

## **EFFECT OF LOAD ON LEG MOVEMENT AND STEP COORDINATION OF THE STICK INSECT *CARAUSIUS MOROSUS***

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### **Summary**

A treadwheel mounted on a torque motor was used to study changes in step coordination as a function of the magnitude and direction of external forces resisting and assisting leg retraction. The apparent contradiction in the literature concerning the shift in the posterior extreme point is shown to depend upon the magnitude of the load. External forces resisting leg retraction generally shift step end-points rostrally and those assisting leg retraction shift them caudally, shifting leg movement so that the active muscles can provide greater force. However, this primary trend reverses for external forces equivalent to or less than the weight of the body. Together, these results indicate that the decision to end stance does not simply depend upon the leg's loading but rather on its efficiency in contributing propulsion. As expected, loads resisting leg retraction decrease retraction speed and increase step period. They also decrease protraction speed, increase swing duration and shift phase relationships of adjacent ipsilateral legs away from alternation towards a metachronal pattern. Moderate to large assisting loads evoke similar changes: they increase swing duration less but shift the phase of middle and rear legs more than the corresponding resisting loads. Thus, by itself, a reduction in the force required during retraction does not transform the gait from metachronal to tripod. The mean phase of contralateral leg pairs is unaffected by load: these legs continue to alternate.

### **Introduction**

One advantage of legged locomotion is the ability to walk on the level and climb up and down vertical surfaces using the same motor apparatus. This ability requires the motor system to produce different patterns of forces in order to propel the body forward and counteract the pull of gravity. Thus, it is not surprising that the load on a leg is one factor affecting leg placement and step timing. This influence has been demonstrated in several different insects and crustaceans using

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various experimental methods (for reviews, see Cruse, 1983; Evoy and Ayers, 1982; Graham, 1985).

Different experimental methods simulate different aspects of the loads actually confronting a walking insect. Some loads change rapidly, such as the resistance incurred in pushing past obstacles or the gravitational components parallel and perpendicular to the direction of locomotion, which vary with orientation. Others, such as inertial forces and the total gravitational force, change more gradually as the insect grows. Thus, loads can appear as inertial terms dependent upon velocity changes, gravitational forces dependent only on the orientation and direction of motion, frictional terms dependent on velocity, or spring-like forces dependent upon some displacement parameter.

For the short-term control of walking, the pull of gravity is probably the most important variable. It influences two parameters. First, it affects the pattern of forces needed to maintain an appropriate distance and orientation between the body and the substratum. This influence includes any gravitational component perpendicular to the substratum as well as torques from components parallel to the substratum. Second, any gravitational component parallel to the direction of locomotion affects the force needed to propel the body.

Thus, the function of the legs can be separated into two parts – forward propulsion and maintenance of an appropriate distance from the substratum. The former is perhaps the simplest to analyse. The influence of load has been investigated in several reports using different methods; in one respect, the results appear inconsistent. On the one hand, the mean end-points of swing and stance (anterior extreme position, AEP; posterior extreme position, PEP) for middle and rear legs shift rostrally when stick insects pull against a constant weight (Bässler, 1977). Similar rostral shifts occur when a stick insect walks up a vertical path (Cruse, 1976). Both situations change support requirements as well as propulsion requirements. Rostral shifts also occur in the AEP and PEP of all three leg pairs when stick insects walk supported over treadwheels subjected to frictional loading (Foth and Graham, 1983*a,b*). On the other hand, unloading in the direction of leg retraction is one factor increasing the probability that a leg will end its stance and begin a swing; thus, an increase in load for an insect supported over a treadwheel causes a caudal shift in the PEP (Cruse, 1985*c*).

Besides these changes in step end-points, several investigators have noted effects of load on step timing. These include intraleg changes in the relative durations of swing and stance and interleg changes in coordination (Evoy and Fournier, 1973; Foth and Graham, 1983*a,b*; Spirito and Mushrush, 1979; reviewed by Cruse, 1983). Qualitatively, increased load shifts the step pattern from an alternating tripod (insects) or tetrapod (crustaceans, arachnids) gait towards a metachronal gait.

Many of the methods used to modify load, particularly for free-walking animals, also influence the forces needed to support the animal. Thus, changes in leg placement or step timing cannot be related unambiguously to one kind of load. The present study presents a detailed analysis of the effects of loading parallel to

the direction of locomotion while the distance between body and substratum was held constant. The primary goal was to investigate the apparent contradiction in the effect of load on spatial parameters of leg movement.

### Materials and methods

Data were collected from adult female stick insects, *Carausius morosus* Br., walking on a treadwheel. The insects were taken from laboratory colonies in Bielefeld. Leg coordination was recorded using previously described methods (Dean, 1984). A holder was attached dorsally to the insect along the tergum of the head, thorax and rostral abdomen. Then the insect was positioned at a natural height (approximately 8 mm) above a light, Styrofoam walking wheel. The wheel (diameter 40 cm, width of walking surface 18 mm, moment of inertia equivalent to 4 g at the rim) was mounted on the axis of a 12 V d.c. motor (RS Components Ltd, type 336-292), which allowed torques of different strengths and direction to be applied. These torques varied the amplitude and direction of the force needed by the insect to maintain a given retraction velocity.

Leg movements in the rostrocaudal direction were registered using a video camera attached to a video interface (Digisector DS-65) in an Apple IIe computer (Godden and Graham, 1983). This system detects the intersection of the femur with a scan line parallel to the long axis of the body and 3–3.5 mm lateral to the subcoxal joint of the middle leg. Leg position is sampled every 40 ms. It is measured with respect to an arbitrary position caudal to the rear leg; the rostral direction is positive. This is the reverse of the usual convention (Bässler, 1972), but the change was made to conform with the intuitive direction of forces representing an increased load. These position values can be converted into femur angles relative to the long axis of the body, but this conversion has several disadvantages. First, the measured position of the leg insertion is inexact because the subcoxal joint is partially hidden beneath the body and because the resolution of the video system is restricted. Second, these angles are not exactly equal to either the angle at the subcoxal joint or the angle to the tarsus because the axis of the subcoxal joint is tilted out of the vertical and the femur rotates about its long axis during a step. For the same reason, the measurement is only approximately proportional to the change in tarsus position. Therefore, in most cases, leg position is presented in terms of video units.

Data were recorded from sixteen animals ranging in mass from 0.80 to 1.22 g. The added torques corresponded to a tangential force at the rim of 4.9, 9.8, 19.6, 29.4 and 39.2 mN. These loads are equal to 0.5, 1.0, 2.0, 3.0 and 4.0 times the force gravity exerts on an animal of average weight. To make this relationship clear, the load conditions will be designated 0.5×, 1.0×, 2.0×, 3.0× and 4.0×. Forces resisting leg retraction, the power stroke of forward walking, are defined as positive. For each animal, walks were recorded first with no added torque. Then the motor was turned on and the voltage adjusted to one of the standard values. Walks were recorded with the motor applying first a positive and then a negative

load. Before testing a new torque, one or more control walks were recorded. For eight animals, the added torques were tested beginning with the smallest and ending with the largest torques. For the others, intermediate forces ( $1\times$  or  $2\times$ ) were tested first, then smaller torques and finally larger torques. Fourteen animals showed steady walking at 8 or more of the 11 torque conditions. In most cases, the animals were stimulated to walk at a more or less steady speed by touching the abdomen.

