

Neuromechanical control of locomotion in larval *Manduca sexta*

An honors thesis for the Department of Biology

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Table of Contents

Abstract	iii
I. Why study soft bodied locomotion?.....	1
Overview.....	1
Figure 1	2
II. Omni-directional robustness of locomotion	3
Introduction	3
Materials and methods.....	4
Results.....	8
Figure 1	8
Figure 2	9
Figure 3	9
Figure 4	10
Figure 5	12
Discussion	12
Figure 6	15
Figure 7	17
Table 1.....	19
Figure 8	19
III. Kinematics of proleg withdrawal.....	20
Introduction	20
Figure 1	21
Materials and methods.....	22
Figure 2	23
Figure 3	27
Results.....	27
Figure 4	28
Figure 5	29
Figure 6	29
Figure 7	30
Figure 8	31
Discussion	31
IV. Summary and conclusions	35
V. Index of terms.....	36
VI. References	37

Abstract

Larval *Manduca sexta*, the tobacco hornworm, are remarkable climbers and crawlers. They have evolved to navigate up, down, and across branched and highly variable terrain: tobacco plants. In order to maintain motion, they must have one or more motor strategies, but they must be able to move in virtually limitless orientations. They are also interesting because *Manduca sexta* are completely soft and lack any rigid support structure. This research explored how larval *Manduca sexta* move in vertical and horizontal orientations. A finding of this research was that the proleg gripping system is used to provide a passive resistance to pitch back. Kinematic study of the proleg withdrawal mechanism was then used to determine how robust release is accomplished. This study found that these animals are able to move the gripping hooks on their feet inward, presumably to release tension on the hooks before withdrawal.

I. Why study soft-bodied locomotion?

Overview

Terrestrial locomotion is a complex and interesting field of study. Organisms have found ways to navigate extremely difficult terrain with little to no error, something very desirable for engineers faced with rough terrain. Previous research has focused on organisms with stiff, articulated skeletons (Goldman et al., 2006; Zaaf et al., 2001; Alexander and Jayes, 1980). Recently, more work has been done to understand the mechanics of soft-bodied locomotion, as the virtually infinite degrees of freedom in these animals provides an attractive model for new robots which would be more robust to damage and to varied terrain when compared with current rigid bodied robots (Laschi et al., 2005). Soft animals are generally categorized as either muscular hydrostats or as simple hydrostatic skeletons. In muscular hydrostats limbs are mostly muscle, such as the octopus arm or elephant trunk (Kier and Smith, 1985; Laschi et al., 2005). In simple hydrostatic skeletons, like the leech and earthworm, the body is a fluid filled cavity surrounded by muscle, and pressure changes are used to move the animal (Kier, 2012; Quillin, 1998; Skierczynski et al., 1996). Larval *Manduca sexta* does not fall neatly into either of these categories. The hornworm consists of a fluid filled cavity surrounded by muscle, but it does not maintain constant volume or internal pressure like a classic hydrostat (Lin and Trimmer, 2010). Another differentiating factor between *Manduca sexta* and most hydrostats are the five articulated proleg pairs that grip the substrate during locomotion. The proleg pairs are remarkable for their passive gripping ability; only one proleg is required to hold the body on a substrate, and with this hold the animal can right itself and fully attach to its substrate. Furthermore, these grippers are entirely passive, and yet during normal crawling they are consistently and robustly withdrawn to ensure the animal moves forward without any hindrance.

Understanding how *Manduca sexta* is able to control locomotion will provide excellent data for the design and construction of robust, mobile, and soft robots, as well as to help understand how a relatively simple neural system produces complex motion. The likely answer is that a large part of the computational control is “offloaded” into the material and morphological properties of the animal itself.

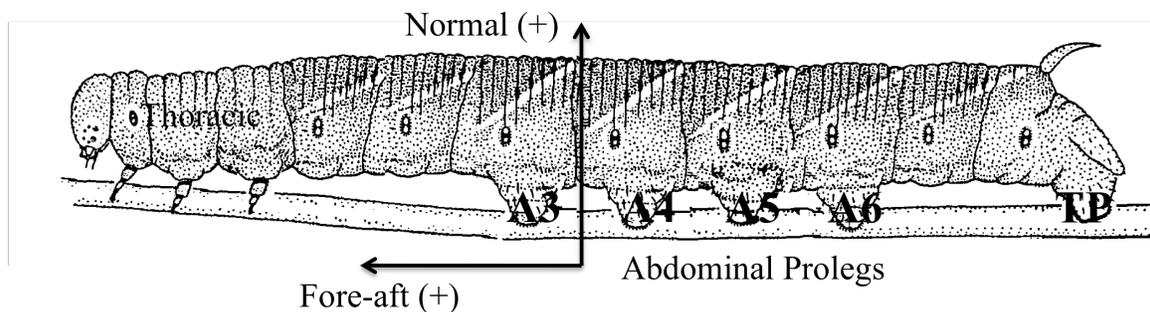


Figure 1: *Manduca sexta*. Important aspects of the animal are labeled. A3-A6 refer to abdominal segments A3-A6, however in this paper the terms A3-A6 will refer to the proleg pairs. Animal is symmetric about the midline, so only one view is needed.

II. Omni-directional robustness of mechanics

Introduction:

To maintain forward progression, terrestrial animals must balance propulsive, gravitational and lateral forces. This is challenging in natural environments because traction is often unpredictable and obstacles interfere with smooth limb trajectories. Most limbed animals can easily maneuver in such environments by continuously adjusting limb movements on a cycle-by-cycle basis and exploiting the passive dynamics of their limbs and body. To cope with a wide range of speeds, most animals adopt different gaits: changes in the rate and patterning of limb movements that help to match mechanical demands to optimal muscle and skeletal performance.

These strategies work well for horizontal crawling, walking, and running in which the gravitational forces are perpendicular to the direction of travel and body mass helps to provide traction through frictional interactions. Even small inclines pose no major mechanical limits for legged locomotion. However, when faced with a steeply sloping surfaces or a vertical wall most animals must adopt a different tactic to climb effectively; bipeds use their arms, fast quadrupeds such as cats use distinctive climbing kinematics. Some animals (*e.g.*, geckos, *Gekko gecko* and *Eublepharis macularius* and cockroaches *Blaberus discoidalis*) are remarkable in being able to employ similar kinematics to move across level ground, climb straight up, or even to run upside down (Goldman et al., 2006; Zaaf et al., 2001). Although the movements are similar in each orientation, the substrate reaction forces are quite different and depend on the gripping and release mechanisms of the feet. These substrate forces (also called ground reaction forces, GRFs) are important for understanding locomotive strategies by individual animals and across different taxa (Alexander, 1992; Alexander and Jayes, 1980; Full and Tu, 1990). For example a parameter in the Fourier series, (the shape factor, which describes the time spent in the air compared with in

contact with the ground, as well as how quickly the animal is moving), can be used to describe the changing profile of bipedal GRFs between walking and running (Alexander, 1992). One can also describe gait transition in terms of energy using Froude number (Alexander, 1976).

However, all current mechanical descriptions of gait transitions between horizontal locomotion and climbing have been based on animals with articulated skeletons, it is not known how soft animals make these transitions. This is of interest for several important reasons. First, although caterpillars can use the same kinematics for vertical climb and horizontal crawl (van Griethuijsen, 2009) these animals are soft and mostly keep their body in tension (Lin and Trimmer, 2010; Lin and Trimmer, 2010) so it is not clear whether force transmission remains identical in different orientations. Second, there is a growing need to understand the mechanisms by which soft animals control their movements in different contexts; these strategies can be exploited for the development of novel moving devices and controls systems (Trimmer, 2008; Saunders, 2011; Laschi, 2009; Lin, 2011).

Caterpillars are particularly useful for carrying out these studies because they crawl and climb using discrete contact points (thoracic legs and abdominal prolegs) and the GRFs at each of these points can be measured using a custom device with multiple sensors capable of recording both axial (direction of locomotion) and normal forces simultaneously.

Materials and methods:

Animals

Second to fifth day 5th instar animals were taken from our colony of *Manduca sexta* raised on a 17:7 light dark cycle at 27°C. Rearing was done based on the protocol described by Bell and Joachim (Bell and Joachim, 1976).

Ground reaction force studies

For all studies second to fifth day 5th instar animals were taken from the colony, weighed and placed into a clear container filled with a variety of wooden substrates on which they crawled spontaneously (Lin and Trimmer 2010). The most active animals were used for experiments.

