very different structurally from the prolegs: they eventually turn into the legs of the adult, and in the caterpillar they are already articulated and possess an exoskeleton. The movements of the thoracic legs in the caterpillar are smaller and less regular than those of the prolegs (Johnston & Levine, 1996*b*). Like the prolegs, both thoracic legs on one body segment are lifted simultaneously, and the wave of steps moves from posterior to anterior. The thoracic legs occasionally take additional steps (extra-step crawling) (Johnston & Levine, 1996*b*), and it has been suggested that they are used (together with the mandibles and antennae) to probe the substrate. Interestingly, extra steps are not seen in late fifth instar caterpillars crawling on a flat surface (Johnston & Levine, 1996*b*). During the late fifth instar, also called the wandering stage, caterpillars actively search for a pupation site. It is not clear why extra steps are not seen in these larvae if their function is indeed to examine the substrate.

Because the thoracic legs persist into the adult stage, the locomotion patterns of these legs can be compared between these two stages. The thoracic legs do change in appearance during metamorphosis: the adult leg is longer and thinner than the larval leg. The most pronounced difference is in the femur, which points downward in the larva, but upward in the adult. The motor neurons to the muscles in the thoracic legs persist from the larval to the adult stage (Kent & Levine, 1988). The larval thoracic legs have a similar locomotion pattern as the prolegs, but in the adult the thoracic legs display no patterns of intersegmental coordination and both left-right alternation and synchrony were observed (Johnston & Levine, 1996*b*). This is in contrast to many adult insects that do display strict inter- and intrasegmental coordination as for example in the tripod gait, which is a very stable gait for a six-legged animal. In a tripod gait, the most posterior and anterior leg on one side of the animal are in stance phase with the middle leg on the other side of the animal, this alternates with a stance phase in the other three legs.

(*d*) *Movement of the prolegs*

Prolegs are only found on the abdominal segments and always in bilaterally symmetrical pairs, including those on the terminal segment. Not all abdominal segments have prolegs, and the number can vary greatly among species. In general, additional pairs are found in caterpillars that crawl compared with those using inching. The proleg is divided into three pseudosegments: the planta, the coxa and the subcoxa (Mezoff *et al.*, 2004) (Fig. 3). The planta is located at the proleg tip, its cuticle is softer and can be drawn in by the principal planta retractor muscle (PPRM) to retract the two rows of hook-like structures called crochets, that are located on the planta in most species. The coxa runs from the edge of the planta to the first fold which acts as an attachment point for the accessory planta retractor muscles (APRM). The subcoxa runs to the next fold (Mezoff *et al.*, 2004). The prolegs on the terminal segment differ anatomically from the prolegs on the other abdominal segments with additional large muscles attached to the base of the appendage. The terminal prolegs (TP, note that TP may also be used to describe the entire terminal body segment) functions in the same way as the other prolegs although, at least in *Manduca sexta*, cannot be shortened to the same extent. However the TP can pivot much more that the other prolegs because there is no attachment point posterior or directly anterior to it (Trimmer & Issberner, 2007) (Fig. 1A). The TP can also be referred to as ‘claspers’.

Detailed kinematic studies of the prolegs in *Manduca sexta* have shown that during the swing phase the prolegs are lifted because the body curves, resulting in the characteristic travelling hump on the back of the caterpillar (Fig. 2). While the prolegs move forward, they do not shorten much. There are two major muscles that are capable of shortening the proleg: the APRM and the PPRM. The PPRM runs from near the spiracle to the tip of the planta whereas the APRM

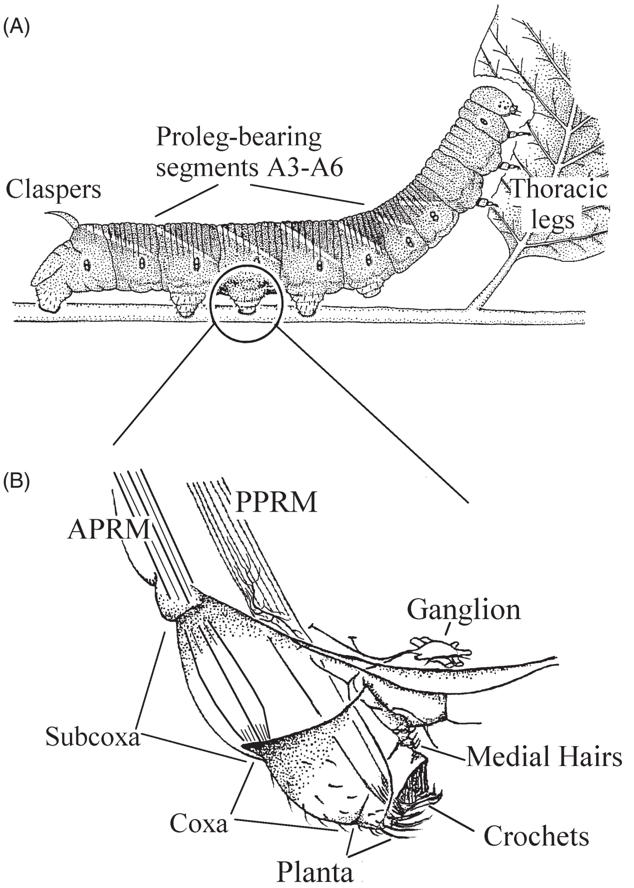

Fig. 3. Anatomy of a *Manduca sexta* proleg. (A) A late fifth instar larva illustrated from the right-hand side. Prolegs are found on abdominal segments 3–6 (A3–A6). The specialized legs on the most posterior segments are called claspers. (B) A single right-hand side proleg illustrated in three-quarter frontal view. The pseudosegments, subcoxa and coxa are named by analogy with segments in the articulated thoracic legs. The tip of the proleg is called the planta and it carries the curved cuticular hooks (crotchets) used for gripping. The principal and accessory planta retractor muscles (PPRM and APRM, respectively) have their origin on the lateral body wall near the spiracle (not illustrated) and insert at the planta and coxa–subcoxa boundary, respectively. The medial hairs are located along the inner surface of the proleg. From Mezoff *et al.* (2004).

