Kinematics of Soft-bodied, Legged Locomotion in *Manduca sexta* Larvae

BARRY TRIMMER* AND JONATHAN ISSBERNER

Department of Biology, Tufts University, Medford, Massachusetts 02155

Abstract. Caterpillar crawling is distinct from that of worms and molluscs; it consists of a series of steps in different body segments that can be compared to walking and running in animals with stiff skeletons. Using a three-dimensional kinematic analysis of horizontal crawling in *Manduca sexta,* the tobacco hornworm, we found that the phase of vertical displacement in the posterior segments substantially led changes in horizontal velocity and the segments appeared to pivot around the attached claspers. Both of the motions occur during vertebrate walking. In contrast, vertical displacement and horizontal velocity in the anterior proleg-bearing segments were in phase, as expected for running gaits coupled by elastic storage. We propose that this kinematic similarity to running results from the muscular compression and release of elastic tissues. As evidence in support of this proposal, the compression and extension of each segment were similar to harmonic oscillations in a spring, although changes in velocity were 70° out of phase with displacement, suggesting that the spring was damped. Measurements of segment length within, and across, intersegmental boundaries show that some of these movements were caused by folding of the body wall between segments. These findings demonstrate that caterpillar crawling is not simply the forward progression of a peristaltic wave but has kinetic components that vary between segments. Although these movements can be compared to

legged locomotion in animals with stiff skeletons, the underlying mechanisms of caterpillar propulsion, and in particular the contribution of elastic tissues, remain to be discovered.

Introduction

The movements of animals with stiff articulated skeletons, such as arthropods and vertebrates, have been studied extensively; they tend to be fast and precise, using joints with a few degrees of freedom and relatively predictable limb kinematics. However, most living animals do not have hard skeletons but instead use fluid and tissue pressure to stiffen the body for muscles to do useful work (Trueman, 1975; Keller and Falkovitz, 1983; Skierczynski *et al.,* 1996; Wilson *et al.,* 1996a, b; Quillin, 1998, 1999; Cacciatore *et al.,* 2000). Without fixed joints, soft-bodied animals are able to move in ways that are difficult or impossible for articulated creatures (Gutfreund *et al.,* 1996, 1998; Matzner *et al.,* 2000). However, this enhanced range of movements must be controlled by an appropriate combination of neural and biomechanical elements. Because there are serious technical challenges to studying movement control in soft animals, comparatively little is known about the interrelationship of these elements.

Caterpillars are particularly intriguing because they are very successful soft-bodied climbers that grasp and navigate in a complex three-dimensional environment. Unlike the bouncing gaits of animals with stiff skeletons or the legless crawling of most worms and molluscs, caterpillar locomotion uses both hydrostatics and powerful abdominal appendages (the prolegs). Apart from some notable behavioral adaptations such as life-line climbing (Brackenbury, 1996) and backward walking/rolling (Brackenbury, 1997, 1999), locomotion in caterpillars is primarily by crawling. Typically, crawling involves bilateral anteriograde dorsoventral undulations with at least two pairs of prolegs in continuous

Abbreviations: APAS, Ariel Performance Analysis System; Abd1-Abd7, abdominal segments 1 through 7; DEO, dorsal external oblique muscle; Jx, segment length, from upper posterior insertion to upper anterior origin, but not including the intersegment membrane; Lx, overall segment length, from upper posterior insertion in one segment to upper anterior origin in the next; Ly, distance from upper posterior insertion to the tip of the proleg in the same segment; T1–3, Thoracic segments 1 through 3; TP, terminal proleg; UI, upper posterior insertion; UO, upper anterior origin.

contact with the substrate. This gait sacrifices the opportunity to cycle the whole body between kinetic and gravitational potential energy as running animals do (Cavagna *et al.,* 1977; Blickhan and Full, 1987; Full and Tu, 1990, 1991; Full, 1993; Full and Farley, 2000; Kerdok *et al.,* 2002). However, the soft tissues of muscular hydrostats could provide a mechanism to store and recover elastic energy in a similar fashion (see also O'Reilly *et al.,* 1997; Sensenig and Shultz, 2003). Another interesting aspect of caterpillar locomotion is that, unlike worms, caterpillars do not have circular muscles and do not have septa dividing the hemocoel. Observers have suggested that caterpillars use longitudinal shortening to power their movements rather than the constriction and elongation exercised by worms (Barth, 1937; Trueman, 1975).

The following study uses three-dimensional kinematics to characterize the abdominal movements of the caterpillar, *Manduca sexta* (Linnaeus, 1763). *Manduca* is an extremely useful model system for studying such soft-bodied locomotion and for examining how neural control and biomechanics are integrated to generate movement ("neuromechanics"). First, the caterpillar has a multidimensional workspace: it can bend, twist, and crumple in ways that are not possible with a rigid skeleton. Second, these movements are achieved through the coordination of concatenated segments, each containing about 70 distinct muscles. Third, despite the complexity of movements and the large number of involved muscles, each muscle is innervated by just one (or occasionally two) motoneuron (Taylor and Truman, 1974; Levine and Truman, 1985) and there are no inhibitory motor units. As a consequence, most of *Manduca*'s movements are controlled by only a few hundred motoneurons whose activity can be monitored using electrodes implanted in the muscles of freely moving animals. Finally, *Manduca* is highly suited to kinematic analysis because it is terrestrial and crawls slowly in a repeating series of steps. One goal of the current study is to compare these stepping movements in a soft-bodied animal with those of walking and running animals that have stiff skeletons. Another goal is to explore in detail how movements differ between body segments and how these movements are coordinated with one another. An understanding of these relationships is important for guiding further studies on the neural and biomechanical control of soft-bodied movements.

Materials and Methods

Raising Manduca

Larvae of the lepidopteran moth *Manduca sexta* were individually raised to the fifth instar after the method of Bell and Joachim (1976). Animals were kept at a constant temperature of 26 °C in a cycle of 17 h light to 7 h dark and fed with an artificial diet. Fifth instar animals were used on the first or second day of the instar.

Manduca *Anatomy*

In external appearance *Manduca* is divided into the head, three thoracic segments, and eight abdominal segments (Fig. 1). The abdomen constitutes over three-quarters of the
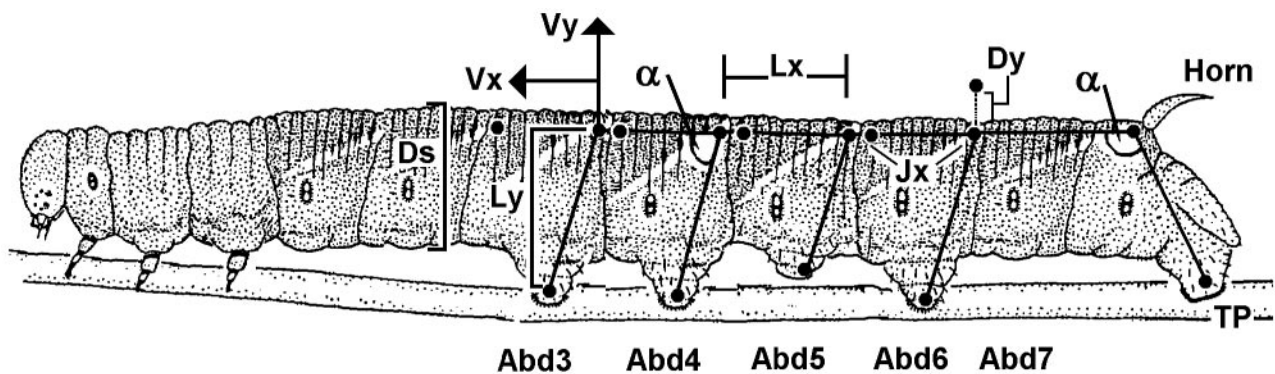
Figure 1. Marker points and parameters used to characterize the kinematics of *Manduca* crawling. Fluorescent beads were placed on the posterior upper insertion (UI) points of the dorsal exterior oblique muscle (DEO) on abdominal segments Abd3 through Abd6 and on the dorsal cuticle at the horn attachment in the most posterior segment. These markers were used to measure the length of the segments (Lx), their vertical (Vy), and horizontal (Vx), velocities, and vertical displacement (Dy). Markers were also applied to the anterior upper origin (UO) of DEO to measure the intrasegmental distance (Jx). Beads applied to the lateral distal margin of the prolegs (including the terminal prolegs, TP) were used to measure proleg movements including retraction (changes in Ly) and swing angle (α). The animals were illuminated with a high-intensity longwave UV lamp and their movements recorded simultaneously from two angles, using a pair of digital video cameras. Video data from each camera captured as 760 pixels $\times$ 480 pixels at a frame rate of 29.97/s were synchronized using flashes produced from an LED and stored in uncompressed format for analysis. Segment diameter (Ds) was measured directly from side-view high-resolution images (3 megapixels) of freely moving caterpillars.

