LOCOMOTORY BEHAVIOR IN THE HAWKMOTH *MANDUCA SEXTA*: KINEMATIC AND ELECTROMYOGRAPHIC ANALYSES OF THE THORACIC LEGS IN LARVAE AND ADULTS

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Summary

During metamorphosis in *Manduca sexta*, muscles and most sensory structures of the thoracic legs undergo extensive changes while the motor neurons that are present in the larva persist into the adult. The main goal of this work was to identify similarities and dissimilarities in thoracic leg movements during crawling in larvae and walking in adults. This information provides a foundation for understanding the extent to which centrally located neural elements are reorganized during metamorphosis to accommodate changes in locomotion. Analysis of electromyographic activity from leg muscles synchronized with video-taped recordings of the leg movements during larval crawling and adult walking revealed differences in cycle periods as well as intersegmental and intrasegmental patterns of coordination. Larval crawling was characterized by synchronous movements of segmental pairs of legs as activity proceeded slowly from the more posterior to the more anterior segments. During crawling, antagonistic muscles maintained a strict reciprocity. In contrast, walking in adults was characterized by fast, alternating movements of the left and right prothoracic legs and more variable coordination patterns in the mesothoracic and metathoracic legs (ranging from synchrony to alternation). In adults, sensory information, possibly associated with the weight-bearing or postural demands of walking on an incline, contributed to a strong dependence between the duration of muscle activity and cycle period and to the extent that the muscle activity overlapped during walking.

Key words: locomotion, insect, metamorphosis, *Manduca sexta*.

Introduction

Insects provide a model in which to examine the neural basis of locomotory and non-locomotory behavior involving the legs. Studies in adult, hemimetabolous insects, such as cockroaches, stick insects and locusts, have provided much information on the anatomy and organization of the central and peripheral nervous systems, as well as information about leg muscles and their control during locomotion (for selected reviews, see Bässler, 1983; Delcomyn, 1985*a*; Gewecke and Wendler, 1985; Cruse, 1990). Although much of the research on insect walking has focused on adults, there is evidence for differences in the leg coordination between adults and earlier stages of development. For example, work by Graham (1972) on first-instar nymphs and adult stick insects revealed a change in stepping pattern during postembryonic development. Similar changes in stepping patterns between larval and adult stages of holometabolous insects, such as moths and beetles, have been proposed (Wendler, 1964; Hoyle, 1976; Kent and Levine, 1988*b*), but have not been formally examined. The thoracic leg system of the hawkmoth *Manduca sexta* provides a suitable model system for examining developmental changes in the coordination of leg movements.

As in other holometabolous insects, *M. sexta* shows extensive changes in morphology, morphometry and behavior during metamorphosis. To accommodate these changes, many embryonic mechanisms, such as neurogenesis, programmed cell death and remodeling of neuronal arborizations and synaptic connections, are employed postembryonically. In addition, much is known about how these mechanisms are regulated and their relationship to the expression of stage-specific behavior patterns in *M. sexta* (for reviews, see Weeks and Levine, 1990; Levine *et al.* 1991). Despite the overall reorganization, many of the motor neurons, such as those that innervate the thoracic legs, persist from the larval to the adult stage (Kent and Levine, 1988*a,b*). Thus, in *M. sexta* we can examine the extent to which changes in identified motor neurons contribute to changes in stepping patterns in larvae and adults. This requires a detailed knowledge of the patterned activity and behavior of the thoracic legs in larvae and adults.

The main goal of this study was to identify the similar and dissimilar features of thoracic leg movements during crawling in larvae and walking in adult moths. This paper describes the

movement and the pattern of motor activity in selected leg muscles of larval and adult *Manduca sexta*. These two types of behavior were compared because they are the major rhythmical events in the thoracic legs at each stage. We describe many features of intersegmental and intrasegmental coordination that distinguish crawling from walking. These data provide a behavioral framework for studying fictive locomotion in isolated nerve cords (Johnston and Levine, 1995) and for examining the reorganization of the underlying neuronal circuits during metamorphosis of the larva into the moth. A preliminary account of some of this work has appeared previously (Johnston and Levine, 1994).

Materials and methods

Experimental animals

Manduca sexta (L.) larvae and adults (Lepidoptera: Sphingidae) were obtained from an established laboratory colony maintained at the University of Arizona and reared on a long-day photoperiod (17h:7h, L:D) at 26 °C and approximately 50–60 % relative humidity. Larvae ate an artificial diet (modified from Bell and Joachim, 1976) throughout the five larval instars. To characterize thoracic leg locomotor activity, animals at three distinct stages were used: feeding larvae, wandering larvae and adults. To characterize crawling in larvae, caterpillars in the second or third day of the final (fifth) instar were selected. In the middle of the fifth instar and prior to pupation, larvae stop feeding and enter a stage known as wandering, named for the robust locomotor activity observed as the caterpillar searches for an adequate site to pupate (Dominick and Truman, 1984). Larvae in their first and second days of wandering were selected to monitor crawling activity; these insects will be termed 'wanderers'. Moths on the first day post-eclosion (i.e. emergence) were chosen to quantify thoracic leg activity in adults.

Video recordings

Larvae were video-taped with a color CCTV camera (Panasonic WV CL700) as they walked on a level, wooden dowel (diameter 0.45 mm) or on a level surface. Wanderers crawled only on the level surface; adults walked up a surface inclined by approximately 45 °. The camera was positioned perpendicular to the lateral surface of the long axis of the body of the insect. Mirrors were used to observe the side of the insect not facing the camera and in some cases to observe the ventral surface during locomotion.

In addition to walking, we evoked walking-like movements in adults, which we refer to as 'airstepping', by suspending the moths in the air with their legs pendent (Giuliani and Smith, 1985; Johnston and Bekoff, 1992). Airstepping is similar to tethered walking and searching movements in cockroaches (Delcomyn, 1973, 1987) in that the insect is suspended in the air and does not support the weight of its body. However, unlike tethered walking, during airstepping the leg movements occur without contacting any subtratum. In addition, the behavioral and motor activity during airstepping in *M. sexta* is distinct from that observed during searching in cockroaches (Delcomyn, 1987).

Video analysis

We selected data that showed repetitive and uninterrupted episodes of crawling or walking for analysis. Video tapes were replayed on a VCR (Panasonic AG 6750A) connected to a color video monitor (Sony PVM 1342Q) and analyzed every 16.7 ms (60 fields s^{-1}) to determine when the pretarsus of each thoracic leg (Fig. 1B,E) made and relinquished contact with the subtratum. In addition, in larvae we determined when the abdominal prolegs (located in abdominal segments 3–6 and in the terminal segment) made and lost contact with the subtratum. From these data, the duration of leg movements and the latency between left and right legs within a segment (intrasegmental latency) and the latencies between legs in different segments (intersegmental latency) were calculated. In larvae, crawling movements typically originated in the most posterior segment of the abdomen and proceeded anteriorly in a peristaltic manner through the abdomen and then the thorax. The legs in each thoracic segment typically took one step (i.e. relinquished and remade contact with the subtratum) for each posterior-to-anterior progressing crawling movement,
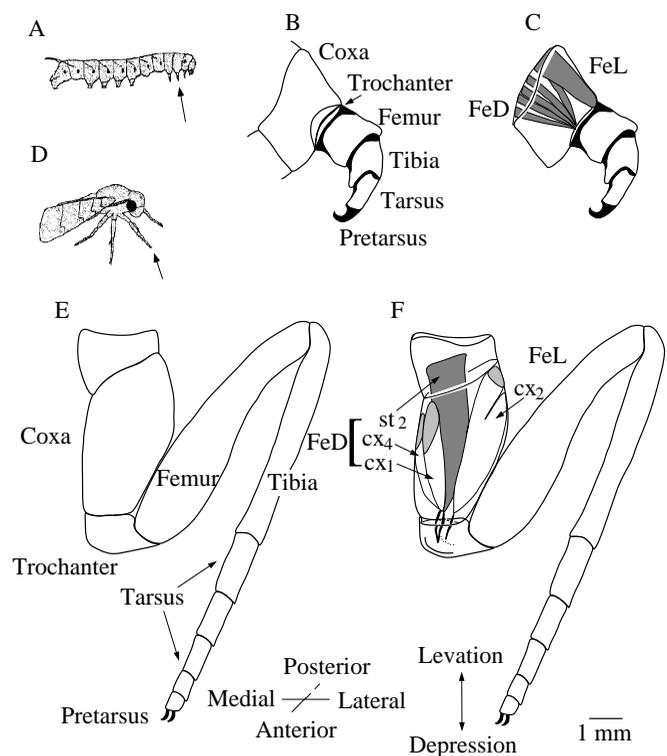

Fig. 1. A larval caterpillar (A) and an adult moth (D) with arrows pointing to a thoracic leg. B and E show the external morphology (without the sensory hairs) of a larval (B) and adult (E) prothoracic leg. (C,F) The femoral levator (FeL) and femoral depressor (FeD) muscles of the coxa examined were using electromyography in larvae (C) and adults (F). (F) st$_2$, sternopedal muscle; cx$_1$, cx$_2$, cx$_4$, coxal muscles (nomenclature based on Eaton, 1974; also see Kent and Levine, 1988*a,b*). Scale bar applies to B, C, E and F only.

although sometimes the legs took more than one step for each crawling movement. We use the term *extra steps* to describe such crawling behavior in larvae. Because we were primarily interested in the locomotor movements of the thorax, a cycle was defined as the period between the right metathoracic pretarsus lifting off the subtratum and the next time that this leg left the subtratum. Thus, in larvae a cycle included the posterior-to-anterior passage of the crawling movement through the three thoracic segments (i.e. metathoracic-to-mesothoracic-to-prothoracic) and then through the abdominal segments (terminal segment, sixth abdominal segment, fifth abdominal segment, etc.).