runs from near the spiracle to half way along the proleg (Fig. 3B). The length of these retractors can be monitored by attaching beads to the muscle origin and insertion points on the cuticle and tracking their movements while simultaneously recording electrical activity of the muscles using EMG. The APRM and the PPRM shorten the proleg during the withdrawal reflex, however during locomotion this does not happen: the muscles do not shorten much beyond their resting length. Slightly before the swing phase of the step, both muscles lengthen, presumably because the crochets are still in contact with the substrate but the body segment has started to lift, effectively stretching the leg. Next the PPRM is activated, resulting in the crochets unhooking and the leg (and both the APRM and PPRM) returning to resting length (Belanger & Trimmer, 2000). Activation of the PPRM, which occurs shortly before the swing phase, is believed to rotate the crochets outward and away from the substrate (Hinton, 1955; Barbier, 1985). The APRM is also activated during locomotion and is thought to resist the lengthening of the leg before it releases from the substrate (Belanger & Trimmer, 2000). Musculature similar to that of *Manduca sexta* was described for the prolegs of the small cabbage white (*Pieris rapae* L.) (Pieridae) (Tsujimura, 1983) and the Chinese oak silkmoth (*Antheraea pernyi* Guer.) (Saturniidae) (Cox, 1989).

The ‘default state’ of the proleg is extended with the crochets rotated towards the midline. There are no muscles that actively extend the proleg, instead extension and adduction appear to be achieved by normal internal pressure and elasticity. Indeed, Mezoff *et al.* (2004) recorded a tendency for reduced pressure in nearby body segments during leg extension. The key factor in proleg extension seems to be relaxation of both the PPRM and APRM: anaesthetized caterpillars always have the prolegs fully extended.

In addition to their role in proleg movements during locomotion, both the PPRM and APRM are involved in the proleg retraction reflex in response to sensory hair stimulation. Several rows of long stiff hairs (the planta hairs) are found on the lateral distal edge of the proleg, close to the planta. Each hair is innervated by a sensory neuron that monitors movement and displacement with some directional selectivity. The projections of the planta hair sensory neurons have been mapped onto the segmental ganglia where some form direct monosynaptic connections to the motorneurons controlling the PPRM and APRM (Weeks & Jacobs, 1987). When the hairs are moved the planta withdraws, initially through activation of the PPRM but also through APRM activation when the stimulus is strong, leading to full proleg retraction (Weeks & Jacobs, 1987). The proleg shortening reflex is more rapid and abbreviated in animals hanging upside down than in animals that are upright (Belanger, Bender & Trimmer, 2000). Other muscles, in particular the VIL muscles, co-contract with the PPRM during the proleg retraction reflex. However, ablation studies show that these VIL muscles are not required for proleg retraction or adduction. It is thought that they contribute to stiffening the ventral body wall during proleg movements (Mezoff *et al.*, 2004).

During crawling most of the lifting of the proleg during the swing phase arises from movement of the body segment rather than proleg retraction. However, when the planta hairs are stimulated during the swing phase of a crawl, the proleg lifts more through increased activation of the retractor muscles (Belanger *et al.*, 2000). This is an assistance reflex used to help the proleg step over obstacles; it has been demonstrated that this reflex can adjust the normal planta trajectory during a single step (van Griethuijsen & Trimmer, 2010). Interestingly, caterpillars can also adjust their proleg kinematics in anticipation of an obstacle. Tactile information from anterior body segments is used to increase the proleg or body segment lift velocity so that the proleg is more likely to land on top of a small obstacle (van Griethuijsen & Trimmer, 2010).

(*e*) *The importance of gripping*

Caterpillars use the strong passive grip of their crochets regardless of orientation. This facilitates retaining hold of a plant moved by wind and resistance to removal by a predator. The prolegs are located underneath the body with a relatively short distance between them, while the centre of mass is located relatively high above the legs (Fig. 1B). This is a mechanically unstable configuration when the caterpillar is right side up. Without a strong grip the caterpillar would be susceptible to toppling over (Brackenbury, 1999). Furthermore, caterpillars such as *Manduca sexta* need a strong grip to support their considerable weight when they are on the underside of leaves (field observations showed that about 78% of caterpillars of this species were on the underside of a leaf) (Madden & Chamberlin, 1945). Active detachment of the crochets during crawling can be achieved with reliability, in any orientation, and on any surface, using only three proleg retractor motor neurons, one innervating the PPRM and two innervating the APRM (Sandstrom & Weeks, 1996).

There are variations in how caterpillars attach themselves to plant tissues. Larvae of the moth family Limacodidae resemble slugs in both appearance and movement and are referred to as 'slug caterpillars' (Crozier, 1923). Their ventral surface is relatively flat and the spinnerets on this surface secrete a wet silk which aids in attachment to smooth leaves. Prolegs are found on A2 and A7 and in some subgroups the larvae have abdominal suckers or ventral membranous pads. Within the Limacodidae there is variation in the extent to which the ventral surface contacts the substrate during locomotion (Epstein, 1996).

(*f*) *Ground reaction forces*

Locomotion in legged animals with rigid skeletons is often described using an inverted pendulum model in which movement of legs during the stance phase of a stride is equated to a mass swinging around a pivot point attached to the ground. The connection between the pivot and the centre of mass can be a fixed-length strut or, in a modified model, a spring-loaded rod (spring-loaded inverted pendulum, SLIP). These models produce reasonably good and general descriptions of exchange of forces in legged locomotion.

The caterpillar provides an exception to the use of such models because the prolegs do not push forward and upwards but instead act as structural supports and produce drag *via* their grip. This effect can be seen in the ground reaction forces measured normal to the substrate and along the direction of travel (Lin & Trimmer, 2010*b*, 2012) (Fig. 4). The ground reaction forces measured along the direction of travel are negative for the TP while in stance, indicating that this leg resists being pulled forward. The more anterior segments and the thoracic legs have a positive ground reaction force in the direction of travel, this means that these segments resist being pulled backward. In effect the caterpillar places the substrate under compression to pull its posterior segments forward using the anterior prolegs and the thoracic legs as anchors (Lin & Trimmer, 2010*b*). Because the caterpillar relies on the stiffness of the substrate instead of hydrostatic pressure it can be described as an 'environmental skeleton' strategy. This explains why caterpillars have difficulty climbing soft rope which buckles under compression (Lin & Trimmer, 2010*a*).