total caterpillar length and provides the principal means of locomotion (Snodgrass, 1961). Abdominal segments three through six (Abd3–Abd6) each have a similar pair of appendages (the prolegs) that grip passively and can be actively unhooked and retracted (Belanger and Trimmer, 2000; Mezoff *et al.,* 2004). The eighth abdominal segment (usually called the terminal segment, TS) has a pair of specialized appendages called the terminal prolegs (TPs), and its musculature and innervation are different from those of the other abdominal segments. A large proportion of the internal body cavity is taken up by the gut, which is surrounded by a continuous (nonsegmented) fluid-filled hemocoele containing other organs. Muscles are attached to the inside surface of the body wall in two main layers, the external and internal muscle groups (Holst, 1934; Barth, 1937). The musculature is complex, with each abdominal body segment containing about 70 discrete muscles with unique attachment points (Eaton, 1988). Most muscles are oriented longitudinally or obliquely (*e.g.,* the dorsal interior oblique muscle runs from the dorsal anterior midline to the lateral posterior margin) and are contained entirely within a body segment. Several of the larger longitudinal muscles (*e.g.,* the dorsal internal medial and ventral internal lateral) attach to the body wall within the posterior margin of one body segment and extend just inside the posterior margin of the next anterior segment.

Removal of the brain

Larvae are quiescent for long periods, particularly after handling, and they frequently interrupt their crawls. To promote spontaneous and persistent crawling, the supraesophageal ganglion (brain) was removed as described by Dominick and Truman (1986). Provided that the neck connectives are not cut, this surgery disrupts transition behaviors (*e.g.,* turns) and evokes continuous locomotion in many insect species (Graham, 1979; Ridgel and Ritzmann, 2005; Ritzmann *et al.,* 2005). In *Manduca,* this lesioning produces premature wandering (Dominick and Truman, 1986) that appears normal (Holst, 1934). However, we have not been able to collect uninterrupted crawl data from a sufficient number of intact larvae to make a statistical comparison. Therefore, all the data reported here are from lesioned *Manduca.* Briefly, first- or second-day fifth instar larvae were anesthetized by chilling on ice for 30 min. A hole ($\sim$ 0.7 mm diameter) was drilled in the upper midsection of the frons, and the brain connectives were cut before the brain was removed with forceps. During this procedure, the body of the larva was held lower than the head to minimize hemolymph loss. The hole was then sealed by swabbing the cuticle dry and placing a small square of lint-free tissue over the hole and gluing it to the head capsule with 2 μl of Vetbond tissue adhesive (a cyanoacrylate glue from 3M

Animal Care Products, St Paul, MN). Animals were typically used 1–2 days postoperatively.

Capture of three-dimensional motion

To visualize motions for kinematic analysis, precisely defined points on the body surface corresponding to muscle attachments (Levine and Truman, 1985) were labeled with fluorescent latex spheres (160 μm diameter, Duke Scientific Corp., Palo Alto, CA; Cat. No. 35-14) glued in place by using silicone rubber clear aquarium sealant (Dow Corning, Midland, MI). Markers were placed on all four "corners" of the dorsal exterior oblique (DEO) muscle (Levine and Truman, 1985) and on the lateral distal surface of the prolegs (Fig. 1). Because the upper and lower muscle attachment points moved in synchrony, the data reported here are from the upper posterior insertion (UI) and upper anterior origin (UO) points alone. The length of DEO, from UI to UO, was made as one measure of segment length (Jx), but this did not include the intersegment membrane. Therefore, overall segment length was defined as the distance from the UI point in one segment to the UI in the next anterior segment (Lx, Fig. 1). Measurements were also made of the distance from UI to the tip of the proleg in the same segment (Ly, Fig. 1) and of the angle between Lx and Ly.

The freely moving larvae were illuminated with a high-intensity longwave ultraviolet lamp (Blak-Ray, Upland, CA; Model B-100A) and their movements recorded using two Canon ZR10 digital video cameras positioned anteriorly and posteriorly about 45° to the axis of movement. Data were analyzed only for sequences of steps in which the markers were clearly visible in both cameras. A small light-emitting diode (LED) and a fluorescent fixed point were always in the field of view for both cameras. A brief (18-ms) LED flash was used to synchronize the recorded data on each camera. Although kinematic studies of hard skeletons often define axes relative to joints and segments, the extreme compliance and compact body of *Manduca* does not present suitable movement planes. Therefore, all movements are described relative to an external three-dimensional calibration frame placed in the view of both cameras with the *x*-axis aligned along the direction of crawling. The *y*-axis describes movements in the dorsoventral plane, and the *z*-axis describes movements in the lateral plane.

Both digital video streams (29.97 frames/s) were transferred, using an IEEE 1394 multimedia connection, to a computer. The video image files were cut to the desired length and the contrast was adjusted to maximize marker visibility using VideoWave software ver. 7.0 (Roxio, Santa Clara, CA). The remaining processing and analysis were carried out using Ariel Performance Analysis System ver. 1.01 (APAS; Ariel Dynamics Inc., San Diego, CA). Recordings were synchronized and trimmed, and the two-dimen-

sional positions of the microspheres in each view were digitized in 7 frames every second. The three-dimensional positions of the moving points were then calculated from the fixed point and calibration frame data by using the APAS direct linear transform module with all filtering turned off. To help visualize the reconstructed movements, these digitized data points were selectively linked to create real-time animated "stick figures" (Fig. 2).