In adults, because of variation in stepping patterns in the metathoracic and mesothoracic legs (see Results section for further details), a cycle was defined as the period between the right prothoracic pretarsus lifting off the subtratum and the next time that this leg left the subtratum. For adults and larvae, the durations of contact (pretarsus-on) and no contact (pretarsus-off) with the subtratum were calculated for each thoracic leg. In adults, pretarsus-on occurs during retraction of the leg and pretarsus-off occurs during protraction of the leg. To determine whether movements of the left and right legs of the same segment or between segments occurred simultaneously, the latency to pretarsus-on and pretarsus-off with respect to the beginning of the cycle was quantified. Phase was calculated from the ratio of latency:cycle period. To determine the proportion of each cycle occupied by pretarsus-off and pretarsus-on movements, normalized durations were calculated from the ratio of duration:cycle period.

Electromyograms

Electromyographic (EMG) recordings were used to provide an indirect measurement of the output of the thoracic leg motor neurons. Larvae and adults were anesthetized by chilling and were maintained on ice throughout the preparation procedure. Wings and scales were removed from moths prior to placement of the electrodes. The ends of the enamel-coated stainless-steel electrode wire (25 or 50 μm diameter) were cut at an angle to remove the insulation only at their tips. Bipolar electrodes were implanted through a small hole in the cuticle of the coxa and positioned parallel to the long axis of the muscles to be investigated (Fig. 1C,F). The electrode wires were routed to the dorsal surface of the insect and secured to the cuticle with a small amount of glue (VetBond, 3M). The long, lightweight wires did not interfere with the locomotor movements, and the animals behaved normally. The electrodes were attached to shielded leads that interfaced with differential a.c. amplifiers (A-M Systems 1700).

To monitor intrasegmental and intersegmental coordination, recordings were made in the thoracic segments from antagonistic muscles of adjacent ipsilateral and contralateral coxae, which act to levate or depress the femur (Fig. 1B,E). After implanting the electrodes, the insects were allowed to recover for 2 h at 26 °C before recordings began. EMG activity was amplified, filtered and stored on a digital Vetter eight-channel recorder (3000A) for subsequent analysis. Following each recording, *post-mortem* dissections were used to confirm the location of each electrode.

One should note that the muscles we refer to as femoral levators and depressors were previously called femoral extensors and flexors (Kent and Levine, 1988*a*). We have adopted the new terminology to remain consistent with the established conventions for defining movements of the femur in other insects and to remain consistent between larval and adult stages in *M. sexta*. For example, previously, extension (i.e. an increase in joint angle) was based on the *medial* surface of the entire larval leg. However, in adults, extension was based on the *lateral* surface of the coxa–trochanter–femur segment and on the *medial* surfaces of the more distal segments of the leg (Fig. 1B,E) (Kent and Levine, 1988*a*). By using different reference surfaces in larval and adult stages to define movements of the femur, it appeared as if muscles located in similar positions in the coxa produced femoral flexion in larvae and femoral extension in adults. Our use of levator and depressor implies movements of the femur relative to the subtratum (see Fig. 1) and does not refer to changes in angular excursions.

In larvae, we recorded EMG activity from the femoral levator (FeL) and from a group of medial femoral depressor (FeD) muscles (Fig. 1C). In adults, femoral depressor (FeD) activity was monitored from sternopedal (st$_2$) and coxal (cx$_1$ and cx$_4$) muscles; femoral levator (FeL) activity was recorded from cx$_2$ (Fig. 1F) (nomenclature taken from Eaton, 1974; also see Kent and Levine, 1988*b*). In this study, we did not determine the patterns of activity for the individual depressors.

EMG analysis

Quantitative analyses of EMG recordings were used to describe and compare the motor patterns during crawling in larvae and during walking and airstepping in adults. We did not collect EMG data from wandering larvae. Data were analyzed using Data-Pac II software (Run Technologies). Except where noted, a cycle was defined from the onset of bursting activity in the right FeL to the onset of the following activity period in the same muscle (recordings were taken from the metathoracic and mesothoracic segments in larvae and the prothoracic segment in adults). From the extracellular recordings of muscle activity, values for cycle period, burst duration, latency, normalized duration (duration/cycle period) and phase (latency/cycle period) were calculated. Within each cycle, the burst duration of a given muscle was defined from the onset of activity to the offset of activity. To quantify whether the onsets in different muscles occurred synchronously, the latency to bursting activity with respect to the beginning of the cycle was quantified. To describe the patterned activity that occurred at the *average* cycle period, mean phase and mean normalized burst durations were calculated.

EMG activity synchronized with movement kinematics

EMG recordings from thoracic legs were synchronized with the video tapes of larvae crawling on a dowel and of adults walking. To synchronize the video display to a concurrent

EMG recording, output from a pulse stimulator (A-M Systems 2100) was used to drive a light-emitting diode (LED) present in the video field of view and simultaneously to generate a d.c. voltage that was recorded on one channel of the Vetter recorder. EMG activity was collected and stored as described above. The LED displayed in the kinematic records was correlated one-to-one with a d.c. voltage trace on the EMG recordings. The period between the relinquishing of contact with the subtratum by the pretarsus of a given leg and the onset of bursting activity in the corresponding femoral levator was quantified. It is important to note that the EMG recordings were taken from specific coxal muscles that move the femur, whereas the behavioral data were based on the gross movements of the entire thoracic leg. Thus, our method for correlating leg movements with the underlying muscle activity provides only an approximate relationship between behavior and EMG recordings. Analyses that include a knowledge of changes in joint angle during walking would be necessary to identify the levation and depression phases of the femur during a step.

Experimental design and statistical analyses

Our results represent data collected from five different animals in each of the following five groups: feeding larvae crawling on a dowel, feeding larvae crawling on a level subtratum, wandering larvae (wanderers) crawling on a level subtratum, adults walking up an inclined surface and adults during airstepping. Locomotor episodes consisting of at least six contiguous steps were analyzed. Ten cycles were chosen for analysis from each of the five animals in each of the five groups stated above. Thus, each animal was represented equally and our sample size was five unless otherwise noted. A critical level of $P<0.05$ and two-tailed probabilities were used to determine statistical significance throughout this study. Averages are given as mean ± standard deviations. Square root transformations were performed on ratio variables (range 0–1.0) prior to statistical testing. When appropriate, one-group t-tests, with a population value of zero, were used to determine statistical significance from zero. The assumptions of both analysis of variance (ANOVA) and regression analyses were formally tested and met. For contrasts between two or more groups, we compared sample means using ANOVA in conjunction with pair-wise comparisons using the Tukey–Kamer HSD *post-hoc* test when appropriate. Regression analysis was used to describe the relationship between burst duration or burst latency and cycle period. Although each of these variables is dependent (i.e. they each vary), we calculated least-square regressions rather than Model II regressions (Sokal and Rohlf, 1995) to facilitate comparisons with other studies. However, analyses based on the Model II approach did not result in different conclusions. In the regressions, the slopes, β, describe the linear relationship between the two variables. The coefficient of determination, r^2, describes the proportion of the variation accounted for by the regression. In all of the scatterplots, coincident points are superimposed. Taking advantage of a procedure in analysis of covariance (ANCOVA) that tests the assumption of homogeneity of slopes, we determined whether specific slopes differed statistically from one another. All statistics were performed using the statistical program Systat for the Macintosh (Systat, Inc.).

Results

Crawling in larvae: behavior

Typically, crawling activity originated in the most posterior abdominal segment and propagated through each segment to the most anterior segment in the thorax. The posterior-to-anterior propagation of activity gave the impression of a wave of muscular activity. As the body advanced during crawling, larvae lifted (levated) and lowered (depressed) their thoracic legs periodically, losing and re-establishing contact with the subtratum.

Our analyses revealed two forms of crawling behavior in larvae moving on a wooden dowel. We termed these single-step crawling and extra-step crawling on the basis of the number of times the thoracic legs relinquished and re-established contact with the subtratum during one posterior-to-anterior wave of crawling. Single-step crawling had an average cycle period of 3.9±0.88 s (N=5) (Table 1) and was characterized by synchronous movements of both the left and right legs of a segment (Fig. 2A). For example, in the stepping patterns shown in Fig. 2A, the left leg of the metathoracic segment had an average phase of 0.0±0.02 (N=5) with respect to pretarsus-off in the right leg, which did not differ significantly from zero (P=0.444). There was also no significant difference in phase between left (0.11±0.02, N=5) and right (0.11±0.03, N=5) legs in the mesothoracic segment (ANOVA, P=0.665) or in the prothoracic segment (left, 0.30±0.03, N=5; right 0.30±0.02, N=5; ANOVA, P=0.644). There was a segmental delay from one thoracic segment to another such that movements in the metathoracic segment preceded those in the mesothoracic by 0.43±0.02 s (N=5) which were followed 0.74±0.02 s (N=5) later by movements in the prothoracic segment (Fig. 2A).