Any moving organism will transmit forces to their environment and hence an organism's environment is always involved in locomotion. So what makes the environmental skeleton different? In terrestrial animals with a rigid skeleton the role of the environment can be as simple as resisting a push-off by a leg. At the other end of the spectrum we find soft-bodied animals such as the octopus or leech that use multiple attachment points to push and pull themselves forward. These animals can also push-off from the substrate because they can stiffen the body thus creating a hydrostatic or muscular hydrostatic skeleton that withstands compression (Huffard, 2006; Calisti *et al.*, 2011). As described above, caterpillars do not have a hydrostatic or muscular hydrostatic skeleton, which may explain why they use two or more anchor points simultaneously and put the substrate under compression for at least part of the stepping motion. We view the environmental skeleton as the extreme end of a spectrum of involvement of the environment in locomotion. Environmental skeletons are probably not limited to caterpillars although *Manduca sexta* is the only animal to date for which such ground reaction forces have been measured during horizontal locomotion.

It is unlikely that the caterpillar always uses this strategy for movement. For example, during searching behaviour almost half of the body is lifted from the substrate; such motion must require stiffening through muscle activation and increased internal pressure. However, by remaining relatively soft a caterpillar can conform to its substrate and remain cryptic.

(4) Neuronal control of locomotion

(*a*) *Description of the nervous system and sensory systems*

The caterpillar central nervous system is distributed throughout the body with major ganglia in the head (the brain, suboesophageal, and frontal ganglia) and a ventral nerve cord consisting of segmental ganglia in the thorax and abdomen connected by thick bundles of axons. Although the brain is important for transitional behaviours in other insects (Bender, Pollack & Ritzmann, 2010) it is not necessary for caterpillars to crawl normally along a simple substrate. In fact, in the early instars removal of the brain or the regions containing the central complex causes *Manduca sexta* to crawl continuously (Dominick & Truman, 1984, 1985, 1986*a*,*b*). Isolated nerve cords without the brain (Johnston & Levine, 1996*a*) or suboesophageal ganglion (Johnston *et al.*, 1999) can

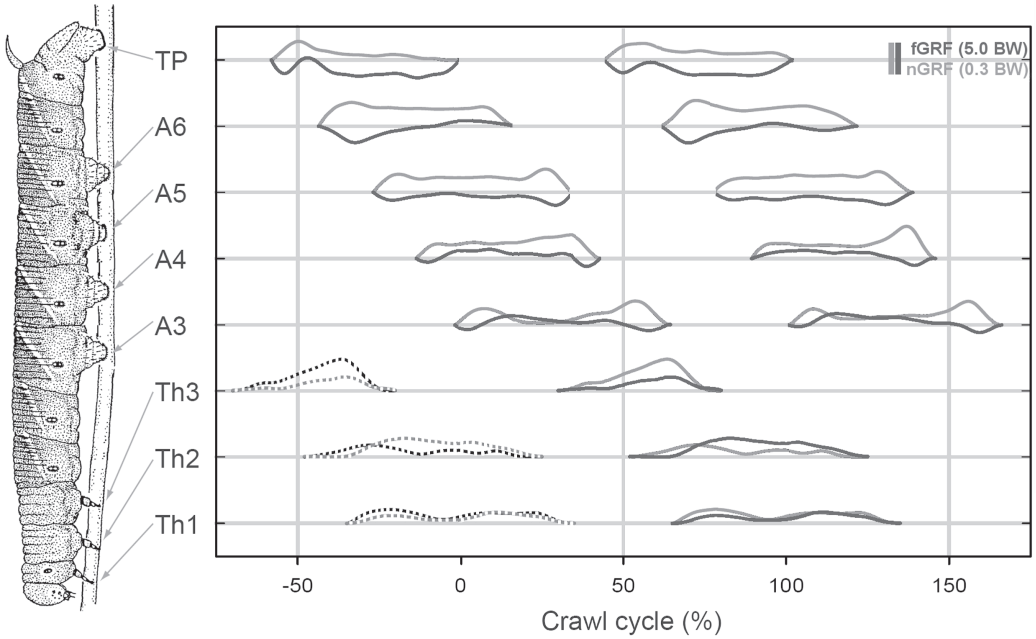

Fig. 4. A simultaneous recording of representative *Manduca sexta* normal ground reaction forces (nGRF, in grey) and fore-aft ground reaction forces (fGRF, in black) for all prolegs (TP to A3) and thoracic legs (Th3 to Th1) during the stance phase of each leg. The graph shows two stance phases per leg and one full crawl. This experiment was not designed to collect thoracic leg GRFs and thoracic legs were not tracked. However, for analytical purposes, one typical recording and its duplication (dotted) are manually inserted on the figure according to the behavioural videos. Timing is expressed as % of the crawl cycle: the start of the crawl is defined as the end of stance/start of swing phase in the TP. The end of the crawl occurs when the swing phase of A3 stops/stance phase starts. All GRF traces are plotted as body weight (BW) as indicated by the reference bars in the upper right. Note that fGRF (scale bar, 5 BW) is much larger than nGRF (scale bar, 0.3 BW), In these experiments the caterpillar was crawling horizontally in a straight line, thus the nGRFs represent the bodyweight loading on the legs. The fGRFs are negative for the posterior legs (TP and A6) and positive for the anterior legs (Th3 to Th1) indicating drag and pull, respectively; the caterpillar puts the substrate under tension. Adapted from Lin & Trimmer (2010*b*).

also be stimulated to produce crawling-like motor patterns (fictive crawling) suggesting that locomotion is largely driven by networks of interconnected neurons in the ventral nerve cord (see Section IV.4*b*).

Each ganglion has a ventral nerve branch on each side that sends signals to the muscles in the ventral side of the animal as well as to muscles in the proleg or thoracic leg if present on the body segment. The dorsal nerve branch is located more anteriorly on the ganglion and runs between the muscles in the ventral part of the animal before branching further and connecting to the dorsal muscles. Sub-branches of both the ventral and dorsal nerve have been described in detail and many of them are named. These branches also contain the dendrites of sensory neurons at the periphery.

Each abdominal body segment contains about 70 muscles, most receiving instructions from one, or occasionally two, motor neurons. The muscles of *Manduca sexta* are not innervated by fast and slow motor neurons, unlike in locusts, nor do they receive inhibitory input although many receive modulatory input from octopaminergic ventral unpaired neurons (Consoulas *et al.*, 1999). Most muscles are named and several consist of a number of muscle fibres. The number of fibres can vary among individuals and among different body segments (C. Metallo, unpublished observation). In addition, many of the cell bodies of these neurons are identified in the ganglia.

