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Combined kinematic and electromyographic analyses of proleg function during crawling by the caterpillar *Manduca sexta*

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Abstract The planta retractor muscles in the prolegs of *Manduca sexta* caterpillars are a frequently-used model system for investigating a number of problems in neurobiology. We have combined kinematic and electromyogram analysis of proleg movements during crawling to examine the roles of these muscles during normal behavior. We found that retractor muscle activity is highly stereotyped, and that the primary function of these muscles is to disengage the crochets at the tip of the proleg for the swing phase of crawling. The duration of activity of the muscles was tightly coupled to the phasing of crawling behavior. The stepping patterns of animals changed to accommodate variations in the substrate, but the relative timing of retractor muscle activity was unaffected. There were no clear correlations between the various properties of motoneuronal input to the muscle (duration of activity, number of spikes, peak frequency of spikes) and the resulting muscle length change. Perhaps because it functions partially as a hydrostat, this may represent a neuromuscular system in which a significant part of the control algorithm is embedded in its morphology.

Key words Insect · Locomotion · Central pattern generator

Abbreviations *APRM* accessory planta retractor muscle · *PG* central pattern generator · *EMG* electromyogram · *PPRM* principal planta retractor muscle

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Introduction

Animal behavior results from the interaction of neural activity, filtered through effectors and biomechanical factors, with the environment. While much is known about this interplay in animals with hard, jointed skeletons (internal and external), less is known about systems that use hydrostatic skeletons (although see Chiel et al. 1992; Wilson et al. 1996; Gutfreund et al. 1998; Cacciatore et al. 2000). Are there similar rules for the control of limbs and posture in these two groups, or does the lack of an articulated skeleton require different control principles?

Caterpillars offer an interesting window onto this problem. Like all arthropods, they possess rigid, articulated legs, but they use a hydrostatic skeleton to support their body, and hydrostatic principles in controlling the prolegs. Prolegs are non-articulated appendages on the abdomen, and they afford a number of advantages as model systems for investigating motor control. Like most arthropod appendages, they are operated directly by a small number of motoneurons, one or two for each of the six proleg muscles. These neurons have been identified and mapped in the central nervous system (Weeks and Truman 1984; Weeks and Ernst-Utzschneider 1989; Sandstrom and Weeks 1996). As the major “limbs” which the animal possesses, the prolegs are obviously important in crawling, the principal means of locomotion for caterpillars. But they are also used in a number of other behaviors, including pre-ecdysis and ecdysis movements (Weeks and Truman 1984), and burrowing (Dominick and Truman 1986). In *Manduca sexta*, the prolegs also exhibit a robust withdrawal reflex, such that deflection of sensory hairs near the distal tip of the leg (“planta hairs”) causes the proleg to retract (Weeks and Jacobs 1987). Synapses in the reflex pathway display a number of forms of activity-dependent modulation, including facilitation, depression and post-tetanic potentiation (Trimmer and Weeks 1991), and the reflex as a whole habituates to repeated stimuli (Wiel and Weeks 1996; Wood et al. 1997).

We would like to understand how these forms of plasticity are incorporated into normal behavior, particularly locomotion. The functions of the thoracic legs during crawling have been examined in detail in both larval and adult *Manduca* (Johnston and Levine 1996a), but there is little information on the activity of the proleg muscles during normal behavior. While it has been shown clearly that fictive crawling behavior can be produced by the isolated central nervous system (Johnston and Levine 1996b), there are nonetheless obvious roles for sensory input in the intact animal. As a first step towards understanding this interplay between sensory input, motor output, plasticity of reflexes, and the successful generation of adaptive behavior, we have characterized the activity of the main retractor muscles of the proleg, the *principal planta retractor muscle* (PPRM) and the *accessory planta retractor muscle* (APRM), during normal locomotion under a variety of conditions. By combining kinematic data on proleg movements with electromyograms (EMG) from the retractor muscles, we have found that the activity of these muscles is extremely stereotyped, and that their main function is to disengage the crochets during crawling. While animals vary their stepping patterns to accommodate differences in the substrate upon which they are crawling, there are no corresponding changes in the activity pattern of the retractor muscles.

Materials and methods

Animals

Larvae of the tobacco hornworm, *M. sexta*, were raised through the first four instars in individual plastic cups at 27 °C under a 17:7 L:D cycle, and fed an artificial diet (Bell and Joachim 1978). We found that animals would crawl more reliably if they were housed in larger quarters after the 4th instar. Accordingly, 5th-instar animals were kept in groups of four to eight in 10-l plastic tubs, with wire frames to crawl on. Male or female animals, on the 2nd or 3rd day of the 5th larval instar, were used in these experiments. All experiments were performed at 25–27 °C.