During crawling episodes showing only single steps, the larval thoracic legs spent most of the cycle in contact with the subtratum (Fig. 2A). On average, the proportion of a cycle that the legs were in contact with the subtratum did not differ statistically (ANOVA, P=0.098) among the metathoracic (0.75±0.04, N=5), mesothoracic (0.68±0.08, N=5) and prothoracic (0.83±0.07, N=5) legs (Fig. 2A).

During extra-step crawling, the thoracic legs took additional steps (Fig. 2B) that were not matched by additional steps in the abdominal prolegs and occurred between successive posterior-to-anterior progressing crawling movements. One, two or all three segmental pairs of thoracic legs could show extra steps. Extra- and single-step crawling had similar cycle periods (Table 1). As in single-step crawling, segmental pairs of legs always moved in synchrony during extra-step crawling and there were segmental delays between stepping movements in adjacent segments regardless of the number of thoracic

Table 1. *Cycle periods based on EMG recordings and kinematic data from locomotory behavior in larvae and adults*

	Cycle period (s)	Percentage of the cycle devoted only to thoracic movements	Thorax only cycle period (s)
Crawling in larvae on a dowel – single steps	3.9±0.88	37±2	1.4±0.39
Crawling in larvae on a dowel – extra steps	3.9±0.93	30±4	1.2±0.29
Crawling in larvae on a flat substratum	2.6±0.88	40±3	1.0±0.30
Crawling in wanderers on a flat substratum	2.0±0.46	34±2	0.68±0.18
Walking in adults on an inclined substratum	0.63±0.11	100	0.63
Airstepping in adults	0.17±0.022	100	0.17

Values are means ± S.D. (*N*=5).
Cycle periods for extra steps during larval crawling were calculated from cycles in which all three thoracic segments took an extra step. The percentage (and absolute time) of the cycle occupied by thoracic movements were determined from the same data set.

Fig. 2. Average stepping patterns for larval crawling on a wooden dowel. Thick lines represent periods when the pretarsus was not in contact with the subtratum (pretarsus-off). Thin lines represent periods when the pretarsus was in contact with the subtratum (pretarsus-on). The onset of a thick line indicates the mean phase value (latency/cycle period) for pretarsus-off. The onset of a thin line indicates the mean phase value for pretarsus-on. The length of a line indicates the normalized duration (duration/cycle period) of the two pretarsal states in a crawling cycle. The standard deviations of the mean phase pretarsus-off (left-hand error bars) and normalized duration of pretarsus-on (right-hand error bars) are shown. Because the normalized duration and phase within a leg are complementary variables, variation in the phase of pretarsus-on and in the normalized duration of pretarsus-off are included in the error bars shown. Data

for right (R) and left (L) legs in each segment are shown. Data shown for the third abdominal segment (Abdomen-3) represent periods when the prolegs were touching (thin line) or not touching (thick line) the subtratum. In all experiments, *N*=5: five animals with 10 measurements per animal. (A) Average stepping patterns during crawling episodes showing only single steps. (B) Average stepping patterns during crawling episodes showing extra steps (*) in all three segmental pairs of thoracic legs.
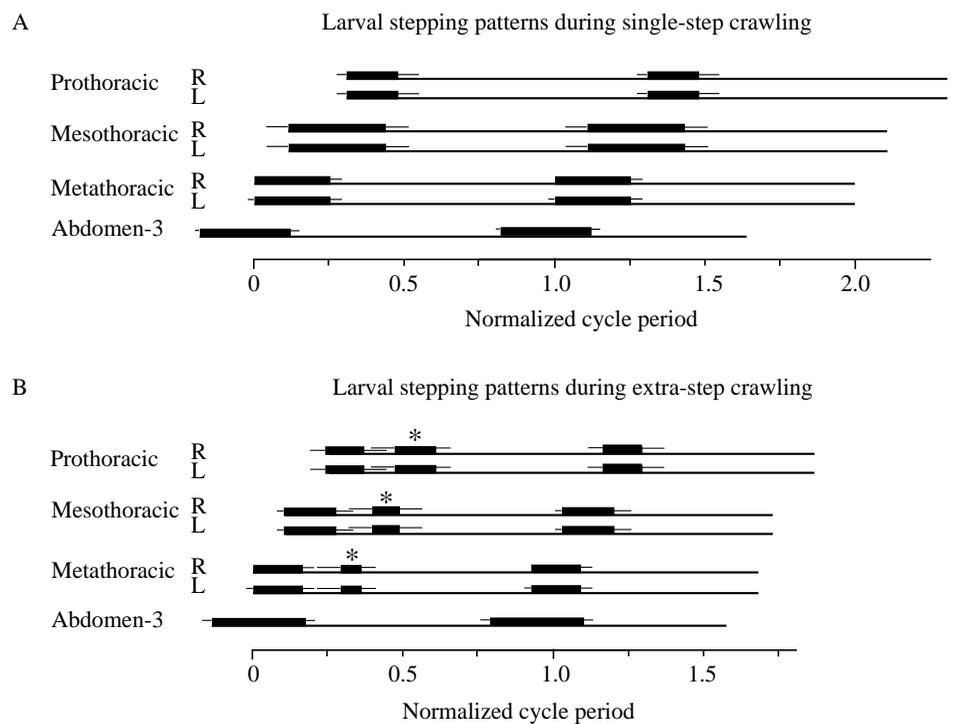
segments involved (Fig. 2B). These data indicate that the thoracic segments and their legs could initiate crawling movements in the absence of observable crawling activity in the abdomen. In addition, each thoracic segment could produce crawling motor activity in conjunction with, or independently from, the other thoracic segments. Thus, intersegmental

coupling was flexible while intrasegmental coupling was maintained when larvae crawled on a wooden dowel.

Crawling in larvae: muscle activity

EMG recordings synchronized with behavioral recordings allowed us to determine an approximate correlation (see
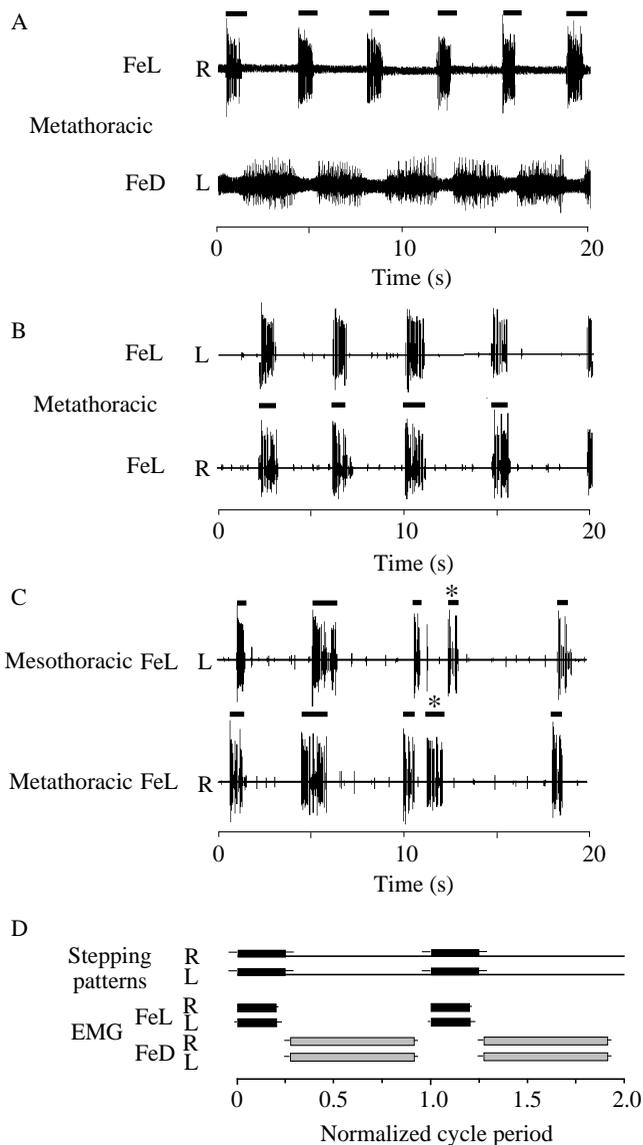
Fig. 3. (A) An example of EMG recordings showing alternation between contralateral levators (FeL) and depressors (FeD) during crawling in a larva. (B) An example of EMG activity showing synchronous bursting in left and right femoral levators during crawling in a larva. (C) An example of EMG recordings showing the delay in levator onset between adjacent segments and the presence of extra steps (marked by the asterisks) in the mesothoracic and metathoracic legs during crawling. The recordings in A–C are from different larvae. The thick lines above each levator burst correspond to when the pretarsus was off the subtratum as determined from the synchronized video recordings. (D) Bar graph summarizing the quantified EMG recordings and the corresponding stepping patterns for larvae crawling on a wooden dowel. Data shown are for the metathoracic legs and are representative for the other thoracic legs (extra steps are not included). Stepping patterns according to Fig. 2 legend. For the EMG recordings, the onset of a bar indicates the mean phase value (latency/cycle period). The length of a bar indicates the normalized duration (duration/cycle period). The standard deviations of the mean phase (left-hand error bars) and normalized durations (right-hand error bars) are shown. $N=5$ animals (10 values per animal).