There are several sources of sensory information that input to the ganglia in each body segment. Direction-sensitive hairs cover the body, several of which are identified and named and their projections known, such as the planta hairs, lateral hairs, and medial hairs (Peterson & Weeks, 1988). A second sensory system exists called the multidendritic complex (MDC) (Grueber & Truman, 1999*a*,*b*; Grueber, Graubard & Truman, 2001). The MDC consists of neurons distributed throughout the cuticle that respond to deformation and possibly to temperature and noxious stimuli. There are three types of neurons in the MDC and they respond to different stimuli. It has been suggested that one type functions as a proprioceptor, providing the central nervous system with information about the position, orientation and posture of the body. There is no direct evidence that the MDC has a role in locomotion although it is thought to act as a source of sensory information during defensive strike behaviour in *Manduca sexta* (van Griethuijsen, Banks & Trimmer, 2013). A subset of similar neurons in the body wall of *Drosophila* spp. larvae have been implicated in the control of normal crawling (Hughes & Thomas, 2007; Song *et al.*, 2007; Heckscher, Lockery & Doe, 2012; Im & Galko, 2012). A third sensory system is the stretch receptor organ (SRO), which is a modified muscle fibre that monitors both its length and changes in its length (Simon & Trimmer, 2009). There are three pairs of SROs in each body segment of *Manduca sexta*, and this organ has also been suggested to function as a proprioceptor. However, ablation studies showed that the SRO is not necessary for locomotion (Simon & Trimmer, 2009).

(*b*) *Central pattern generators in Manduca sexta*

Central pattern generators (CPGs) consist of neurons that can produce a rhythmic pattern of activation that resembles the motor programs underlying rhythmic behaviour. Once switched on, the motor pattern can be produced in the absence of sensory information. CPGs exist for many rhythmic behaviours. CPGs for locomotion often occur in each body segment in elongate animals, with activation of the CPG time-phased between the segments. Changing the phase can result in a gait change or even a different type of locomotion (e.g. crawling to swimming in the salamander) (Delvolvé, Bem & Cabelguen, 1997). To study CPGs one can use a 'fictive' approach, in which the nervous system (or the part containing the neurons involved in the CPG) is dissected out of the body and stimulated electrically or chemically to start the CPG. The signals arising in the motor neurons or even the activity of individual cells can be measured. A CPG for locomotion in *Manduca sexta* has been identified and studied using pilocarpine which stimulates muscarinic acetylcholine receptors (Johnston & Levine, 1996*a*; Johnston *et al.*, 1999), and atropine (a muscarinic receptor antagonist). The output was compared to muscle activity patterns in intact animals; the rhythms produced by the isolated nerve cord were symmetrical as in locomotion (both legs on one segment are lifted at the same time). Dorsal muscles were activated before ventral muscles in both the isolated nerve cord and during locomotion. Activation patterns travelled from posterior to anterior through the thoracic segments as seen in the intact animal. However the CPG rhythm was slower than the travelling wave in locomotion. Due to the technical difficulty of recording directly from neurons in a moving, intact and soft-bodied animal, CPGs in *Manduca sexta* have only been confirmed in fictive crawling.

(5) Muscular control of locomotion

(*a*) *EMG studies*

Much of what is known about the patterns of muscle activation during locomotion and related behaviours in caterpillars comes from EMG recordings using bipolar nichrome electrodes. These electrodes are inserted through a small puncture hole in the caterpillar's cuticle. Caterpillars have two layers of muscles: a layer of oblique muscles immediately below the cuticle and under this a layer of longitudinal muscles (see Section IV.1). The wires are inserted < 1 mm deep and held in place by superglue or Vetbond. To target the longitudinal muscles, the wires can be inserted a little deeper. Bipolar electrodes are generally placed near attachment points of muscles to minimize motion artefacts. In most species EMG signals are a measure of the summed neural activity to a muscle (Gielen, 1999) which provides information about the onset, stop and duration of muscle stimulation (Simon *et al.*, 2010*a*). In *Manduca sexta* the single-neuron innervation of muscles also allows the EMG to be interpreted in terms of motor neuron firing rates (Belanger & Trimmer, 2000; Metallo *et al.*, 2011), a procedure that requires sophisticated algorithms in many other species (Nawab, Chang & De Luca, 2010). EMG demonstrated that the large ventral (VIL) and dorsal muscles (DIM), which are located in the inner muscle layer, are active simultaneously (Simon *et al.*, 2010*a*), in contrast to models of caterpillar locomotion in many textbooks. It was also demonstrated that the shortening and lengthening patterns recorded in the proleg muscles during locomotion do not translate directly to activation or relaxation of these muscles (Belanger & Trimmer, 2000).

Nichrome bipolar wires have the limitation that they are much stiffer than the tissues of the caterpillar. This can result in tissue damage during recordings and also to movement of the wire relative to the muscle, reducing the signal to noise ratio. Recently, a flexible multi-electrode array was developed (Metallo *et al.*, 2011) that suffers less motion artefact. The array has several recording sites which makes it possible to match motor activities to individual muscles (Metallo *et al.*, 2011). Studies to record the activities of individual muscles in caterpillars during locomotion are currently in progress.

(*b*) *Work-loop studies*

The strain and stimulus cycling experienced by an individual muscle during crawling can be measured using synchronized kinematic and EMG recordings. Muscle length during locomotion is calculated from video recordings, tracking small fluorescent beads glued to indentations in the cuticle that correspond to the muscle attachment points. EMG data are recorded using bipolar wires. Muscles can then be removed from the body and subjected to *in vivo* strain cycles using a lever-arm ergometer while receiving electrical stimuli *via* an intact motor neuron of the same duration and frequency as recorded from the EMG in the intact animal. This has been carried out for the VIL. VIL contraction occurs as the muscle shortens, but the muscle typically experiences its highest stress as it is being re-lengthened (Woods, Fusillo & Trimmer, 2008) (Fig. 5A). The function of the muscle during crawling is made clearer by plotting either muscle force or stress as a function of length for a complete strain cycle. In such a 'work-loop' plot, the muscle is performing work when the direction of the loop (representing elapsed time) is counterclockwise, with the muscle under greater stress during shortening than