Video recordings

Using a S-VHS resolution camcorder (Canon ES-4000), animals were videotaped from the side as they crawled on level wooden dowels of various diameters: 4 mm, 8 mm, or 12 mm. While most measurements were based on this side view, some measurements were also made using a mirror positioned at 45° to the camera's focal plane that gave an image of the ventral side of the animal.

Sequences of uninterrupted crawling that lasted at least five steps were selected for analysis. Initial and final steps in crawling bouts were not included in the analyses. Videotapes were either replayed frame-by-frame using a VCR (JVC HR-S5400U) and color monitor, or digitized and analyzed on a PC using commercially-available software (VideoPoint software from Lenox Software, Lenox MA). In either case, data were analyzed at a temporal resolution of 33 ms (based on the video rate of 30 frames/s), and a spatial resolution of approximately 0.15 mm (corresponding to one pixel in the digital video). The positions of a number of surface landmarks and points previously marked with ink on the animal (see Fig. 1) were recorded in each frame, and subsequently used to determine the motion of the proleg. We also recorded the

time at which the proleg made contact with, and released from, the substrate, in order to determine the relative timing of steps. Since the origins and insertions of the retractor muscles are visible on the external cuticle, the degree of muscle shortening for each muscle was approximated by measuring the distance between these points in each video frame of interest. Note that this measure is only an approximation of muscle length, as it also includes any change in the lengths of the muscle tendons, and compliance in the apodemes. For comparisons between animals, these values were normalized by dividing them by the muscle's rest length, calculated as a mean of five or six measurements in quiescent animals.

Electromyography

Electromyographic (EMG) recordings were used to determine the activity of muscles underlying proleg movements. Animals were anesthetized by chilling on ice and maintained on a chilled metal block during electrode implantation. Bipolar electrodes were fashioned from Formvar-coated Nichrome electrode wire (A-M Systems, Everett, Wash.), 25 µm in diameter. The wires were cut to remove insulation only at the tip, inserted through a small hole in the cuticle close to the origin of the muscle of interest, and positioned next to the muscle. A small amount of NewSkin (Medtech Labs, Jackson, Wyo.) was used to fix the electrodes and seal the insertion hole. The electrodes were then routed to the dorsal surface of the animal and secured with cyanoacrylate glue. A single 50-µm wire, bared of insulation for about 3 mm at the tip, was inserted into the last abdominal segment to serve as a ground electrode. Up to four pairs of electrodes, plus a ground lead, were implanted in any animal. Based on kinematic measurements of animals before and after electrode placement, neither the implantation process nor the presence of the electrodes interfered with normal crawling behavior (data not shown).

The electrodes were connected to shielded leads which led to differential a.c. amplifiers (A-M Systems model 1700). Signals were recorded at a bandwidth of 100 Hz to 5000 kHz, with a 60-Hz notch filter, and stored on magnetic tape for subsequent analysis. To synchronize EMG data with video data, a square-wave voltage pulse was recorded with the EMG signals, and simultaneously used to drive a light-emitting diode in the video field of view.

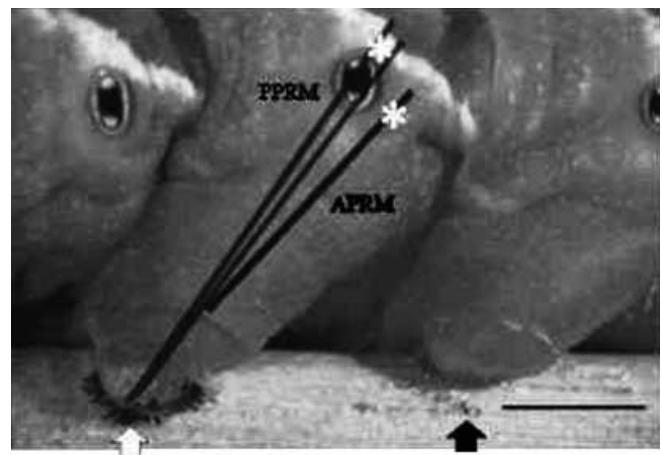


Fig. 1 Lateral view of a proleg of *Manduca sexta* showing the approximate courses of the principal and accessory planta retractor muscles (PPRM and APRM, respectively) from their origins to insertions, and the electromyogram (EMG) recording sites (asterisks). The white arrow shows the crochets, visible here as the planta is pulled outward from the proleg just prior to a step. The black arrow shows the planta hairs contacting the substrate. (The tips of several hairs have been coated with ink for visibility.) Scale bar: 2 mm

Some EMG records were analyzed by playing them back onto a chart recorder and measuring by hand. The remainder were digitized at 20 kHz using hardware from RC Electronics or Axon Instruments Inc, and analyzed using custom software written for MatLab (The MathWorks, Natick, Mass.).