For studies of thrust and drag during horizontal crawling (fGRF-horizontal), animals weighing between 1.43g-2.96 g were placed on a beam with a single embedded fore-aft force transducer (Harvard Apparatus, Model 60-2995). Animals (n= 7) were observed while crawling along this beam and the timing of each leg contact with the sensor was manually noted into the force data file. Signals were recorded at 100 Hz, processed using a low pass filter at 30 Hz, and extracted from this data by matching continuous deviations from zero force to each proleg pair contact. 30 traces were collected for each proleg pair. Recordings of GRFs normal to the direction of travel (nGRF-horizontal) were made by allowing animals (n=4, weight between 1.6 and 2.5g) to crawl along an array of cantilever beams and strain gauges configured to be sensitive in a single direction (Lin and Trimmer 2010).

For studies of vertical crawling, a custom built *Manduca* force beam array capable of measuring both fore-aft (fGRF-vertical) and normal forces (nGRF-vertical) was mounted to a camera tripod so that its orientation could be easily adjusted, and bubble levels were attached to ensure a perfect vertical orientation. The animals (n=6), weighing between 1.67 and 2.82g were placed on the beam and forces during the climb were recorded at 100 Hz synchronized with a simultaneous video recording of the leg contacts on the array (Lin and Trimmer, 2010). Signals were processed using Matlab 2010b's (Mathworks) smooth function with parameters 'rlowess' and window=30 divided by recording length. This smoothed the data using a lowess filter, and it

removed noise introduced by nearby electrical equipment. Drift was generally linear and could be compensated using averages covering one second at the beginning and end of the recording. For two sets of data drift was clearly non-linear and was corrected by subtracting a strongly filtered signal (rlowess using a window of 1000/recording length) from the original signal. The ground reaction forces for each proleg pair was identified by looking at the continuous recordings for force exerted at each beam and matching deviations from zero to proleg contacts observed in simultaneously recorded video data. The magnitude of forces observed was normalized to percent of animal bodyweight and percent step length in the y and x directions respectively.

To determine the contribution of both single leg pairs and the abdomen as a whole to the overall step cycle, data was averaged using Matlab 2010b (Mathworks). The data were normalized to a percentage of time in contact with the ground by dividing the observed force by the total animal weight and using a spline function to fit each trace to windows of 100 consecutive data points (each representing 1/100th of a step). The thrust as a percentage of bodyweight for each proleg pair contact was calculated by summing all positive values and all negative values separately. Total normal forces from each contact pair were taken together as one average value.

Predicting the fGRF-horizontal from fGRF-vertical data

If *Manduca* uses the same strategy to crawl and climb it is expected that the fore-aft climbing forces will have the same profile as those seen during horizontal crawling plus the load created by the animal's weight. Based on this hypothesis the fGRF-horizontal was predicted by

subtracting the mean nGRF-horizontal from the mean fGRF-vertical data and comparing this to empirical fGRF-horizontal data.

Behavioral observations

Healthy third day 5th instar animals were recorded using color digital video while moving on a single hanging strand of suture and on three suture stands braided together. To prevent an animal from using its thoracic legs, a fourth day 5th instar animal was sedated and its thoracic legs were bonded together using cyanoacrylate glue (Gorilla brand). It was allowed to recover for 3 hours, and then placed onto a rigid vertical substrate where its behavior was observed.

Animals were debrained by removing their brain with forceps through a hole drilled into the head capsule as outlined by Dominick and Truman (1986). Modifications were made as described in Trimmer and Issberner (2007). Animals were anesthetized on ice, placed in a custom built clamp that isolated the head above a test tube, while allowing the body to hang down into an ice-water bath. A small hole was drilled into the front of the frons, and the brain was excised using forceps. Surgical injuries were sealed with Gorilla brand cyanoacrylate glue. Animals were allowed to recover overnight and their behavior was observed ~40 hours postoperatively. Each debrained animal was then dissected posthumously to ensure that the brain had been removed. Prior to each debraining surgery, a sham surgery was conducted where a hole was drilled, but no excision performed. This served as a paired control for each animal.

Results:

Proleg-specific ground reaction forces

Ground reaction forces during *Manduca* crawling were found to be highly variable with standard deviations almost equal in size to the average values. The general shape of each proleg pair GRF trace was consistent throughout trials in the same orientation. During vertical crawling, axial (fore-aft) ground reaction forces show much higher positive values than those observed during horizontal crawling, almost never exerting drag force (figures 1, 2). The individual contributions of each leg also maintained a similar trend when comparing the proportion of thrust generated by each leg, specifically greater thrust was contributed by A3 and A4, and less thrust/greater drag from A5, A6 and the TP.

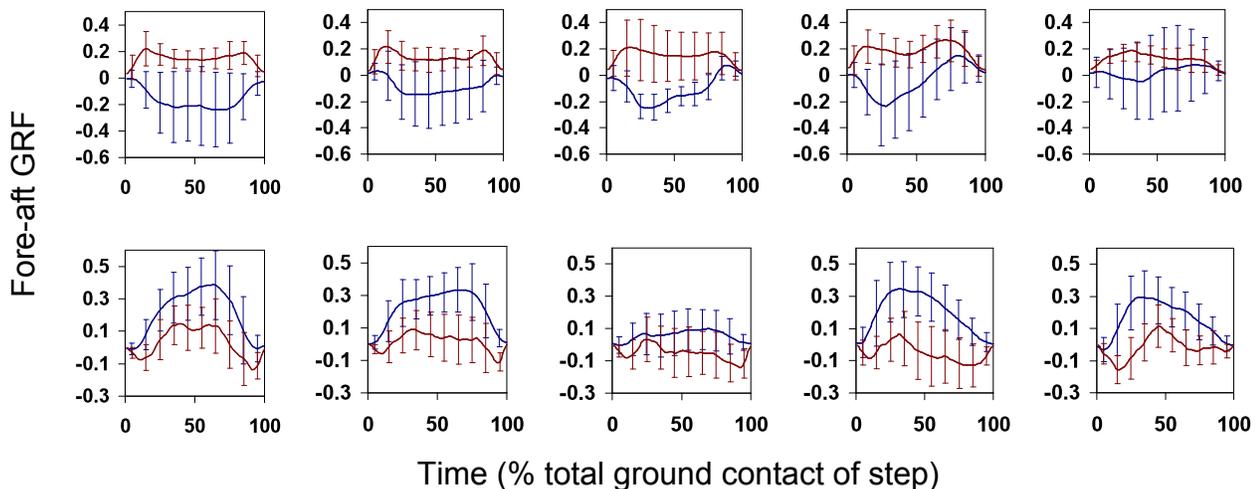


Figure 1: Normalized ground reaction force traces for each pair of prolegs from *Manduca sexta* crawling and climbing. Top plots represent normal forces, bottom plots represent axial forces. Each red line is the mean force during horizontal crawls, blue lines represent vertical crawling. Error bars represent standard deviation and are drawn at intervals of 10% from 5% crawl to 95% crawl. Each mean trace is derived from 30 traces gathered from 6 animals climbing vertically, and 7 animals moving horizontally.

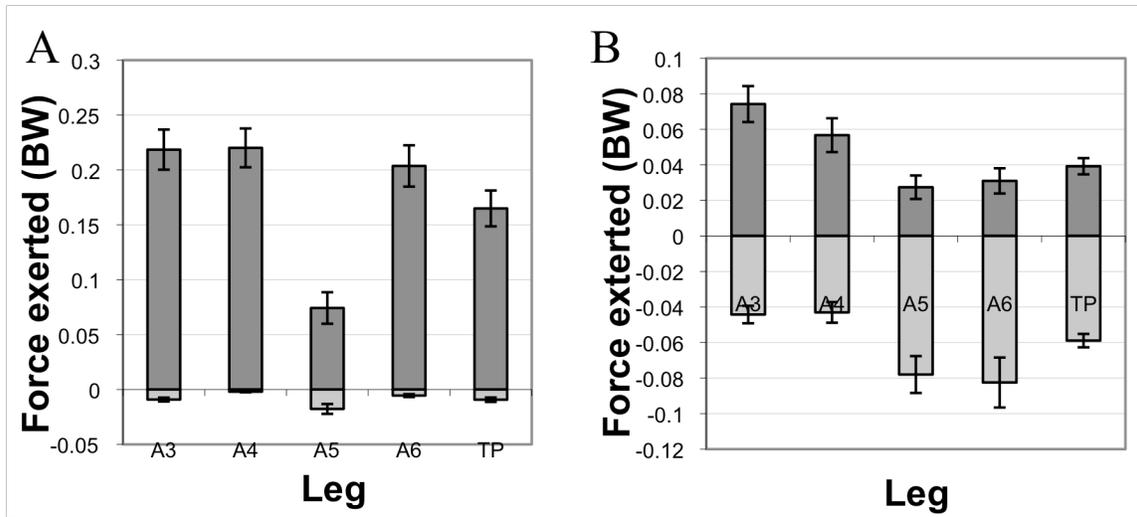


Figure 2: The distribution of proleg-specific fGRFs during crawling and climbing. The force profiles for each proleg were integrated by summing all thrust (positive values) and drag (negative values) during the entire stance. Mean values (\pm SE) were calculated by taking the mean ground reaction force of 30 single leg recordings taken from 7 animals in horizontal, and 6 animals in vertical crawl. Data is reported as a percentage of the total body weight exerted throughout the contact time. (A) Horizontal crawl: average net force during one crawl cycle is -7.8% of bodyweight. (B) Vertical crawl: in one crawl cycle, average net force on all abdominal prolegs is 83.8% of bodyweight.