Materials and methods: EMG activity synchronized with movement kinematics) between the intersegmental and intrasegmental patterns of muscle activity and the movements of the legs during crawling in larvae. Femoral depressor (FeD) activity occurred mainly during pretarsus-on and alternated with femoral levator (FeL) activity that occurred during the pretarsus-off phase of crawling (Fig. 3A). On average, the normalized duration of FeL activity (0.20 ± 0.02, $N=5$) was significantly less ($P=0.016$) than the duration of FeD activity (0.64 ± 0.04, $N=5$) (Fig. 3D). In parallel to the behavioral data described above, the EMG recordings showed synchronous activity between agonistic muscles in left (L) and right (R) legs (Fig. 3B). For example, in the metathoracic segment, the phase relationships between left FeL activity (0.00 ± 0.02, $N=5$) with respect to right FeL activity did not differ significantly from zero ($P=0.704$). In addition, the intersegmental phase delay between FeL activity in adjacent segments (e.g. metathoracic and mesothoracic, 0.12 ± 0.06, $N=5$) and the occurrence of single- and extra-step crawling (Fig. 3C) based on EMG recordings also supported the behavioral data. Thus, the motor activity of these femoral muscles accurately represents leg movements during crawling on a dowel by larvae. Although burst duration and cycle period could vary (see below), the average motor profile and stepping pattern are shown in Fig. 3D. Notice that, on average, the activity of FeL and of FeD do not overlap.

Larvae commonly displayed long episodes of uninterrupted crawling during which the cycle period could vary step by step. To account for the variability in the duration of motor activity, we regressed FeL and FeD durations against their corresponding cycle periods. Crawling episodes that showed extra steps were not included in this analysis. Regression analysis showed that the activities of FeL and FeD muscles were differentially affected by changes in step frequency. FeL duration increased only slightly with increases in cycle period (Fig. 4A), whereas FeD duration showed a strong dependence on changes in cycle period (Fig. 4B). The regression relationships were maintained even when the cycle period was adjusted by omitting the portion of the cycle devoted to abdominal activity (not shown). In addition, on the basis of kinematic data, pretarsus-off duration remained stable over the range of cycle periods while pretarsus-on duration showed a strong dependence on cycle period (not shown).

To determine whether the timing (i.e. onset) of levator and depressor activity was phase-dependent or time-dependent, we regressed the latency to FeL or FeD onset with respect to the onset of a cycle against their corresponding cycle periods (Fig. 4C–E). A statistically significant slope for the regression was interpreted as indicating phase dependency. A non-significant relationship was interpreted as indicating time dependency for the onset of motor activity. The onset of levator activity showed a weak, yet significant, dependence on phase (Fig. 4C). In contrast, the onset of depressor activity showed a dependence on time (Fig. 4D). For intersegmental activity, the onset of levator activity also showed a dependence on time and not on phase (Fig. 4E). Insufficient data were available to
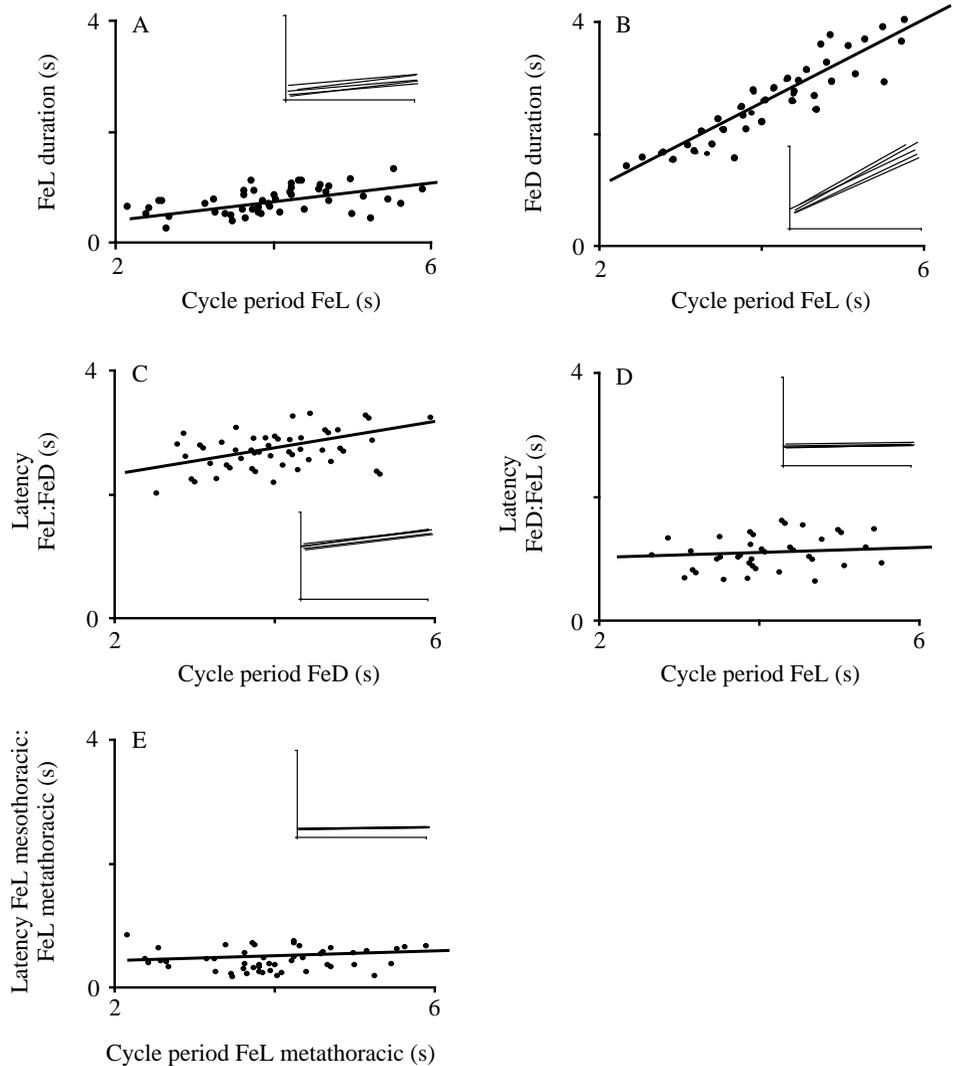
Fig. 4. Regression analyses for activity duration (A, B) or latency to activity onset (C–E) *versus* cycle period for the activities of FeL and FeD for larvae crawling on a wooden dowel. Each plot contains 50 data points: 10 values from each of five animals. Coincident points are superimposed. The individual regressions for each animal (shown in the insets) do not differ statistically from one another. In plots C–E, latency FeL:FeD corresponds to the latency to the onset of bursting in FeL with respect to the onset of the cycle defined by activity onset in FeD. In these regressions, the slopes, β, describe the functional relationship between the two variables. The coefficient of determination, r^2, describes the proportion of the variation explained by the regression. The probability value, P, is for the slope. (A) β=0.12, r^2=0.30, P=0.0011. (B) β=0.82, r^2=0.60, P=0.0035. (C) β=0.20, r^2=0.52, P=0.043. (D) P=0.5224.

determine phase- or time-dependence for the intersegmental activity in femoral depressors.

Larvae and wanderers crawling on different subtrata

It is known that changes in subtratum influence the precise coordination of the legs during walking (Spirito and Mushrush, 1979; von Holst, 1943). To examine the influence of subtratum and developmental stage on leg coordination, larvae and wanderers were allowed to crawl on a flat surface. We did not examine wanderers crawling on a dowel because they could not maintain their grip on a dowel owing to the degeneration of the proleg system that occurs prior to pupation (Weeks and Truman, 1984). The pattern of intrasegmental and intersegmental coordination among the thoracic legs was not affected by changing the subtratum upon which the insects crawled. Bilateral synchronous movements, characteristic of crawling on a dowel, were maintained during crawling on a flat surface. However, we never observed extra steps when larvae or wanderers crawled on a flat surface (20–30 cycles examined from each of five larvae). The intersegmental delays between

thoracic segments during crawling on a flat surface were similar to those for crawling on a dowel. The delay between movements in the prolegs of the third abdominal segment and the metathoracic legs in wanderers differed statistically from the delay for larvae crawling on different subtrata (Tukey's HSD, larvae on a flat surface *versus* wanderers P=0.005, larvae on a dowel *versus* wanderers P=0.002, larvae on a flat surface *versus* larvae on a dowel P=0.223). Differences in subtratum and developmental stage significantly affected the cycle period of thoracic leg movements during crawling in larvae (Table 1). The fastest cycle period occurred in wanderers, followed by larvae crawling on a flat surface and then larvae crawling on a dowel.

