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The Effect of Amputation and Leg Restraint on the Free Walking Coordination of the Stick Insect *Carausius morosus*

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Summary. The stepping patterns of intact, amputated and leg restrained first instar stick insects were examined by analysing video tape records of their free walking behaviour. Amputation produced changes in the relative timing of protraction movements both along and across the body axis. Restraint of individual front or rear legs produced walking behaviour similar to that of the amputee animal but restraint of middle legs caused a breakdown in the coordination of front and rear legs. The changes in behaviour produced by leg autotomy and restraint were used to test certain assumptions of a model for generating the step pattern of these insects and to investigate how the tonic influence of proprioceptive input might be incorporated into the model.

Introduction

Plucking the legs from insects suggests an act of vindictive boredom rather than enthusiastic research but such an attack is not particularly harmful to the stick insect and a quantitative description of the changes in behaviour which occur can provide insight into the structure of the system used by the animal to coordinate its leg movements in walking. The nymph readily releases or autotomises a leg held for a few seconds by the experimenter and is immediately capable of a fully coordinated walk after the removal of not more than one leg from each side. A new leg is constructed within a few moults and even in the adult the permanent loss of one leg from each side does not appear to impair its walking ability to any great extent.

The most comprehensive quantitative study of the effect of leg amputation is that of Hughes (1957) on the cockroach. This has been followed by a more detailed analysis of the coordination changes in some pro- and mesothoracic amputees by Delcomyn (1971). Wendler (1964) examined the coordination

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changes produced by mesothoracic autotomy under zero weight conditions by supporting an adult stick insect above a treadmill. This, and much of the earlier work, has been comprehensively reviewed by Wendler (1966) and Wilson (1966).

In the earliest work on locomotion frequent mention is made of insects walking with their legs protracting simultaneously in diagonal pairs (Wilson, 1966). This implies that the phase relation for legs of the same segment is not exactly 0.5 (180°). This temporal asymmetry in stepping is not mentioned in the papers by Hughes (1957) and Wendler (1964) although asymmetry is present in their data. In both cases the effects are small compared to those found in the free walking adult stick insect and the asymmetries were probably assumed to be small sample errors. As the present work will show a strong asymmetry is present and is directly related to the position of the leg removed in single leg amputees.

The purpose of the present investigation was to examine the role of gross sensory input on the coordination patterns of stick insect nymphs by comparing the step patterns before and after amputation, paying particular attention to temporal asymmetry between the leg steps on right and left sides of the animal. The free walking step pattern of adult stick insects is asymmetrical in the timing of leg movements across the body (Graham, 1972), and in the seven adults examined the average phase of right legs on left was always less than 0.5. In first instar nymphs no right or left asymmetry was detected on average but the model proposed to explain the timing of leg movements in both the adult and first instar suggested that asymmetry might be expected in certain amputee configurations. In addition to the autotomy experiments some legs were temporarily restrained by attaching them to the body. In this situation it is assumed that the afference from the restrained leg is present but abnormal. The coordination changes produced by restraint of the legs often differed from those produced by autotomy.

Materials and Methods

First instar nymphs were chosen for this work because, at this stage of growth, the animal is quite active under daylight conditions and rarely enters the unresponsive thanatotic state that frequently occurs in the adult. Furthermore, the nymph walks at all times with the body lifted well clear of the ground so that the legs provide both propulsion and support for the body.

The first instar nymphs used in this study ranged in age from a few hours to a few days. Free walking behaviour was recorded on magnetic tape by means of a Shibaden HV-15S television camera and an SU-700 video tape recorder and played back frame by frame for analysis of the stepping patterns. The scanning head of the recorder rotated at 25 revolutions per second and produced one 310 lines per inch image every 20 ms. This is equivalent to a conventional filming speed of 50 frames/s. The animals walked freely over a graph paper surface moved below the camera lens and shadows were used to estimate the position of the body relative to the walking surface. Using the maximum magnification, with a reasonable field of view for tracking the movements of the animals, the protraction of legs was easily observed.

Legs were removed by gripping them at the mid-point of the femur with a pair of forceps until autotomy occurred. The first leg autotomised within a few seconds but the next leg to be removed might be retained for up to 3 or 4 min before being finally released.

In restrained preparations a leg was prevented from moving normally by attaching the tibia to either the head, thorax or abdomen with Dow Corning Silastic 732 RTV. This flexible adhesive does not appear to harm the cuticle even when applied for a period of a month or more. It forms a tight bond which resists movement of the leg and can be stripped off after an experiment without producing any noticeable damage. Front legs were attached to the dorsal surface of the head capsule, middle legs were held parallel to the body axis pointing forwards, and rear legs were fixed to the abdomen pointing towards the rear. The position of the front legs was unusual, but for middle and rear legs the positions were typical of the thanatotic position of the nymph.

Before each experiment the animal was inspected carefully for damage which might affect leg coordination such as the absence of tarsal hooks. Then the nymph was run beneath the camera to give a sequence of 20–30 steps. If this sequence showed no abnormalities on slow replay the animal was passed through the experimental procedure. In restrained experiments the animal was retested after removing the adhesive from the legs. Although the legs did not appear to be damaged by the adhesive the animal frequently autotomised a leg held immobile for a period of more than 10 min.

Results

The results are given in three separate sections. The first gives data for the intact first instar nymphs and includes some of the results from an earlier paper on walking coordination in intact animals (Graham, 1972). The second contains the results of the amputation study and the third describes the changes in behaviour which occurred when certain legs were prevented from moving normally.

As a short hand notation for the operations carried out on a particular animal an amputee is denoted by the letter A, and I is used to indicate that some legs of an animal have been restrained or immobilised. The particular legs operated upon are given in parentheses using R and L for right and left relative to the animal and numbering the legs 1 to 3 from front to rear. Thus an animal with a left mesothoracic leg removed is labelled A (L2) and an animal with both rear legs immobilised is labelled I (R3, L3) or alternatively I (3, 3).

At least two animals were examined for each amputation. No systematic differences between individuals were found and the data are representative of at least four members of each amputation class if both right and left mirror image animals such as A (L1) and A (R1) are included for each amputation. The results for certain amputations are presented in the form of a step pattern and three phase histograms. The phase histograms show the time relationship between two neighbouring legs on the right side (upper positive histogram), the left side (lower positive histogram) and between them the time relationship between right and left legs of the most posterior intact segment (negative black histogram). A second step pattern in each figure shows either another sequence from the same animal, a pattern from the same animal at a later time, a sequence from another animal with the same amputation or the step pattern of a mirror image amputee. The three histograms are arranged vertically so that the timing of leg pairs having one leg in common may be compared. This particular arrangement was chosen to show the right-left symmetry of the step patterns and permit rapid identification of diagonal leg pairs which tend to step simultaneously.