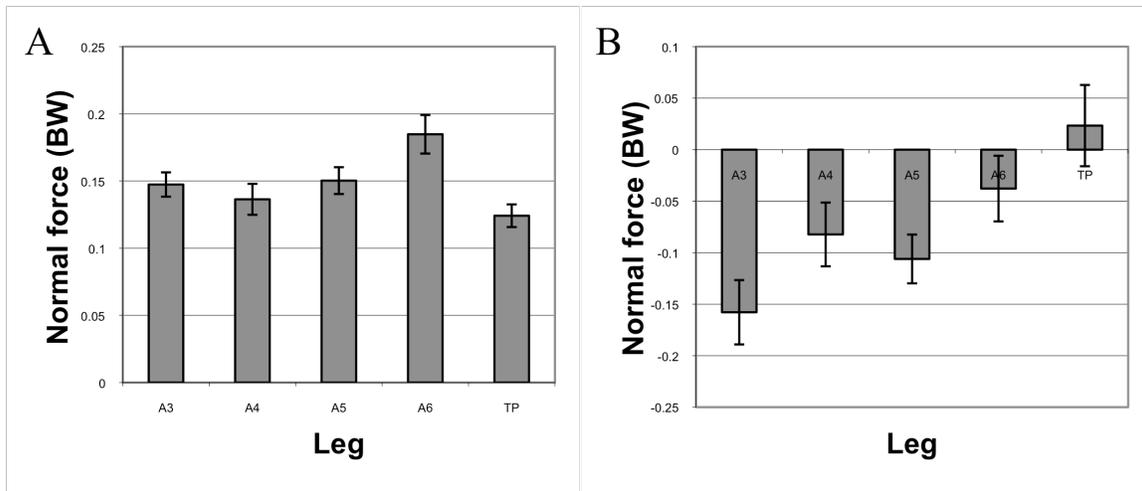


Figure 3: Average normal forces observed in climbing and crawling animals, for each proleg pair, $n=30$. Error bars represent standard error. A) horizontal crawl, B) vertical crawl. Standard error of close to 2 times the average value in vertical crawl shows the extreme variability in this behavior. General characteristics to note: normal force is always positive in horizontal crawling, and in vertical crawling shifts from more negative to less negative to positive along the body toward the posterior.

Subtracting horizontal crawl normal forces from axial ground reaction forces in the vertical direction results in a calculated fore-aft ground reaction force for horizontal crawling that assume vertical crawling is mechanically equivalent to normal crawling with bodyweight shifted from the normal direction to the fore-aft direction (Equation 1).

$$fGRF_{Horizontal} = fGRF_{Vertical} - nGRF_{Horizontal} \quad (1)$$

Plots of this calculated force have been overlaid with average fGRF plots; each calculated plot falls largely within the standard deviations. In the cases where calculated traces fall outside the standard deviations, they do so in the stance phase (figure 4, Bottom). Additionally, the calculated plots fall within the observed range of natural variation (figure 4, top).

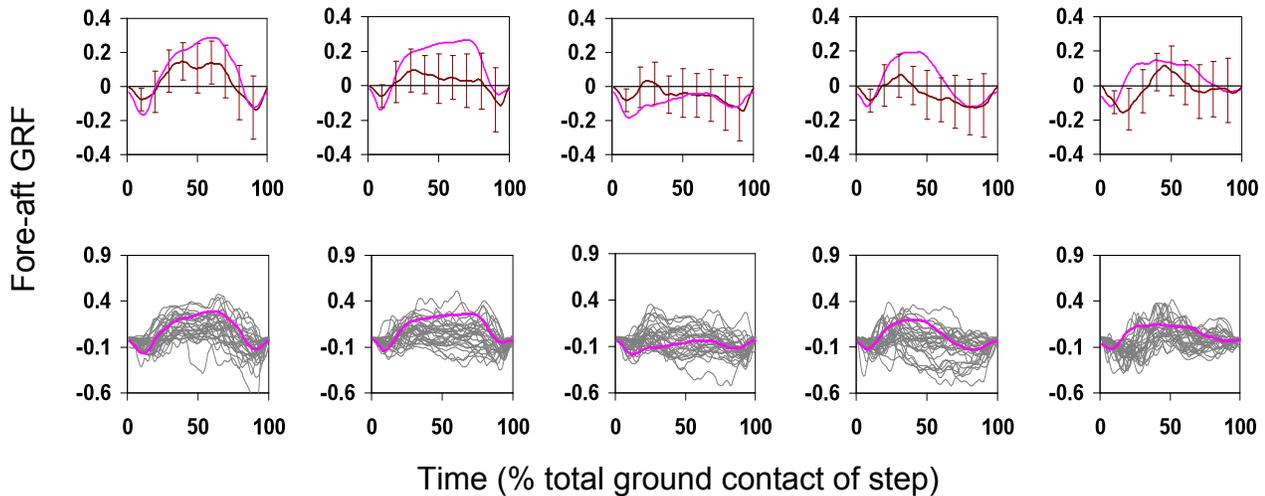


Figure 4: (Top) Predicted and average fore-aft ground reaction forces during horizontal crawling. Pink line is the force trace (n=30) calculated using Predicted $fGRF_{horizontal} = fGRF_{vertical} - nGRF_{horizontal}$. Red lines show actual average force with standard deviation (error bars). (Bottom) Predicted values for GRF traces plotted over 30 actual traces (from n=7 animals). The predicted traces fall with the range of the natural variation in forces, but they are generally higher than the mean.

Experiments on crawling and climbing have shown that the animals move quasi-statically, and there are no detectable (within the sensitivity of the equipment) inertial forces.

Therefore, net ground reaction forces equal to 0% bodyweight during crawling, and 100% bodyweight during climbing. This leads us to sum the forces in the fore-aft direction in both horizontal and vertical crawling (Equations 2, 3).

$$\text{Horizontal:} \quad F_{\text{fore-aft, abdominal}} + F_{\text{fore-aft, thoracic}} = 0 \% \text{ BW} \quad (2)$$

$$\text{Vertical:} \quad F_{\text{fore-aft, abdominal}} + F_{\text{fore-aft, thoracic}} = 100 \% \text{ BW} \quad (3)$$

During horizontal crawling the average total force produced by the prolegs consists of a drag equal to 7.8% of the bodyweight. During climbing, the average total force generated by the prolegs is 83.8% of the bodyweight (figure 1,2). Applying equations 2 and 3 yields a net thrust of 7.8% BW in horizontal crawling, and a net thrust of 16.2% BW in climbing.

Negative normal forces observed during climbing are highly variable. A trend of more negative force in the more anterior segments exists, however the forces are highly variable in both shape (figure 1) and total force exertion (figure 3).

Combining all forces into a single quasi-static free-body diagram for climbing and crawling caterpillars yields a balanced free body diagram in horizontal crawling (figure 5A). In vertical crawling, the resultant balanced forces imply a moment that would cause the animal to pitch back (figure 5B). This is not observed *in vivo*, and therefore the averaged data in vertical crawling does not fully describe vertical crawling.