Walking in adults: behavior

Although the main mode of locomotion in adult *M. sexta* is flight, adult moths also displayed spontaneous walking when placed at the bottom of an inclined surface. Adults would also walk on a level surface, but much tactile stimulation was needed to initiate walking and it was uncommon to observe

Fig. 5. (A) An example of stepping patterns for an adult *M. sexta* walking on an inclined surface. Note that the abscissa is in real time, not normalized time as shown in Fig. 2. Thick lines represent periods when the pretarsus was not in contact with the subtratum (pretarsus-off). Thin lines represent periods when the pretarsus was in contact with the subtratum (pretarsus-on). The onset of a thick line or a thin line represent the beginning of pretarsus-off or pretarsus-on periods, respectively. The length of a line indicates the duration of the two pretarsal states in a walking cycle. (B) Contiguous cycle periods plotted against elapsed time showing the extent of ipsilateral and bilateral frequency coupling during walking in adults. Left legs are filled symbols and right legs are open symbols. (C) Frequency distributions of phase relationships between pretarsus-off in the left leg with respect to pretarsus-off in the right leg in each thoracic segment during walking in adults. A phase of 0.5 indicates a pattern of alternation between left and right legs within a segment. Data are from five animals with 10 measurements per animal.
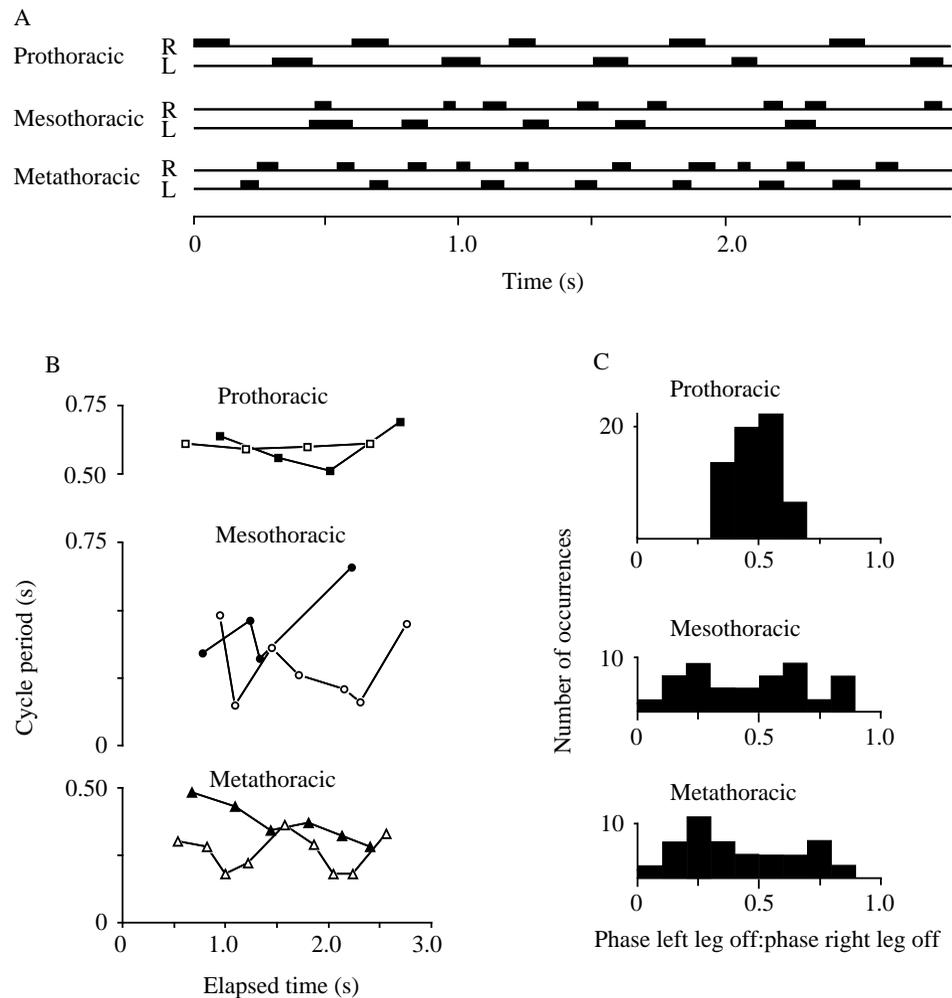
bouts of many consecutive steps without turns or stops. We did not determine whether walking on an inclined surface differed from walking on a horizontal surface. It has been shown that cockroaches use a different gait when walking up (or down) an inclined surface in comparison with walking on a horizontal surface (Spirito and Mushrush, 1979). An example of stepping patterns for each segmental pair of legs during walking in an adult is shown in Fig. 5A. Over the entire range of cycle periods examined (range 0.45–1.03 s; 0.63±0.11 s, $N=5$ animals) we were unable to see consistent patterns of *intersegmental* coordination, such as tripod gait, tetrapod gait or integral fraction coordination (i.e. 2:1, 3:2, etc.; von Holst, 1939), as described in other insects (for reviews, see Hoyle, 1976; Graham, 1985). Within an episode of walking, discrete occurrences of intersegmental coordination could be detected, but consistent patterns failed to emerge.

It was obvious from such data (Fig. 5) that legs in adjacent segments and segmental pairs of legs could step at different frequencies. By plotting cycle periods from contiguous steps against elapsed time (Graham, 1978*a,b*), we sometimes detected evidence for intersegmental and intrasegmental frequency coupling during walking in adults (Fig. 5B). In *M. sexta* and other animals, ipsilateral coupling was observed between the metathoracic and mesothoracic legs, during which the ipsilateral legs in these two segments showed parallel changes in cycle period over time (i.e. frequency coupling) (Fig. 5B). However, steps between ipsilateral legs did not occur in a consistent manner (e.g. 2:1, 1:1), and the order in which the thoracic legs stepped relative to one another varied. Systematic absences in steps as described by Graham (1978*a,b*) were not apparent.

Frequency coupling between contralateral legs in different segments and between segmental pairs of legs in the metathoracic and mesothoracic segments was not apparent in any of the data that we examined (Fig. 5B). We were also unable to detect any phase coupling based on analysis of phase response curves (Stein, 1976; Chasserat and Clarac, 1983), which examined whether the cycle-to-cycle variability in the timing of movements in one leg was influenced by, or directly related to, the current timing of the step cycle in another leg during walking. However, the prothoracic legs showed strong frequency coupling that occurred over a narrow range and maintained a 1:1 movement relationship (Fig. 5A,B). In contrast to the variable phasing between the mesothoracic and metathoracic legs, the phase relationship between the left and right prothoracic legs during walking in adult moths occurred over a discrete range, with the average phase indicating a pattern of alternation (0.47±0.06, $N=5$) (Fig. 5C). Because of

its consistent pattern of alternation and 1:1 bilateral coordination (i.e. absolute coordination according to von Holst, 1939), we focused our EMG analyses on the prothoracic legs.

Walking in adults: muscle activity

We monitored the activity from muscles in the prothoracic coxa that acted either to levate or to depress the femur (Fig. 1D–F). Examples of EMG activity of the prothoracic legs during walking in adults are displayed in Fig. 6A,B. During walking, FeL was active throughout the entire portion of the step cycle that the leg was not in contact with the subtratum and remained active during much of the time the leg was in contact with the subtratum (Fig. 6A). The onset of FeL activity in left and right legs alternated, but the duration of levator activity in the two legs overlapped. In addition, there was much overlap between FeL and FeD activity in the same leg or in bilateral legs and a phase delay between their onsets (Fig. 6B). Fig. 6C represents the average duration and phase relationships characteristic of walking in adults. In addition to the bilateral alternation, there was substantial overlap in the activity of contralateral agonists (e.g. left and right levators) and ipsilateral antagonists (e.g. left FeL and left FeD). The average normalized durations of levator (0.85 ± 0.04, $N=5$) and depressor (0.80 ± 0.04, $N=5$) activity each occupied the majority of the cycle and did not differ statistically from one another (ANOVA, $P=0.221$).

Because of the close proximity of the muscles in the coxa, it was possible that the recording electrode picked up activity in adjacent muscles. The possibility of cross-talk was one explanation for the multiple spike heights present in the EMG recording and the extensive overlap between levator and depressor activity (Fig. 6A,B). However, careful analysis of the recordings at expanded time scales did not reveal any evidence for cross-talk (i.e. time-locked spikes from electrodes in different muscles in the same leg). Moreover, the degree of overlap between FeL and FeD was reduced dramatically during airstepping (see below; Fig. 8). Thus, we concluded that the duration and latency values were not contaminated by cross-talk. The different spike heights shown in Fig. 6 could reflect the recruitment of multiple motor neurons innervating the levator and depressor muscles. On the basis of biocytin backfills into the ganglia from specific nerve branches, there is evidence for polyneuronal innervation in FeD and FeL (K. Oanh-Phan, R. M. Johnston, C. Consoulas and R. B. Levine, unpublished data).