Table 1. Parameters of the experimental results

Expt.	*N*	Right phase	*n*	Left phase	*n*	Across phase	*n*	Fig. No.
A(0) gait I	4	R3:R2 0.52, 0.9368,	60	L2:L3 0.48, 0.9266,	60	R3:L3 0.51, 0.9021,	64	1 a
A(0) gait II	2	R3:R2 0.63, 0.9482,	44	L2:L3 0.37, 0.9464,	43	R3:L3 0.35, *0.65*,	44	1 b
A(R1)	3	R3:R2 0.64, 0.9194,	32	L2:L3 0.36, 0.9241,	29	R3:L3 0.39, 0.8510,	28	
A(L1)	3	R3:R2 0.63, 0.8994,	43	L2:L3 0.39, 0.8116,	47	R3:L3 0.63, 0.7708,	43	4 a
A(R2)	2	R3:R1 0.62, 0.6254,	30	L2:L3 0.39, 0.8279,	36	R3:L3 *0.35*, 0.65,	35	5 a
A(L2)	2	R3:R2 0.60, 0.8279,	28	L1:L3 0.37, 0.6467,	29	R3:L3 0.31, *0.62*,	26	5 a
A(R3)	2	R2:R1 0.66, 0.8505,	70	L1:L2 0.38, 0.7988,	70	R2:L2 0.79, 0.4969,	70	
A(L3)	3	R2:R1 0.63, 0.9265,	21	L1:L2 0.32, 0.9564,	21	R2:L2 0.23, 0.8919,	21	4 b
+5 m		R2:R1 0.64, 0.9445,	31	L1:L2 0.30, 0.9445,	30	R2:L2 0.15, 0.9140,	31	
A(R1, L3)	2	R2:R3 0.33, 0.9074,	35	L1:L2 0.34, 0.8743,	37	R2:L2 0.70, 0.8930,	35	5 b
A(L1, R3)	2	R2:R1 0.71, 0.8894,	28	L3:L2 0.73, 0.8889,	32	R2:L2 0.31, 0.7904,	28	5 b
A(R1, L2)	2	R3:R2 0.72, 0.8675,	27	L1:L3 0.35, 0.8423,	26	R3:L3 0.51, 0.8372,	28	
A(L1, R2)	2	R3:R1 0.70, 0.9251,	31	L2:L3 0.28, 0.9452,	31	R3:L3 0.53, 0.8295,	31	
+48 h		R3:R1 0.69, 0.8979,	27	L2:L3 0.21, 0.9079,	33	R3:L3 0.49, 0.8489,	28	
A(R2, L3)	2	R1:R3 0.30, 0.9297,	34	L2:L1 0.80, 0.9298,	34	R1:L1 0.58, 0.9140,	34	6 b
+48 h		R1:R3 0.29, 0.9542,	32	L2:L1 0.78, 0.9326,	32	R1:L1 0.57, 0.9482,	33	6 b
A(L2, R3)	3	R1:R2 0.22, 0.8692,	55	L3:L1 0.76, 0.9705,	54	R1:L1 0.48, 0.9072,	55	
A(R1, L1)	2	R3:R2 0.71, 0.8964,	65	L2:L3 0.31, 0.8785,	62	R3:L3 0.49, 0.7388,	63	
A(R2, L2)	3	R3:R1 0.78, 0.9413,	39	L1:L3 0.25, 0.9441,	41	R3:L3 0.50, 0.8800,	40	6 a
A(R3, L3)	2	R2:R1 0.77, 0.8981,	24	L1:L2 0.24, 0.9570,	26	R2:L2 0.52, 0.8948,	24	
×48 h		R2:R1 0.71, 0.9047,	15	L1:L2 0.23, 0.9690,	15	R2:L2 0.48, 0.9317,	15	
I(R1)	1	R3:R2 0.60, 0.9513,	30	L2:L3 0.44, 0.9303,	28	R3:L3 0.48, 0.9195,	29	7 a
I(R3)	1	R2:R1 0.63, 0.9230,	16	L1:L2 0.36, 0.9445,	17	R2:L2 *0.40*, 0.70,	17	7 a
I(R2)	1	R1:R3 0.42, 0.3572,	13	L3:L2 0.69, 0.6594,	13	L3:R3 0.58, 0.1746,	12	8 a
I(R2, L2)	2	R3:R1 0.45, 0.0843,	22	L1:L3 0.46, 0.4085,	26	R3:L3 0.44, 0.4081,	24	8 b
I(R3, L3)	1	R2:R1 0.66, 0.8181,	18	L1:L2 0.35, 0.8387,	18	R2:L2 0.52, 0.7727,	18	7 b

N denotes the number of animals used in each experimental series. Three numbers are given for each phase analysis: the first is a mean phase or modal value; the second is either a concentration (4 figure decimal) or the alternative mode of a bimodal distribution (2 figure decimal); the third is n the number of steps used for the analysis. On the extreme right a figure number is given for some experiments. Notice that the dominant mode of a bimodal distribution is italicized and that the bimodal statistical parameters are given separately in Table 2. See Appendix for comparative statistical tests

The mean or mode values of phase for all animals are given in Table 1, including the data for the mirror image amputees. In all amputations the phase histograms and step patterns can be used to represent the behaviour of the mirror image amputee by transposing right and left symbols in the figures. Where statements are made in the text concerning similarities or differences between the timing or phasing of protractions in different amputations these were confirmed by the statistical tests defined in the appendix.

Step Patterns for Intact First Instar Nymphs

Figure 1a shows the typical leg coordination for a first instar nymph under daylight illumination. In this mode of walking front and rear legs on the same side protract simultaneously and, from an earlier paper on walking in these

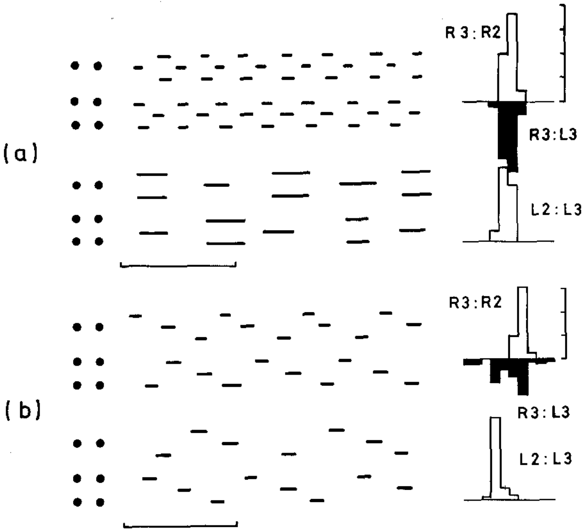


Fig. 1. a Intact nymph using gait I. **b** Intact nymph using gait II. The step patterns are arranged according to the Wilson (1966) convention R3, R2, R1, L3, L2, L1 from top to bottom. 1 s bar: time scale. The black bars in the step pattern show the duration of the protraction stroke. Leg arrangement, viewed from above with the head up, on the left. Filled circles intact legs; a cross shows where a leg has been autotomised; restrained legs in parentheses. The three histograms described in the text are shown on the right. The base extends from a phase of 0.0 to 1.0; the frequency in units of 10 on the ordinate

animals (Graham, 1972), this behaviour is described as gait I and has the following characteristics. The coordination pattern is independent of step frequency. The mean value of the phase of R3 on L3 is 0.51 ± 0.07 and the step pattern is symmetrical about the body axis. The mean phase of nearest neighbour legs on the same side is also close to 0.5 and 2 sets of 3 legs protract alternately producing the well known tripod step pattern.