The records of leg movement *versus* time were stored on diskette and then plotted. Sequences of steady walking were identified in the plots and the points marking the transitions between swing and stance were re-entered into an Apple IIe using an Apple graphics tablet. Total numbers of steps for each leg ranged from 60 to 82 and 330 to 368 for  $4\times$  assisting and resisting leg retraction, respectively, to about 3400 for control walks. These step data were used to calculate statistics for step end-points, durations of swing and stance, step period and phase relationships of ipsilateral and contralateral leg pairs.

Several statistical tests were used to evaluate overall trends and pair-wise differences. Parametric tests (*t*-test, Watson-Williams two-vector comparison) were used for the latter purpose because the distributions were approximately normal and *N*, the number of steps, was large. A non-parametric test (Spearman rank correlation coefficient,  $\rho$ ) was used to evaluate trends in means, because *N*, the number of load conditions, was small and the independent variable, load, was not normally distributed. However, parametric regression coefficients were also calculated to provide a measure of the rate of change with load.

## Results

Stick insects subjected to loads equivalent to or less than the weight of the body walked with a coordination not obviously different from that of control walks (e.g. Fig. 1A,B,C). In most cases, step period was short and the coordination was approximately that of alternating tripods. Applied forces equivalent to 3 or 4 g, much exceeding the weight of the insect, caused several changes which varied with the direction of the applied force.

Large forces resisting leg retraction reduced the speed of retraction and lengthened the step period (Fig. 1E). They also caused the range of leg movement to shift forward. In some insects, this forward shift was accompanied by a change in the targeting behavior, the coordination mechanism that guides the swing movement of a leg to an end-point close to the position of the adjacent rostral leg

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Fig. 1. Examples of step coordination under different applied torques. The six traces show the movement of the femur as registered by the video system. Leg protraction (Pro) and retraction (Ret) correspond to upward and downward changes, respectively, in the trace. Legs are numbered from front to back and designated as left or right. The load conditions are as follows: (A), control walk, no added load; (B), 4.9 mN ( $-0.5\times$ ) assisting; (C), 9.8 mN ( $1\times$ ) resisting; (D), 9.8 mN ( $-1\times$ ) assisting; (E), 29.4 mN ( $3\times$ ) resisting; and (F), 29.4 mN ( $-3\times$ ) assisting.

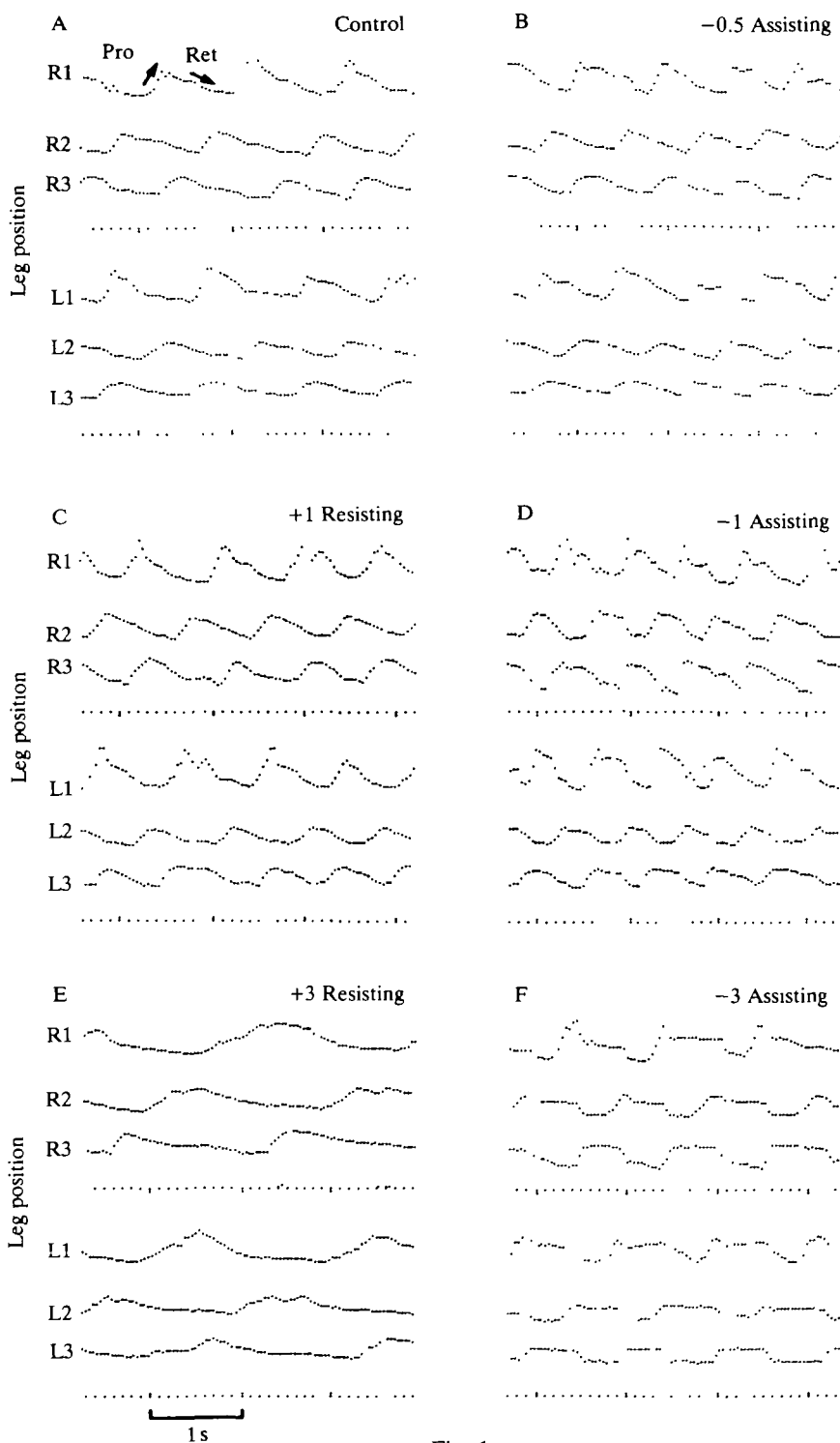


Fig. 1

(Cruse, 1979; Dean and Wendler, 1983). Qualitatively, moderate loads appeared both to increase the consistency of the targeting and to decrease the distance between the anterior extreme position (AEP) of the moving leg and the position of the target leg. The largest loads appeared to shift the swing end-point still further; the middle leg often swung medially onto the top surface of the wheel instead of grasping its edge. Over the course of a walk, they also caused the swing movements of front legs, and to a lesser extent those of middle legs, to become quite slow and prolonged (Fig. 1E). These slow protraction movements appeared cramped and the tarsus tapped down onto the wheel several times during its swing forward.

Large forces assisting leg retraction were qualitatively more disruptive than those resisting retraction. The insects were less willing to walk at all and often preferred to walk backwards, i.e. against the load. The insects were also more likely to lose control of the wheel under these conditions. These assisting torques caused the range of leg movement to shift caudally. The ipsilateral legs adopted a metachronal pattern and the contralateral legs stepped in diagonal pairs (Fig. 1F). Forward progression was quite uneven (Fig. 1F and, to a lesser extent, Fig. 1D). The normal oscillation in forward progression (Graham, 1983) was exaggerated; each stance contained a period of extremely rapid retraction preceded and followed by periods of little or no retraction. Thus, each step cycle contained two periods of rapid retraction, coinciding with the swings of each rear leg and the contralateral middle leg.

The quantitative analysis of spatial parameters confirmed these qualitative observations. Several trends were evident. Because the individual variation was considerable, these trends will be illustrated with results from one animal (Fig. 2A) as well as the overall average (Fig. 3).