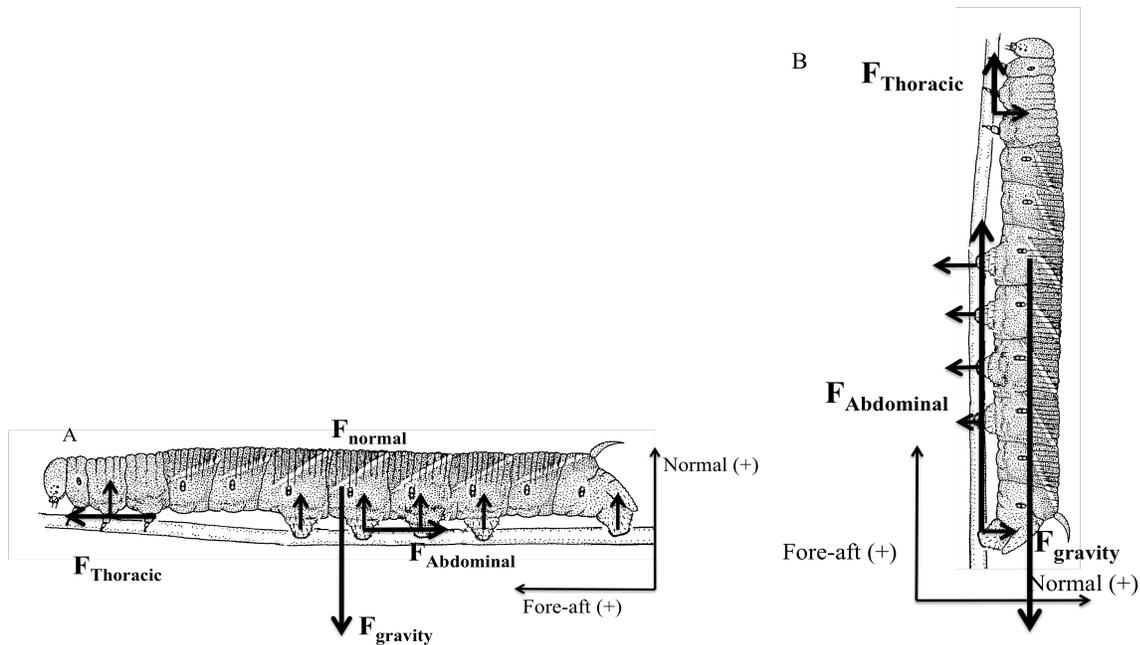


Figure 5: Free body diagrams describing the average forces on *Manduca sexta* during the average crawl. Axes show positive directions. Thoracic leg forces were calculated assuming quasi-static motion, such that in the horizontal crawl, $F_{\text{net}}=0\%$ body mass and in vertical crawling $F_{\text{net}}=100\%$ body mass. As such, in horizontal crawls, $F_{\text{th, fore-aft}}=0.078\text{BW}$, $F_{\text{th, normal}}=0.743\text{BW}$. In vertical crawls, $F_{\text{th, fore-aft}}=0.167\text{BW}$, $F_{\text{th, normal}}=0.36\text{BW}$. This result balances forces, but also implies a moment between 1 and 5 $\text{BW}\cdot\text{mm}$ of pitch back torque unaccounted for. (Specific values based on a range of body geometries used for calculation).

Discussion:

A general form for horizontal crawls

Recent work on the climbing methods of *Manduca sexta* demonstrated that during horizontal crawling, the animal keeps its body in tension and transmits forces through the substrate to the rest of its body, termed the environmental skeleton theory (Lin and Trimmer 2010). The impact, pivot and drag phase observed in horizontal crawling are consistent with this hypothesis (figure 1). This study looked at a much larger dataset of horizontal crawling, and demonstrated that while the general shapes are maintained, there is a large degree of variability in the shape of each proleg GRF trace (figures 1, 2). In contrast, rigid bodied animals of both similar weights and of much larger weights exert ground reaction forces are highly reproducible

and largely speed dependant (Alexander 1992, Full and Tu, 1990; and Goldman et al. 2006). Given this contrast, the slow movement and highly deformable nature of *Manduca* likely accounts for the observed variability.

Variation in ground reaction forces makes it difficult to define normal horizontal crawling in *Manduca*. While individual leg GRF data is variable, general trends have been found by averaging traces. When crawling horizontally, the abdominal section tends to produce a net drag force. The abdomen is maintained in tension with the rest of the body through the thoracic leg contacts, and thrust is transmitted from the thorax to the abdomen through the substrate. Further experiments focusing on the thoracic legs will be necessary to confirm that they exert the same amount of thrust as the abdomen does drag.

On the difference between horizontal and vertical crawling

Due to their relatively simple nervous system and the natural requirement for navigating up and around highly branched tobacco plants, it makes sense for *Manduca* to use the same neural pattern and gait during both horizontal crawling and vertical climbing. Kinematics studies support this theory because while there is a significant difference in the kinematics of climbing and crawling, the difference is not large enough to differentiate between the gaits of separate animals in the same orientation (van Greithuijsen and Trimmer 2009). Although at first glance, horizontal and vertical GRF data appear very different, the actively generated forces underlying motion are in fact the same, differences arise because of the way horizontally and vertically oriented animals differentially account for torque and gravity.

Calculated traces based on vertical fore-aft data and horizontal normal data are difficult to distinguish from actual horizontal fore-aft traces (figure 2). Since these calculated traces are

based on averages, the fact that they tend to be on the higher side of observed fGRF data is important. When vertical, the abdomen is supporting a larger amount of the body weight compared to the amount it supports in horizontal crawling because the large, open gut cavity allows the gut to slide down to the posterior of the body. Accounting for the addition of bodyweight through equation (1), the abdomen produces similar levels of net drag during horizontal and vertical locomotion. The animal is maintaining similar tension between the thorax and abdomen in both crawling modes.

Analyses of the average vertical crawl show that little to no actual drag force is produced during vertical crawl cycles. Therefore the climbing animal is not tensioning itself by exerting opposing forces on the substrate. Instead, it appears that the weight of the animal itself is enough to provide the necessary tension for the animal to climb. *Manduca* can easily climb highly flexible braids of string without having to curl up large amounts of string beneath them. They appear to only have problems when crawling along this type of substrate (Lin and Trimmer 2010). *Manduca* are clearly maintaining tension in their body while moving, but they seem to accomplish this by allowing gravity to tension their body. No matter their orientation, they follow the same movements, and the strength of their muscles overcomes additional gravitational force in the vertical orientation.



Figure 6: (A) 2nd day 5th instar with its thoracic legs bonded together. The animal is able to climb this rigid substrate only while thoracic legs are pressed against the substrate and generating frictional force during crawl cycle. (B) Animal able to climb a highly flexible substrate without any difficulty. (C) Animal attempting to climb a highly rigid substrate where it is unable to grip the substrate with its thoracic legs. This has caused it to fall down the substrate a small amount during each crawl cycle.

The largest observed difference in the crawls is in the normal forces. Normal forces are (in general) in the opposite direction in climbing caterpillars. This is due to the pitch-back torque on the body from gravity. The center of mass pulls the animal away from the wall, and the passive grip of the prolegs counters this action.

The results also demonstrate the extreme variability in the force distribution during climbing, and thus the robustness of the crawling pattern. Since approximations of torque on the animal yield a net pitch back moment about the center of mass, averaging steps from 30 separate crawl cycles for each “general” trace has introduced error. The variability in the crawl was not sufficiently accounted for with only thirty single leg ground reaction forces per proleg pair. The observed trends are still valid, however exact numbers for amount of force generated are only approximations useful for understanding the motor strategy used by *Manduca*, not predictors of exact forces given a certain crawl. The normal forces in the vertical direction are especially variable, with standard deviations often equal to or larger than the average force generated. However, the trend observed in normal forces of climbing caterpillars, in conjunction with the relatively less variable fGRF measurements demonstrates that the animal does not need to alter

its motor pattern to achieve this opposite direction grip. It is achieved by the properties of the legs instead.

The thoracic legs

Up until this point, the thoracic legs have been generally ignored for locomotion studies. This was done because the animals have been observed crawling relatively normally without the thoracic legs (Lin and Trimmer 2010). Current data on the total force contributions of the abdominal prolegs show that while the thoracic legs are intact, the abdomen does not produce enough force to move the animal forward and on average produces a decelerating effect. This effect is pronounced in both horizontal crawling and climbing, however it seems to be more important in the vertical orientation. Over one full cycle of climbing, the animal on average would fall down the substrate while the thoracic legs fail to grip, and this has been observed (figure 6C). On the other hand, the animal is capable of climbing a highly compressible substrate, probably due to the ability of the animal to use gravity to maintain tension and resist buckling (figure 6B). The animal can produce some thrust and move up a vertical substrate while the legs are hampered (figure 7), but the exact amount of frictional force produced by the animals thoracic segment and mandibles makes it unclear as to whether or not *Manduca* can climb without their thoracic legs. It is possible that when the legs were removed, the mandibles were used as a substitute. One trend that is clear is that kinematics of climbing are changed by the removal of the thoracic legs in the vertical direction; as seen in figure 7, preparations like this show slower paces and only one leg in swing phase at a given time.



Figure 7: Prolegs of an animal with thoracic legs removed. Shown here walking with A4 in swing as it is able to walk up the rigid substrate using only the prolegs. As can be seen from the proximity of A3 and A4, this gait does not resemble normal crawling.