Figure 1b shows an alternative step pattern which can be adopted by the nymph when walking slowly. The nymph turns to right and left frequently when using this gait, but the upper step pattern of Figure 1b shows one of three rare sequences in which the animal almost walks in a straight path. In this mode front and rear legs on the same side do not protract simultaneously and the step pattern is one in which diagonal pairs of legs tend to protract at the same time when the animal is walking straight with right and left sides weakly coupled. This type of walk constitutes a different kind of coordination pattern to that of gait I and is referred to as gait II. In first instar animals the coupling between right and left sides in gait II is very weak and there is no strongly maintained phase relationship between legs in the same segment. The phase histogram for R3 in Figure 1b consists of three relatively straight walking sequences and shows a slight tendency towards two alternative asymmetrical step patterns. The two modes occur at phases of 0.35 and 0.65 (see Table 1) and indicate that this animal had a slight preference for walking with either R2 and L3 or L2 and R3 stepping as a pair. In Figure 1b the upper step pattern is changing from R2–L3 preferred to L2–R3 preferred. The lower pattern shows a more typical sequence for this gait in which right and left sides are operating independently at different step frequencies. In the adult animal a gait resembling gait II is used exclusively but the simultaneous protraction of R3 and L2 is strongly preferred.

It must be stressed that gait I, in first instars, appears in approximately 95% of the walks examined and represents the dominant gait in these animals when filmed under daylight conditions. The alternative walk, gait II, is a rare ocurrence but appears to be associated with feeding and can sometimes be induced by smearing the walking surface with leaf sap.

The distinction between gait I and gait II is shown most clearly in Figure 2 where the average elapsed time or lag between the onset of protraction in leg 3 and the next protraction of leg 1 on the same side (${}_3L_1$) is plotted as a function of step period (P). As an alternative to such a graph approximate values of the lag ${}_3L_1$ can be derived directly from the phase histograms of Figure 1a. Here the typical phase of L2 on L3 is 0.49 ± 0.06 (Table 1) and by the definition of phase

$$\frac{{}_3L_2}{P} = 0.49.$$

It can be seen from the step patterns that ${}_3L_2 \approx {}_2L_1$ and thus ${}_3L_1 = 0.98$ P. The calculated lag ${}_3L_1$ is approximately equal to P and the gait is type I as shown in Figure 2.

However, in Figure 1 (b) the typical phase of L2 on L3 is 0.37 ± 0.05 (Table 1). By similar reasoning the calculated value of ${}_3L_1$ in this case is only 0.74 P and is appreciably less than the step period as shown in Figure 2 for gait II. Thus examination of the ipsilateral timing of leg movements gives an indication of the extent to which a given walk is like gait I or gait II. In addition, examination of the phase of legs in the same segment shows whether the gait is symmetrical as in Figure 1a or tends towards one of the two alternative asymmetries shown in Figure 1b.

In the lower part of Figure 2 the duration of the protraction stroke for a mesothoracic leg is plotted as a function of step period. This shows that protraction duration is proportional to period in gait I but is independent of period in gait II and provides another means of distinguishing between gait I and gait II when the animal is walking slowly.

Step Patterns for Amputees

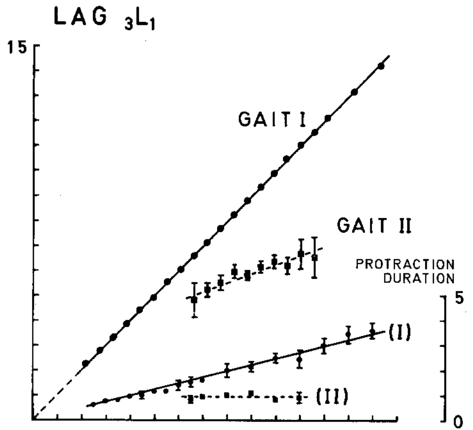
The amputees are divided into four classes based on the major changes in behaviour produced by the amputations in each class.

Class I includes all single leg pro- and metathoracic amputees.

A(R1) denotes a right prothoracic amputee and this class also includes A(L1), A(L3) and A(R3).

Class II contains right and left mesothoracic amputees A(L2) and A(R2).

Class III consists of the two amputees A(R3, L1) and A(L3, R1).



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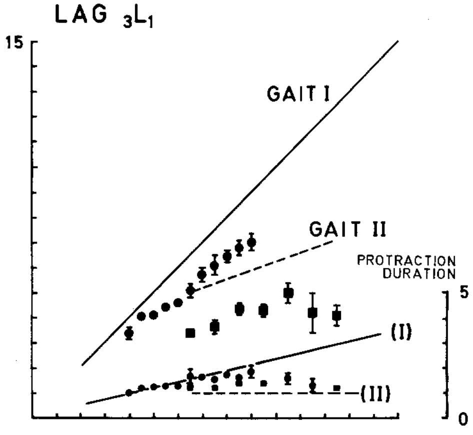

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Fig. 2. Lag $_3L_1$ and protraction duration versus period for an intact nymph. The lag and protraction duration are the means of a number of observations plotted against a class interval of period. Circles: gait I: rectangles: gait II. Vertical bars: the standard error of the mean where this is greater than the symbol size. N.B. The data are selected measurements at constant velocity to avoid distortions produced by acceleration and deceleration (for details see Graham, 1972). Units: 100 ms

Fig. 3. Lag$_3L_1$ and protraction duration versus period for leg amputees A(L3), A(R3) and A(3, 3). Circles: the intact side of A(L3) and A(R3) combined. Rectangles: data for A(3, 3) obtained by multiplying the mean value of $_2L_1$ by 2. Unbroken and broken lines: gait I and gait II, respectively, for the intact nymph. Vertical bars: standard error of the mean. Units 100 ms

Class IV contains all the remaining two leg amputees such as A(1, 2), A(2, 3), A(1, 1), A(2, 2), A(3, 3). Remember that only one leg is removed from each side.

Class I Amputees A(1) and A(3)

The removal of a prothoracic or metathoracic leg produces a significant change in the ipsilateral timing of the legs on both the intact and amputated side. Figure 3 shows a lag ($_3L_1$) versus period plot for the intact sides of the amputees A(R3) and A(L3) (circles). A similar graph can be constructed for the amputees A(R1) and A(L1) and in both types of amputation the mean value of $_3L_1$ at each period is appreciably less than P. Thus the typical gait I behaviour of the nymph is converted to a gait II type walk by the removal of one leg. Below a step period of 0.6 s $_3L_1$ for A(R3) and A(L3) appears to be an extension of the intact gait II data to higher step frequencies. At lower speeds $_3L_1$ for the amputee is greater than that observed in gait II in the intact animal but is still too small to produce gait I.

Figure 4a shows typical step patterns for the front leg amputee A(L1), and a comparison of the histograms with those in Figure 1 shows that the amputee has adopted one of the asymmetrical alternatives shown for gait II in Figure 1b in which legs R2 and L3 tend to step simultaneously. Table 1 shows that the

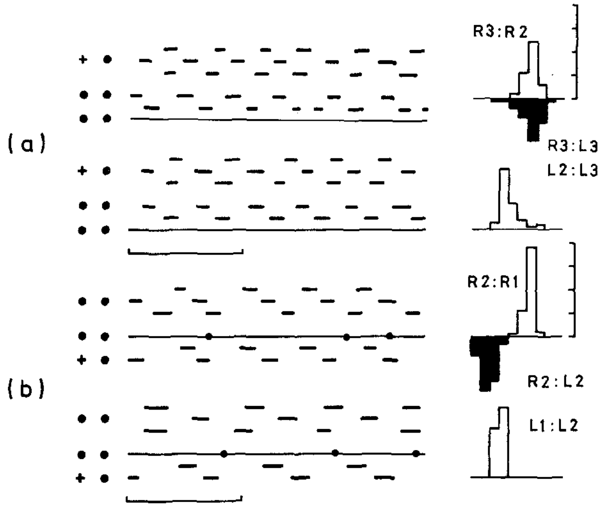

Fig. 4. **a** Amputee A(1), Class I. Step patterns for two A(L1) nymphs. Symbols and units as in Figure 1. **b** Amputee A(3), Class I. Two consecutive step patterns from the same animal A(L3) with an interval of 5 min. Filled circle on the line marking the autotomised leg: the timing of forward movements of the coxal stump

mirror image amputee A(R1) adopts the alternative asymmetrical pattern in which L2 and R3 tend to protract at the same time. Similar behaviour occurs in the metathoracic amputee A(L3) shown in Figure 4b although, with this amputee and the mirror image, the asymmetry across the body axis is reversed and more extreme.