The primary trend was a linear shift in the mean AEP and PEP in the opposite direction to and roughly proportional to the magnitude of the applied force (e.g. all but L1 AEP and L3 PEP in Fig. 2A). For individual animals, this positive correlation was significant at  $P < 0.05$  to  $P < 0.001$  (Spearman rho, one-tailed) in about two-thirds of the cases (6 legs of 15 animals; the one animal omitted was tested in only 5 torque conditions). The trend was most consistent in middle legs (50 of 60 cases: AEP and PEP of both middle legs in 15 animals), weaker in rear legs (40 of 60 cases) and weakest in front legs (30 of 60 cases). For middle and front legs, the trend was equally frequent in AEP and PEP (middle leg: 27 AEP *versus* 23 PEP, front leg 14 AEP *versus* 16 PEP); for rear legs, it was more often significant in the AEP than in the PEP (25 *versus* 15 cases).

The across-animal averages demonstrate five features related to this primary trend and segmental differences therein. First, the load-induced changes were larger, more consistent and more nearly monotonic in middle and rear legs (Fig. 3). In these legs, the primary trend was significant for both AEP and PEP. The size of the shift, measured as the difference between maximum and minimum values, ranged from 29 % of control step amplitude (R2 PEP) to 66 % of control step amplitude (R3 PEP). In regressions of mean values *versus* applied force,

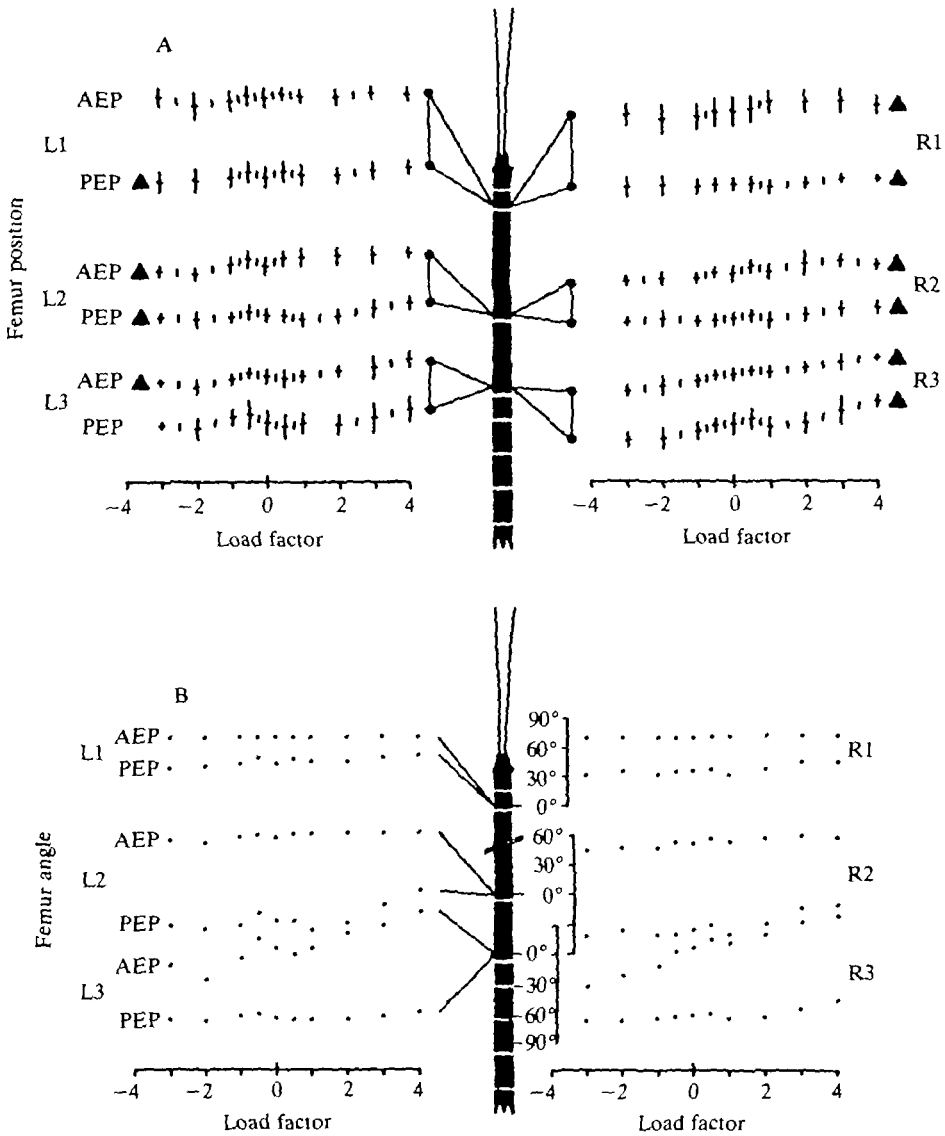


Fig. 2. Mean anterior extreme position (AEP) and posterior extreme position (PEP) for one animal as a function of applied torque. Means and standard deviations for step end-points of all six legs are plotted as a function of load. The rostrocaudal position of the femur is given both in video units (A) and in angles relative to the long axis of the body (B). The location of the leg insertion is indicated by the schematic drawing of the animal. A leg at right angles to the body corresponds to  $0^\circ$  in B. Positive loads resist leg retraction. The numbers of steps in the different means for this animal range from a minimum of 24 in the  $-3\times$  group and 34 in the  $4\times$  group to 272 in the control group. The short vertical lines between means indicate pair-wise differences significant at  $P < 0.05$  in *t*-tests. A triangle at the left or right end of a set of means in this and later figures shows that the overall trend is significant at  $P < 0.05$  in a Spearman non-parametric test and indicates whether the correlation is positive ( $\blacktriangle$ ) or negative ( $\blacktriangledown$ ).

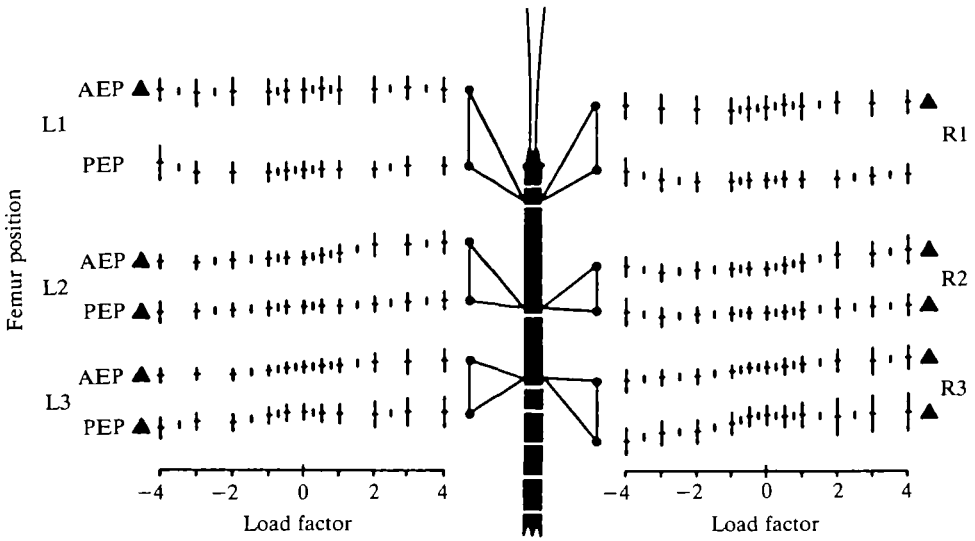


Fig. 3. Mean AEP and PEP as a function of applied torque: across-animal averages. The format is that of Fig. 2. Means for each end-point and each animal were normalized to make the value for the control walk a constant. The number of steps in the across-animal averages in this and later figures is lowest for the  $-4\times$  condition (59–82); otherwise it ranges from 332 to 1674 for different load conditions and up to 3479 for control walks.

coefficients were between  $0.076\pm 0.026$  (R2 PEP) and  $0.202\pm 0.067$  (R3 PEP) video units per mN (equivalent to  $0.74\pm 0.26$  and  $1.98\pm 0.65$  video units per g).