Limited GRF data on the thoracic legs is currently available because the set of experiments presented here has focused on the abdominal prolegs, what is available confirms the calculated GRF (from equation 2) because it is consistently a positive force. The small size in irregularity of thoracic steps makes it difficult to gather single thoracic leg pair data.

Furthermore, some probing behavior has been observed in continuous crawling, which makes it difficult to resolve clean and continuous thoracic fGRF traces. What is known is that total forces in the animals must sum to 0% or 100% of the bodyweight, depending on orientation. The only unknown contact points are the thoracic legs. Because net force is known, we can infer the contacts at the thoracic segment produce 7.8% and 16.2% bodyweight in horizontal and vertical crawling respectively. The thoracic legs are necessary for normal crawl, and they serve a similar purpose in both crawling modes.

Modifications to experimental protocols in order to develop specific GRF on the thoracic legs are currently being developed.

Behavioral observations confirm conservation of motor strategy

Debrained animals are capable of moving in both the horizontal and vertical plane without any apparent difficulty. We observed that vertical movement remains easily accomplished and in fact appears to continue as long as the substrate continues. If the animals were using a different pattern during vertical crawling, they would likely be unable to crawl vertically once the brain was removed. In addition, debraining usually interrupts transition behaviors in insects (Graham 1979; Ridgel and Ritzmann 2005; Ritzmann et al. 2005). This suggests that if there were a difference in the motor patterns required for vertical and horizontal motion, debrained animals would get stuck shifting between orientations. 5 animals were successfully debrained, all 5 were able to accomplish transitions from horizontal to vertical crawling, therefore we can conclude that horizontal and vertical crawling are accomplished through the same motor pattern (table 1, figure 8).

Table 1: Results of debraining experiments. All animals are 2nd day 5th instar, and those that survived were able to continue from a horizontal onto a vertical surface.

Animal number	Ability to transition 90°(healthy)	Ability to transition 90°(sham surgery, 10 hrs postop)	Ability to transition 90°(debrained, 46 hr. postop)
1	Yes	Yes	Yes
2	Yes	Yes	(Animal died during surgery)
3	Yes	Yes	Yes
4	Yes	Yes	Yes
5	Yes	Yes	Yes
6	Yes	Yes	(Animal died during surgery)
7	Yes	Yes	Yes



Figure 8: Debrained animal (46 hr. postop) transitioning from horizontal (not shown) to sideways to vertical orientation. Motion occurs in all three directions (forward, up, and to the right). Each frame represents +6 seconds.

III. Kinematics of proleg withdrawal

Introduction

Grip has been shown to be important to the climbing abilities of a variety of animals. Cockroaches actively dig in small hooks on the ends of their legs while running (Frazier et al. 1990) and geckos utilize a unique gripping system while climbing, exploiting van der Waals forces to climb surfaces (Zaaf et al. 2001). Grip is also an important subject from a practical standpoint, as novel systems found in nature have served as the basis for engineering man-made gripping devices (Kim et al. 2007).

Initial work on ground reaction forces has highlighted the importance of the gripping system in *Manduca sexta*. By exploiting the passive, robust grip of the prolegs, animals are able to use the same mechanics in both climbing and crawling. In climbing, the proleg grip resists pitch back forces generated by the animal's bodyweight; in consequence, the resulting mechanics are the same as those in horizontal crawling (see section II). The grip is also remarkable for its failsafe design. Grip is passive. Activation in the principal planta retractor muscle (PPRM) and accessory planta retractor muscle (APRM) are responsible for the release of the crochets, small hooks that grip the substrate (Belanger and Trimmer, 2000)

The proleg itself is divided into three segments: the subcoxa, the coxa, and the planta (Mezoff et al, 2004). On the boundary of the planta there is a set of fine hairs called the planta hairs, which can be used to stimulate reflexive proleg withdrawal. Along the medial edge of the proleg there is a set of small hard hooks used for Velcro™-like gripping, this grip can be actively turned off through the activity of the two muscles which go to the leg, PPRM and APRM (Belanger and Trimmer, 2000). For close up of the anatomy, see figure 1.

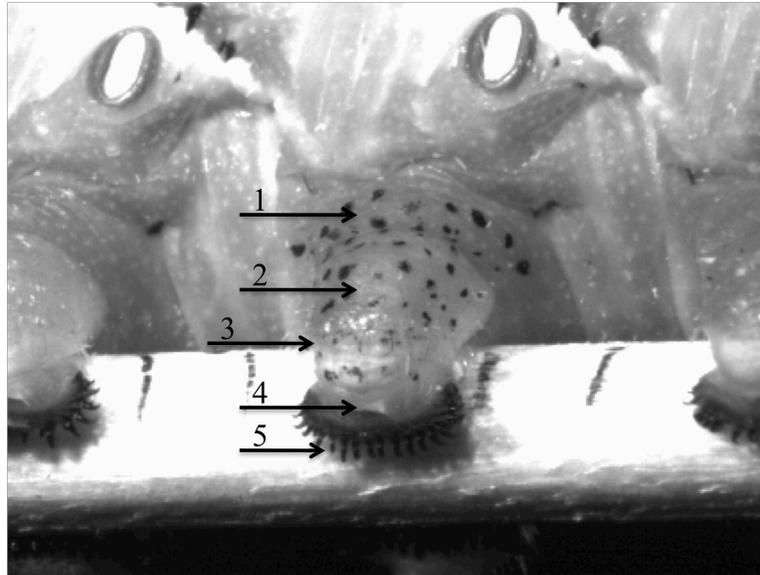


Figure 1: Proleg sections in experimental animal, proleg A4. 1) Subcoxa. 2) Coxa. 3) Planta hairs 4)Planta, arrow highlights insertion of PPRM. 5) Crochet hooks. Black dots are not natural and were added in as per materials and methods. Also visible are legs A5(left), A3(right), and the spiracles (large white dots).

The prolegs are a commonly studied system, as they are a useful model for habituation and sensitization experiments. For this reason, most previous work on proleg withdrawal in *Manduca sexta* focused on the reflexive withdrawal of the proleg (Belanger et al. 2000; Wiel et al. 2001). Some work has been done to characterize the phasing and kinematics of proleg withdrawal in intact, naturally crawling systems (Belanger and Trimmer, 2000; Mezoff et al., 2004), although these studies lacked the resolution to track the exact paths of the specific segments of the proleg. Additionally, due to the folding of the proleg, it is difficult to capture the exact kinematics. This is because points often become obscured in the leg's folding. These experiments characterize the movement at a much higher resolution than previous studies. Through motion tracking, it was found that the crochet hooks move differentially from the rest of the proleg, initially moving medially toward the substrate, which helps crochet hook release.

Materials and Methods

Animals

Healthy 2nd and 3rd day, 5th instar animals were taken from our colony of *Manduca sexta* raised on a 17:7 light dark cycle at 27°C. Rearing was done based on the protocol described by Bell and Joachim (Bell and Joachim, 1976).

Motion capture setup

Motion capture experiments were recorded using Vicon Motus software and four Basler 602f cameras under visible light. These cameras were outfitted with Computar-Ganz 3.3x zoom lenses. Calibration was accomplished using a fifty-two point calibration frame custom built out of acrylic. This frame was built by aligning a 1.5mm thick piece of acrylic 3cm x 4cm, with a 3cm x 4cm template. The template was built with 13 holes spaced out such that they evenly covered the field of view (Figure 2). After aligning the template and the acrylic using alignment pins in the corners, small dots were made on the acrylic by sticking a pin through the holes in the template. This made small holes in the face of the acrylic. The holes were then filled with ink. The procedure was repeated four times resulting in four identical pieces of acrylic with all 13 points marked. The depth of the acrylic offset each set of points, allowing for variation of the calibration frame in the z-direction.



Figure 2: Calibration frame. Dimensions show sizes. Frame constructed from four pieces of acrylic approximately 1.2 mm thick each. Dots are spaced on each piece at (0,0), (2,0), (4,0), (0,2), (2,2), (4,2), (0,4), (2,4), (4,4), (1,1), (1,3), (3,1), (3,3) (All measurements in millimeters). There are four dots deep. Each dot was made with a pinprick filled with ink. All acrylic was aligned using the pins in the four corners.

Cameras were focused on a single wooden dowel 2.1 mm in diameter. The dowel was positioned on a stand in a low friction track in order to slide it in and out of the same position without shaking the cameras. Calibration was done with a single image of the calibration frame taken immediately before or after the crawl was recorded.