Data analysis

The movements of larvae were described by length changes in Lx and Jx; displacements of UI in horizontal (Dx) and vertical (Dy) planes; the velocity of UI in horizontal (Vx) and vertical (Vy) directions and acceleration along the horizontal axis (Ax). These values for different body segments are indicated by subscripts (*e.g.,* the length of abdominal segment 4 is named Lx_{A4}). Although *z*-axis data was used for three-dimensional reconstructions, crawling is largely confined to the *x*- and *y*-axes, so *z*-axis data are not reported in the current analysis. To examine the relationship between these parameters, the data were interpolated at 1-ms intervals and small systematic changes in data offsets were removed (de-trended) by subtracting a lowess-filtered baseline (smoothing parameter of 0.8) (Porges and
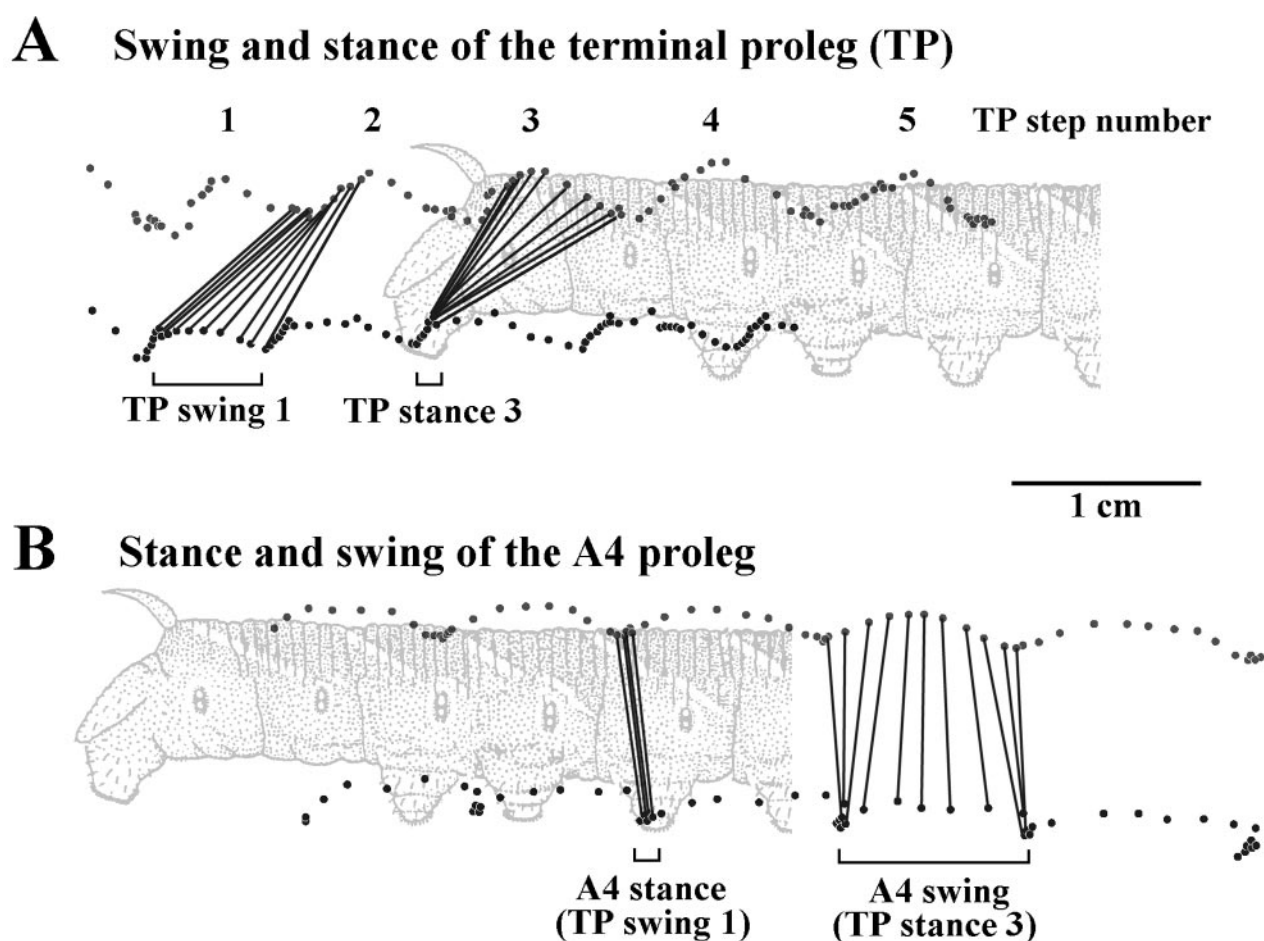

Figure 2. Movements of the terminal prolegs and body segments are distinct from those in the midbody segments. (A) The positions of the terminal segment dorsal marker (upper trace) and the terminal proleg (TP, lower trace) are tracked at 7 points per second. Steps have been numbered successively from the first complete swing phase. Straight lines have been drawn between points taken at the same time, during both a swing phase (TP swing 1) and a stance phase (TP stance 3) to illustrate the continuously changing angle of the TP and the pivoting movement of the segment during stance. An outline of a caterpillar at the onset of TP stance is superimposed as a reference. (B) The positions of the Abd4 dorsal marker (upper trace) and the proleg in Abd4 (A4P; lower trace) are tracked at the same times as those shown in (A). Straight lines have been drawn between points taken at the same time, during both A4P stance (coincident with TP swing 1) and A4P swing (coincident with TP stance 3). The A4P remains essentially vertical during a step and is stationary during stance. An outline of a caterpillar during A4P stance is superimposed as a reference.

Bohrer, 1990). The phase relationship between parameters was then determined by cross correlation (Matlab ver. 6.0, Mathworks, Natick, MA). The overall period of the step cycle was measured as the time between the primary cross-correlation peak and its first harmonic. The lag (or lead) time between parameters was identified as the time of the peak of the primary cross-correlation coefficient. In cases where markers were visible for only a limited portion of the crawl, the peak correlation coefficient dropped below 0.5; such events were excluded from the calculation of overall phase differences. To avoid variation caused by the initiation and cessation of locomotion, the first and last steps of each crawl were omitted from the analyses.

The swing phase of each segmental step was defined by an increase in the horizontal velocity of the proleg; stance phase was the near-zero velocity period between these sharp velocity peaks (*e.g.,* Fig. 3). To calculate the duty factor, a threshold velocity (5% to 10% of the peak velocity) was set above which the proleg velocity increased monotonically for at least 300 ms at the start of the swing; the duration above this threshold was defined as swing phase and that below it the stance. The average crawling speed was estimated from the mean of Vx_{A4} and the average step length by dividing Dx_{A4} by the number of steps taken. The extent of segment shortening and the peak-to-peak amplitude of the ratio Jx/Lx were measured from the amplitude of a modified sine wave ($y = y_0 + a \sin(2 \pi t/b + c)$ fitted to the data for each crawl. The diameter-to-length ratio (Ds/Lx) of abdominal segments was measured directly, using ImageJ (ver. 1.35a; National Institutes of Health, Bethesda, MD), from side-view high-resolution images of freely moving caterpillars. All mean values are reported with the standard error of the mean (SEM).