The load-induced changes in the front legs were less monotonic and the overall shifts smaller. The curves have an asymmetrical U-shape with mean positions for moderate assisting loads or no loads more to the rear and mean positions for larger loads in either direction more forward (Fig. 3). A linear dependence upon load over the whole range was significant only for the AEPs. Differences between maximum and minimum values were between 8% of step amplitude (L1 AEP) and 14% of step amplitude (R1 AEP). Regression coefficients were correspondingly smaller:  $0.023\pm 0.017$  and  $0.057\pm 0.032$  video units per mN ( $0.23\pm 0.17$  and  $0.56\pm 0.31$  video units per g). For loads between  $-2\times$  and  $+4\times$ , all four step end-points of the front legs showed a significant positive correlation. Within this range, regression coefficients were between  $0.029\pm 0.026$  and  $0.100\pm 0.032$  video units per mN ( $0.28\pm 0.25$  and  $0.98\pm 0.31$  video units per g).

Second, with increasing load, the mean AEPs of middle and rear legs moved farther forward than did the PEPs of the front and middle legs, respectively, resulting in a decreased separation of adjacent ipsilateral legs. This change indirectly reflects the qualitative improvement in the consistency of the targeting behavior noted above. (Quantitative measurements of the actual spatial separation were not feasible.)

Third, the measured changes in femur position corresponded to considerable



changes in femur angle relative to the long axis of the body (Fig. 2B); in the across-animal average, these were as large as  $60^\circ$ . The largest shifts in leg angle were for the AEP of the rear leg and the PEPs of front and middle legs: these are the positions for which the femur is more nearly at right angles to the body, so a given change in tarsus position corresponds to a larger change in angle.

Fourth, when resisting and assisting loads were compared separately to the control values, loads resisting retraction elicited larger changes in front and middle legs, whereas loads assisting retraction elicited larger changes in rear legs.

Finally, the relationship between step end-points and applied load was usually not monotonic, even when the primary trend was significant. One non-linearity was simply variability in the means, which was particularly evident for the largest load conditions. The second, more systematic non-linearity was restricted to small torques, where it constituted a secondary trend running counter to the primary trend. Reversals in the primary trend meant that step end-points shifted in the opposite direction to the change in loading, producing regions of negative correlation between mean position and load (e.g. L3 PEP for  $-0.5\times$ , 0 and  $+0.5\times$  in Fig. 2A). This secondary trend was weaker than the primary trend. Leg L3 in Fig. 2A is a particularly prominent example. More often, reversals in the primary trend that were significant in pair-wise comparisons were restricted to one or a few torque levels and the loads for which the reversal occurred varied from animal to animal. Thus, the secondary trend is not particularly evident in the across-animal average (Fig. 3). It became clearer when the linear trend was removed with a linear regression for each animal before computing the across-animal average (Fig. 4). This negative correlation was present for AEPs and PEPs of middle and rear legs for loads within the range  $-2\times$  to  $2\times$ . The total amplitude of this secondary trend amounted to between 2.7% (R2 PEP) and 22% (R3 PEP) of control step amplitudes. For front legs, the residual relationship tended to be U-shaped with more forward positions for increasing loads in either direction.

The changes in step end-points produced small and variable changes in step amplitude (Fig. 5). Differences between maximum and minimum average values ranged between 15% of control step amplitude (R3) and 25% of control step amplitude (L2), less than the shifts in end-points. Changes were most consistent for middle legs. A significant trend was present in data from 15 of 30 middle legs (15 animals), all showing a positive correlation with load (increasing step amplitude as load changed from assisting to resisting). Moreover, the amplitude calculated from the average step end-points from all the animals (Fig. 5) also showed a significant positive correlation ( $\rho > 0.7$ ,  $P < 0.05$ , regression coefficients  $< 0.088 \pm 0.013$  video units per mN). The pattern was less consistent for front and rear legs. Significant trends occurred in individual cases, but the direction of the correlation was not consistent (10 of 30 rear legs, 7 negatively and 3 positively correlated; 12 of 30 front legs, 7 negatively and 5 positively correlated). As a result, amplitudes calculated from the end-points in across-animal averages were also variable; correlations, both positive, were significant only for one rear and one front leg.

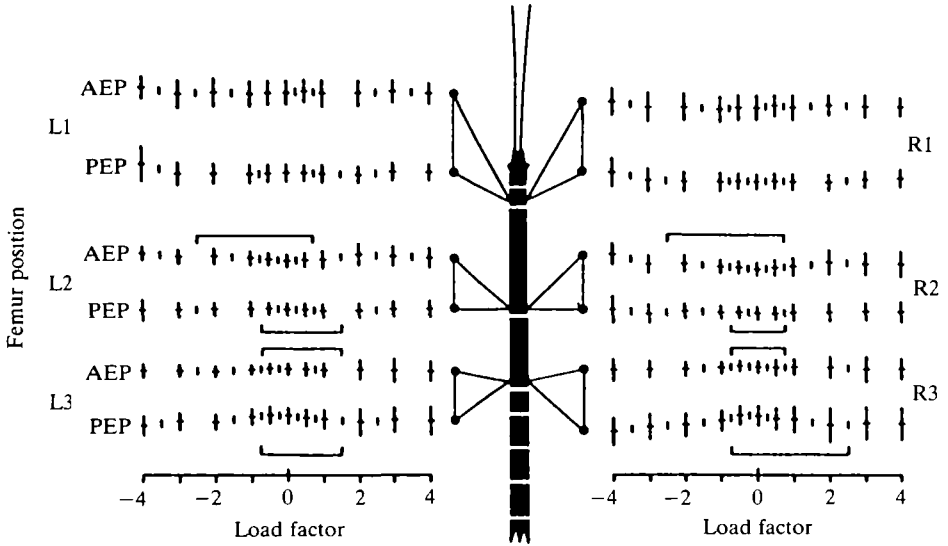


Fig. 4. Mean AEP and PEP as a function of applied torque after removing the primary trend. The format is that of Fig. 2. The linear trends in the means for each step endpoint and each animal were removed with a linear regression. The adjusted means were normalized according to the value for the control walks and then averaged. The horizontal brackets above and below means for the middle and rear legs enclose the regions of negative correlation discussed in the text.

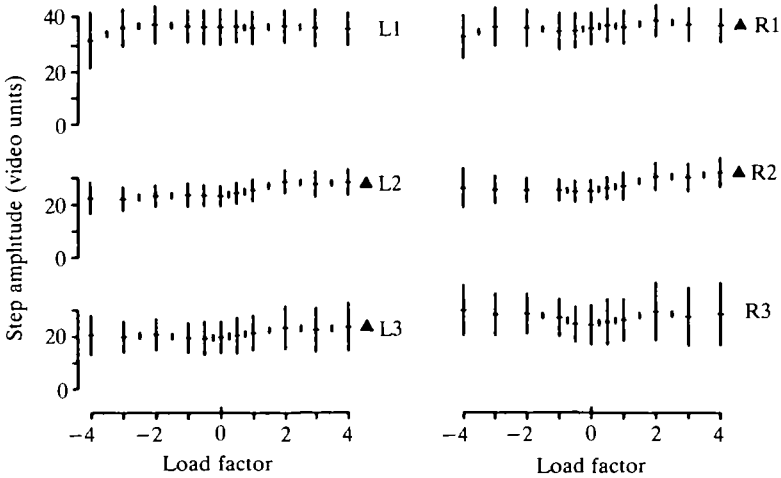


Fig. 5. Mean step amplitude as a function of applied torque. The format is similar to that of Fig. 2. The baseline for each leg pair is plotted on the left. The step amplitude shown here represents the difference between the mean AEP and mean PEP in an across-animal average computed without normalization.