In order to track detailed motion, prolegs were marked using between 35 and 62 small ink dots from a Staedtler 0.1 mm drawing pen. Animals were sedated on ice and then restrained over a cold block with A4 exposed. A4 was then marked by hand under a dissection scope. In order to improve the permanence of the ink on the proleg, the leg was cleaned using acetone prior to marking. This removed the waxy layer on the cuticle and significantly improved marker permanence. To further improve permanence, legs were dried with compressed air immediately after marking. The exact number and location of dots was random (see figure1), however no animal was tracked without at least three locations at the base of the crochet hooks, three

locations on the subcoxa, and three locations on the very tip of the coxa. After animals had been marked, they were given at least one hour to recover from the procedure before a trial was recorded.

Trial procedure

Proleg withdrawal was studied in the right A4 proleg under free crawling conditions by allowing “intact” animals to crawl across the dowel. When A3 came into view of the cameras, an 8-10 second recording was taken at 200 fps. This recording was cropped to only show A4 from just before A5 liftoff to full withdrawal of A4. Recordings that did not include a centralized view of A4 were rejected and deleted. Full withdrawal was defined as the point at which the planta appeared to be no longer moving with respect to the rest of the proleg. Recording and synchronization of cameras was done using Vicon Motus 9.2 (Vicon corporation, CO). This system was designed for rigid bodied kinematics, so recordings were exported to the DLTcal5 and DLTdv5 suite, which tracked the deformable aspects of the leg more easily (Hedrick, 2008).

3D reconstruction

DLTcal5 and DLTdv5 were used to reconstruct the three-dimensional coordinates of each tracked marker. This software uses an eleven coefficient direct linear transform (DLT) to establish the location and orientation of each camera view in space. After coefficients were calculated, a reconstruction of the calibration frame was used as a control to ensure accuracy of the calibration. The parameters were then applied to each view of the proleg withdrawal, and points were tracked through a combination of manual clicking and automatic pattern recognition in DLTdv5. Raw locations of each point were filtered in DLTdv5 using a Monte Carlo approach to smooth out any shake due to digitization error. The filtered data was output to Matlab 2010b

(Mathworks) for analysis. Full 3D reconstructions of the full A5 release to A4 withdrawal period were completed for 3 animals, and one withdrawal per animal. For more details on the reconstruction approach used, see Hedrick, 2008.

Data analysis

Each withdrawal was characterized as the removal from the substrate to a fully withdrawn position where the planta had been entirely folded into the proleg. All phasing was calculated as relative to the A5 release-A4 release interval. All reference time points in the crawls were defined through manual frame-by-frame analysis of crawls.

Surface areas were calculated using the Delaunay function in Matlab 2010b (Mathworks). This fit a triangular mesh over the data in the X-Z plane that was further extrapolated to the Y plane based on the y values at each point. The areas of the resulting triangles were then calculated and summed. Use of the X-Z plane for Delaunay fit ensured the smoothest fit to the data based on manual observation of fit. The number and location of points in each prep were variable, and comparisons of surface area changes were only made within single animal preps.

For differential measurements between segments of the animal, points were binned based on the segment of the proleg being tracked. Instead of binning based only on the segment a point corresponds to, an additional section was added to differentiate the bases of the crochets that were tracked. Furthermore, when a point was close to the coxa-planta boundary (within about 1mm), it was always placed into the planta grouping (Figure 3A). Length and rotation were measured by averaging the location of the crochet hook points and averaging the location of the subcoxa points and taking a change in offset and a change in length during proleg withdrawal (Figure 3B).

The path of each tracked point was calculated as a single three- dimensional path, where frame-by-frame changes in the point's location in space corresponded to its path. Analysis of

withdrawal was flattened to remove variation in one of three planes when movement in one direction was considered relatively uniform, and unnecessary to understanding the behavior. For example, analysis focused on the crochet hooks focused on the Y-Z plane because the hooks are simple hooks that engage orthogonally to the caterpillar's direction of motion. Therefore, during unhooking the animals do not move significantly in the X direction, and any motion in the X direction is not biologically meaningful. In order to characterize the initial direction of movement, the angle between the initial point and the average of points 3 through 8 was measured. This angle was defined to always be positive and greater than ninety degrees. This meant that angles such as -10 and 10 degrees were measured as 350 and 370 degrees respectively, improving the ease of averaging data and also accurately getting the direction of movement (Figure 3). Due to variability between the animals orientation with respect to vertical, all crawls were normalized to have a thirty-degree angle between horizontal and the line through the most medial point tracked (a crochet), and the most lateral point tracked on the subcoxa.

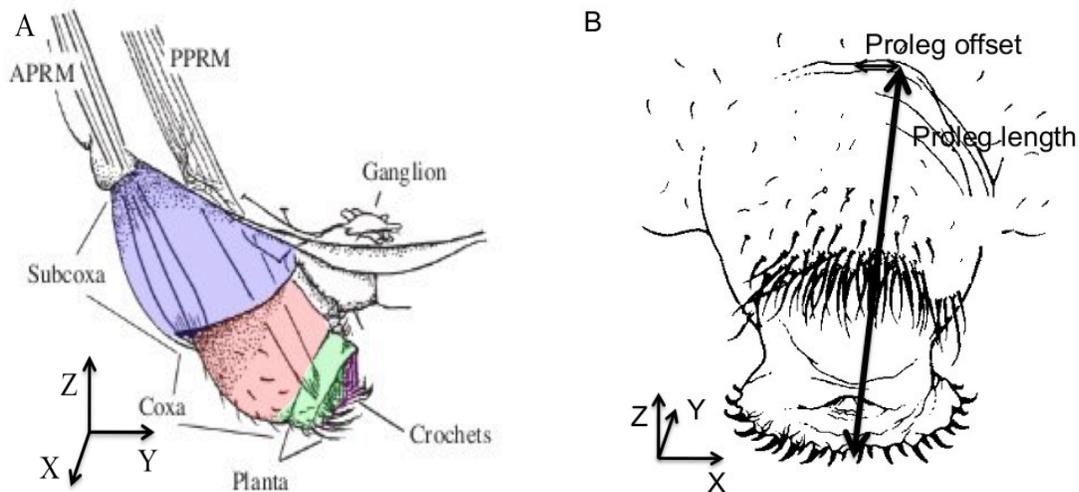


Figure 3: A) Schematic of *Manduca sexta* proleg, from Mezoff et al. 2004. Color added. Each color represents a different binned segment during analysis. As can be seen, the lowest segments of the coxa were binned with the planta. The coordinate system shown is conserved throughout this section of the paper. In measurement of initial direction, all angles were taken in the Y-Z plane, where the positive Z-axis was taken as zero degrees, and along the positive Y was called

360 degrees. B) Square on view of proleg, from Peterson and Weeks, 1988. Annotations added. Shows metrics used to determine proleg shift and lengthening.

Results

In freely crawling animals, proleg withdrawal is characterized by an expansion of the leg surface following the release of the posterior leg (Figure 4). Following A4 liftoff, the surface area of the leg significantly decreases as the planta is pulled into the leg. The phase order of expansion and shrink was consistent within all preps and the final tracked surface area (proleg withdrawn) was always lower than surface area in stance phase. (Figure 4).

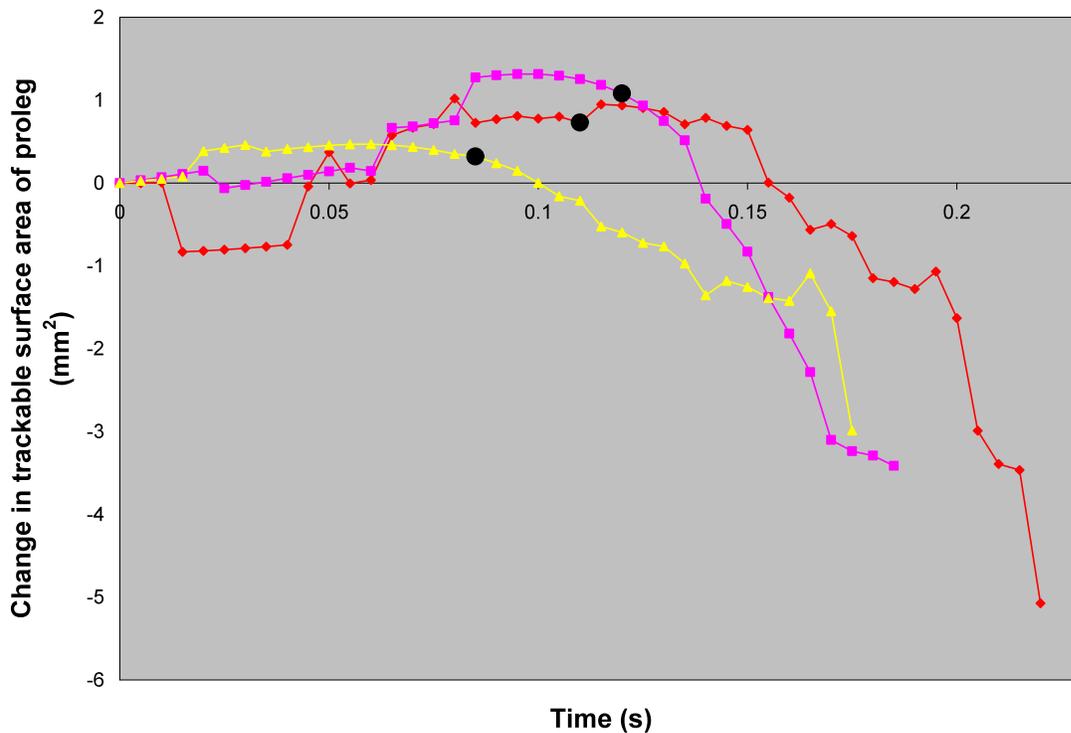


Figure 4: Surface area change of proleg A4 as a function of time. T=0 is A5 liftoff, each trace ends when the crochet points could not be tracked. Each trace represents a single withdrawal from a different animal, black circles represent A4 liftoff. In each trace, the proleg increases in surface area before A4 liftoff occurs.