The movement of coxal stumps was sometimes observed in these amputees and the step patterns in Figure 4b show the time at which a coxal stump moved forward in a protraction stroke. Protractions of the stump occurred at irregular intervals and did not always appear at the correct time for appropriate phasing with the other legs.

In summary, front or rear single leg amputations produce a well coordinated gait with a change in ipsilateral timing and alterations in contralateral timing which produce asymmetrical step patterns. These leg amputations are equivalent to removing a leg from an intact animal using gait II and increasing the strength of right-left coupling to produce a strongly coordinated gait using only one of the two alternative asymmetrical step patterns. The particular asymmetry observed depends upon which leg is removed.

Class II Amputees A(2)

Inspection of the step pattern in Figure 5a shows that removal of a mesothoracic leg caused a phase shift of 0.5 in the relative timing of legs 1 and 3 on the amputated side and similar changes to those observed in Class I on the intact side. Table 1 shows that this change in timing always occurred when a mesothoracic leg was autotomised. The phase of R3 on L3 in Figure 5a is bimodal showing that both asymmetrical patterns are possible in A(R2) with a phase

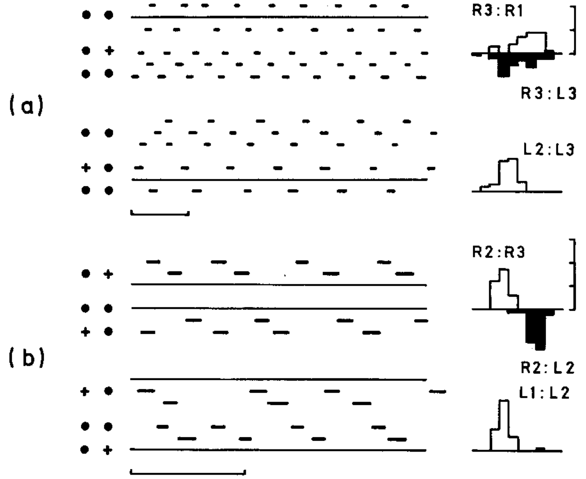

Fig. 5. **a** Amputee A(2), Class II. Step patterns for A(R2) and A(L2) are both starting sequences and show abnormalities in the relative timing of leg movements at the beginning of each walk. Notice reduction of a factor 2 in time scale. Phase histograms are for A(R2). **b** Amputee A(1, 3), Class III. Step patterns for A(R1, L3) and A(L1, R3). Notice that the latter starts with a sharp right turn. Phase histograms are for A(R1, L3)

for R3:L3 of 0.3 preferred. Reference to Table 1 shows that the mirror image amputee favours the alternative asymmetry and the typical step sequence for A(L2) is shown in the second pattern of Figure 5a. Notice that in this amputation front legs occasionally protract simultaneously. No movements of coxal stumps were observed but in these amputations the coxae were held in a position at right angles to the thorax and small amplitude movements would be difficult to detect with any certainty.

Class III Amputees A(3, 1)

Figure 5b shows that in the amputee A(R1, L3) the changes in ipsilateral timing are analogous to those observed in Class I but there is reversal in asymmetry. Reference to Table 1 shows that A(R1) walks with $R3:L3=0.39$ and A(L3) walks with $R2:L2=0.23$. Surprisingly, removal of both legs from one animal does not give $R2:L2\approx 0.3$ but 0.7 and the asymmetry of A(R1, L3) is the reverse of that for either A(R1) or A(L3). This observation is confirmed by the mirror image amputee and shows clearly that effects of amputation are not additive as one might have expected.

In the second step pattern of Figure 5b an example of a turn to the right is shown at the beginning of the sequence for A(L1, R3) but most of the step patterns for these animals consisted of straight walks with a coordination similar to that shown by the phase histogram for A(R1, L3) with the appropriate right-left reversal of asymmetry (see Table 1). The coordination of these amputees is quite different from all other two leg amputees in that it shows a clearly defined asymmetry in the step pattern similar to that observed in Class I.

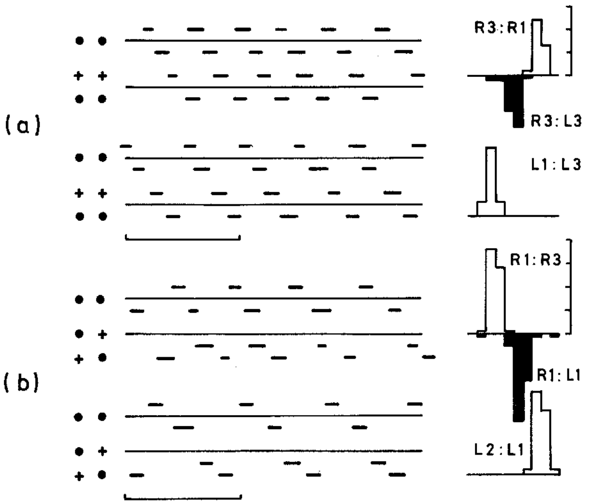

Fig. 6. a Amputee A(2, 2), Class IV. Step patterns for two different animals. **b** Amputee A(2, 3), Class IV. The first step pattern for A(R2, L3) was recorded immediately after autotomy and the second 48 h later

Class IV Amputees A(1, 2), A(2, 3), A(1, 1), A(2, 2) and A(3, 3)

In these amputees the ipsilateral timing is similar to that of gait II but contralateral timing is such that the step pattern is symmetrical about the body axis. In Figure 6a and 6b the phase of right on left has a modal value close to 0.5 and examination of Table 1 shows that in all amputees in this class the step patterns are symmetrical. In A(1, 1) the contralateral phase histogram is rather broad but in the remaining amputees right and left sides are rigidly coupled into a symmetrical step pattern.

It is impossible to determine proper values of $_3L_1$ for these amputees due to the absence of a leg from each side. However, $_3L_2 \approx {}_2L_1$, and we can calculate lag $_3L_1$ values for A(3, 3) by multiplying $_2L_1$ by 2 for each side of the body. These artifical values and the protraction duration for mesothoracic legs, are plotted as a function of period in Figure 3 (squares) to illustrate the gait II character of the walk in these amputees. Across the body coordination is similar to that for gait I (phase 0.5).

Differences in Behaviour between Animals with the Same Amputation

Figure 4b shows an example of step patterns from two different animals. In all cases separate phase histograms for individual animals did not differ significantly and the data from all animals with the same amputation was combined. Bimodal distributions for legs in the same segment do not imply that one animal had one asymmetry and another had the alternative asymmetry, for in every case where a bimodal histogram occurred the animal for which most steps were recorded showed both alternative asymmetries, and the step patterns of other animals contributed to one or both modal peaks so that the original preference was maintained.