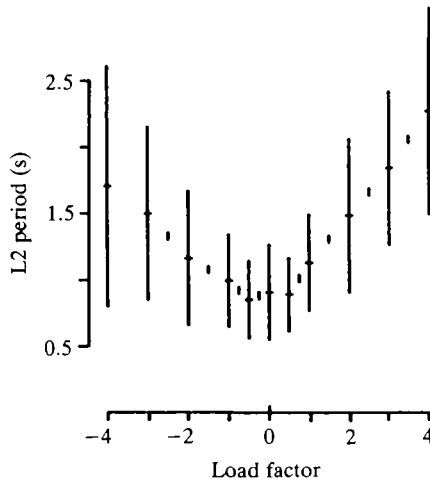


Fig. 6. Means and standard deviations for the step period of the middle leg as a function of applied load.

Changes in load produced several changes in intraleg step timing. Step period, which was considered in detail for middle legs, showed a slightly asymmetrical, U-shaped relationship to applied force (Fig. 6). Minimum periods for most animals occurred for loads of less than  $\pm 0.5\times$ ; the minimum in the overall average occurred for  $-0.5\times$  (assisting retraction). For each load value, the mean period was longer for the resisting load than for the assisting load. The increases in period with increasing load magnitude were almost linear; the regression coefficients were  $0.037\pm 0.002$  and  $-0.022\pm 0.002$   $\text{s mN}^{-1}$  ( $0.36\pm 0.02$  and  $-0.22\pm 0.02$   $\text{s g}^{-1}$ ) for resisting and assisting loads, respectively. The corresponding mean periods for the largest loads amounted to 265% and 199% of the mean for control walks.

Analogous changes occurred in the swing durations of all three legs (Fig. 7A), but in many animals the curves for assisting loads were relatively flat or, particularly for the rear leg, continued to decline. This led to a significant positive correlation over all loads for L3 in 10 animals and L1 in 8 animals. In contrast, swing duration of L2 and step period were significantly correlated with load in only 5 of 15 and 3 of 15 animals, respectively. In the U-shaped curves for across-animal averages, minimum swing durations occurred at  $-2\times$  to  $-3\times$  (assisting), 0 to  $-0.5\times$  (assisting) and 0 load for L3, L2 and L1, respectively. The mean swing durations of all three legs were longer for resisting than for the corresponding assisting loads, even when differences in mean period were taken into account. Compared to the control condition, the maximum increases were 160% and 127% (L3), 172% and 113% (L2) and 208% and 111% (L1), respectively. For all three legs, mean swing durations for assisting loads changed little for loads up to  $-2\times$  and then increased more rapidly. In contrast, mean swing durations for resisting loads increased almost linearly with load and period. Changes in the mean swing velocity, computed from the mean amplitude and mean swing duration in the overall averages, were dominated by the changes in duration (Fig. 7B); corre-

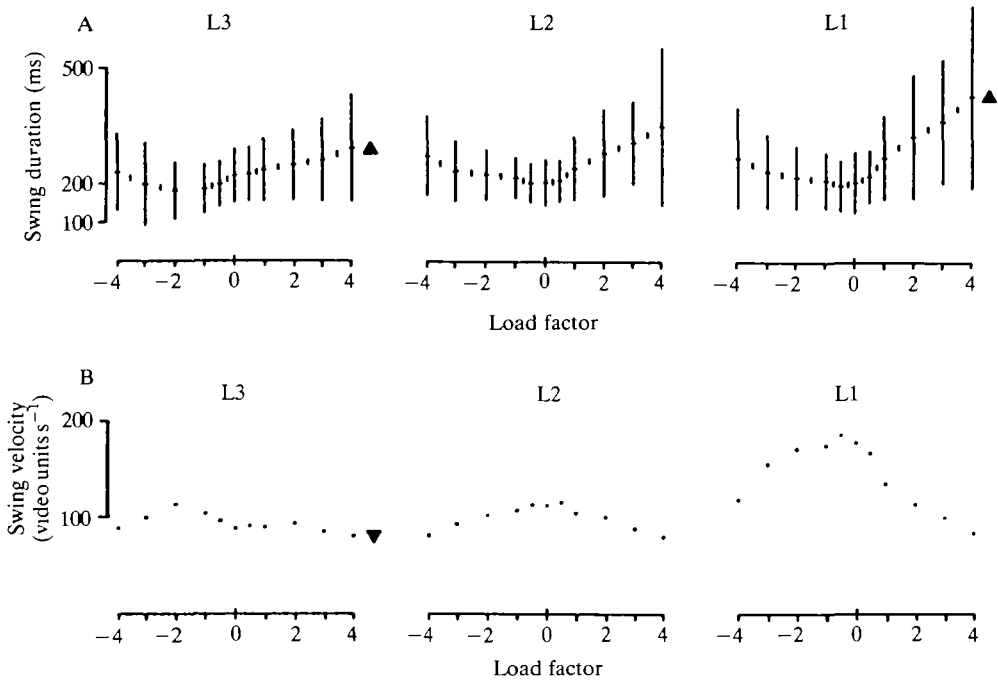


Fig. 7. Mean duration and speed of swing as a function of applied torque. Data are shown for the three left legs. (A) Mean values and standard deviations for swing duration. The vertical dashes between means indicate pair-wise differences significant at  $P < 0.05$  in  $t$ -tests; triangles at the end of a set of means indicate the direction of overall correlations significant at  $P < 0.05$  in a Spearman non-parametric test. (B) Average speed of swing is calculated here as the mean step amplitude, shown in Fig. 5, divided by the mean duration shown in A.

lations with amplitudes were weak. Within each load condition, swing durations typically showed only a weak correlation with step period but the variation from animal to animal was considerable. This was investigated for the middle legs. For loads less than  $2\times$  in magnitude, regression coefficients for swing duration on step period within individual animals ranged from 0.238 to not significantly different from zero. They changed little with load condition: means of the regression coefficients for 13 animals ranged between 0.073 and 0.093 for loads between  $+3\times$  and  $-1\times$ . The maximum occurred for  $-0.5\times$ . Mean coefficients were smaller ( $<0.05$ ) for more negative loads and larger (0.123) for  $+4\times$ .