To account for the variability in duration of muscle activity, we regressed the durations of FeL and FeD activity of the prothoracic legs against their corresponding cycle periods.
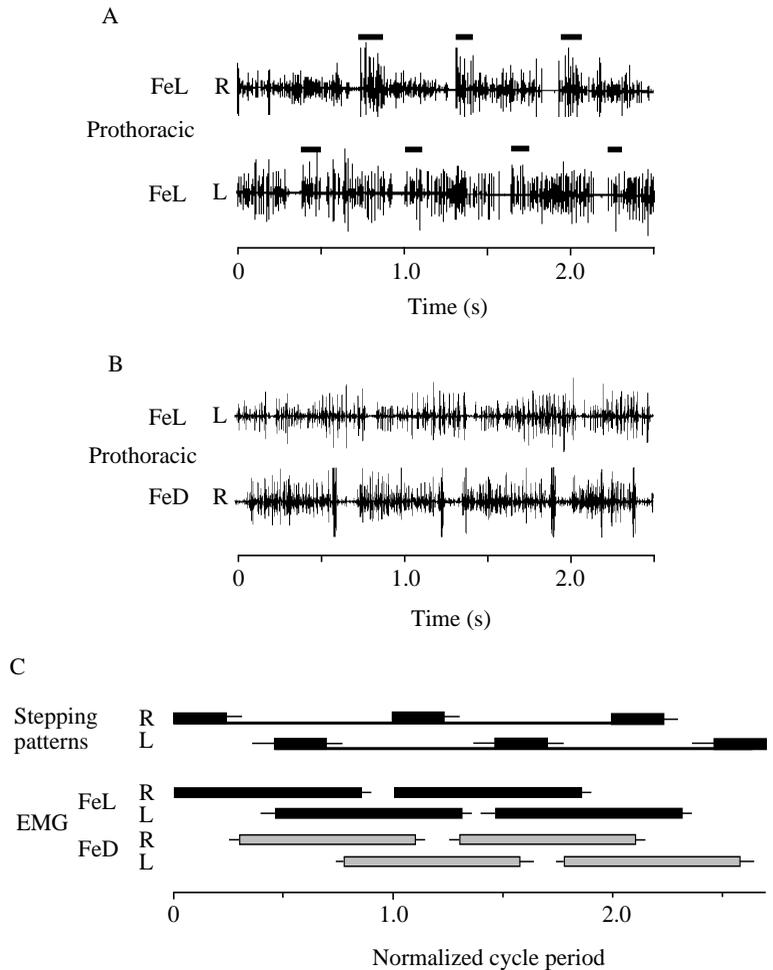

Fig. 6. (A,B) Examples of EMG activity synchronized with video recordings (A only) of walking in two different adult moths. Synchronization according to legend to Fig. 3. (C) Bar graph summarizing the quantified EMG recordings and the corresponding stepping patterns during walking in adults. Stepping patterns according to legend to Fig. 2 and EMG data according to legend to Fig. 3.
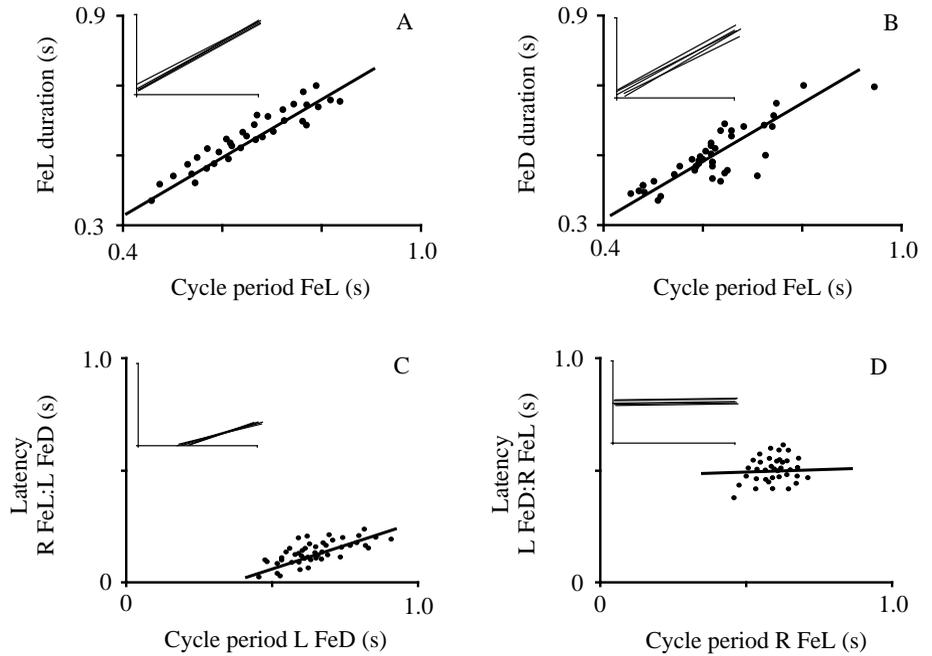
Fig. 7. Regression analysis for the burst duration (A,B) or latency to burst onset (C,D) *versus* cycle period of FeL and FeD activity during walking in adults. See legend to Fig. 4 for definitions and details. Each plot contains 50 data points: 10 values from each of five animals. Coincident points are superimposed. The individual regressions for each animal (shown in the inserts) do not differ statistically from one another. (A) β=0.84, r^2=0.92, P=0.0001. (B) β=0.80, r^2=0.80, P=0.0001. (C) β=0.42, r^2=0.59, P=0.0264. (D) P=0.5056.

Both FeL and FeD burst durations showed a strong and similar dependence on cycle period (Fig. 7A,B). Increases in cycle period were accompanied by increases in the duration of both FeL and FeD activity. In a similar manner, we regressed latency to burst onset against cycle period to determine whether the timing of activity was phase-dependent or time-dependent. The timing of FeL activity showed a significant dependence on cycle period (Fig. 7C). In contrast, the timing of FeD activity was independent of cycle period (Fig. 7D). These results indicate that the timing of levator activity was phase-dependent and the timing of depressor activity was time-dependent.

Airstepping in adults: muscle activity

The medium on (or in) which the leg movements occur has been shown to influence coordination between segmental pairs of legs and the duration of motor activity in some arthropods (Miller, 1972; Hoyle, 1976; Reingold and Camhi, 1977; Spirito and Mushrush, 1979; Delcomyn, 1987). We analyzed EMG activity from adult prothoracic legs during airstepping to determine the extent to which the lack of contact with the subtratum influenced bilateral coordination. In addition to lacking sensory inputs associated with a stance phase, other sources of altered or unique sensory inputs, such as postural demands or changes in motion-dependent feedback, could influence the motor patterns during airstepping. Unlike the continually modulated output with variable left–right phasing commonly seen during searching movements in cockroaches (Delcomyn, 1987), airstepping in *M. sexta* is characterized by well-defined bursts of activity and regular alternation between left and right legs. Examples of motor activity during airstepping and the average coordination pattern of motor activity are shown in Fig. 8. In contrast to walking, during
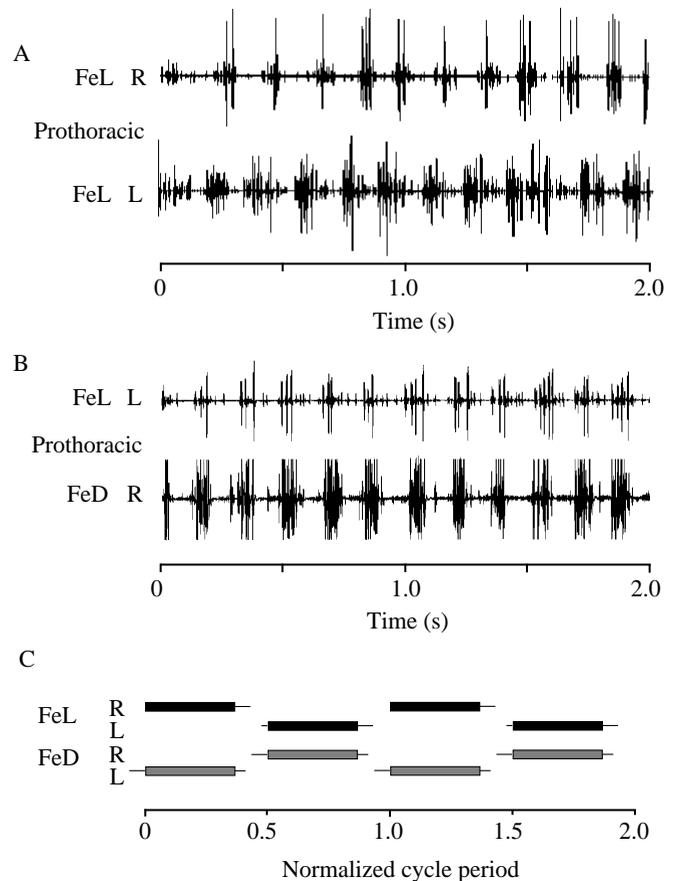

Fig. 8. (A,B) Examples of EMG activity during airstepping by the prothoracic legs of two different *M. sexta* adults. (C) Bar graph summarizing the quantified EMG recordings during airstepping in adults. EMG data according to legends to Fig. 3.

airstepping, the durations of FeL and FeD activity were shorter (Fig. 8A,B) and the cycle period was faster (0.17±0.02 s, *N*=5). Despite the changes in duration and cycle period, the phasing between left and right agonists continued to alternate during airstepping (e.g. left FeL with respect to right FeL: 0.50±0.04, *N*=5) and did not differ significantly from the corresponding phasing during walking (Tukey's HSD, *P*=0.466) (Figs 6C, 8C). In addition, as in walking, the normalized duration of FeL activity (0.38±0.06, *N*=5) was similar to the duration of FeD activity (0.38±0.06, *N*=5) (ANOVA, *P*=0.001) (Fig. 8C). However, the normalized burst durations during airstepping were significantly shorter than either FeL (ANOVA, *P*=0.001) or FeD (ANOVA, *P*=0.001) burst durations during walking (Figs 6C, 8C).