Experimental design and statistical analyses

The kinematic and EMG data reported here consist of eight step cycles from each of eight animals under each set of conditions. Averages are given as mean \pm standard deviation (SD). A critical level of $P < 0.05$ and two-tailed probabilities were used throughout this study. Comparisons between two or more groups used ANOVA, followed by pair-wise comparisons using the Newman-Kewls post-hoc test where appropriate. Ratio variables were transformed using square root transformations prior to testing (Sokal and Rohlf 1995). For the experiments on the effects of substrate on crawling behavior, each animal was tested on each dowel size. Hence, this is a repeated measures design, with step number and dowel size representing within-individual effects, and was treated accordingly.

For kinematic data, the start of a cycle was defined as the release of the substrate by the terminal claspers. Cycle period was then the time between this reference point in subsequent steps, and latencies of movement of the prolegs were calculated relative to this. Normalized values were obtained by dividing either duration or latency values by the cycle period.

For EMG records, we calculated duration of activity, number of spikes per burst, maximum frequency of spikes per burst (based on the interspike intervals), average spike frequency (number of spikes/burst duration), normalized duration (duration/cycle period) and onset phase (latency/cycle period).

Results

The prolegs and crawling behavior

The prolegs are nonarticulated evaginations of the abdominal body wall (Fig. 1). At the tip of the proleg is the planta, composed of cuticle less rigid than the sides of the proleg, and bearing an array of curved cuticular hooks, the crochets. These are used by the animal to grip the substrate. Their attachment to many surfaces is strong enough that attempts to remove the animal forcibly (from the dowels used in this study or from a finger) will often tear the crochets from the proleg before they loosen from the substrate. The crochets are shaped and arranged on the planta such that a slight retraction of the planta causes them to disengage from the substrate. Conversely, contact by the planta with the substrate causes the crochets to hook into the surface.

Since there are no extensor muscles for the prolegs, they are operated via a combination of hydrostatic pressure and the activity of intrinsic muscles. In cold-anesthetized animals, which are essentially flaccid and therefore have negligible hydrostatic pressure, the "rest" position of the prolegs is extended and close to fully adducted, the tips almost meeting along the animal's midline. In this state, the prolegs are still rigid enough to bear the animal's weight, and show very little compression into the body wall if the animal is balanced on their tips. (Note that this is no longer the case for animals several days into the 5th instar; the considerable gain in

mass is then sufficient to compress the prolegs and cause the animal to rest its venter on the substrate.)

While the basic mechanics of crawling in caterpillars have been described by a number of authors (Kopeck 1919; Barth 1937; Hinton 1955; Hughes 1965; Weevers 1965; Dominick and Truman 1986; Johnston and Levine 1996a; Brackenbury 1999), a brief description will facilitate presentation of the results (see Fig. 2). Crawling is primarily an abdominal behavior, in which the prolegs function as claspers. A wave of longitudinal contractions begins posteriorly, and progresses forward. Contraction of the dorsal portion of a particular segment leads that of the ventral portion. This results in each body segment being alternately lifted by the contraction of the dorsal portion of the next anterior segment, and then moved forward and lowered by the contraction of the ventral portion. In most cases, there is very little shortening of the prolegs, as determined by the distance between the origins and insertions of PPRM and APRM (see below). Indeed, most of the proleg stepping movement is produced by the abdomen, with the prolegs being "carried along". Depending on the step and the substrate, the planta may or may not be retracted completely into the proleg, and there may or may not be abduction of the prolegs during the step.

On average, this pattern of coordination results in a stepping pattern in which the prolegs are recruited in a posterior to anterior progression (Fig. 3). Significant transitions in the pattern appear to be the onset of stance in A8, which is roughly simultaneous with the onset of

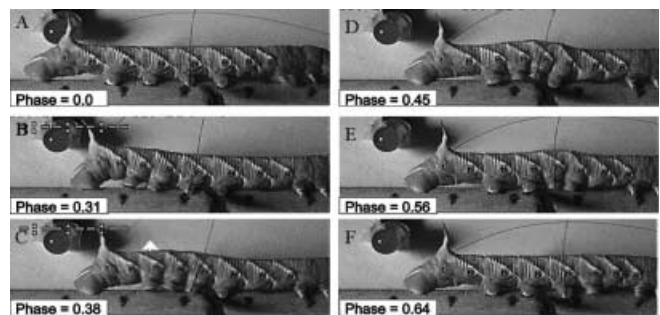


Fig. 2A–F Example of a 5th-instar *M. sexta* crawling on a dowel. The cycle period for this step was 2.1 s, and the relative phase during the step for each figure is indicated. The two solid black lines indicate the distances between the segment boundaries of the third, fourth, and fifth abdominal segments. The dashed black line indicates the approximate length and orientation of PPRM. The arrowhead in A indicates the crochets, which are visible as a black band at the tip of each proleg. A step cycle begins with dorsal shortening of the terminal segment. The terminal claspers are then lifted from the substrate (A), and a wave of longitudinal contractions progresses anteriorly (B). The dorsal portion of a given segment shortens first, the proleg detaches from the substrate (D), and then the ventral portion of the segment shortens to bring the segment forward, while the dorsal portion of the next anterior segment contracts (E). Note that the crochets are withdrawn into the tip of the proleg during the swing phase (D, E), emerging just before contact with the substrate at the end of swing (F). The marks on the dowel occur every centimeter