Changes in coordination of adjacent ipsilateral legs showed a similar pattern. The relationship between mean phase and load followed an inverted U-form both for middle and rear legs and for front and middle legs. The maximum values occurred for the control condition or for  $-0.5\times$  assisting (Fig. 8): in the overall average, the values were  $148\text{--}156^\circ$  for the phase of the middle leg in the cycle of the rear leg and  $186\text{--}189^\circ$  for the phase of the front leg relative to the middle leg. The curves for front and middle legs were quite symmetrical but, for a given load,

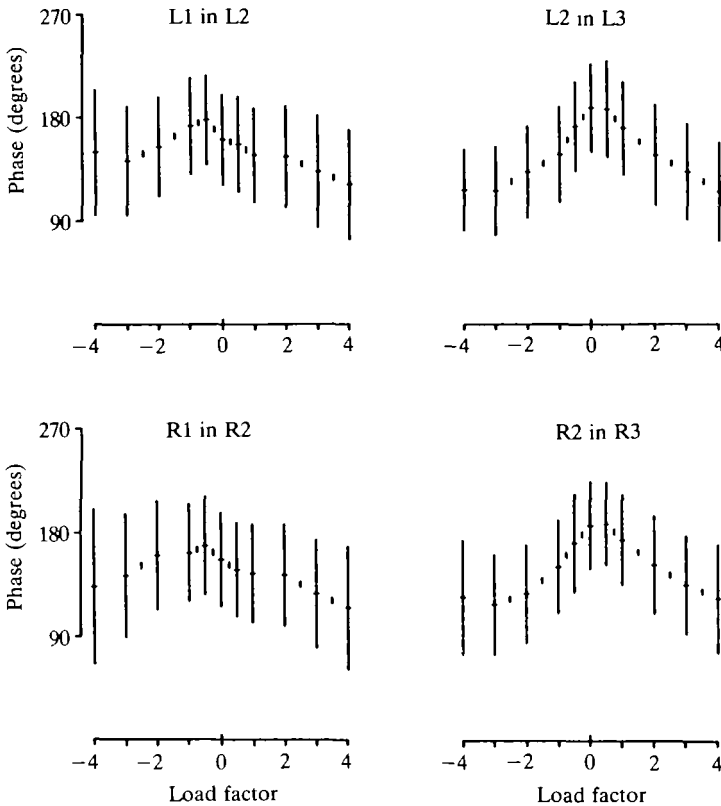


Fig. 8. Mean ipsilateral phase values as a function of applied torque. The mean phase and the circular standard deviation [ $81^\circ \times \sqrt{1-k}$ , where  $k$  is the concentration parameter or length of the mean vector] are plotted *versus* applied torque. The vertical marks between mean values indicate differences significant at  $P < 0.05$  in a Watson-Williams two-vector comparison.

the mean phase tended to be slightly lower for the resisting load. For larger loads, this difference disappeared when corrected for differences in step period (Fig. 9). Ipsilateral middle and rear legs showed the opposite pattern: mean phases were lower for assisting loads. Correction for differences in mean step period increased the difference for loads of opposite direction. In contrast to the other curves, the relationship for assisting loads was quite non-linear: for the largest load the mean phase approached the linear relationship for the resisting loads.

The relationship between step period and the duration of the metachronal sequence, the lag from one step by the rear leg to the next step by the ipsilateral front leg (3Lag1), was examined in 13 animals. Means of the within-animal regression coefficients for 3Lag1 on step period ranged between 0.62 and 0.59 for loads of  $\pm 1\times$ . They were lower for larger loads in either direction but, in each case, the coefficient for positive loads was larger than that for the corresponding negative load. The across-animal mean lags showed a similar pattern. The mean

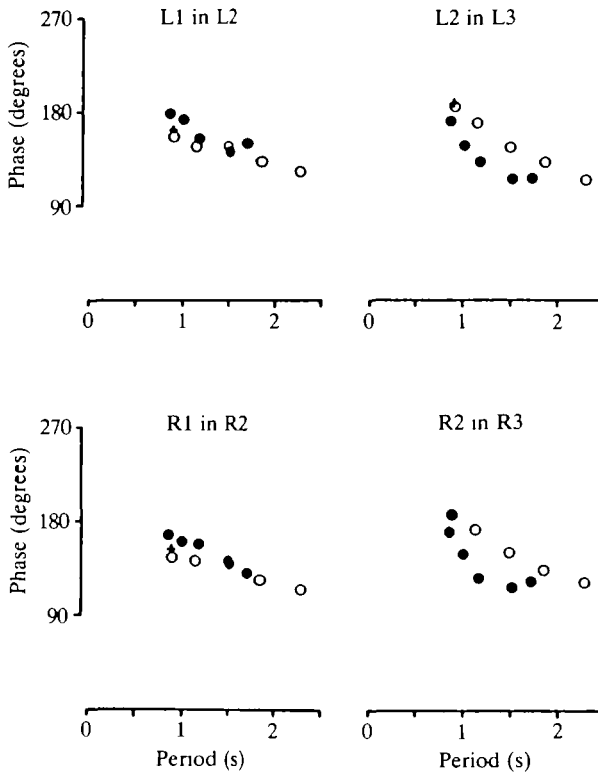


Fig. 9. Mean ipsilateral phase values as a function of step period. For each load condition, mean phase is plotted *versus* the mean period. Control (+), resisting (O) and assisting (●) loads.

3Lag1 was longer in relation to the mean period for the assisting than for the resisting loads. However, the difference was small; regression coefficients for the mean 3Lag1 on mean period were  $0.36 (\pm 0.03)$  and  $0.51 (\pm 0.02)$  for assisting and resisting loads, respectively.

Contralateral phase values were little affected by the loads: in all cases, mean values were near  $180^\circ$  and no significant relationships with load or step period were apparent (Fig. 10).

### Discussion

Effects of load have been studied using various methods. The torque motor used in the present experiments facilitated tests of graded loads in both directions and thus allowed a systematic examination of changes in leg placement and interleg coordination. The motor added a constant force which modified the force required for propulsion but did not affect the force required to maintain the distance between the insect and the substratum. A direct result of such loads is to change the activation of the power muscles. Increased resistance leads to increased

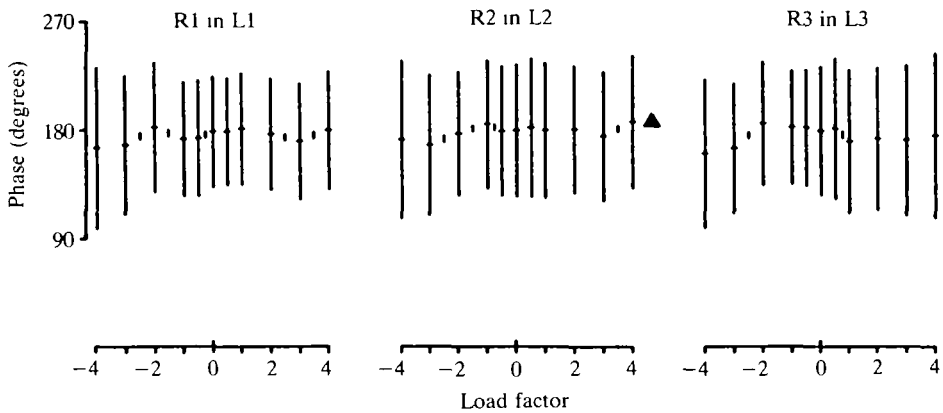


Fig. 10. Mean contralateral phase values as a function of load. The mean phase and the circular standard deviation [ $81^\circ \times \sqrt{1-k}$ , where  $k$  is the concentration parameter or length of the mean vector] are plotted *versus* applied torque. The vertical marks between mean values indicate pair-wise differences significant at  $P < 0.05$  in a Watson-Williams two-vector comparison.

activation of the power muscles in the cockroach (Pearson, 1972) and in the stick insect (Graham, 1985; J. Dean, unpublished results). The change is mediated by both intraleg and interleg mechanisms (Cruse, 1985a; Pearson, 1972). These loads on the propulsive mechanism also influence spatial and temporal parameters of stepping. These changes were the focus of the present study; they provide clues to the underlying control mechanisms. The discussion will begin with the spatial parameters.

Effects of load have been studied in numerous arthropods, but spatial parameters have been quantitatively studied only in the stick insect. This literature contains an apparent contradiction concerning the PEP: increased loading parallel to the body axis has been reported to produce both rostral and caudal shifts under different conditions (Bässler, 1977; Cruse, 1985c; Foth and Graham, 1983a). The present experiments show that the magnitude of the load determines the direction of the shift.