Correlates of energy exchange and work

This study did not attempt to make force measurements from the caterpillar; nor is it possible to define the center of mass of each moving body part during continuous crawling. However, by assuming that each point is associated with a fixed mass (*M*), various correlates of energy exchange and force were calculated from the kinematics. Gravitational potential energy (E_p) was represented by Dy ($E_p = Mgh$), and kinetic energy (E_k) in each plane was considered proportional to Vx and Vy ($E_k = MV^2/2$). Furthermore, the storage of elastic potential energy (E_s) can be derived from the displacement of a point from its equilibrium position against a spring constant (*K*) ($E_s = Kx^2/2$). In *Manduca,* each segment was described as a spring moving in the *x*-axis with E_s proportional to Lx. Because both ends of a segment are in concurrent motion, the spring E_k is proportional to the difference in Vx between the anterior and posterior points. It should be noted that the use of a spring constant in this
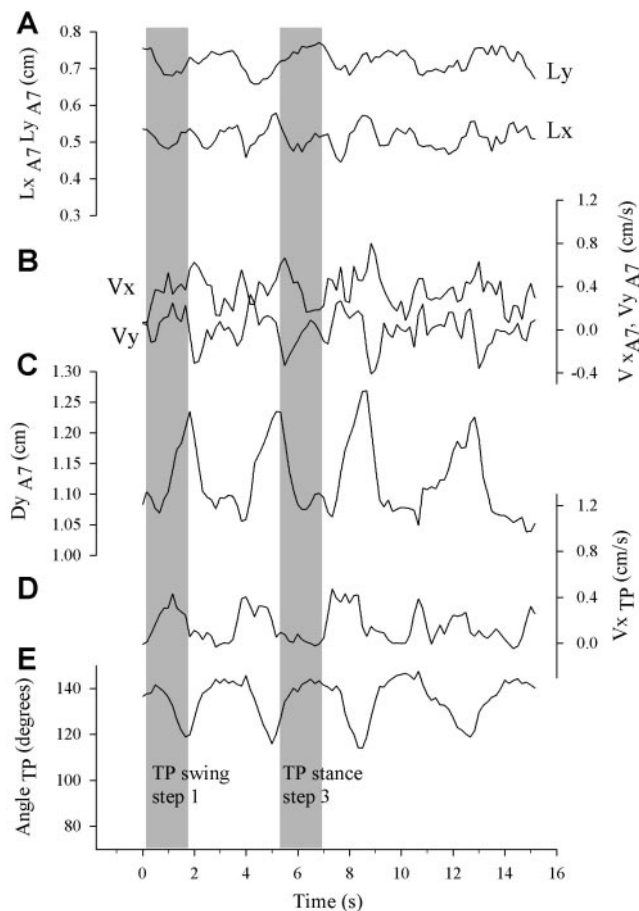
Figure 3. Kinematic parameters of the posterior segments (Abd7) and terminal prolegs (TP) during a typical crawl. Each panel represents parameters measured during four complete steps in the middle of a single crawl for the animal shown in Fig. 2. (A) Changes in the length of Abd7 and the terminal segment (Lx) were relatively small and not tightly correlated with the crawl cycle. Much of the cyclical shortening and lengthening of the posterior segments occured along the line (Ly) from the dorsal margin to the tip of the terminal proleg (TP). (B) Changes in the horizontal (Vx) and vertical (Vy) velocities of the Abd7 segment were not smooth oscillations but consisted of relatively rapid, short periods of acceleration and deceleration phase-locked to changes in Lx and the swing cycle of the TPs. (For clarity of illustration, the values for Vx_{A7} have been displaced upward by 0.2 cm/s.) (C) The segment vertical displacement (Dy_{A7}) slightly preceded changes in its horizontal velocity (B, Vx_{A7}) but lagged behind the increase in horizontal velocity of the TP (D, Vx_{TP}). (E) During stance, the TP was held extended behind the dorsal horn; at each step it rotated so that it became more vertical by 20°. The most vertical orientation (at about 120° in this example) occurred at the peak vertical displacement (see C). The gray bars are the periods of TP swing phase (step 1) and TP stance (step 3), as illustrated in Fig. 2A.

analysis is not meant to imply that springs are discrete physical structures in *Manduca,* but only that some aspects of its motion have spring-like properties. Unless otherwise stated, the values reported are the mean and standard error of a multi-step crawl (three to six analyzed steps) in nine animals (total 42 steps).

Results

General description of crawling

Manduca larvae crawl using an anteriograde wave of segmental steps. Each crawl began at the terminal segment and proceeded anteriorly through the abdomen as a series of phase-delayed steps in sequential segments, ending at the metathoracic segment. From this point the crawl continued as an elongation of the thoracic segments accompanied by a forward extension of the head. Although the thoracic legs are used to steady the cantilevered anterior segments, they are not necessary for propulsion and their denervation or surgical removal does not alter the basic crawling movements (Kopec, 1919; Holst, 1934). The kinematic analysis therefore concentrated on the movements of the abdominal segments. Each step in the proleg-bearing segments was composed of a stance phase, during which the prolegs were attached to the substrate by the cuticular hooks (crochets); and a swing phase, during which the crochets were unhooked from the substrate, raised, and moved forward along with the segment as part of a crawl. As many as three segments were in different stages of the swing phase at any one time. As noted previously, the onset of swing phase in Abd5 coincided with the onset of stance phase in the terminal segment, and onset of swing phase in Abd3 coincided with the onset of stance phase in Abd6. The mean crawling velocity was 0.28 ± 0.03 cm/s, and the mean step period (sum of swing and stance) for the prolegs in Abd4 was 2.91 ± 0.09 s. The duty factor (fraction of the step cycle in stance) for the prolegs in Abd4 was 0.53 ± 0.025 ($n = 37$ steps, 9 animals), which was not significantly different from the time spent in swing phase ($P = 0.223$, paired Student's *t* test). However, for the terminal prolegs (TP), the duty factor was 0.41 ± 0.029 ($n = 31$ steps in 8 animals), which was significantly different from the swing duration ($P = 0.003$, paired *t* test) and from the stance duty factor in Abd4 ($P = 0.0017$, *t* test, degrees of freedom = 57).

Kinematic differences between the posterior and midbody segments

Stance and swing. Movements of the posterior segment Abd7 and TP were distinct from those of the midbody abdominal segments. When the TP was in stance, the terminal segment continued to move forward (Fig. 2A). In contrast, when the Abd4 proleg (A4P) was in stance, the Abd4 segment remained stationary (Fig. 2B). The swing phases were also distinct, with the TP rotating within the plane of forward movement but the A4P remaining perpendicular throughout its step. Examples of these movements are quantified in Figures 3 and 4.

Posterior segment kinematic. In the posterior part of the animal there was very little change in the length of the
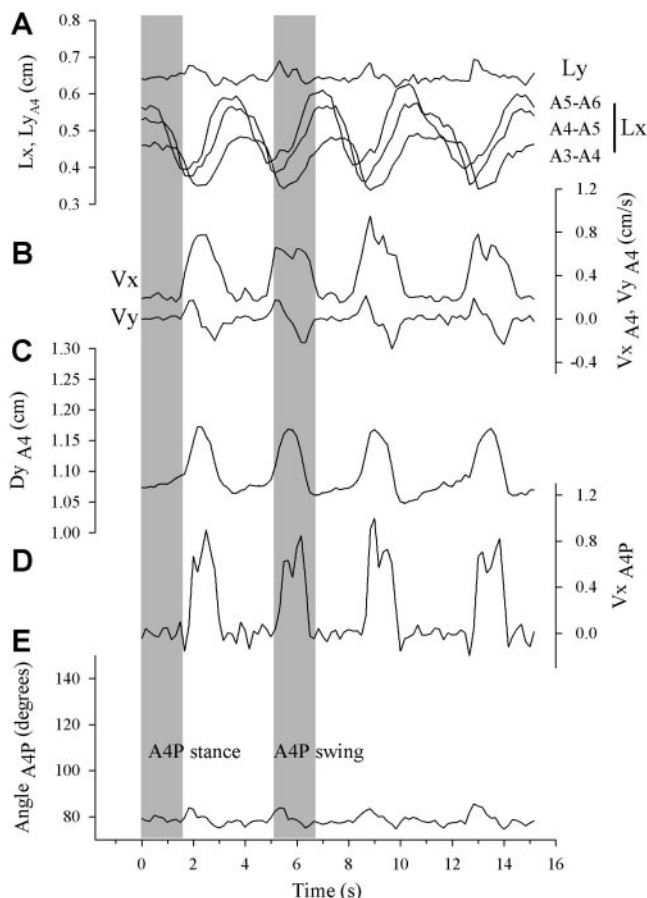
Figure 4. Kinematic parameters of the mid-abdomen segment (Abd4) and its prolegs (A4P) during a typical crawl. Each panel represents parameters measured during four complete steps in the middle of a single crawl from the same animal and bout of crawling shown in Figs. 2 and 3. (A) Changes in the length of the mid-abdominal segments (Lx) compared to Ly_{A4}. Lx varied cyclically, with waves of shortening that progressed in a posterior-to-anterior direction during each crawl cycle. The distance from the tip of the Abd4 proleg to the dorsal margin of Abd4 (Ly) changed very little each step, showing that the proleg lifts primarily because of segment elevation rather than proleg retraction. (B) Changes in the horizontal (Vx) and vertical (Vy) velocities of the Abd4 segment were cyclic and phase-locked to the step cycle. Peak vertical velocity (upward and downward) occurred at the start and end of each swing phase and at the beginning and end of the sustained peak in horizontal velocity. (For clarity of illustration, the values for Vx_{A4} have been displaced upwards by 0.2 cm/s.) (C) The segment vertical displacement (Dy_{A4}) was also highly correlated to changes in the segment velocity (see B) and to the increase in horizontal velocity of the proleg (D, Vx_{A4}). (E) During stance, the proleg was held directly ventral to the segment, and unlike the movement of the TP (Fig. 2), this angle remained constant throughout the crawl. The gray bars are the periods of A4P stance and swing as illustrated in Fig. 2B.