Changes in Step Patterns with Time Following Amputation

In most instances animals were filmed at varying intervals for up to 1 h after amputation. However, some animals were again filmed 48 h later. Reference to Table 1 shows that with only two exceptions there were no significant differences between runs performed immediately after amputation and those performed up to 2 days later. The exceptions were the contralateral phase of R2:L2 for A(L3) which altered significantly (at the 10% level) over an interval of 5 min, and the ipsilateral relation of L2:L3 for A(L1, R2) which changed significantly (at the 10% level) in 48 h. No systematic changes were observed and it is assumed that such changes probably occurred by chance. It is concluded that with the possible exception of the first few steps, no learning took place in these amputation experiments.

Restrained Leg Experiments

Figure 7a shows step patterns for two animals in which one leg was temporarily restrained. The contralateral phase histogram for I(R1) has a mean value of 0.48 but the mean values for the phase of R3:R2 and L2:L3 differ significantly from 0.5. Thus the walking pattern appears to contain components of both gait I and gait II. At the beginning of the upper step sequence the nymph walked with gait I and this resulted in two successive impacts between the mouth parts and the walking surface as shown by the x symbols. The animal protracted R2, L3 and L1 simultaneously and appears to have attempted to stand on the two legs L2 and R3. The weight of adhesive fixing leg R1 to the head probably contributed to the imbalance and the animal fell over towards the right front. After the second fall the animal appeared to solve this problem either by making the lag ${}_3L_1$ on the left smaller than the step period on that side or by dividing the protraction of R2 or L3 into two parts to avoid lifting all components of the tripod R2, L3 and L1 simultaneously. However, later runs produced similar falls to those just described at the beginning of each new walk.

The step pattern for I(R3) in Figure 7a and reference to Table 1 show that this animal walked with gait II. The femur of the restrained leg R3 attempted to protract at regular intervals and these movements were correctly phased with respect to the protractions of the other legs. The pattern of stepping only superficially resembled that of the amputee and differed from it in two important respects. Firstly, comparison of I(R3) and A(R3) in Table 1 shows that I(R3) could adopt either asymmetry while A(R3) did not, and secondly, the values for the two alternative asymmetrical modes were closer to normal gait II behaviour in I(R3) and did not show such extreme asymmetry as A(R3) or A(L3).

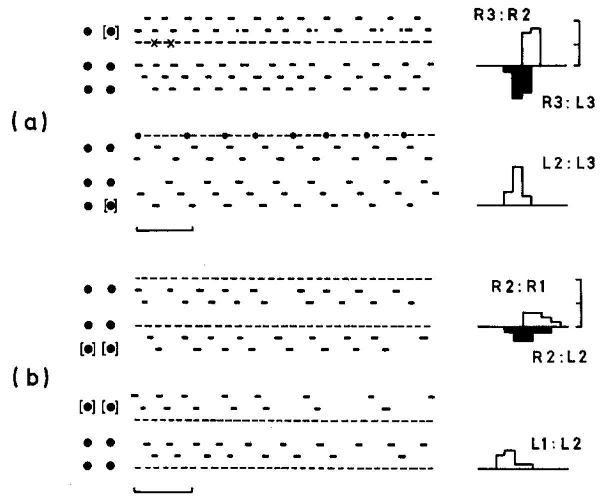

Fig. 7. **a** Restrained leg animals I(R1) and I(R3). The x symbol marks the times at which the mouth parts hit the walking surface. Filled circle on the broken line showing the restrained leg: the timing of strong twitches in the restrained leg. The phase histograms are for I(R1). **b** Restrained leg animals I(1, 1) and I(3, 3). The phase histograms are for I(3, 3)

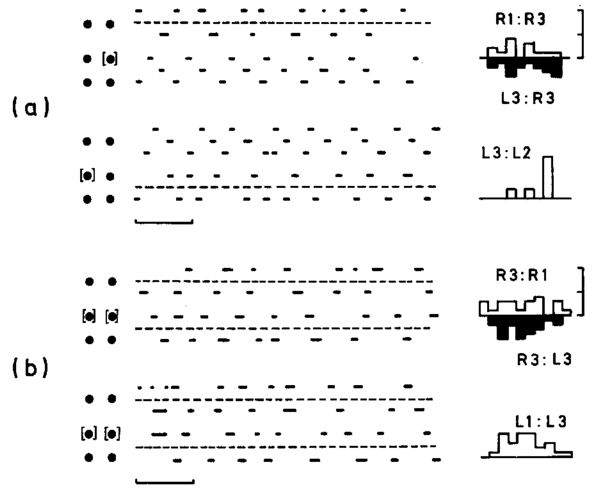

Fig. 8. **a** Restrained leg animals I(R2) and I(L2). Notice the poor coordination on the restrained side. The phase histograms are for I(R2). **b** Restrained leg animals I(2, 2). Step patterns are for two different animals. Notice the rear legs never step with a phase in the range 0.9 to 1.1

Figure 7b shows step patterns for I(3, 3) and I(1, 1). In both examples a gait II ipsilateral pattern was used and the phasing of leg movements was similar to the amputees A(3, 3) and A(1, 1).

When a mesothoracic leg was restrained Figure 8a shows that the animal behaved quite differently from the amputee in that front and rear legs were very weakly coordinated. This poor coordination was restricted to the immobilised side although the relatively weak movements of the rear leg on the immobilised side caused the animal to walk in a crab fashion with the immobilised side lagging behind which caused some unusual double protractions on the intact side. Figure 8b shows two step patterns for I(2, 2). No strongly preferred phase relation existed for ipsilateral legs when a mesothoracic leg was restrained. Front and rear legs on the same side occasionally attempted to protract together causing a movement of the body to the rear but there was some measure of coordination still present across the body for R3 and L3 never protracted simultaneously in the 53 steps recorded.

Statistical analysis of the data for these animals (see Table 3) showed that half the phase histograms for I(R2) and I(2, 2) were not significant. The phase histograms for L3:L2 in I(R2) and for R3:L3 and L1:L3 in I(2, 2) all show a maximum which is just significant at the 5% level. Possibly more data would show that all the relationships were weakly significant. However, it is clear that restraint of a middle leg produces a dramatic reduction in the coordination of front and rear legs on the operated side and what little coordination is left tries to prevent contralateral and ipsilateral legs from stepping simultaneously.

The movements of rear legs were small in amplitude and they did not appear to be protracting in a normal manner. It is possible that some of the protractions recorded in Figure 8 were not real protractions but merely forward movements of the legs following release of the tarsus when the leg is over extended. The front legs appeared to be operating vigorously but the rate of progress was extremely slow compared with the equivalent amputee. This was clearly demonstrated when one of the insects used for Figure 8 was observed over a period of 35 min. After 12 min of slow forward movement and occasional rests, the animal began to walk in a crab movement towards the left side with front and rear legs protracting alternately. A short while later the insect walked with a gait equivalent to an L2, R2 amputee. Subsequent inspection of this animal showed that both mesothoracic legs had been autotomised.

Discussion

In intact first instar nymphs the distinction between gait I and gait II is well defined at low speeds. Under the experimental conditions used here the intact nymph almost always uses gait I. However, it has been possible to record gait II by persuading the animal to take very long walks of the order of 10–30 min or smearing leaf sap on the walking surface. The animal rarely transfers from one gait to the other during one episode of walking and the difference in the average value of lag ${}_3L_1$ for the two gaits is large at low speeds.