The primary change in step end-points is a shift in the direction of the applied load. This result confirms numerous reports that increased loads induce rostral shifts in both AEP and PEP. Such shifts in the PEP occur under some conditions when an insect pulls against a constant force. Rostral shifts are observed in middle and rear legs when a stick insect pulls against a load equivalent to about twice the weight of the insect (Bässler, 1977). The PEP changes are small and not significant when the insect climbs a vertical path, pulling against its own weight (Cruse, 1976). Both situations change the requirements for support as well as for propulsion, so it is not clear which factors contribute to the rostral shift. However, rostral shifts are also elicited by loads parallel to the direction of locomotion, whether these be frictional and velocity-dependent (Foth and Graham, 1983a) or constant force (present experiments). Thus, the load on the propulsive mechanism is one factor.

However, vertical loading also has a small influence on the PEP (Jander, 1985). Different support requirements could explain why the changes reported here, expressed as percentage of step amplitude, are smaller than those reported by Bässler (1977) and the pattern of changes in the three segments is different from that described by Bässler (1977) or Cruse (1976).

In contrast to these reports, Cruse (1985c) observed a caudal shift in the mean PEP when the load on the test leg was maintained by letting the leg retract on a spring-mounted platform moving slowly to the rear. In normal walking, a leg that reaches a caudal position and reduces its force of retraction is rapidly unloaded as the other legs propel the body forward. The spring-mounted platform presumably hinders this unloading. Cruse concluded that decreasing load and caudal leg position combine to determine the end of stance. This interaction has also been demonstrated in cockroaches and cats (Pearson and Duysens, 1976; reviewed by Grillner, 1981).

The resistive loads applied in the present experiments should behave like the spring. To the extent that the added loads cannot be fully assumed by the other legs, they should increase the load on legs nearing their normal PEP. The intersegmental coactivation mentioned above may further increase the strain measured by leg proprioceptors. Thus, in analogy with the results of Cruse (1985c), the resistive loads should prolong the stance and shift the PEP caudally. The secondary trend identified here for middle and rear legs is in accord with this expectation. The experimental situation studied by Bässler (1977) is similar in some respects to the one used here, but the single load tested was approximately twice the weight of the animals and therefore outside the range eliciting caudal shifts. In all three experiments, the load actually experienced by a leg nearing its PEP is unknown. This useful information is most easily accessible when loads are applied to a single leg, but Cruse did not measure spring deflections.

Foth and Graham (1983a) studied diverse frictional loads including several less than the weight of the animals, but they only mentioned rostral shifts of step end-points. The reason may lie in the nature of frictional loads. Unlike the torques used here, the magnitude of a frictional load varies with velocity. In the stick insect, velocity oscillates during the course of each step (Graham, 1983). Thus, the frictional loads should exert a large effect during the velocity peaks, which occur in the middle of stance, and a small effect during the velocity minima, which occur at the end of stance. Hence, leg unloading and PEP should not be greatly affected.

The rostral shift of the PEP under large resistive loads could have a simple explanation in muscle characteristics. In general, the maximum force that a muscle can generate depends upon its length and, hence, for the retractor muscles, on the extent of retraction. As expected, the isometric force parallel to the long axis of the body does decrease for more caudal tarsus positions (Cruse, 1985b). Peak values were about 10 mN in all three segments, so a maximum isometric force of about 60 mN could be produced when all six legs are forward. Non-isometric forces will be less: Cruse (1976) measured peak values of 1–2 mN for animals walking freely. Preliminary measurements of the retractor force when the nerve is



electrically stimulated also indicate that maximum force decreases when the leg is more retracted (J. Dean, unpublished results). Thus, these results suggest that the PEP shifts forward under large loads because the muscle is unable to continue retraction. In contrast, under small to medium loads, normal increases in muscle force are apparently sufficient for retraction to continue past the usual PEP.

Pearson (1972) presented a simple model for the step pattern generator in which load monitored by proprioceptors, presumably the strain-sensitive campaniform sensilla, generates positive feedback for power muscles and inhibits swing initiation. This model predicts that increased load should shift the PEP caudally, a prediction not in accord with the primary trend reported here and in the literature. The present results suggest that leg loading and leg position influence the PEP in a more sophisticated way. The nervous system appears to monitor both the load on the leg and the success of the muscles in moving the leg. If progress comes to a halt, then legs in positions with a poor mechanical advantage step to more effective positions. This adjustment is in accord with the suggestion that the step pattern generator attempts to maintain an appropriate leg velocity during walking (Cruse, 1985*b*; Dean and Cruse, 1986). This notion also fits with physiological results in the cockroach demonstrating a negative feedback circuit from strain-sensitive campaniform sensilla onto the power muscles (Zill and Moran, 1981). Vertical loads modify step timing in an analogous manner. If a leg is still effectively supporting the body, then its stance is prolonged and its PEP shifted to the rear (Jander, 1985).

The changes in AEP are similar to those in the PEP. Forward shifts in the AEP under increased resistive loads have been noted previously (all legs, Cruse, 1976; Foth and Graham, 1983*a*; middle and rear legs, Bässler, 1977). This was also the case for the loads used here. For middle and rear legs, these shifts could be a consequence of the targeting mechanism, the spatial coordination between adjacent ipsilateral legs (Cruse, 1979; Dean and Wendler, 1983). Because the rostral leg retracts less far as the resistance increases, the target it provides for the swing of the caudal leg also shifts forward. This would explain why the shifts in the AEPs of middle and rear legs are at least equal to the shifts in the PEPs of the adjacent rostral legs. Two other factors may also play a role. First, the AEP would automatically shift forward if the step pattern generator simply maintains a more or less constant swing activity for a given central excitation, as suggested by Pearson (1972). Second, the muscle activation during swing and stance may covary, as suggested by Cruse (1983). When the applied force resists leg retraction, then the protractors are also more strongly activated, owing to intra- and interleg influences (Bässler, 1979; Cruse and Saxler, 1980). This linkage would explain decreases in swing duration observed by Foth and Graham (1983*a*). It would also account for two further observations: (1) changes in the AEP generally exceeded those in the PEP of the adjacent forward leg, and (2) large resisting loads often caused middle legs to over-extend the swing and step onto the surface of the wheel.

Shifts in step end-points of front legs were less monotonic. Results for resisting

loads resemble those for middle and rear legs. The targeting mechanism cannot be invoked to explain the rostral shifts in the AEPs, but these would result if protractor activity remains unchanged or co-varies with retractor activity. The anomalous rostral shifts in the AEP for large assisting loads may indicate a general increase in activity associated with an increased coactivation of retractors and protractors during stance. Together, the rostral shifts in the AEP and PEP may allow the front legs to serve as struts to brake the assisting load. Changes in the stepping of the downhill leg in cockroaches walking up or down a slope have been interpreted in this way (Spirito and Mushrush, 1979).

Functionally, the shifts in the AEP are the counterpart to the shifts in the PEP. They move the AEP and the step movement as a whole towards configurations providing better mechanical advantage for dealing with the load. The limited force provided by the muscles can be used more effectively because it is applied more nearly opposite to the direction of the load. This would also explain the pattern of changes in different legs. The front and middle legs, which normally move in a range rostral to the leg insertion, exert larger forces than do the rear legs during supported walking on a treadmill (Cruse, 1985*b*) and unsupported walking up a vertical path (Cruse, 1976). They also show larger rostral shifts for resisting loads. In contrast, the rear legs, which normally move in a range caudal to the leg insertion, show larger caudal shifts for assisting loads.