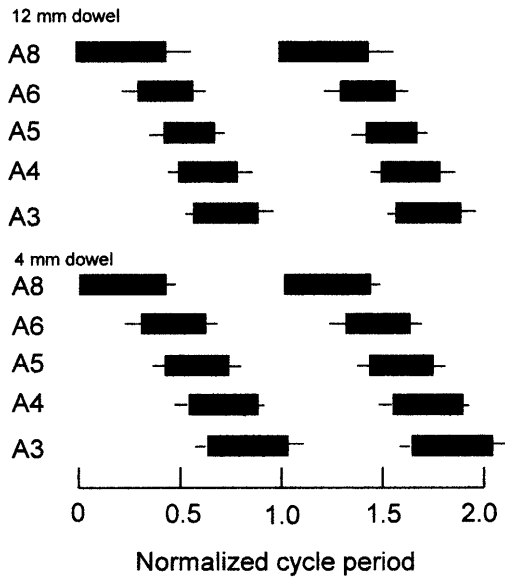


Fig. 3 Average stepping patterns for all animals in this study crawling on the 4-mm or 12-mm dowels. The *bars* indicate the duration of swing phase for each of the prolegs ($A_{3,4,5,6,8}$), measured as a proportion of the step cycle length (*x-axis*). The *start of the bar* indicates the mean time of onset of swing, the *preceding thin line* indicates the standard deviation of the onset mean, the *length of the bar* indicates the mean duration, and the *trailing thin line* indicates the standard deviation of duration. There is no standard deviation for the onset of swing in A_8 , as it was arbitrarily chosen to define cycle onset

swing in A_5 , and the onset of stance in A_6 , which is synchronous with the onset of swing in A_3 (Fig. 3).

Muscle activity underlying proleg movements during crawling

The two planta retractor muscles, PPRM and APRM, are the only muscles in a position to significantly shorten the proleg. They are active in phase with proleg stepping movements, becoming active just prior to swing (Fig. 4). Their activity is approximately synchronous, with PPRM slightly leading APRM. Closer examination of the length of either of the retractor muscles during a step, however, shows that neither muscle shortens much below its rest length. Rather, just prior to lifting off of the substrate, the muscles are stretched by the movement of the body segment anterior to the segment in question (Fig. 5). The onset of muscle activity is roughly synchronous with the beginning of this stretch. This stretch continues until the crochets release from the substrate, initiating the swing phase. The muscles do not shorten significantly during this activity, on average just returning to their approximate rest length. (An exception to this occurs during the proleg withdrawal reflex, Belanger et al. 2000) The activity is generally sufficient to withdraw the planta slightly into the proleg (e.g., Fig. 2C, D). The EMG activity ends just prior to contact with the substrate, presumably to allow the planta to be in a position for the crochets to engage the substrate.



Fig. 4 A EMG activity of PPRM and associated proleg movements during six steps by an animal crawling on a wooden dowel. The video image shows the side view of the animal, along with the ventral view reflected in a mirror below the dowel. (Note that the video information partially obscures the ventral view.) Superimposed on this are lines joining the origin and insertion of PPRM in each video frame as the animal crawled. Note the lengthening just prior to, and the slight rotation of the proleg during, each swing phase. The *row of circles along the bottom of the image* indicates the movement of the proleg tip in the horizontal plane (adduction/abduction), as measured in the reflected ventral view over the same steps. The EMG trace below the image shows PPRM activity over the same steps. The *light gray lines* join equivalent time points on the EMG trace with kinematic data in the image. Markings on the dowel indicate centimeters. B Examples of two PPRM bursts (from different animals) at a fast sweep speed. Scale bars for EMG traces: A 1 mV, 2 s; B 0.5 mV, 0.2 s

An obvious shortcoming of our data is that most of the kinematics data represent measurements made in the vertical plane. However, this appears to be the main component of proleg movements during crawling, at least on the substrates we examined. In most cases, the lateral excursion – abduction – of the proleg tip during a step was less than 0.1 mm, the spatial resolution of our data (Fig. 5A). To further minimize the possibility of introduced errors, any steps which showed significant abduction were not included in this study.