segment or the terminal prolegs (Fig. 3A, Lx_{A7}, Ly_{A7}). Although the segment velocities (Vy_{A7} and Vx_{A7}, Fig. 3B) increased and decreased with each step, these peaks were superimposed on a rapidly changing baseline. Also, in Abd7, each cycle of vertical displacement (Dy_{A7}, Fig. 3C) was preceded by TP moving forward (Vx_{TP}, Fig. 3D) and rotating (Fig. 3E) about $30°$ (mean $32.2 \pm 1.2°$, $n = 9$ crawls).

Midbody segment kinematics. In contrast to Abd7, the mid-body segments shortened on each step (Fig. 4A, Lx), with shortening greatest in Abd6 and least in Abd4 (Fig. 5). The horizontal (Vx_{A4}) and vertical velocities (Vy_{A4}) varied in phase, with peaks in Vy_{A4} (upward and downward) at the beginning and end of the sustained peak in Vx_{A4} (Fig. 4B). As the midbody segments lifted (Fig. 4C, Dy_{A4}), they also moved forward with no phase delay (Fig. 4B, D, Vx_{A4}, Vx_{A4P}; see below). The prolegs rotated very little relative to the horizontal plane (mean change $8.9 \pm 0.40°$, $n = 9$ crawls, Fig. 4E), and there was negligible retraction (Fig. 4A, Ly_{A4}).

Intersegmental displacement. The wave of vertical displacement moved forward (Fig. 6A) with a phase delay of 30–35° (about 0.24 to 0.29s) between each of the segments Abd6 to Abd3 (Fig. 6B, C). However, segment Abd6 lifted and moved forward at the same time as Abd7 (phase delay <10°, Fig. 6).

Phase relationship between vertical displacement and horizontal velocity

For each midbody segment, Vx peaked during the swing phase with a duration that substantially overlapped the change in Dy (Fig. 4). This association was consistent for all
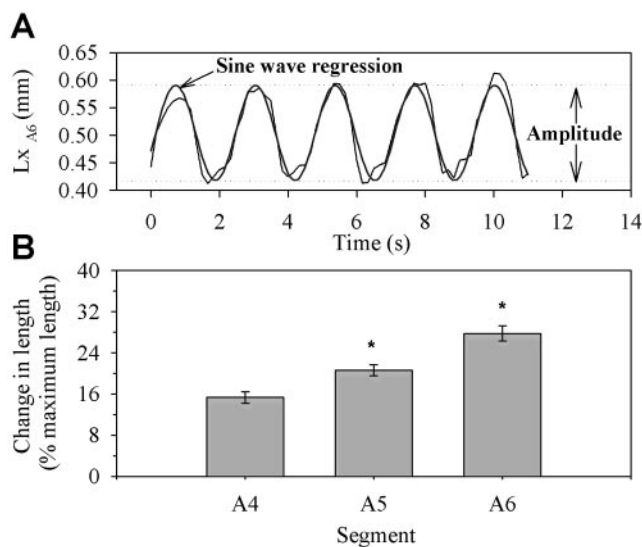
Figure 5. Length changes during a crawl vary by segment. (A) The magnitude of length changes in each segment were measured by fitting a modified sine function $y = y_0 + a \sin(2 \pi t/b + c)$ to each record of Lx. This example from one animal shows that the sine wave ($y = 0.504 + 0.087\sin(2 \pi t/2.324 + 5.897)$) is a good fit to changes in Lx_{A6} ($r^2 = 0.931$) with a peak-to-peak amplitude of 0.174. (B) The length change in each body segment was expressed as a percentage of the maximum stance phase length, and the mean changes ($\pm$ SE) were calculated for nine animals. The length change in Abd5 was significantly greater than that in Abd4, and that in Abd6 was significantly greater than that in Abd5 (*, both $P < 0.001$, paired t test). Length changes in Abd7 were too small and variable to be included in this analysis (see Fig. 2A).
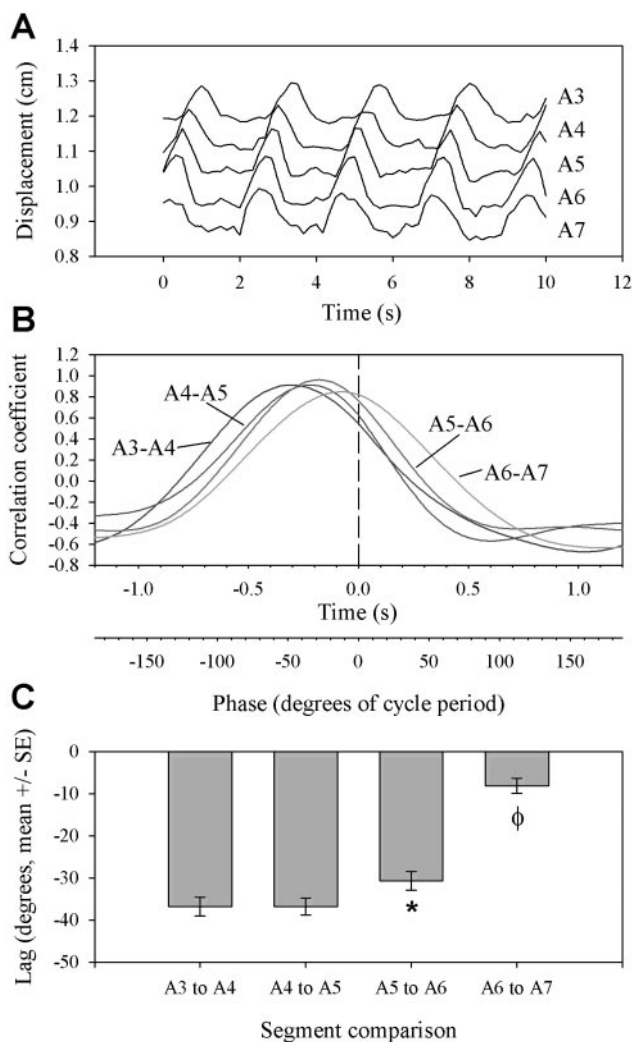
Figure 6. Phase relationship of movements in different body segments during crawling. Because segment shortening is indistinct in Abd7, the relative timing of the anteriograde wave was compared between segments by using the vertical displacement (Dy). (A) Changes in Dy for each body segment are shown for a single crawl. (For clarity of illustration, the initial displacements have been offset from one another by approximately 0.1 cm.) Changes in Dy in each segment maintained a fixed phase relationship throughout the crawl. (B) The fidelity and magnitude of this phase shift was measured by cross correlating each pair of recordings (data from A). Phase delay was measured from the peak of the correlation, which was typically greater than 0.8. (C) The mean segment-by-segment phase shift was calculated for nine animals. There was no significant phase difference between movements in the anterior proleg-bearing segments ($P = 0.999$, paired t test, Abd3 to Abd4 compared with Abd4 to Abd5). The phase difference between Abd5 and Abd6 was significantly less than that between Abd4 and Abd5 (*, $P = 0.027$, paired t test). The phase difference between Abd6 and Abd7 was very small and significantly different from that between Abd5 and Abd6 (Φ, $P < 0.001$, paired t test).