In contrast to walking, the phase relationships between contralateral antagonists showed that the onset of bursting occurred simultaneously. For example, during airstepping, the phase values indicated that right depressor activity (0.50±0.04, *N*=5) and left levator activity (0.50±0.04, *N*=5) occurred synchronously (Tukey's HSD, *P*=0.664) (Fig. 8C). During walking, there was a phase delay between the right depressor (0.31±0.04, *N*=5) and the left levator (0.47±0.06, *N*=5) (Tukey's HSD, *P*=0.008) (Fig. 6C). In addition, during airstepping ipsilateral FeL activity and FeD activity no longer overlapped as they did during walking in adults.

Unlike in walking, the variability in the burst duration of either levators or depressors in airstepping adults could not be related to changes in cycle period. Regression analysis demonstrated only non-significant relationships between burst duration and cycle period for airstepping (Fig. 9A,B). This suggested that the strong linear relationship between burst duration and cycle period characterizing walking was due to

modulation from unidentified sources of sensory inputs associated with the stance phase or other sources of sensory input. However, the dependence of the timing of burst onset in airstepping paralleled the results characterizing walking such that the timing of FeL activity showed a dependence on phase and FeD activity showed a dependence on time (Fig. 9C,D). Thus, the timing of bursting activity in the prothoracic legs in adults is independent of contact with the subtratum.

Discussion

Comparisons of crawling in larvae and walking in adults

The primary purpose of this study was to describe the locomotory behavior and pattern of motor activity during crawling in larvae and walking in adult moths. Our main findings were that crawling and walking differed in cycle period, intersegmental coordination and intrasegmental coordination. Briefly, larval crawling is characterized by synchronous movements between left and right segmental pairs of legs, which proceed slowly from posterior to anterior segments. In contrast, walking in adults is characterized by fast, alternating movements of the left and right prothoracic legs and more variable coordination patterns (ranging from synchrony to alternation) in the mesothoracic and metathoracic legs. These differences are not surprising given the change in overall body and leg morphology between the larval and adult stages in *M. sexta*. In addition, these differences may reflect the extent to which the centrally located, neural circuits that produce thoracic leg activity in each stage have been reorganized during metamorphosis (Johnston and Levine, 1995).

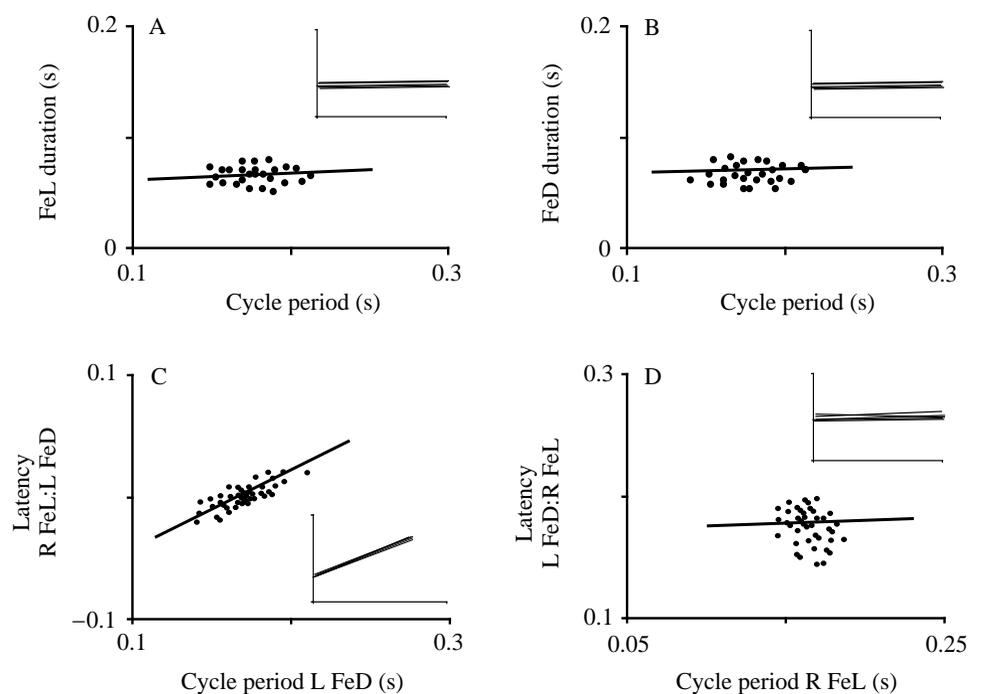Adult walking is substantially faster than larval crawling.

Fig. 9. Regression analysis for burst duration (A,B) or latency to burst onset (C,D) *versus* cycle period for FeL and FeD activity during airstepping in adults. See legend to Fig. 4 for definitions and details. Each plot contains 50 data points: 10 values from each of five animals. Coincident points are superimposed. The individual regressions for each animal (shown in the insets) do not differ statistically from one another. (A) *P*=0.2647. (B) *P*=0.4149. (C) *β*=0.62, *r*²=0.71, *P*=0.0006. (D) *P*=0.6124.

However, when the cycle period of crawling is adjusted to represent only the frequency of thoracic activity, the cycle periods for crawling in wandering larvae fall within the range for walking in adults. It is not known what induces the higher frequency in wandering larvae *versus* feeding larvae or whether wanderers and adults use the same mechanisms for establishing frequency. These data reveal, however, that early in the wandering period the larval nervous system has the potential to produce coordinated, rhythmical movements of the thoracic legs at a frequency similar to that of adult movements. It will be interesting to determine whether the frequency of leg movements is influenced by the actions of the steroid hormone 20-hydroxyecdysone, which induces wandering (Dominick and Truman, 1986*b*) and plays a number of other roles during postembryonic development in *M. sexta* (for a review, see Weeks and Levine, 1990).

The extent of intersegmental coordination during locomotion differed between larvae and adults. During crawling in larvae, thoracic leg movements could occur in the absence of any observable crawling activity in the abdominal segments. Furthermore, stepping could occur in one, two or all three pairs of thoracic legs. Thus, these data suggest that the mechanisms that generate crawling movements in the thoracic legs can be uncoupled from one another and can also be uncoupled from the mechanisms that generate crawling movements in the abdomen. The production of extra steps by larvae crawling on a wooden dowel may reflect sources of sensory modulation that are not experienced by larvae and wanderers crawling on a flat, level surface. In addition, extra steps may be correlated with sensory modulation associated with changes in stride length, although we did not measure this variable.

Unlike larvae, intersegmental coordination during walking in adults is highly variable. The variability in stepping patterns appears to be associated mainly with the variable intrasegmental movements in the mesothoracic and metathoracic legs. In contrast, the prothoracic legs produce very consistent stepping patterns. It has yet to be shown whether more consistent patterns of intersegmental coordination are expressed by the mesothoracic and metathoracic legs in adults in other situations, such as walking on a level surface or during airstepping where the requirements for balance and weight support are reduced (see below).

Larvae and adults also show differences in the patterns of intrasegmental coordination used during crawling and walking. Larvae produce only synchronous movements between left and right legs within a segment. In contrast, the phase relationships for pairs of legs during walking in adults are quite variable and range from synchrony to alternation. However, this variability is only seen for the mesothoracic and metathoracic legs; the prothoracic legs always produce a reliable pattern of alternation between the left and right legs. Furthermore, during airstepping in adults, the consistent alternation between left and right legs in the prothoracic segment is not affected by the absence of various types of sensory inputs, such as those associated with weight-bearing and force generation. Thus, mechanisms producing intrasegmental coordination appear to constrain the output to bilateral synchrony in larvae and bilateral alternation in the prothoracic legs of adults.