Because PPRM is innervated by only one motoneuron, it may have a straightforward control system, and so we attempted to determine if any basic activity measures were predictive of changes in muscle length. Thus, we looked for correlations between the duration of muscle activity, the number of muscle spikes (which equals the

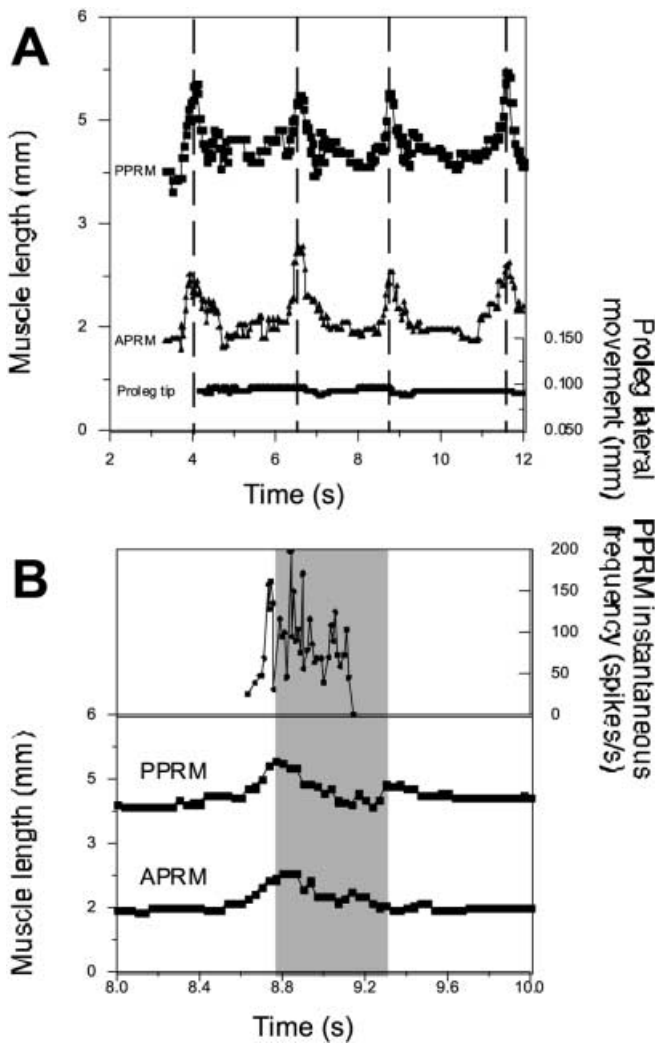


Fig. 5 **A** Changes in muscle length (as measured by the distance between muscle origin and insertion) for PPRM and APRM as a caterpillar crawled on the 4-mm dowel. The lateral movement of the proleg tip, indicating abduction and adduction, is also shown. **B** Single step from **A** at an enlarged time scale to show the relationship between PPRM activity and muscle length. The *gray box* indicates the duration of swing for this step

number of action potentials in PPR), the maximum and average frequency of firing and the resulting muscle movements (Fig. 6). The only significant correlations were between the duration of PPRM activity and the duration or magnitude of swing phase for a particular proleg. There was a statistically significant correlation between the average spike frequency of PPRM and the degree of muscle contraction (Fig. 6G), but since the coefficient of determination was only 16%, and the relationship was in the direction opposite to what one would expect, we did not consider it biologically meaningful.

Timing of proleg movements

Johnston and Levine (1996b) have convincingly demonstrated the existence of a central pattern generator

(CPG) which can produce the basic elements of the crawling pattern in *Manduca* caterpillars. However, their measures of motor output, recordings from nerve roots, did not allow a characterization of the activity of individual muscles. Given the strongly abdominal nature of crawling behavior, it seemed worthwhile to determine if the activity of the proleg muscles was tightly regulated by this CPG, or was instead driven by sensory inputs associated with stepping, such as the stretch of the proleg prior to swing or loading of the proleg at the onset of stance. Accordingly, we performed regression analyses of the activity of the muscles against the cycle period (Fig. 7). The duration of activity of both muscles was strongly dependent on the cycle period, with variation of the cycle period for crawling accounting for almost 80% of the variability in muscle burst duration. Thus, both muscles are tightly coupled to the phase of crawling activity.