steps across all body segments, but there were small segment-specific differences in the phase relationship of Vx and Dy. In the mid-abdominal segments Abd4 and Abd3, Dy and Vx were essentially in phase, with a lead (Abd4),

and lag (Abd3) of about 10° (<100 ms, Fig. 7). In Abd7, Abd6, and Abd5, the rise in Dy preceded changes in Vx by 28–33° (200–250 ms, Fig. 7). This shift in phase was also found in measurements of Dy and Vx for the prolegs (TP lead 278 ± 135 ms, A4 lag −68 ± 12.5 ms, $n = 10$ animals, $P = 0.002$, paired t test).

Body segment compression and extension during crawling

When the animal was at rest between bouts of crawling, the diameter/length ratio (Ds/Lx) of the proleg-bearing abdominal body segments (Abd3–Abd6) was 1.404 ± 0.042 ($n = 20$ segments in 5 larvae). When *Manduca* was not feeding, the head and thorax curled ventrally, and abdominal segments Abd1 and Abd2 were compressed such that
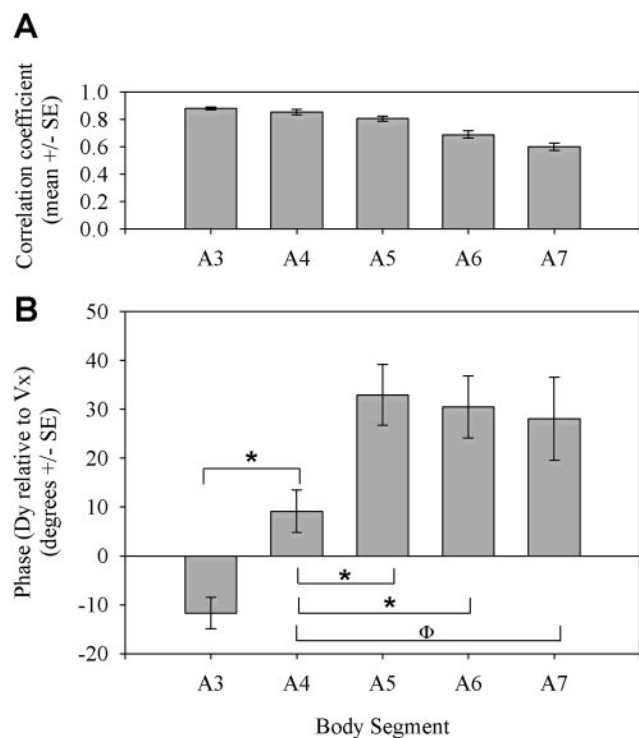
Figure 7. Phase relationship between vertical displacement and horizontal velocity. The phase relationship between Dy and Vx for each body segment was calculated from the time of the peak of the cross-correlation function for movement of the upper insertion point of the dorsal interior oblique muscle (DIO). (A) The strength of this correlation decreased progressively from Abd3 to Abd7, mainly because Vx was less cyclic in the more posterior segments (*e.g.,* Fig. 2). (B) The timing of changes in Dy and Vx varied with segment. The phase differences between Abd3 and Abd4 were significantly different (*, $P < 0.001$, paired t test), but both were small (a lag of 11.7° for Abd3 and lead of 9.1° for Abd4, corresponding to <100 ms), so that Dy and Vx were essentially in phase in these segments. In Abd5, Abd6, and Abd7, the rise in Dy preceded changes in Vx by 28–33° (200–250 ms), with no significant difference between the segments ($P = 0.727$ Abd6 to Abd7 and $P = 0.376$ from A5 to A6). The phase difference in Abd4 was significantly different from Abd5, Abd6 (*, $P < 0.001$), and Abd7 (Φ, $P = 0.019$), $n = 9$ animals.

the Ds/Lx ratio was about 2. In cantilevered "reaching" movements, segments Abd1 and A2bd stretched but Ds/Lx never decreased below 1.2. During crawling, the segments changed shape primarily by compressing and elongating. Within each body segment, the maximum shortening corresponded approximately to peak velocity and the middle of the proleg swing period; hence changes in Lx were approximately in phase (within 11°) and negatively correlated with Vx (Fig. 8). Therefore, the movement of a body segment during each swing phase involved an initial compression as it accelerated forward, followed by an expansion back to resting length as the segment decelerated. The small but statistically significant change in phase direction in segment Abd6 (where forward movement preceded shortening) suggests a transition in motive force (see Discussion).

The length changes in each segment could involve two components: body wall deformation and folding of the intersegment membrane. This possibility was examined by measuring the length of a segment within the segmental boundaries (Jx; from upper origin to the upper insertion points of muscle DEO) and expressing it as a proportion of the segment length, Lx. For an evenly stretching elastic material, this value should remain constant, but in *Manduca* it varied cyclically and was antiphasic to the segment length (Fig. 9A). Hence, as Lx lengthened, the relative length of Jx diminished (by 8.80% ± 0.73%, $n = 27$ segments, 9 animals); this implies an unfolding or disproportionate stretching of the intersegmental membrane. This finding was confirmed by inspecting crawling *Manduca* on slow-motion video. As each segment moved forward, its leading edge collapsed into the next anterior segment.

Spring and elastic properties of the abdominal segments

The movements of each body segment can be treated as a spring that is itself moving forward periodically. Hence the difference in velocity between one end of a segment and the other provided a simplified measure of the spring properties of the segment. To test whether the cyclic compression of each segment resembled harmonic motion of a sprung mass, the differences in Vx at each end of a segment were compared with changes in Lx (Fig. 9B). In all cases, changes in the Vx difference preceded Lx changes by about 0.6 s with a mean phase lag of 73.03° ± 3.17° ($n = 23$ segments from 7 animals); true harmonic motion would have a lag of 90°. The lag was smallest (least like harmonic motion) in Abd4 and greatest in Abd6 (Fig. 9C). Velocity differences and length changes in segment Abd7 were not well correlated (coefficients were all below 0.60).