During crawling, the proleg maintains its length until withdrawal, when the proleg begins to shorten. Proleg length remains approximately equal between A5 liftoff and A4 liftoff (Figure 5), although the leg is generally pivoting forward in this time shifting the leg from upright to a tilted forward position (Figure 6). At approximately the point where the animal releases its leg, length begins to decrease. After release, the length continues to decrease until the planta is fully withdrawn. Both tilt and length phasing are variable, and the point within the crawl where A4 begins to shorten is not conserved. Furthermore, the phasing of the angle shift is variable, although the top of the leg is always shifted forward prior to A4 liftoff.

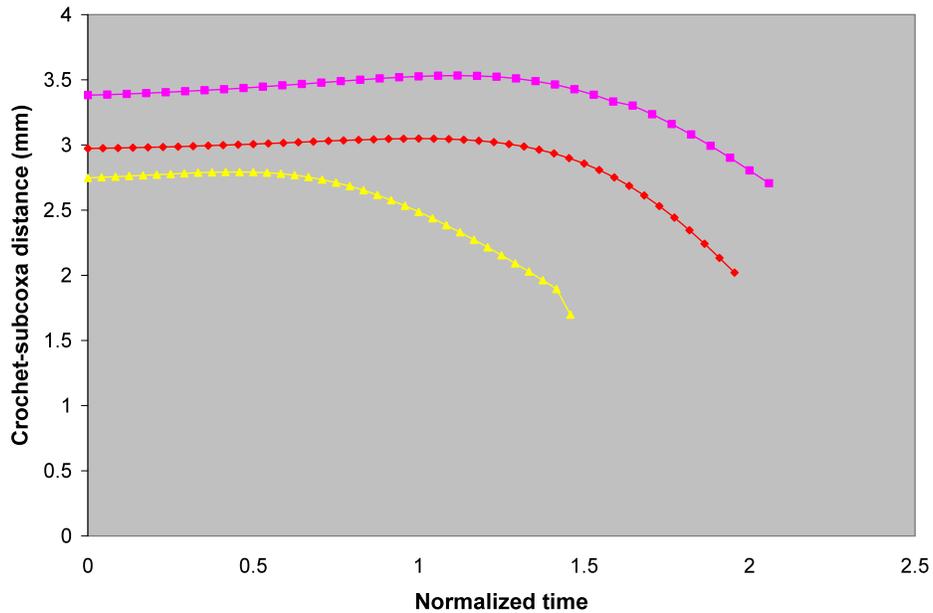


Figure 5: Distance change between crochet hooks and the subcoxa on proleg A4 between A5 liftoff and full withdrawal of the planta. Data are normalized to the A5-A4 period where when the time scale equals one, A4 release occurs and time zero is A5 release. Each trace is from a unique crawl in a unique animal. Length remains approximately constant until A4 withdrawal, when the proleg shortens.

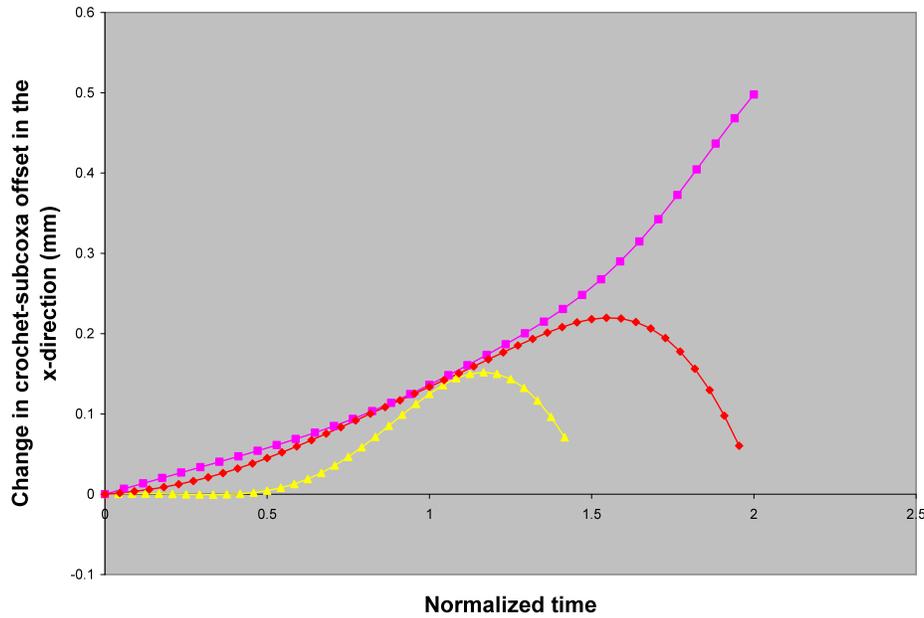


Figure 6: Average distance in the X-direction (direction of crawling) between average tracked subcoxa and average crochet hook point. This metric roughly corresponds to forward shift of the leg at the body, and thus the tilt of the leg. The data are normalized to the A5 release-A4 release period, where A5 release is at time zero and A4 release occurs at time 1. All distances are normalized to zero and thus reflect change.

When the proleg is broken down into its respective pieces, differential movement of the crochet hooks is observed. Separate tracking of the crochet hook bases, subcoxa, coxa, and planta in the Y-Z plane found that in the initial motion following A5 release the crochets are moved inward toward the midline. The rest of the proleg is on average, moved outward, away from the substrate (Figures 7,8). Inward motion occurs for about approximately half of the gripped phase between A5 liftoff and A4 liftoff; at this point, the crochet hooks stop and move in the other direction with the rest of the leg.

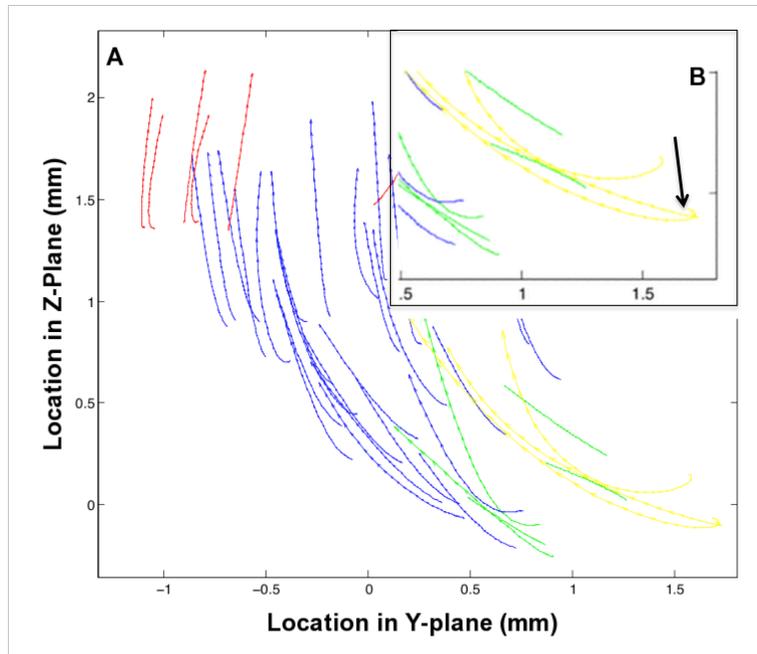


Figure 7: A) Full leg view of the paths taken through the YZ plane by tracked points. Paths show data from A5 liftoff to full withdrawal. Sections are color coded with yellow for the crochet hooks, green for the planta, blue for the coxa, and red for the subcoxa. Arrowheads in a path represent $1/200^{\text{th}}$ of a second interval. B) Magnification highlights the inward motion of the crochet hooks at the start of the crawl. This behavior is consistently seen in the crochet hooks, and also occasionally occurs in the planta. Crochet hooks begin to move with the rest of the proleg approximately half way between A5 liftoff and A4 liftoff.