Behavioral variability

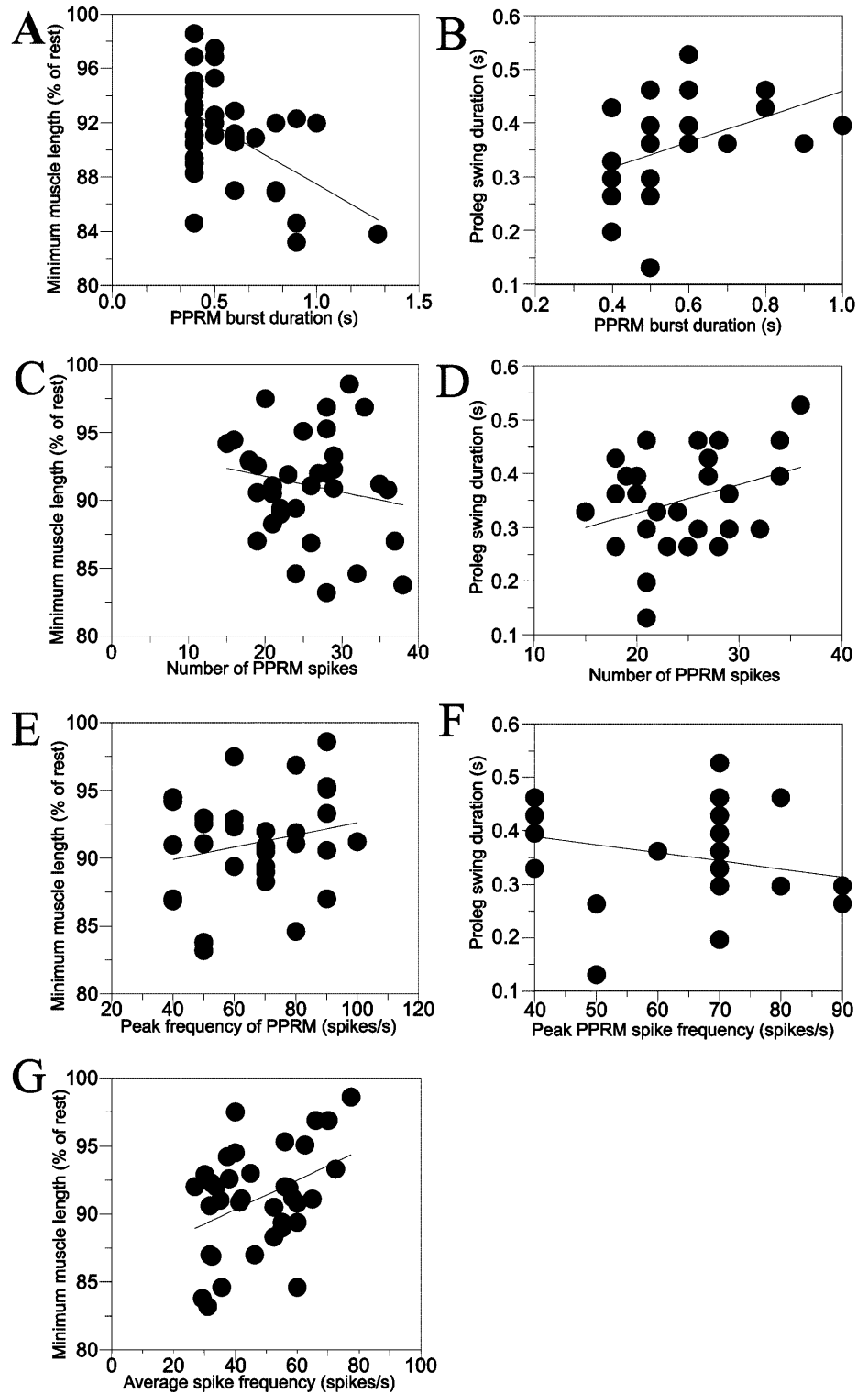
How much variability is there in the normal functioning of the prolegs? To answer this question, we videotaped caterpillars crawling on substrates of different curvatures, and measured the stepping patterns of the prolegs and the associated muscle activity. The three dowel sizes (4 mm, 8 mm, and 12 mm) approximated the range of substrates that animals would encounter on plants in the field.

While the basic stepping pattern was the same across the dowel sizes, there were significant effects of substrate size on some aspects (Fig. 3). Because there were no significant differences between the behavior on the 8-mm and 12-mm dowels, we only present results for the 4-mm and 12-mm dowels here (Fig. 8). The cycle period was significantly longer on the small dowel (3.19 ± 1.60 s versus 2.14 ± 1.52 s, $P < 0.01$), and the absolute onset time of steps by individual legs within a cycle was increased. In contrast, the relative phase of onset was unchanged by variation in the substrate, but the relative duration of swing phase was increased on the small dowel. For example, the absolute onset time for swing by the proleg on segment 5 (A5) was 1.37 ± 0.46 s on the small dowel, and 0.90 ± 0.38 s on the large dowel ($P < 0.05$). The relative onset times were 0.43 ± 0.1 s on the small dowel, and 0.42 ± 0.1 s on the large one, but the relative durations were 0.23 ± 0.1 s and 0.32 ± 0.1 s ($P < 0.001$). Interestingly, the lengthening of swing phase on the small dowel was not accompanied by a concomitant change in the activity of the retractor muscles. Indeed, when the values were normalized to account for the differences in cycle period, the average activity patterns of PPRM and APRM were identical on the large and small dowels.