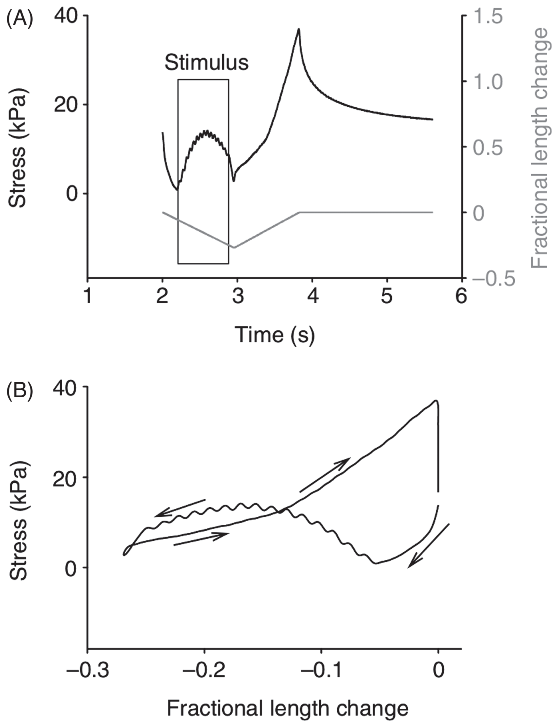

Fig. 5. (A) Time course of stress and length change of a 4.6 mm third abdominal segment ventral interior lateral (VIL) muscle subjected to a simplified consensus crawling strain cycle and stimulus. The muscle is shortened by 28% in 0.95 s and re-lengthened in 0.87 s. (B) Work-loop during the crawling cycle in (A). The loop does not close during strain cycling, but stress returns to nearly starting values during a time interval representative of that seen between strain cycles. From Woods *et al.* (2008).

during lengthening (Fig. 5B). The area within this loop represents the work performed (with the muscle acting as an activator). In the case of the VIL, the muscle performs positive work while at its shorter lengths. As the muscle returns to its initial resting length, however, muscle stress is actually greater during lengthening than during shortening, resulting in a clockwise loop representing work dissipated (acting like a damper, the muscle resists being stretched) (Woods *et al.*, 2008) (Fig. 5B). While it may seem counterintuitive that a large locomotory muscle produces net negative work, the explanation may lie in the use of the environmental skeleton discussed above and the need to put the substrate in compression.

V. TOWARDS A NEW MODEL OF CATERPILLAR CRAWLING

Barth (1937) proposed a theoretical model of caterpillar crawling. His model included anti-phase activation of dorsal and ventral muscles, creating a peristaltic wave to drive the body forward. Subsequent studies however demonstrated that dorsal and ventral muscles are co-activated (Simon *et al.*, 2010*a*). Recent work shows that muscles used in caterpillar crawling can function as activators or as dampers (Woods *et al.*, 2008) while activation of motor neurons driving major muscles is now known to overlap in more than four body segments (while swing phase occurs in 2–4 body segments simultaneously) (Simon *et al.*, 2010*a*). These findings are in agreement with a tension-based model of crawling or the use of an environmental skeleton. Additional evidence for an environmental skeleton comes from the ground reaction forces measured in *Manduca sexta* (Lin & Trimmer, 2010*a*,*b*). In addition the gut is known to move forward in the midbody segments while these are still in contact with the substrate: such visceral-locomotory pistoning could play an active role in crawling (Simon *et al.*, 2010*b*). Crawling is a very robust movement with few differences between horizontal and vertical locomotion (van Griethuijsen & Trimmer, 2009). This phenomenon can be explained by the use of a strong grip independently of the orientation of the body. Locomotion in the caterpillar is thought to be regulated by a CPG (Johnston & Levine, 1996*a*; Johnston *et al.*, 1999), although direct evidence in intact animals is lacking.

To summarize, we now have a much more complex picture of caterpillar crawling than was described by the original models of legged peristalsis. Caterpillars keep their body in tension for much of the crawl cycle while controlling the timing of grip release from the stiff substrate. This allows the body to remain relatively soft and conformable. Internal tissues such as the gut may also play a mechanical role in transferring forces and movements from one part of the body to another. Consequently, body segments do not necessarily conserve their volume and motor control can be distributed by both mechanical and neural mechanisms. This strategy is in marked contrast to the traditional models of soft animal locomotion that generally assume that hydrostatic pressure plays a critical role.

VI. CONCLUSIONS

(1) Caterpillars use different locomotion strategies of which crawling and inching are the most prominent. Crawling has been studied in the most detail.

(2) Caterpillar crawling was originally modelled as a type of hydrostatic locomotion, however several lines of evidence suggest that caterpillars do not function as classic hydrostats: they do not have a fixed volume; their body plan is not similar to that of a hydrostat or a muscular hydrostat; and pressure measurements do not necessarily correlate with specific movements.

(3) In *Manduca sexta* crawling consists of crawls, each of which is a series of steps starting at the posterior end of the body and travelling to the first pair of thoracic legs. Crawls can overlap; multiple travelling waves of steps exist simultaneously. The stepping movements of the prolegs overlap; in each travelling wave 2–3 adjacent legs are lifted simultaneously. To increase speed during normal forward

locomotion, the time between crawls is reduced, but the timing of the steps remains similar.

(4) In the midbody region of the *Manduca sexta* caterpillar, the gut moves independently from the surrounding tissues during locomotion. This phenomenon seems to occur in other lepidopteran species and is expected to contribute to forward locomotion.

(5) The timing of the movements of the prolegs within a crawl is unaffected by the orientation of the caterpillar's body relative to gravity.

(6) The thoracic legs display a less coordinated stepping pattern than the prolegs and it has been suggested that they function to explore the substrate.

(7) The prolegs play an important role in *Manduca sexta* crawling. They have a strong grip and act as anchors during stance. They do not shorten much during crawling; instead the entire body segment is lifted.

(8) The prolegs display a withdrawal reflex; this reflex is context dependent and functions as an assistance reflex during locomotion.

(9) Measurement of ground reaction forces during crawling has demonstrated that the *Manduca sexta* caterpillar puts the substrate under tension, equivalent to using it as an environmental skeleton. The simultaneous activation of muscles in multiple body segments is consistent with this model.

(10) A central pattern generator is likely to regulate crawling in *Manduca sexta*; fictive crawling can be initiated in preparations of the nerve cord, but the use of a CPG still needs to be confirmed in intact animals.