In every "one leg" amputee the changes in mean or mode of the ipsilateral phase histograms show that an amputation produces a reduction in the lag ${}_3L_1$ compared to the step period for the legs on the intact side so that the new step pattern resembles gait II in the intact animal. On the amputated side ${}_3L_1$ cannot always be measured except when movements of the coxal stump occur but in every amputee the lag $_3L_2$ or $_2L_1$, as derived from the mean or mode of the ipsilateral phase histogram, between nearest neighbour legs, is without exception equal to or less than that observed in gait II for the intact insect (Table 1). Thus a transition to gait II ipsilateral timing appears to be an automatic consequence of the removal of a leg from one or both sides and the effect is most pronounced on the amputated side. Clearly this is a reasonable response on the part of the animal to the loss of one leg because gait I is obviously unsuitable for a five legged animal. This is demonstrated by I(R1) in Figure 7a. This animal falls forward because it attempts to step onto a tripod of legs when only two of them are able to take up their correct position. The use of gait II immediately solved this difficulty. However, occasionally the animal used an alternative solution and divided the protraction of R2 into two parts. This permits R2 to be used in support of the body at a time when the set L1, R2, L3 would normally be protracting together. It appears that this is a less satisfactory solution as the step frequency of R2 is reduced and coordination is disturbed.

In mesothoracic amputees the change in timing of the prothoracic leg produced by autotomy is large and the phase of this leg relative to the metathoracic leg is altered by 180° to a phase of 0.5. Wendler (1966) in his study of the adult mesothoracic amputee showed that this change in coordination is not produced by an increase in the vertical loading of the remaining legs because the insect was supported above a treadmill in his experiments. He also showed that this coordination pattern did not exist anywhere in the step frequency range of the intact animal. Thus an entirely new coordination pattern is brought about by the autotomy of a mesothoracic leg. It is not the loss in afference from the femur, tibia or tarsus that produces this coordination change, for the addition of a balsa prosthesis to the coxal stump re-establishes the intact coordination pattern (Schaller, unpublished; see Wendler, 1966). It seems most likely that it is upthrust on the coxa itself which permits the normal intact coordination pattern to occur. Thus an input from sensory structures in the coxa-thorax joint appears to be essential to the maintenance of those intersegmental pathways which control the normal timing of leg movements in the intact animal.

Movements of the mesothoracic stumps were not seen in the present work but this is not surprising in view of the problem of observing small amplitude movements and the difficulty in placing any markers on the coxae. On rare occasions when the front of the thorax touched the surface vigorous strokes of the autotomised prothoracic coxa were observed but at other times no movement was detected. In the metathoracic coxae small protraction movements were observed and these are shown in some of the step patterns. When they occurred they were usually correctly phased relative to the movement of other legs and support the observation of Wendler (1964) that weak oscillatory activity occurs even in the absence of proprioceptive feedback from the autotomised part of the leg.

The major observations of the present work that $_3L_1$ is decreased by amputation and asymmetrical patterns appear in some amputees is supported by the earlier work of Hughes (1957) on the cockroach *Blatta orientalis*. In general

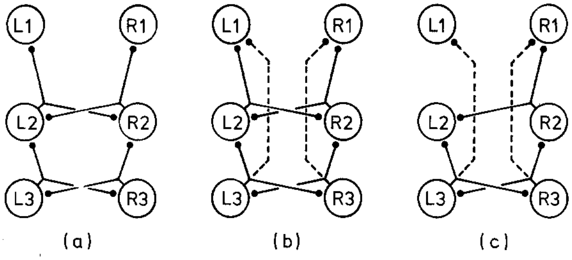

Fig. 9a–c. Model configurations for the timing of leg movements. Circles represent each leg oscillator and the delay unit used to generate a lag. The inhibitory pathways show which legs have their timing modified by these delays. **a** The model constructed to simulate the behaviour of intact first instar nymphs and adults. **b** Revised model to include the changes in step pattern produced by mesothoracic autotomy. **c** Suggested changes in functional pathways produced by autotomy of a left middle leg

his data are insufficient to assign any great significance to the observed asymmetries and changes in ${}_3L_1$ but they are present in the step patterns given and are in qualitative agreement with the present results. In the one example of a right mesothoracic amputee a phase histogram for R3:L3 is given for the results of 7 runs on one animal (Hughes, 1957) (Fig. 4). In this animal 4 runs out of 7 gave modes of 0.4 or less and one of the remaining ones had a mode of 0.7. These results are similar to those for the stick insect nymph A(R2) in Figure 5a even to the extent of showing the same preferred asymmetry.

Before discussing the behaviour of individual amputees I wish to consider some of the assumptions made in an earlier model of walking coordination in intact nymphs and adults, for some of the stepping patterns obtained from first instar amputees provide support for these assumptions.

The model is described in detail in a recent paper (Graham, 1977a), and the proposed coordination pathways are shown in Figure 9a. Briefly, the major assumptions were as follows. It was assumed, from the difference in step frequency on right and left sides for the adult stick insect when turning and the weak coupling between right and left sides of the nymph when walking with gait II, that each side of the walking system could function independently. In the present work coupling across the body is strong and all the amputees show well defined phase relationships between the legs of the same segment in straight walking. The uncoupling or independent operation of right and left sides is relatively infrequent but it does occur during deliberate turning movements (Fig. 5b).

It was also assumed that "inhibitory" coupling between right and left sides only occurred in meso- and metathoracic segments. Thus, a gait in which front legs step simultaneously is permissible in the model. Such a gait is used by the amputees A(R2) and A(L2) in Figure 5a. No examples were found of R3 and L3 stepping simultaneously and such stepping is even excluded in animals which have both middle legs immobilised and show very weakly coordinated behaviour of the legs. The contralateral legs of meso- and metathoracic segments sometimes protract simultaneously during turning movements but the right and left sides are presumed to be uncoupled at this time. It was also assumed that ipsilateral and contralateral nearest neighbour delays are approximately equal in duration for the intact animal. In all the single leg amputees except A(3) the lag across the body is approximately equal to the ipsilateral lag between adjacent legs. In A(3) it is only half this value.

The assumptions of the model regarding coupling pathways between oscillators are confirmed by the single leg amputee behaviour in one further respect for the model assumes a hierarchy in natural oscillator frequency in which posterior legs control the protraction timing of anterior legs. This implies that the rear legs are the source of timing for the whole system in an intact animal. Therefore, if the removal of the lower part of the leg influences the operation of the delay or leg oscillator in the model the most significant effects should be found in rear leg amputations. This prediction is supported by the observation that more extreme changes in asymmetry occur in the rear leg amputees A(3) than in the front and middle leg amputees A(1) and A(2).

The models of Wilson (1967) and Wendler (1968) bear a superficial resemblance to the one used here, but the assumptions on which they are based are markedly different. In the former an excitatory connection is postulated along the body axis and an inhibitory one giving a phase of 0.5 is assumed to act across the body. No interaction configuration for all the legs is given and no attempt is made to determine the behaviour of the proposed model. In the Wendler model a phase of 0.5 across the body is produced by an unspecified mechanism and a similar but not identical system of interactive paths is assumed. Unfortunately, insufficient information is presented on the nature of the coupling to allow independent simulation of this model but the author argues against the concept of hierarchy in oscillator frequency.

In contrast, the present model depends strongly upon a hierarchy of inherent frequency in the oscillators to produce the metachronal rhythm, and differences in frequency across the body to produce the asymmetrical step patterns found in free walking insects. For further discussion of these models and a detailed experimental examination of the assumptions used in the Graham (1972) model see Graham (1977a and b).