Discussion

We had two major aims in performing this study. First, what are the basic patterns of activity of the planta

Fig. 6A–G Correlations between PPRM burst properties and the resulting proleg movements. Each of the correlations is based on the data used to generate Fig. 7A. **A** $r^2=0.25$, $\beta = -8.71$, $P=0.002$. **B** $r^2=0.19$, $\beta=0.24$, $P=0.02$. **C** $P=0.28$. **D** $r^2=0.11$, $\beta=0.005$, $P=0.09$. **E** $P=0.22$. **F** $P=0.14$. **G** $r^2=0.16$, $\beta=0.11$, $P=0.02$



retractor muscles during normal behavior? Second, how are these basic patterns of activity modified by the animal to meet the demands of a variable environment? Given the extensive use of these particular muscles as models for a number of aspects of neurobiology, these data are essential to understanding the functional significance of developmental changes, and of physio-

logical responses measured in reduced preparations. In particular, studies of adaptive plasticity of proleg reflexes (Trimmer and Weeks 1991; Wiel and Weeks 1996; Wood et al. 1997) require estimates of the normal variability of muscle activity, to determine the behavioral significance of changes superimposed upon this normal activity.

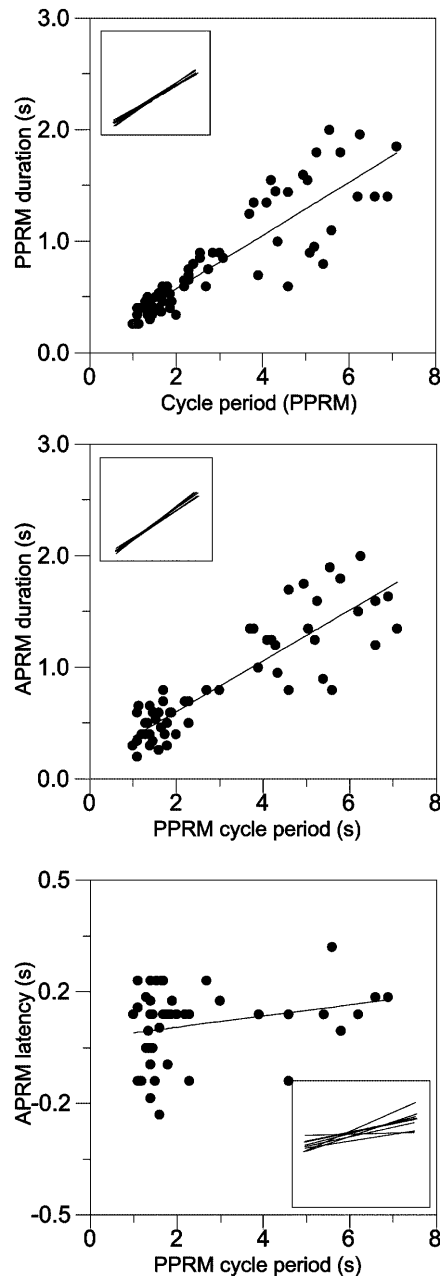


Fig. 7 Regression analyses for activity duration (A, B) and latency to activation (C) versus cycle period of the activity of PPRM and APRM in animals crawling on the 4-mm wooden dowel. Each plot contains 64 data points, representing 8 steps from each of 8 animals. Coincident points are superimposed. The insets show individual regressions for each animal. The coefficient of determination, r^2 , describes the proportion of the variability in the dependent variable which is attributable to variation in the independent variable. The slope of the regression line, β , describes the apparent functional relationship between the two variables. A $r^2=0.78$, $\beta=0.24$, $P<0.0001$. B $r^2=0.77$, $\beta=0.24$, $P=0.008$. C $r^2=0.06$, $\beta=0.02$, $P=0.09$

Our basic finding is that the use of the planta retractor muscles during crawling by *Manduca* caterpillars is highly stereotyped. The planta retractor muscles are not particularly involved in retracting the prolegs during stepping. Instead, their major role is that of disengaging

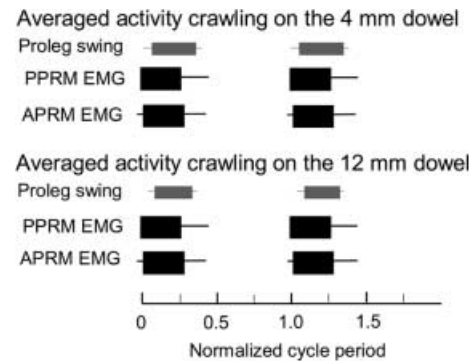


Fig. 8 Averaged muscle activity patterns and proleg movements for animals crawling on two different dowel sizes. Gray bars show the duration of proleg swing, while the black bars show the duration of muscle activity. Leading thin lines indicate standard deviation of onset, length of bars indicates mean duration, and trailing thin lines indicate standard deviation of duration