Discussion

The results of this kinematic analysis of *Manduca* locomotion provide detailed information about the displacements, dimensions, and velocities of different body seg-
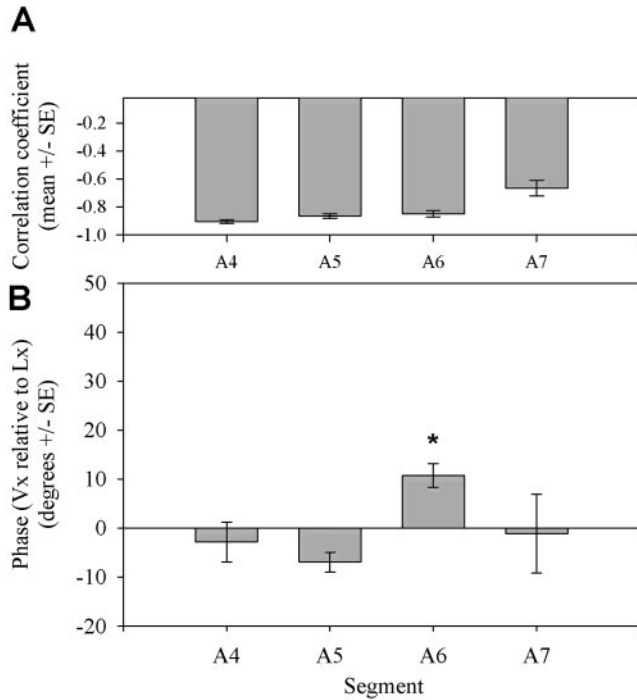
Figure 8. Segment velocity is negatively correlated with segment length. (A) Changes in Vx for each body segment were negatively correlated with Lx. The correlation was largest in segments Abd4 to Abd6 and significantly smaller in Abd7 ($P = 0.016, 0.018,$ and $0.019,$ compared to A4, A5, and A6, respectively, paired t test, $n = 9$). (B) Although changes in Vx occurred at about the same time as changes in Lx (phase was within 11°), the phase difference in segment Abd6 was significantly different from more anterior segments (*, $P = 0.009$ Abd4 to Abd6, $P < 0.001$ Abd5 to Abd6, paired t test, $n = 9$), with changes in Vx preceding changes in Lx. Because of its relatively high variability, the means phase in Abd7 was not significantly different from that of any of the other segments ($P = 0.510, 0.377, 0.892$ compared to Abd4, Abd5, and Abd6, respectively, paired t test, $n = 9$).

ments relative to one another and to the crawl cycle. The following discussion will (1) compare the stepping movements of different body segments to those in other legged animals, (2) compare *Manduca's* anteriograde movement with models of worm peristalsis, and (3) describe a general and comprehensive approach to modeling these movements through neural and biomechanical control.

(1) Kinematics of stepping

Legged locomotion with a stiff skeleton. Locomotion in most legged animals can be described quite accurately with mechanical models of a bouncing or swinging center of mass (Dickinson *et al.,* 2000; Full and Farley, 2000). There is enormous complexity in the details of how such motions are accomplished, but in general, walking resembles the action of an inverted pendulum, with the center of mass lowest during mid-stance and changes in horizontal kinetic energy out of phase with changes in gravitational potential

energy (Full, 1993; Full and Farley, 2000). Running resembles the action of an oscillating sprung mass, with the center of mass highest during swing and the kinetic and gravitational potential energies in phase with one another (Full and Tu, 1990, 1991). During running, energy is stored and
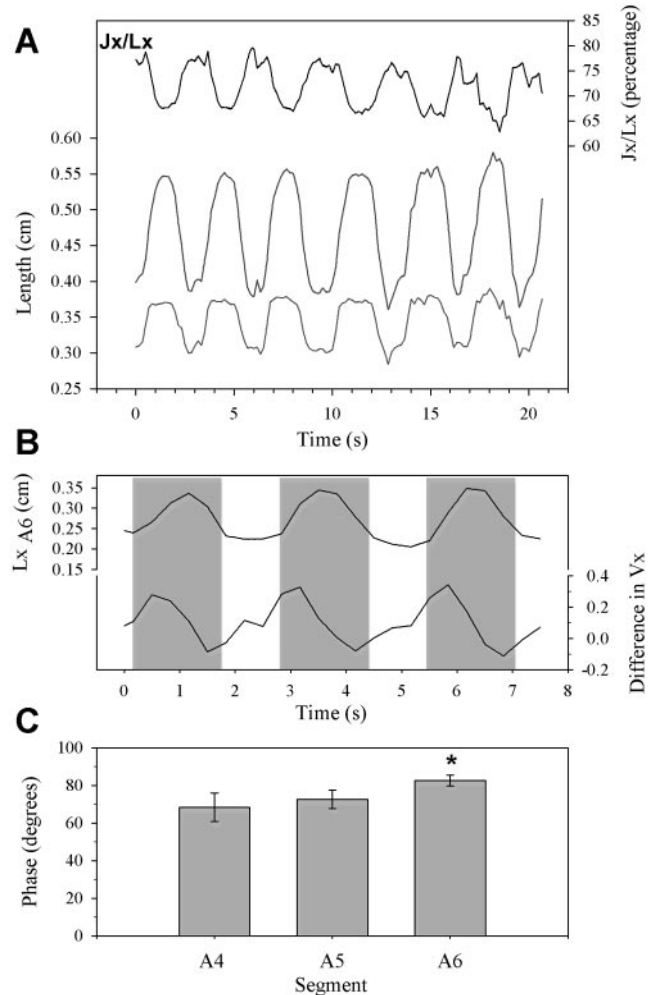
Figure 9. Segments length changes are not uniformly elastic. (A) The length of a segment (Lx) measured across the anterior intersegment boundary varied more than the intrasegment length (Jx) between boundaries. Hence the ratio Jx/Lx was not constant (as predicted for a uniformly elastic material) but varied cyclically by about 10% of Lx. Because the Jx/Lx ratio was smallest when Lx was largest, the change was caused by a disproportionate increase in expansion at the intersegment boundary. The data shown here are from segment Abd6. (B) The movement of a point relative to more posterior parts on a segment could be described as spring-like. In this example of three steps in a single crawl, the peak change in velocity of the anterior margin of Abd6 relative to the posterior margin (difference in Vx) occurred during the lengthening of the segment (Lx_{A6}), about 90° out of phase. Gray bars indicate the duration of the swing phase. (C) The phase delay between changes in Vx and Lx (measured from the peak of the cross correlation) was greatest in segment Abd6 and significantly less in Abd5 (*, $P = 0.012$, paired t test, $n = 7$). There was no significant difference in phase between segments Abd4 and Abd5 ($P = 0.633$) or Abd4 and Abd6 ($P = 0.140$). Vx and Lx in segment Abd7 were not well correlated (coefficients < 0.60) and were not included in this analysis.

recovered in tendons or other elastic tissues (Cavagna *et al.,* 1977; Blickhan and Full, 1987; Alexander, 1991; Full *et al.,* 1991; Kerdok *et al.,* 2002). Walking and running have also been distinguished by measuring differences in the duty factor. This is the proportion of a step during which the limb is in contact with the ground; values are about 0.6 during walking and 0.35 during running (Alexander and Jayes, 1980).

Stepping movements in Manduca. The overall stepping pattern in *Manduca* (Belanger and Trimmer, 2000) is similar in most respects to that of other caterpillar species (Kopec, 1919; Barth, 1937; Holst, 1943; Casey, 1991; Brackenbury, 1999). However, the present study reveals that movements in the midbody are different from those in the posterior segments.

Midbody segments. The midbody segments (*e.g.,* Abd4) move in symmetrical arcs as they lift and swing forward. The prolegs remain perpendicular to the axis of movement, only pivoting slightly at the transitions from swing to stance and stance to swing. Although the duty factor of midbody prolegs is typical of a walking gait, changes in vertical displacement and horizontal velocity are in phase with one another, as expected for running animals. This motion is consistent with the interchange of kinetic and potential energy through the storage and release of elastic energy. However, running gaits are not necessarily defined by their mechanism. For example, fast-moving cockroaches are classified as runners by the pattern of ground reaction forces, the short duty cycle of their steps, and their in-phase fluctuations in gravitational potential energy and kinetic energy (Full and Tu, 1991). However, the potential energy changes are exceedingly small (peak values of 2–4 μJ) compared to the horizontal kinetic energy (330–360 μJ). Therefore, the role of elastic storage in cockroaches is unlikely to be the same as that in a galloping horse where potential energy and kinetic energy are roughly the same magnitude (Alexander and Jayes, 1983). Clearly, similarities in the overall stepping kinematics of these species are not explained by energy exchange alone.