The Mesothoracic Amputee

The model used to describe the behaviour of the intact animal (Fig. 9a) is not able to simulate the removal of a mesothoracic leg other than to predict that the nymph might walk like an intact animal with this leg absent in either gait I or one of the two stable asymmetrical forms of gait II. Another possible alternative would be to assume that the ipsilateral pathway would be broken on the amputated side and leg 1 would operate independently of the other legs. In the stick insect none of these possibilities occurs. In the cockroach, Delcomyn (1971) has shown that the first of these alternatives is possible, but both the cockroach and the stick insect most frequently adopt a new walking pattern in which legs 3 and 1 on the amputated side step alternately rather than simultaneously as in gait I.

In order to expand the original model for intact animals (Fig. 9a) to include the behaviour patterns of the mesothoracic amputee an additional delay pathway must be introduced. In the new configuration of the model (Fig. 9b) the prothoracic legs may be controlled by either the meso- or metathoracic delay oscillators. If the influence of the new pathway is inserted into the step pattern of the front legs for an intact animal, it can easily be seen that the new pathway produces no change in the intact coordination. However, this new pathway does produce a more rapid adoption of a normal pattern during start sequences and improves the overall stability of the model. When the left mesothoracic leg is autotomised a breakdown in the pathway L2 to L1 is assumed as shown in Figure 9c. The protraction of the prothoracic leg is then timed by the action of the metathoracic leg and both L1 and the stump of L2 will receive the same timing information causing them both to protract simultaneously. Such a pathway is equivalent to that suggested by Wendler (1966) to explain the synchronous movement of the mesothoracic stump and prothoracic leg. The steps of L1 and L3 then alternate with a phase of approximately 0.5 in gait I or slightly less if gait II is adopted.

Such an interaction between leg 3 and leg 1 appears to provide much weaker coupling in the adult than the short 3–2 and 2–1 pathways, for Wendler (1966) found that the relative timing of front and rear legs is unstable in the absence of a middle leg, and periods of changing phase or glide occurred at regular intervals. In free walking first instar nymphs such departures from a fixed phase relationship are rare and occurred most often at the beginning of a walking sequence (Fig. 5a). In a total of 65 steps only 5% were of an atypical phase as compared with perhaps 30% in the adult walking on a treadmill.

Right-left Asymmetry in "Single Leg" Amputees

The nature of the model proposed to describe the protraction timing of intact stick insects makes asymmetry a natural consequence of the adoption of gait II. Therefore, if amputation produces gait II all amputees might be expected to walk with asymmetrical step patterns. The experimental results show that this is only true for "single leg" amputees and the "two leg" amputee A(1, 3).

In adult stick insects the independence of right and left sides when making turns suggested that either the right or left side might control the timing of leg movements, and the terms right and left "drive" were defined to denote the two alternative asymmetrical patterns that could appear in the model behaviour. The use of the word "drive" in an earlier paper, Graham (1972), was unfortunate for "drive" carries with it the impression of increased activity on the "driving" side. However, the model is based upon an "inhibitory" interaction and, consequently, the side having the lower frequency controls the timing of the more active side and the word "timing" will be used in place of "driving" in an attempt to reduce this confusion. See Graham (1977a) for a complete description of the model structure.

Figure 10 shows the two alternative patterns in a simulation of the intact animal using gait II. The model step patterns (upper left) show the delay or "barrier to protraction" as a dotted line constraining the step pattern to one of the two alternative forms of asymmetry. Figure 10a shows the patterns of

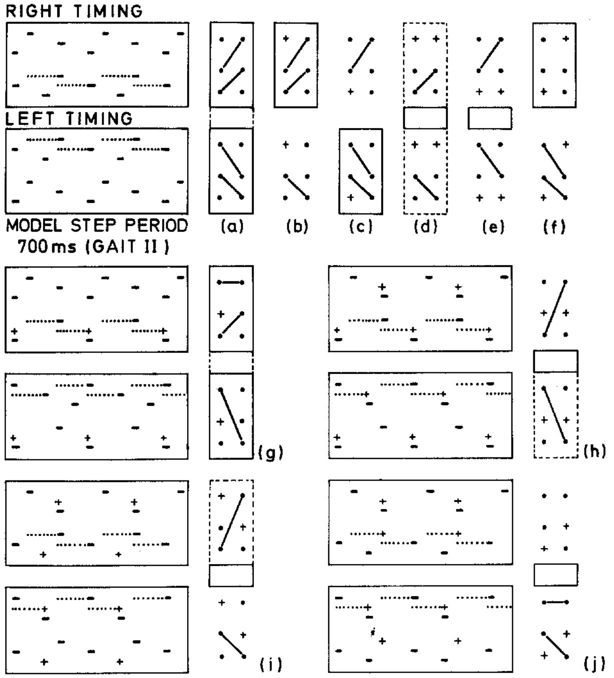

Fig. 10a–j. Predicted step patterns for the intact insect A(0) and the amputees A(L1), A(L3), A(1, 1), A(3, 3), A(R1, L3), A(L2), A(2, 2), A(L1, R2) and A(R2, L3). A simulation of the model configuration in Figure 9a or 9b showing the two alternative "*right*" and "*left timing*" step patterns for a first instar nymph using gait II with ${}_3L_1 = 560$ ms and $P = 700$ ms. Dotted line: duration of the contralateral inhibitory delay responsible for "timing". Patterns on the right: leg configuration for different amputees. Filled circle: an intact leg; cross: the position of the autotomised leg. The simultaneous protraction of two legs is shown by connecting them with a bar. Enclosure of the leg pattern within a box implies frequent use of the pattern; boxes between the alternative patterns indicate that a symmetrical gait is used. A dashed box implies that the pattern appears occasionally. **a** Two alternative asymmetrical patterns for an intact nymph using gait II. **bm c, d, e, f** leg patterns for various amputees. **g, h, i, j** step patterns for middle leg amputees derived from a simulation using the model configuration of Figure 9b

leg movement found in an intact animal for a step period of 700 ms and bars link those legs which tend to protract simultaneously. In the "right timing" example the oscillator system representing the legs on the right side has a slightly smaller input than the left. Therefore, the right side operates at a lower step frequency and controls the timing of the left via inhibitory delays giving a stable asymmetrical step pattern in which the phase of R3:L3 is 0.6. The "left timing" example gives R3:L3 = 0.4. These two alternative stable states derived from the model in Figure 9a can be used to predict the step patterns for all amputees other than those with a mesothoracic leg removed. For each amputee the appropriate set of steps may be deleted from the basic pattern and the stepping pattern for the remaining legs in each amputee is shown on the right of the figure. Comparing the data for A(L1), see Table 1, with the simulated behaviour shown in Figure 10b shows that removal of a left front leg produces a "left timing" step pattern. The pattern observed most frequently in the animal is enclosed in a box.

How Does the Walking System Decide Which of the Two Alternative Patterns it Should Adopt?

The choice of "right" or "left timing" may be at the discretion of the animal so that the actual choice represents a high level decision to use one of two alternative step patterns. However, as there are no examples of an inconsistent choice it may be that an automatic response is built into the walking system itself to compensate for the loss of propulsive thrust caused by the amputation. As an example of how such an automatic system might work, the loss of one leg might be expected to produce a continuous turn towards the amputated side if all the other parts of the system are unchanged. In an attempt to prevent this, the remaining legs on the amputated side could respond automatically by increasing their rate of stepping. Thus, in attempting to compensate for the lost leg the oscillator frequency increases on the amputated side, and the intact side then becomes responsible for the timing of all leg movements. A similar argument can be used for the preferred asymmetry in the mesothoracic amputee A(2). However, in this amputee both asymmetrical modes appear which suggests that perhaps middle legs contribute less to propulsion than front legs and the removal of a mesothoracic leg does not create a strong turning tendency.