(11) The muscles and several motor neurons involved in crawling have been identified in *Manduca sexta*. Each muscle receives input from usually one, but occasionally two, neurons. Due to the caterpillar's soft body, muscle length is not related to muscle activation. The dorsal and ventral muscles are co-activated. Work-loop studies revealed that the ventral interior longitudinal muscle in *Manduca sexta* functions as an activator during part of the stride, while functioning as a damper during other times.

(12) A new model of caterpillar crawling includes: (*i*) movement of the gut during locomotion; (*ii*) use of the substrate as a medium to transmit muscle forces; (*iii*) use of muscles as activators and as dampers; and (*iv*) the ability to use the same basic movements regardless of body orientation due to the strong grip provided by the prolegs and the presence of a central pattern generator (CPG).

VII. ACKNOWLEDGEMENTS

The authors would like to thank William A. Woods for feedback on the section on work-loops and permission to use the figures. The authors would also like to thank Cinzia Metallo for feedback on the section on EMGs. Original work contributing to this review was supported by the National Science Foundation IOS-7045912.

VIII. REFERENCES

Barbier, R. (1985). Morphogenèse et évolution de la cuticule et des crochets des fausses-pattes, au cours du développement larvaire de *Galleria mellonella* L.(Lepidoptera, Pyralidae). *Bulletin de la Société Zoologique de France* **110**, 205–221.

Barth, R. (1937). Muskulatur und Bewegungsart der Raupen. *Zoologische Jahrbücher. Abteilung für Anatomie und Ontogenie der Tiere* **62**, 507–566.

Belanger, J. H. & Trimmer, B. A. (2000). Combined kinematic and electromyographic analyses of proleg function during crawling by the caterpillar *Manduca sexta*. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology* **186**, 1031–1039.

Belanger, J. H., Bender, K. J. & Trimmer, B. A. (2000). Context dependency of a limb withdrawal reflex in the caterpillar *Manduca sexta*. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology* **186**, 1041–1048.

Bell, R. A. & Joachim, F. A. (1978). Techniques for rearing laboratory colonies of the tobacco hornworm, *Manduca sexta* and pink ballworms. *Annals of the Entomological Society of America* **69**, 365–373.

Bender, J. A., Pollack, A. J. & Ritzmann, R. E. (2010). Neural activity in the central complex of the insect brain is linked to locomotor changes. *Current Biology* **20**, 921–926.

Bernays, E. A. & Woods, H. A. (2000). Foraging in nature by larvae of *Manduca sexta*-influenced by an endogenous oscillation. *Journal of Insect Physiology* **46**, 825–836.

Brackenbury, J. (1996). Novel locomotory mechanisms in caterpillars: life-line climbing in *pinotia abbreviana* (Tortricidae) and *Yponomeuta padella* (Yponomeutidae). *Physiological Entomology* **21**, 7–14.

Brackenbury, J. (1997). Caterpillar kinematics. *Nature* **390**, 453.

Brackenbury, J. (1999). Fast locomotion in caterpillars. *Journal of Insect Physiology* **45**, 525–533.

Calisti, M., Giorelli, M., Levy, G., Mazzolai, B., Hochner, B., Laschi, C. & Dario, P. (2011). An octopus-bioinspired solution to movement and manipulation for soft robots. *Bioinspiration & Biomimetics* **6**, 036002.

Carter, D. & Locke, M. (1993). Why caterpillars do not grow short and fat. *International Journal of Insect Morphology and Embryology* **22**, 81–102.

Casey, T. M. (1991). Energetics of caterpillar locomotion: biomechanical constraints of a hydraulic skeleton. *Science* **252**, 112–113.

Chapman, R. F. (1998). *The Insects: Structure and Function*. Cambridge University Press, Cambridge.

Consoulas, C., Johnston, R. M., Pflüger, H. J. & Levine, R. B. (1999). Peripheral distribution of presynaptic sites of abdominal motor and modulatory neurons in *Manduca sexta* larvae. *Journal of Comparative Neurology* **410**, 4–19.

Cox, S. C. (1989). The electrical and mechanical properties of the proleg retractor muscle of the Chinese oak silkmoth larva. *Physiological Entomology* **14**, 265–272.

Crozier, W. J. (1923). On the locomotion of the larvae of the slug-moths (Cochlidiidae). *Journal of Experimental Zoology* **38**, 323–329.

Delvolvé, I., Bem, T. & Cabelguen, J. M. (1997). Epaxial and limb muscle activity during swimming and terrestrial stepping in the adult newt, *Pleurodeles waltl*. *Journal of Neurophysiology* **78**, 638–650.

Dominick, O. S. & Truman, J. W. (1984). The physiology of wandering behaviour in *Manduca sexta*. I. Temporal organization and the influence of the internal and external environments. *Journal of Experimental Biology* **110**, 35–51.

Dominick, O. S. & Truman, J. W. (1985). The physiology of wandering behaviour in *Manduca sexta*. II. The endocrine control of wandering behaviour. *Journal of Experimental Biology* **117**, 45–68.

Dominick, O. S. & Truman, J. W. (1986*a*). The physiology of wandering behaviour in *Manduca sexta*. III. Organization of wandering behaviour in the larval nervous system. *Journal of Experimental Biology* **121**, 115–132.

Dominick, O. S. & Truman, J. W. (1986*b*). The physiology of wandering behaviour in *Manduca sexta*. IV. Hormonal induction of wandering behaviour from the isolated nervous system. *Journal of Experimental Biology* **121**, 133–151.

Douglas, M. M. (1986). *The Lives of Butterflies*. University of Michigan Press, Ann Arbor.

Epstein, M. E. (1996). Revision and phylogeny of the limacodid-group families, with evolutionary studies on slug caterpillars (Lepidoptera: Zygaenoidea). *Smithsonian Contributions to Zoology* **582**, 1–102.

Full, R. J. (1997). Invertebrate locomotor systems. In *The Handbook of Comparative Physiology* (ed. W. Dantzler), pp. 853-930. Oxford University Press, Oxford.