Finally, larvae and adults differ in their patterns of antagonistic muscle activity within a leg. In both larval crawling and adult walking (prothoracic legs only), antagonistic muscles of the same leg alternate with one another. However, crawling shows a brief period of levator activity that cleanly alternates with a longer period of depressor activity. In contrast, during walking, the levator and depressor bursts are equal in duration and show large periods of overlapping activity. In isolated larval nerve cords, the absence of phasic sensory input does not influence the normalized duration and phase of motor activity during fictive crawling (Johnston and Levine, 1995). However, the strict reciprocity between femoral levators and depressors and the uniform reduction in their durations during airstepping in moths suggest that, in the adult stage, phase and burst durations are influenced by sources of sensory inputs, such as those associated with the weight-bearing and postural demands during walking. These data also indicate that the duration of femoral levator and depressor activity in adults is modulated to the same extent or possibly in tandem under these conditions. Regression analysis of burst duration and cycle period in adults supports the idea that both levator and depressor activity is regulated in a similar manner in the presence or absence of sensory feedback related to weight bearing. The symmetrical nature of levator and depressor activity may reflect the properties of the central circuits that control the prothoracic legs.

In contrast to adults, analysis of activity duration *versus* cycle period relationships suggests that depressors and levators are differentially controlled during crawling in larvae. This feature of crawling is typical of terrestrial locomotion in many animals (reviewed by Grillner, 1981; Jacobson and Hollyday, 1982; Bässler, 1986*a*). Work in various vertebrate systems has shown that the asymmetrical relationship between antagonistic muscles is a product of sensory modulation associated with weight-bearing during the stance phase of walking and is not a feature of the underlying neural circuits producing the patterned locomotor activity (e.g. cat, Grillner and Zangger, 1979; turtle, Lennard and Stein, 1977; chicken, Bekoff *et al.* 1987). This may also apply to our data on crawling in caterpillars. Kent and Levine (1988*a*) showed that sensory bristles covering the larval thoracic legs influence the activity of leg motor neurons in *M. sexta*. Although sensory information plays a prominent role in establishing and maintaining many features of the leg activity during locomotory behavior in various insects (Delcomyn, 1985*b*; Bässler, 1986*b*) and probably influences larval leg movements in *M. sexta*, our observations of fictive crawling in isolated nerve cords suggest that the levator–depressor asymmetry is a feature of the central circuits that generate patterned motor output during crawling in larvae (Johnston and Levine, 1995).

In addition to the differences described above, we also identified common features for the control of motor activity in larvae and adults. In both larvae and adults, the onset of levator

activity is phase-dependent and the onset of depressor activity is time-dependent. Perhaps the premotor neural elements that produce this feature in larvae survive metamorphosis and are re-used in a similar manner in the generation of adult walking. Knowledge of the behavioral and motor profiles for crawling in larvae and walking in adults, as described in this paper, will facilitate our work on the functional significance of the changes in central and peripheral elements of the thoracic legs during postembryonic development.

The differences in leg coordination between larval and adult stages of development may involve changes on several levels. For example, the adult legs support a new complement of external receptors and internal proprioceptors (Kent and Griffin, 1990) as well as the persistent larval internal proprioceptors (C. Consoulas, R. M. Johnston and R. B. Levine, unpublished data). Thus, behavioral context and alterations in sensory feedback may contribute to the generation of the new adult locomotor patterns. Differences between the properties of larval and adult muscles and their innervation patterns may also contribute. Changes in the dendritic morphology of the leg motor neurons (Kent and Levine, 1988*a,b*, 1993) may be linked to the loss of larval, and the acquisition of new adult, synaptic inputs from sensory neurons and interneurons (Levine and Truman, 1982; Levine, 1986; Jacobs and Weeks, 1990; Sandstrom and Weeks, 1991; Waldrop and Levine, 1992; Streichert and Weeks, 1995). Interneurons play a key role in organizing the premotor inputs that recruit functional subsets of leg motor neurons and, thus, produce different coordination patterns in different types of behavior (Siegler, 1985; Laurent and Burrows, 1989; Burrows, 1992). Postembryonic neurogenesis in *M. sexta* adds new interneurons to the thoracic ganglia during metamorphosis (Booker and Truman, 1988; Witten and Truman, 1991) and may provide novel inputs to the existing thoracic motor neurons. Finally, alterations in the biophysical properties of the thoracic motor neurons may contribute to developmental changes in thoracic leg activity (Hayashi and Levine, 1992).

Comparisons with other insects

Crawling in insect larvae has been examined on many levels, including locomotor performance and energetics (Casey, 1991; Joos, 1992), coordination of abdominal activity (Kopec, 1919; Barth, 1937; von Holst, 1939; reviewed by Hughes, 1965) and proprioceptive reflexes (Weevers, 1965) during crawling, and the role of descending inputs and hormones on the generation of crawling (Dominick and Truman, 1984, 1986*a,b*). However, other than this report, we are aware of only one other study that has examined the behavior of the thoracic legs during crawling in insect larvae (Wendler, 1964). Wendler's work on crawling in beetle larvae (*Cantharis fusca*) shows both similarities with and differences from our data. *M. sexta* larvae produced only synchronous movements between segmental pairs of thoracic legs even on different substrata and during the wandering phase of larval development. However, although beetle larvae prefer to use synchronous movements, they also produced variable phase relationships as well as alternating

movements between left and right legs of the same thoracic segment during crawling (Wendler, 1964).

In contrast to larvae, adult insects have been the subject of numerous studies examining the behavioral and motor profiles of the thoracic legs during walking and related behavior patterns (Bässler, 1983; Delcomyn, 1985*a*; Gewecke and Wendler, 1985; Graham, 1985). Although many insects show both inter- and intrasegmental coordination in the form of a tripod gait during walking, it is not the only stepping pattern expressed (Delcomyn, 1985*a*). Various studies have shown that gait depends on the speed of walking. For example, insects that walk quickly, such as cockroaches, show a more variable, tetrapod gait during slow walking and a tripod gait at the more common faster walking speeds (Delcomyn, 1985*a*). Other insects that walk slowly, such as stick insects, show a continuous gradation of tetrapod stepping over most of their range and a tripod coordination at the fastest walking speeds (Graham, 1972; Bässler, 1983). In *M. sexta*, we were not able to detect a consistent gait at any frequency.

In adult *M. sexta*, grasshoppers (Graham, 1978*a,b*) and locusts (von Holst, 1943), legs in adjacent segments can step at different frequencies relative to one another during free walking. However, unlike *M. sexta*, in other insects the differences in frequency can occur acutely, resulting in missing steps (Graham, 1978*a,b*), or in a regular manner, producing continuous cycles of walking where one leg takes more steps than the other (e.g. 3:2, 2:1; Foth and Bässler, 1985; also see Chasserat and Clarac, 1980, 1983). With the exception of the strong bilateral coupling between prothoracic legs, walking in adult *M. sexta* lacked intersegmental and intrasegmental coordination when the pattern was examined by accounting for cycle-to-cycle variability in frequency and phasing.

Our inability to detect a consistent pattern during walking in *M. sexta* does not indicate that adult moths are unable to produce more stereotyped, patterned stepping during walking and may reflect the demands, such as postural instability, of walking on an incline. Interlimb coordination appears to be more varied and complex when walking on an inclined surface in both cockroaches (Spirito and Mushrush, 1979) and locusts (von Holst, 1943). We also noticed that ipsilateral frequency coupling, if it occurred, was usually present only on one side of the moth. Although we selected for analysis animals that appeared to walk straight, this result may indicate a differential load on each side of the body (Foth and Graham, 1983*a,b*; Dean, 1991) as might occur during gradual turning. More consistent stepping patterns may be produced when adult moths walk on a flat surface or under the regulated environment of treadmill walking (Bässler, 1983; Chasserat and Clarac, 1983; Graham, 1985). In addition, experiments that measure spatial (joint angles and elevation) and geometric (e.g. Cruse *et al.* 1983; Cruse, 1990) variables may reveal coordination mechanisms that are not apparent from temporal features alone. The variable pattern defining walking in adult *M. sexta*, however, may reflect constraints on the system other than those imposed by our experimental approach. Because flight is the main mode of locomotion and escape in adult

moths, there has probably been little selective pressure for establishing a precise, yet modifiable, intersegmental coupling that is characteristic of the walking of other insects (e.g. stick insects). Furthermore, the large abdomen of adult moths probably influences the coordination patterns produced during walking on various substrata.

Despite the lack of consistent coupling among the three thoracic segments, the stepping patterns of the prothoracic legs were highly regular in adult *M. sexta*. The regulation of step duration for the prothoracic legs showed the same trend as that seen in other insects using a tetrapod gait; the duration of movements during the swing phase remained relatively stable, whereas the duration of movements during the stance phase varied in a linear manner with changes in cycle period (e.g. Burns, 1973; Delcomyn, 1973; Graham and Wendler, 1981). Unlike the strong reciprocity between antagonistic muscles characteristic of walking in other insects (Delcomyn and Usherwood, 1973; Graham and Wendler, 1981), antagonistic muscles of the same leg showed a high degree of overlapping activity during walking in adult *M. sexta*. Many of these differences and other variations in walking strategies used by many insects, including *M. sexta*, as well as other arthropods, may be related to morphological differences and functional specializations of the thoracic legs (Full, 1993; Cruse, 1990).

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