the crochets from the substrate to allow the proleg to be carried along by abdominal movements. This role has been suggested before (Weeks and Truman 1984), but earlier studies did not combine kinematic and EMG data to demonstrate it positively. The fact that the proleg is actually stretched by abdominal movements until the planta retractors can be activated shows quite clearly this role for the retractors. In this context, it is interesting to note that caterpillars and cockroaches have evolved opposing strategies for controlling their distal “claws”. Cockroaches activate the retractor unguis muscle to flex their tarsal claws and engage them with the substrate at the onset of stance (Frazier et al. 1999), while disengagement is driven by the recoil of elastic elements in the tarsus when the muscle relaxes at the onset of swing. Conversely, *Manduca* uses muscle activation at the onset of swing to disengage the crochets, while the muscle relaxation prior to stance allows the crochets to engage the substrate. Although speculative, it is likely that these differing control strategies reflect the differing lifestyles of the two animals: The “default” position of crochet attachment for caterpillars is probably adaptive in that it makes it less likely for the animal to be dislodged from the substrate, which is most often also its food supply. The rapid running of cockroaches, on the other hand, places a premium on speedy mechanisms for foot placement.

Relationships between EMG and proleg movement

The lack of a significant correlation between any simple measures of motoneuronal output and muscle contraction (Fig. 6) is probably not surprising in this system. Because there are no extensor muscles in the proleg, the functional antagonist for the planta retractors is hydrostatic pressure. We did not measure the internal pressure in the proleg during stepping, so the retractor muscles are operating against an unknown (to us) force. Having said that, Watson and Ritzmann (1998) found in

running cockroaches a linear relationship between mean motoneuron activity and average joint velocity, but no clear relationship between the properties of a specific motor burst and the resulting joint velocity. They speculate that a large part of the variability in individual burst properties represents cycle-by-cycle adjustments of activity in the face of sensory inputs. An alternative hypothesis for the caterpillar is that the retractor muscles are simply a "low-tolerance" system, and that a tight coupling between motoneuronal output and muscle movement is not required. It is clear from the experiments using different substrates that identical retractor motor patterns can underlie quite different stepping patterns. This may represent an efficient design for the animal – what could be expensive calculations of muscle activity to accommodate substrate variation have been "off-loaded" onto the compliance of the hydrostatic skeleton. As long as the coupling of the disengagement and re-enabling of the crochets during swing is properly maintained (witness the tight correlation between retractor activity and cycle period), the fine details of retractor activity don't matter. Why do it this way? Casey (1989) has shown that caterpillars are energetically very inefficient during locomotion, and so any mechanism to minimize energy consumption would be useful. Furthermore, Full and Koditschek (1999) have recently advanced general hypotheses of the control of locomotion for legged animals. They argue that, in many legged organisms, variable speed locomotion is dominated by central (CNS) control, whereas fast, rhythmic locomotion is more a product of biomechanical effects (muscle stiffness, tendon elasticity, etc) operating against the environment. While they did not consider animals with hydrostatic skeletons, the apparent control system for the prolegs is in agreement with their hypothesis that control strategies can be simplified by "building them into" the morphology of the system. It also suggests that adaptive reasons other than processing speed can determine whether the emphasis is on central or peripheral control.

A second reason for the simplicity of the proleg control system is suggested by the observation of an anonymous reviewer that caterpillars are more like "worms with paddles", than like typical legged animals. It would be particularly interesting to compare caterpillars with onychophorans, which have a similar morphology and locomotion, but are in a different phylum (Barnes 1980). This would begin to answer the question asked in the introduction, "does the lack of an articulated skeleton require different control principles?"

Changes to accommodate substrate variation

Our findings of variation in the stepping pattern of caterpillars to accommodate changes in substrate are similar to those of earlier workers (von Holst 1943; Spirito and Mushrush 1979; Johnston and Levine 1996a). The basic effect is an increase in the frequency of

stepping on surfaces having a large radius of curvature relative to the animal. The fact that the swing phase is increased in animals crawling on a more stick-like substrate can be attributed directly to the shorter distance the proleg has to move before it reencounters the substrate. That the muscle activation patterns in the different conditions are identical highlights the role that the planta retractors are playing – disengaging the planta for swing phase.

Insights into circuitry from proleg movements

Our results on stepping patterns offer some useful insights into the circuitry underlying interlimb coordination. Most notably, the apparently significant transitions during stepping – onset of stance in A8 occurring with the onset of swing in A5, and the onset of stance in A6 with the onset of swing in A3 – can both be viewed as a "stance" signal which is sent forward three ganglia. Thus there are likely to be interneurons spanning this distance which are part of the CPG. The onset of activity in the planta retractor muscles coinciding with the stretch of those same muscles is probably too fast to represent a reflex activation of the muscles, and so most likely represents a common synaptic drive to the longitudinal muscle motoneurons and the retractor motoneurons. The fact that the crawling CPG can be activated in isolated chains of ganglia by the application of pilocarpine (Johnston and Levine 1996b) makes these attractive hypotheses to test.

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