Gielen, S. C. (1999). What does EMG tell us about muscle function? *Motor Control* **3**, 9–11.

van Griethuijsen, L. I. & Trimmer, B. A. (2009). Kinematics of horizontal and vertical caterpillar crawling. *The Journal of Experimental Biology* **212**, 1455–1462.

van Griethuijsen, L. I. & Trimmer, B. A. (2010). Caterpillar crawling over irregular terrain: anticipation and local sensing. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **196**, 397–406.

van Griethuijsen, L. I., Banks, K. M. & Trimmer, B. A. (2013). Spatial accuracy of a rapid defense behavior in caterpillars. *The Journal of Experimental Biology* **216**, 379–387.

Grueber, W. B. & Truman, J. W. (1999*a*). Development and organization of a nitric-oxide-sensitive peripheral neural plexus in larvae of the moth, *Manduca sexta*. *The Journal of Comparative Neurology* **404**, 127–141.

Grueber, W. B. & Truman, J. W. (1999*b*). Sensory responses and dendritic projections define distinct functional groups of dendritic arborization neurons in *Manduca sexta*. *American Zoologist* **39**, 134A.

Grueber, W. B., Graubard, K. & Truman, J. W. (2001). Tiling of the body wall by multidendritic sensory neurons in *Manduca sexta*. *The Journal of Comparative Neurology* **440**, 271–283.

Hartmann, T. (2004). Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. *Planta* **219**, 1–4.

Heckscher, E. S., Lockery, S. R. & Doe, C. Q. (2012). Characterization of *Drosophila* larval crawling at the level of organism, segment, and somatic body wall musculature. *The Journal of Neuroscience* **32**, 12460–12471.

Hinton, H. E. (1955). On the structure, function, and distribution of the prolegs of the Panorpoidea, with a criticism of the Berlese-Imms theory. *Transactions of the Royal Entomological Society of London* **106**, 455–540.

Holst, E. (1934). Motorische und tonische Erregung und ihr Bahnenverlauf bei Lepidopterenlarven. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **21**, 395–414.

Huffard, C. L. (2006). Locomotion by *Abdopus aculeatus* (Cephalopoda: Octopodidae): walking the line between primary and secondary defenses. *Journal of Experimental Biology* **209**, 3697–3707.

Hughes, G. M. (1965). Locomotion: Terrestrial. In *The Physiology of Insecta* (Volume 2) (ed. M. Rockstein), pp. 227–253. Academic Press, Inc., New York.

Hughes, C. L. & Thomas, J. B. (2007). A sensory feedback circuit coordinates muscle activity in Drosophila. *Molecular and Cellular Neuroscience* **35**, 383–396.

Im, S. H. & Galko, M. J. (2012). Pokes, sunburn, and hot sauce: *Drosophila* as an emerging model for the biology of nociception. *Developmental Dynamics* **241**, 16–26.

Johnston, R. M. & Levine, R. B. (1996*a*). Crawling motor patterns induced by pilocarpine in isolated larval nerve cords of *Manduca sexta*. *Journal of Neurophysiology* **76**, 3178–3195.

Johnston, R. M. & Levine, R. B. (1996*b*). Locomotory behavior in the hawkmoth *Manduca sexta*: kinematic and electromyographic analyses of the thoracic legs in larvae and adults. *The Journal of Experimental Biology* **199**, 759–774.

Johnston, R. M., Consoulas, C., Pflüger, H. & Levine, R. B. (1999). Patterned activation of unpaired median neurons during fictive crawling in *Manduca sexta* larvae. *The Journal of Experimental Biology* **202**, 103–113.

Joos, B. (1992). Adaptations for locomotion at low body temperatures in eastern tent caterpillars, *Malacosoma americanum*. *Physiological Zoology* **65**(6), 1148–1161.

Kent, K. S. & Levine, R. B. (1988). Neural control of leg movements in a metamorphic insect: sensory and motor elements of the larval thoracic legs in *Manduca sexta*. *The Journal of Comparative Neurology* **271**, 559–576.

Kopec, S. (1919). Lokalizationsversuche an Zentralen Nervensysyteme der Raupen und Falter. *Zoologische Jahrbücher. Abteilung für Allgemeine Zoologie und Physiologie der Tiere* **36**, 453–502.

Levine, R. B. & Truman, J. W. (1982). Metamorphosis of the insect nervous system: changes in morphology and synaptic interactions of identified neurones. *Nature* **299**, 250–252.

Levine, R. B. & Truman, J. W. (1985). Dendritic reorganization of abdominal motoneurons during metamorphosis of the moth, *Manduca sexta*. *The Journal of Neuroscience* **5**, 2424–2431.

Lin, H. T. (2011). *Biomechanical strategies for locomotion in soft-bodied animals*. PhD Thesis: Tufts University.

Lin, H. T. & Trimmer, B. A. (2010*a*). Caterpillars use the substrate as their external skeleton: a behavior confirmation. *Communicative & Integrative Biology* **3**(5), 471–474.

Lin, H. T. & Trimmer, B. A. (2010*b*). The substrate as a skeleton: ground reaction forces from a soft-bodied legged animal. *The Journal of Experimental Biology* **213**, 1133–1142.

Lin, H. T. & Trimmer, B. A. (2012). A new bi-axial cantilever beam design for biomechanics force measurements. *Journal of Biomechanics* **45**, 2310–2314.

Lin, H. T., Dorfmann, A. L. & Trimmer, B. A. (2009). Soft-cuticle biomechanics: a constitutive model of anisotropy for caterpillar integument. *Journal of Theoretical Biology* **256**, 447–457.

Lin, H. T., Slate, D. J., Paetsch, C. R., Dorfmann, A. L. & Trimmer, B. A. (2011). Scaling of caterpillar body properties and its biomechanical implications for the use of a hydrostatic skeleton. *The Journal of Experimental Biology* **214**, 1194–1204.

Madden, A. H. & Chamberlin, F. S. (1945). *Biology of the Tobacco Hornworm in the Southern Cigar-tobacco District*. Technical Bulletin No. 896. United States Department of Agriculture, Washington.

Metallo, C., White, R. D. & Trimmer, B. A. (2011). Flexible parylene-based microelectrode arrays for high resolution EMG recordings in freely moving small animals. *Journal of Neuroscience Methods* **195**, 176–184.

Mezoff, S., Papastathis, N., Takesian, A. & Trimmer, B. A. (2004). The biomechanical and neural control of hydrostatic limb movements in *Manduca sexta*. *The Journal of Experimental Biology* **207**, 3043–3053.

Miller, J., Janzen, D. & Hallwachs, W. (2006). *100 Caterpillars*. Belknap Press of Harvard University Press, Cambridge.