Previous studies have shown that temporal parameters are affected by loads in two related ways. One change affects the duty cycle of each leg by altering the ratio of swing and stance durations. The second change modifies the phase relationships in such a way as to place more legs on the ground at any one time.

Data on swing-stance ratios under loads of different kinds are available for many arthropods. Because swing duration varies with period in the common alternating tripod and tetrapod gaits of insects and crustaceans, any change in swing duration must be considered in relation to step period. In general, increasing loads decrease the swing duration relative to the stance duration for a given step period, thereby increasing the time the leg is grasping the wheel and contributing forward propulsion (reviewed by Cruse, 1983; Graham, 1985). This is true for loads of different kinds, including loading parallel or perpendicular to the body axis, weights added to the body, and frictional and inertial loads (Evoy and Fournier, 1973; Foth and Graham, 1983*a*; Graham, 1981; Pearson, 1972).

The effect of loading is particularly evident in the adult stick insect because this animal shows two qualitatively different gaits. When the insect walks supported on a light treadmill, swing duration varies with step period (Graham, 1981, 1985). When the wheel is heavy or a light wheel is subjected to frictional loading, the swing duration is reduced to the minimum observed in the tripod gait and remains independent of period (Foth and Graham, 1983*a*; Graham, 1981).

In the present experiments, swing durations in control experiments were already near minimum, owing to the inertia of the treadmill plus motor. Typical means were about twice the figure of 100 ms given by Graham (1972) for free walking, but the difference probably relates to the different swing motion required for the

'wheel. Swing duration was not further shortened by resistive loads. In fact, it increased almost linearly with load. The swing velocity of rear legs remained nearly constant, while that of front and middle legs decreased, particularly for moderate to large loads. For large resistive loads, the swing of front and middle legs appeared cramped. The protractor activation presumably remains maximal, so the increased duration and decreased speed must have another cause. One candidate is an inability of the retractors to relax normally after a strong contraction during stance. The swing velocity in locusts is quite sensitive to changes in the residual stiffness of the antagonists (Wolf, 1990). This would explain the cramped appearance of some middle and front leg swings under large loads.

The behavior of front and middle legs during these cramped swings suggests the presence of separate subunits in the step pattern generator for the control of leg elevation and leg protraction. The leg often tapped the wheel several times during the course of its swing, as if the motor program for setting the leg down continued at a normal pace.

Unexpectedly, swing duration under assisting loads also increased, albeit less markedly. Small to moderate assisting loads should reduce the activation of the retractors during stance, which, in turn, should reduce the activation of the protractors during swing, leading to increases in swing duration and decreases in velocity. The observed changes were small and in the opposite direction. The unexpected increase in velocity could reflect an early onset in protractor activity needed to brake the wheel at the end of stance: thus, protractor tension would build up prior to the actual start of swing. For large assisting loads, swing duration increased, amplitude changed little and velocity decreased. Two factors may contribute to the decreased velocity. First, muscle stiffness could be higher in both protractors and retractors if significant co-contraction occurs during stance. Second, the protractors might be more subject to fatigue when they are also active during stance.

Swing duration was at best weakly related to swing period under all load conditions; regression coefficients were less than those reported for the tripod gait (Graham, 1972). If low load biases the coordination in the direction of the tripod gait (Foth and Graham, 1983*a*), then regression coefficients should be larger for assisting loads than for resisting loads. This was true only for the smallest assisting load relative to the control condition.

Thus, the changes in swing duration do not entirely fit the pattern expected from the literature on the stick insect. In particular, assisting loads did not induce a pronounced change from metachronal to tripod coordination. The inertia of the wheel, which is considerably above that of the animal, is probably the cause. Measurements on other arthropods show no change in some cases (Barnes, 1977; Macmillan, 1975; Grote, 1981), an increase in others (Evoy and Fournier, 1973) and a decrease in yet others (Pearson, 1972; Spirito and Mushrush, 1979). These diverse results have been attributed to different load conditions (Cruse, 1983).

Increased inertial and frictional loads change ipsilateral step coordination by

shortening the lag for a given step period (Graham, 1981; Graham and Cruse, 1981; Foth and Graham, 1983*a*; reviewed by Graham, 1985). This change underlies the transition from the tripod gait, used by larval stick insects and fast-walking insects in general, to the metachronal gait, used by the adult stick insect (Graham, 1981, 1985). Together with the increased duty cycle, this gait change places more legs in contact with the ground at any one time and therefore increases the force available for propulsion.

The constant force loads applied here elicited similar changes in ipsilateral coordination. Adjacent ipsilateral legs alternate in the tripod gait and in fast walks with the metachronal gait (Wendler, 1964; Graham, 1972). This was true for the relatively fast walking under small or no loads. In the metachronal gait, the step of the rostral leg occurs earlier in the cycle of the adjacent caudal leg and the mean  $3Lag1$  increases less rapidly than the period. This was true for walks under moderate and large loads in either direction. If low loads induce a shift towards metachronal stepping, then the assisting loads might also be expected to have this effect. This was not the case; in fact, mean phases were farther from the tripod values than they were for resisting loads.

Regression coefficients for  $3Lag1$  on period are reported to be about 0.33 for free-walking adults and 0.23 for adults walking under high frictional loads compared to 0.90 for low frictional loads (Graham, 1972; Foth and Graham, 1983*a*). Regression coefficients calculated for each load condition in the present experiments were intermediate. Moreover, they tended to be lower for the assisting loads than for the resisting loads, which again does not fit with the expected bias in favor of the tripod gait when load is reduced. The high inertia of the wheel plus motor may explain this difference. However, the differences are small and these comparisons must be treated with caution because the regression coefficients depend upon the variance of the step periods in the samples.

A second gait change sometimes related to loading is the tendency for contralateral leg pairs to step in synchrony when loads are high (Graham, 1985; von Holst, 1943). Since much of the active muscular force for forward propulsion is provided by front and middle legs (Cruse, 1976, 1985*b*), synchronous stepping by these leg pairs can increase the available force. In the present experiments, the frequency of such gallops did not increase noticeably.

One final clue to the nature of the control system is provided by the profile of the retraction movement under large assisting loads. The reduction in retraction velocity associated with transitions between swing and stance is again indicative of velocity control during stance. These pauses appear to be part of the motor program. Assisting loads should decrease rather than increase the pause at the start of stance if it were simply due to low-pass filter characteristics of the muscles.

Overall, the insects handled large resisting loads better than large assisting loads, presumably because the former leave the responsibility for the stance movement with the retractors, the normal power muscles. One contributing factor, the tendency to walk backwards under assisting loads, may reflect the negative geotaxis of the stick insect (Bässler, 1965; Wendler, 1965).

In summary, the performance of the neuromuscular system controlling walking in the stick insect is little affected by load changes corresponding to different orientations relative to gravity: the step pattern remains more or less constant. The large loads tested here exceed those due to gravity. The stick insect may encounter such large resistances in the form of fixed or compliant obstructions, but such loads may better be met by strategies other than increasing the force, e.g. avoiding the obstruction. The large loads help to explore the function and the limits of the control system. The changes in leg placement can best be interpreted as adjustments to continue leg retraction when the leg is efficiently providing propulsion and to shift the leg movement into ranges where the muscles are most effective. These changes and the changes in the profile of the retraction velocity support the suggestion that the control system attempts to maintain a set velocity during stance. As a whole, the data provide a quantitative basis for the extension of kinematic models of leg coordination (e.g. Cruse, 1983; Dean, 1991; Graham, 1977; Wendler, 1968) to include dynamic parameters.

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