Hence, although the kinematics of midbody movements in *Manduca* can be compared to other gaits, the underlying mechanisms are extremely different from those of stiff-skeleton animals. To a first approximation (ignoring vertical speed and possible changes in mass), a *Manduca* body segment of 1-g mass moving vertically 0.1 cm each step and horizontally at 0.6 cm/s (Fig. 4) will have a peak potential energy of 10 μJ and peak kinetic energy of 0.018 μJ. This is a massively disproportionate amount of potential energy (relative to kinetic energy) compared to either the cockroach or the galloping horse. Evidently such descriptions of mechanical energy exchange are inadequate to explain stepping movements in *Manduca.* It is therefore of utmost importance to also examine the mechanisms of *Manduca* crawling by measuring ground reaction forces, body pressure changes, and muscle activation patterns.

Terminal segment. Despite these limitations, it is interesting that the kinematics of the posterior body segments in *Manduca* is different from that of the other abdominal segments. At the start of a step the TP usually extends posteriorly, making an obtuse angle with the longitudinal dorsal line of the rest of the caterpillar. When the crochets release, this angle decreases about 30° until the crochets re-attach and the TP re-extends. The maximum angle change corresponds to the end of the TP swing phase and to the maximum vertical displacement of the posterior body segment (Figs. 2A and 3E, C). The TP therefore appears to pivot around its attachment point, as expected for the inverted pendulum motion of a legged walker (Cavagna *et al.,* 1977; Dickinson *et al.,* 2000). This similarity is also seen in the offset between movements in the vertical plane and the horizontal velocity of the posterior segments. However, in *Manduca* the phase delay between these correlates of gravitational and kinetic energy is relatively small ($\approx$30°) and more consistent with a walking gait that incorporates elastic components such as that in walking frogs (Ahn *et al.,* 2004)). The TP also has a significantly shorter duty factor (about 0.4) than other prolegs. This duty factor probably reflects the comparatively small amount of direct propulsion that can be developed by the prolegs, which are not extended by active increases in pressure (Mezoff *et al.,* 2004).

(2) Caterpillars are not worms with legs

In many hydrostatic animals, significant changes in shape are accomplished by alterations in the length and radius of fixed-volume segments. For example, in an earthworm such as *Lumbricus terrestris,* the segments have a diameter/length ratio between 2 and 5 (Quillin, 1999), which ensures that small changes in segment length (using longitudinal muscles) produce forceful and relatively large increases in diameter to aid in burrowing (Wainwright, 1988; Quillin, 1998). At the other end of the geometric relationship, hydrostatic animals that use small changes in diameter to generate large and fast changes in length have very low diameter/length ratios (*e.g.,* the long thin tentacles of squid have a ratio of 0.1; Kier and Leeuwen, 1997). In addition, many species of legless worms must expand the body to provide anchor points, and this determines the direction of the propagating wave of contraction (Trueman, 1975). It is therefore interesting that the typical diameter/length ratio for *Manduca* segments is close to unity, the most "neutral" region of the relationship. The shortening of longitudinal muscles will have similar (but reciprocal) effects on both the length and diameter of a body segment. This simple geometric observation supports the contention that caterpil-

lar propulsion is driven predominantly by contractile shortening rather than hydrostatic elongation (Barth, 1937; Snodgrass, 1961).

The locomotion of many legless animals such as worms alternates hydraulic extension powered by circular muscles with shortening mediated by longitudinal muscles (Keller and Falkovitz, 1983; Skierczynski *et al.,* 1996; Wilson *et al.,* 1996a, b; Quillin, 1998, 1999; Cacciatore *et al.,* 2000). Such movements can be modeled as peristaltic waves or as longitudinal and transverse waves propagating though uniform elastic material (Keller and Falkovitz, 1983; Dobrolyubov, 1986; Wadepuhl and Beyn, 1989; Dobrolyubov and Douchy, 2002; Steigenberger, 2003). Although such models produce plausible movements, the kinematics have not been compared directly to the living animals, and the underlying assumptions are not necessarily appropriate for legged crawlers such as *Manduca.* For example, caterpillars do not have a divided hemocoel or circular muscles like annelids (Snodgrass, 1961), and they are able to grip and climb in any orientation. Another obvious deviation is that the kinematics of the propagating wave in *Manduca* changes as it moves forward. This difference might be explained by the proleg gripping system, the nonuniform segments (Abd7, for example, does not have prolegs), or by viscous damping—none of which are considered in the theoretical models.

A different approach (for horizontal motion) is to treat each segment as a spring that compresses and expands once for each step. As predicted for an oscillating sprung mass that is also moving forward, the phase relationship between changes in segment length and the velocity differences at each end of a segment in *Manduca* were very spring-like (Fig. 9B, C). This spring-like motion does not, however, necessarily imply the presence of discrete springs in *Manduca;* rather, it arises from the elastic properties of soft tissues. Typically, systems of two or more coupled sprung masses exhibit complex oscillations with phase planes that are chaotic. The (almost) harmonic motion of *Manduca* body segments therefore implies strong (perhaps critical) damping, such that each step during a crawl is mechanically independent of the previous steps. Such damping could easily be provided by the body wall of *Manduca,* which stretches easily at low strains and becomes stiffer as it is stretched (Alexander *et al.,* 1999). This pseudo-elastic property (elasticity depends on the strain and whether the material is being loaded or unloaded; Ogden and Roxburgh, 1999) is shared with *Manduca* muscles which, in addition, exhibit viscoelasticity, changing stiffness as a function of their stretch rate (Dorfmann *et al.,* 2007; Woods *et al.,* unpubl. data). Both tissues are likely to contribute some of the spring-like properties in each body segment. It is therefore important to include both elasticity and viscous damping in any mechanical models of caterpillar crawling. Another factor in the wavelike movement of *Manduca* is the thickness and elasticity of its contoured segments. The results presented here show that folds occur at segment boundaries such that shortening is not uniform along the body wall. This folding probably changes with the degree of body turgor, itself a function of food intake, water balance, and muscle tonus. These structural complexities are expected to be important in determining the movement produced by each muscle as it contracts.

(3) Toward a neuromechanical model of soft-bodied locomotion

Although elasticity and spring-like kinematics appear to contribute to *Manduca's* movements, any models based on coupled and damped springs are likely to be overly simplistic. Many additional factors are essential for normal locomotion. Clearly caterpillar crawling is initiated and maintained by the phasic activation of muscles (Dominick and Truman, 1986; Belanger *et al.,* 2000), which itself involves a central pattern generator (Johnston and Levine, 1996a, b) and sensory feedback (Holst, 1934; Lowenstein and Finlayson, 1960; Weevers, 1965; Johnston *et al.,* 1999). However, the work described here highlights the particular importance of soft tissues in generating complex movements. Indeed, there is an apparent paradox that animals such as *Manduca,* which have a remarkable three-dimensional workspace and virtually unlimited degrees of freedom, manage to control their movements with relatively simple central nervous systems. Although it is possible that such animals dedicate relatively more of their central computing power to coordinate movements, it is also conceivable that the intrinsic material properties of soft tissues (pseudoelasticity, viscoelasticity) form a self-adjusting part of the motor system. Because natural selection for behavior operates on the system as a whole (Chiel and Beer, 1997), such complex biomechanics could actually lower the need for precise central coordination. This hypothesis is testable with an appropriate model system. We are currently developing constitutive models of *Manduca* tissues that will be used in a mathematical simulation of the mechanical system (Dorfmann *et al.,* 2007). This will be combined with electrophysiological data on the patterns of neural output to individual muscles and with an accurate structural representation of the body shape, to begin building a more complete understanding of soft-bodied, legged locomotion.

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