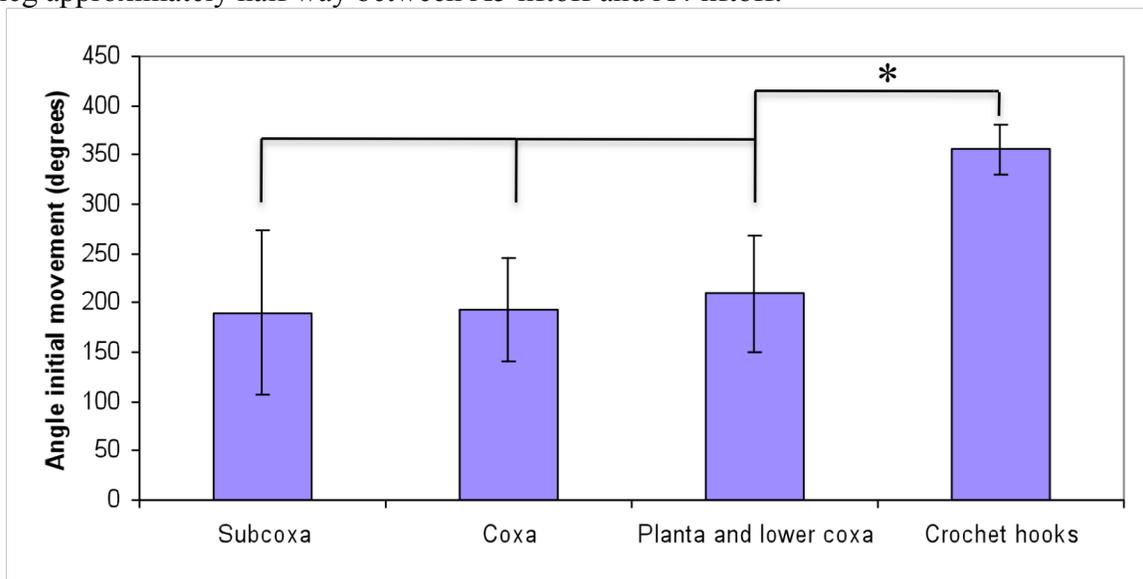


Figure 8: Average initial angle of motion by section. Movement at 0 and 360 degrees is movement toward the animal's midline and toward the substrate. 180 degrees is movement laterally away from the midline. Crochet hook motion is significantly different from all other segments, (ANOVA with Tukey's post hoc test, $df=3\ 132$, $F=20.849$, $p<0.005$).

Discussion

The overall kinematics of proleg withdrawal

Previous work on freely moving animals demonstrated that retraction of the prolegs is preceded by an increase in proleg basal pressure (Mezoff et al. 2004). The Mezoff et al. study focused on restrained animals because during free crawls they were unable to differentiate between specific proleg pressure changes and noise from other moving body segments. The current finding that surface area increases approximately 0.1s prior to proleg withdrawal in A4 confirms the hypothesis that in natural crawling, the proleg withdrawal is preceded by an increase in pressure, as in order to expand the leg, either surface area must increase, or the animal must modulate stiffness of the leg to allow for increased expansion. The increase in pressure could come from the large, fluid filled gut sliding forward ahead of the crawl cycle as A5 is contracted (Simon et al. 2010). This would fill the proleg with fluid just before it retracts. This hypothesis is attractive because it explains part of how retraction is consistently maintained with no extensor muscles to release proleg grip.

The swelling in the prolegs is also interesting in the context of proleg length. A4 does not change length during stance phase, although it does shift forward, implying a pivot over the leg. Belanger and Trimmer (2000) showed that during crawling, PPRM and APRM do not shorten, but rather lengthen just prior to proleg retraction. Given that these muscles lengthen by approximately 1 mm, the constant length maintained in the proleg is unexpected. Since proleg length remains constant, the body wall must develop tension as the origins of PPRM and APRM move forward with the animal. This passive development of tension likely pulls the leg forward, moving it up to the next location. Furthermore, tension would account for the net drag force observed during the last part of the A4 stance phase (see section 1, Lin and Trimmer 2010). The sharp drop off in proleg length following the retraction of A4 from the substrate suggests that

once retraction does occur, the leg passively shortens as PPRM and APRM return to their resting lengths. Tension in the body wall must then be relieved during the next step as A4 adducts and returns to resting length (not measured). Since the neural activity in PPRM decreases during this period (Belanger and Trimmer, 200), the proleg pair is not actively moved forward; it is passively pulled along by the rest of the animal.

The crochet hooks

Reconstructing the paths of the crochet hooks has shown that prior to retraction, the hooks are moved medially toward the substrate. Simultaneously the rest of the proleg begins to retract laterally and upwards. Movement toward the midline is useful to the animal because it will unload any tension on the crochet hooks. The crochets are extremely effective grippers; the grip from one leg can hold a hanging animal. Furthermore, when pulled away from a tightly gripped substrate, the crochet hooks will sometimes be torn from the animal before grip is released. Therefore, it is important that the grip be fully disengaged prior to swing phase. In fact, in animals that have lost proper retraction in one proleg, crawling is severely inhibited and the animal often gets stuck (SCV, unpublished observations). The motion of the legs inward before release occurs is akin to putting slack into the line of a grappling hook prior to pulling it down off of an attachment.

Medial motion of the crochet hooks consistently stops at approximately the same time as surface area increase, (t-test, $df=12$, $t=1.3471$, $p=0.203$). In individual preps, close relation was observed twice, however in the third the pressure increase preceded inward motion terminus. The increase in pressure, which corresponds to surface area expansion, probably helps to ensure that the unloaded legs are kept away from the substrate, preventing them from accidentally adhering a second time post-retraction.

The proleg release can now be characterized by phasing. As A5 releases, some as yet unknown mechanism causes the crochet hooks to move medially, either rotating or unloading their grip on the substrate. Simultaneously, the body rocks forward stretching PPRM and APRM (Belanger and Trimmer, 2000) while maintaining leg length. After about 100 ms, the leg begins to expand due to higher pressure, this expansion coincides with lateral movement of the crochet hooks, as they are released from a gripping position, as well as with PPRM firing (Belanger and Trimmer, 2000). The hooks release, and tension developed in PPRM and APRM returns the leg to resting length through a shortening of the proleg. The proleg then swings forward, and adduction occurs as described by Mezoff et al. 2004.

Future directions

Inward motion is occasionally seen in other low parts of the proleg, not just the planta. Furthermore, the mechanism of medial motion is still unknown. A focused reconstruction with higher spatial resolution (more zoom), tracking only the planta and crochet hooks, is necessary to elucidate the exact mechanism of unloading. It will also be important to track the hook tips as well as the hook bases, to determine if there is rotation as well as unloading. By understanding the detailed motion of the hooks and the planta, mechanistic explanations for the release can be found.

IV. Summary and conclusions

The hypotheses explored in this paper were two fold:

- I. Motor strategy and mechanics are the same in both climbing and crawling, and there is no active change that occurs to account for differential pull of gravity.
- II. Proleg release occurs through a robust process that allows the crochet hooks to disengage from the substrate cleanly and without exerting negative drag force, this release occurs as a result of the specific proleg movement associated with crawling.

Ground reaction forces in climbing and crawling are variable and only the shape of the force appears to be highly stereotyped. Exploration into differences between GRF measurements in varied orientations has shown that when gravity and pitch-back torque are accounted for, the resulting force distributions are essentially the same, confirming hypothesis (I).

In detailed motion capture experiments, differential movement of each proleg segment reflects a tendency for the crochet hooks to move toward the substrate, helping them to disengage. This confirms that the animal has a mechanism designed to control the specific movement of the crochet hooks, and thus grip.

These observations will be helpful for future experiments further understanding caterpillar locomotion, as well as to provide inspiration for motor strategies to control novel soft-bodied robots.

Index of terms

GRF	-	Ground reaction force
fGRF	-	Fore-aft ground reaction force
nGRF	-	Normal ground reaction force
PPRM	-	Principal planta retractor muscle
APRM	-	Accessory planta retractor muscle
A3	-	Abdominal segment 3 proleg pair
A4	-	Abdominal segment 4 proleg pair
A5	-	Abdominal segment 5 proleg pair
A6	-	Abdominal segment 6 proleg pair
TP	-	Terminal proleg pair
F_{th}	-	Force exerted on the thoracic legs
F_{abd}	-	Force exerted on abdominal prolegs

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