Montgomery, S. L. (1982). Biogeography of the moth genus Eupithecia in Oceania and the evolution of ambush predation in Hawaiian caterpillars(Lepidoptera: Geometridae). *Entomologia Generalis* **8**, 27–34.

Montgomery, S. L. (1983). Carnivorous caterpillars: the behavior, biogeography and conservation of Eupithecia (Lepidoptera: Geometridae) in the Hawaiian Islands. *GeoJournal* **7**, 549–556.

Morris, C. E. (1983*a*). Efflux of nicotine and its CNS metabolites from the nerve cord of the tobacco hornworm *Manduca sexta*. *Journal of Insect Physiology* **29**, 953–959.

Morris, C. E. (1983*b*). Efflux patterns for organic molecules from the CNS of the tobacco hornworm *Manduca sexta*. *Journal of Insect Physiology* **29**, 961–966.

Morris, C. E. (1983*c*). Uptake and metabolism of nicotine by the CNS of a nicotine-resistant insect, the tobacco hornworm (*Manduca sexta*). *Journal of Insect Physiology* **29**, 807–817.

Morris, C. E. (1984). Electrophysiological effects of cholinergic agents on the CNS of a nicotine-resistant insect, the tobacco hornworm (*Manduca sexta*). *Journal of Experimental Zoology* **229**, 361–374.

Murray, C. L., Quaglia, M., Arnason, J. T. & Morris, C. E. (1994). A putative nicotine pump at the metabolic blood–brain barrier of the tobacco hornworm. *Journal of Neurobiology* **25**, 23–34.

Nawab, S. H., Chang, S.-S. & De Luca, C. J. (2010). High-yield decomposition of surface EMG signals. *Clinical Neurophysiology* **121**, 1602–1615.

Peterson, B. A. & Weeks, J. C. (1988). Somatotopic mapping of sensory neurons innervating mechanosensory hairs on the larval prolegs of *Manduca sexta*. *The Journal of Comparative Neurology* **275**, 128–144.

Pierce, N. E. (1995). Predatory and parasitic Lepidoptera: carnivores living on plants. *Journal of the Lepidopterists Society* **49**(4), 412–453.

Powell, J. A. (2009). Lepidoptera. In *Encyclopedia of Insects*, pp. 559–587. Elsevier Science & Technology, Oxford.

Quillin, K. J. (1998). Ontogenetic scaling of hydrostatic skeletons: geometric, static stress and dynamic stress scaling of the earthworm *Lumbricus terrestris*. *The Journal of Experimental Biology* **201**, 1871–1883.

Sandstrom, D. J. & Weeks, J. C. (1996). Novel dual innervation of a larval proleg muscle by two similar motoneurons in the tobacco hornworm *Manduca sexta*. *The Journal of Experimental Biology* **199**, 775–791.

Self, L., Guthrie, F. & Hodgson, E. (1964). Adaptation of tobacco hornworms to the ingestion of nicotine. *Journal of Insect Physiology* **10**, 907–914.

Simon, M. A. & Trimmer, B. A. (2009). Movement encoding by a stretch receptor in the soft-bodied caterpillar, *Manduca sexta*. *The Journal of Experimental Biology* **212**, 1021–1031.

Simon, M. A., Fusillo, S. J., Coleman, K. & Trimmer, B. A. (2010a). Motor patterns associated with crawling in a soft-bodied arthropod. *The Journal of Experimental Biology* **213**, 2303–2309.

Simon, M. A., Woods, W. A. Jr., Serebrenik, Y. V., Simon, S. M., van Griethuijsen, L. I., Socha, J. J., Lee, W. K. & Trimmer, B. A. (2010b). Visceral-locomotory pistoning in crawling caterpillars. *Current Biology* **20**, 1458–1463.

Slansky, F. Jr. & Feeny, P. (1977). Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. *Ecological Monographs* **47**, 209–228.

Snodgrass, R. E. (1961). The caterpillar and the butterfly. *Smithsonian Miscellaneous Collections* **143**, 1–51.

Song, W., Onishi, M., Jan, L. Y. & Jan, Y. N. (2007). Peripheral multidendritic sensory neurons are necessary for rhythmic locomotion behavior in *Drosophila* larvae. *Proceedings of the National Academy of Sciences* **104**, 5199–5204.

Stamp, N. E. & Casey, T. M. (eds) (1993). *Caterpillars: Ecological and Evolutionary Constraints on Foraging*. Chapman & Hall, New York.

Sugiura, S. & Yamazaki, K. (2006). The role of silk threads as lifelines for caterpillars: pattern and significance of lifeline-climbing behaviour. *Ecological Entomology* **31**, 52–57.

Taylor, H. M. & Truman, J. W. (1974). Metamorphosis of the abdominal ganglia of the tobacco hornworm, *Manduca sexta*. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology* **90**, 367–388.

Taylor, C. R., Caldwell, S. L. & Rowntree, V. J. (1972). Running up and down hills: some consequences of size. *Science* **178**, 1096–1097.

Trimmer, B. & Issberner, J. (2007). Kinematics of soft-bodied, legged locomotion in *Manduca sexta* larvae. *The Biological Bulletin* **212**, 130–142.

Tsujimura, H. (1983). Anatomy of external structure and musculature of abdominal segments in the larva of the lepidopteran *Pieris rapae crucivora*. *Journal of Morphology* **177**, 191–203.

Vogel, S. (2003). *Comparative Biomechanics: Life's Physical World*. Princeton University Press, Princeton.

Weeks, J. C. & Jacobs, G. A. (1987). A reflex behavior mediated by monosynaptic connections between hair afferents and motoneurons in the larval tobacco hornworm, *Manduca sexta*. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **160**, 315–329.

Weeks, J. C. & Truman, J. W. (1984). Neural organization of peptide-activated ecdysis behaviors during the metamorphosis of *Manduca sexta*. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **155**, 407–422.

Wigglesworth, V. B. (1950). *The Principles of Insect Physiology*. Methuen & Co, London.

Woods, W. A. Jr., Fusillo, S. J. & Trimmer, B. A. (2008). Dynamic properties of a locomotory muscle of the tobacco hornworm *Manduca sexta* during strain cycling and simulated natural crawling. *The Journal of Experimental Biology* **211**, 873–882.

(*Received 18 May 2012; revised 10 November 2013; accepted 12 November 2013*)