If this argument is applied to the removal of a metathoracic leg, assuming that the rear legs are as important as front legs in propelling the body over the ground, then the amputee A(L3) should exhibit "right timing" as the left side is expected to become more active in order to compensate for the loss of leg L3. This is not observed experimentally and reference to Table 1 shows that extreme "left timing" occurs in this amputee. This apparently contradictory behaviour can perhaps be explained by the special significance of the rear legs in the model since they are responsible for the timing of all anterior legs. Examination of the model behaviour shows that small changes in the natural oscillator frequency of front or middle legs cannot be detected because the observed leg frequency is determined by the timing of posterior legs unless the change is sufficiently great to cause a breakdown in the coupling. Thus it is only in the rear legs that the direct effect of amputation on the natural frequency of the leg oscillator and its associated delays can be seen.

When a rear leg is amputated the model can qualitatively predict the step pattern if it is assumed that amputation produces a reduction in the natural frequency of this leg oscillator. This causes the amputated side to take over the timing of all leg movements so that left leg amputation produces a "left timing" step pattern. The observation that the animal does not turn strongly to the amputated side suggests that metathoracic legs are much less important than front legs in propelling the body.

One further significant change produced by rear leg amputation is a decrease in the lag across the body giving an exaggerated asymmetry to the step pattern. Again, changes of this kind would not be detected in front or middle leg amputations as the right-left timing is already established by the posterior legs.

The suggestion of a tractor role of front legs, support role of middle legs and support plus timing role for rear legs is only one of degree, for it is clear that rear legs can push strongly under certain circumstances. However, in walking on a horizontal surface the proposed dominance of front legs in propulsion would assist in maintaining directional stability and is consistent with the changes in asymmetry observed in "single leg" amputees. Clearly, such a hypothesis represents only one of a number of possibilities and without information on leg position and the trajectory of the walk it cannot be proven. However, it does provide a set of rules for predicting the asymmetry that results from a single leg amputation.

Recent work on adult stick insects by Cruse (1976) shows by direct measurement of forces that front legs are not used in propulsion but for exploring movements. This use of front legs as "feelers" has been seen only occasionally in 1st instars. In the walking sequences used in this work all legs appear to be fully engaged in walking movements.

Right-left Asymmetry in "Two Leg" Amputees

The amputee A(1, 3) presents an interesting problem because the experimental results for the amputees A(R1) and A(L3) both suggest that "left timing" should appear in the A(R1, L3) step pattern. However, Table 1 shows that the two separate amputations are not additive and "right timing" is used exclusively by this amputee. The simulated step patterns of the model in Figure 10b, 10e and 10f show that a theoretical prediction based on the amputees A(R1) and A(R3) is somewhat impractical. If "left timing" were used in the amputee A(R1, L3) both leading legs would have to protract at approximately the same time and the animal would be forced to balance on the most posterior pair of legs L2 and R3. Such a step pattern is clearly an impossible one and the opposite timing is adopted by this amputee so that the remaining legs all protract at different times. This appears to be an example of an amputation in which higher centres may override the automatic system suggested earlier by reducing the input to the side on which a front leg is amputated when a rear leg has already been removed. Unfortunately in this amputee no records of the walk were obtained immediately following the amputation. It is possible that this particular amputee might experience problems in coordinating its leg movements immediately following autotomy. However, even this change of step pattern in response to the amputation of the second leg could be "built in" to the protraction timing system although the cues necessary to produce this change in timing are quite complex and imply a subtlety and sophistication outside the scope of the present model. This seems to be an extremely interesting amputation deserving of further study as one would expect that, for a straight walk, there must be a significant reduction in the amplitude of leg movements on the side with the rear leg amputated.

In the "two leg" amputees, other than A(1, 3), the removal of one leg from each side produces a symmetrical step pattern in every case. The reason for such a gait is shown in Figures 10d, e, h and i. We can see that the use of either of the asymmetrical patterns would cause the animal to attempt to stand on only two legs. One way of avoiding the difficulty of balancing on two legs is to adopt a symmetrical step pattern in which each leg protracts at a different time. Reference to Figure 10e shows how this can be achieved. If the step frequency on the left side is slightly reduced in the "right timing" example the protractions of R1 and L2 no longer occur simultaneously and each leg completes its protraction separately. However, when sufficient drift has occurred the legs will adopt the "left timing" pattern and then L1 and R2 will protract simultaneously. This shows that although a symmetrical step pattern can be produced by the model the gait is inherently unstable and a right or left "timing" pattern will appear in response to any maintained inequality in the inputs to right and left sides. This results in a bimodal histogram such as that shown in Figure 1b.

It is possible to produce a symmetrical phase relation in the model by using almost equal but fluctuating inputs on right and left sides but this would give a broad phase histogram limited by the two alternative asymmetrical patterns. This is not a satisfactory explanation since most of the "two leg" amputees show sharp and well defined phase peaks for legs in the same segment. The mean values in every example other than A(1, 3) do not differ significantly from 0.5 and the distribution about the mean is of the same order as that for step patterns which are phase locked and suggests that either the duration of the contralateral delay is increased relative to that of the ipsilateral delay in order to lock the system into a symmetrical configuration or some additional coordination system is present.

An increase in the complexity of the model to permit an inequality in the delays along and across the body appears to be essential if a quantitative simulation of the behaviour of all the amputees is required. However, at least half the amputee step patterns can be simulated by the original model with the one modification of direct forward coupling between front and rear legs on the same side in order to produce the step patterns of the mesothoracic amputee.

The Effect of Leg Restraint on Coordination

The step patterns of I(R1) and I(R3) suggest that the animal does not adequately compensate for the absence of proper movement in the restrained leg and walks with either gait I coordination and frequently falls, divides the protraction stroke into two parts or uses gait II. Only gait II or autotomy produces a well coordinated step pattern in the remaining legs. In I(R3) both the asymmetry and the delays across the body are similar to those of the intact unrestrained animal using gait II and it seems that even an incorrect afference from the restrained leg does not produce the special changes found in an amputation of this leg.

The observation that I(R1) often falls forward at the beginning of a walk is of considerable interest. Examination of the leg coordination in an A(L1) amputee (see Fig. 10b) shows that the animal responds to the amputation by adopting gait II and protracts L1 and R2 at a time when L2 and R3 have completed approximately 1/3 of their retraction strokes and L3 has completed 2/3 of its stroke. The center of gravity in the first instar is considerably further forward than that of the adult animal and is in front of the line joining the mesothoracic leg articulations. Therefore, when the leg pair L1–R2 is protracted the weight of the animal should tip the body forward preventing a normal protraction stroke. This does not occur in the amputee and as it takes only 24 ms for the head to reach the ground one must conclude that the rearmost leg L3 prevents the body from tipping forward. The leg must rapidly reverse the action of its musculature from that of pushing to pulling while L1 and R2 are being protracted. It seems most probable that a simple resistence reflex comes into effect to compensate for such unusual movements of the body relative to the walking surface.

In the restrained animal I(R1), where the leg is not actually removed, the animal tips over because the leg L3 leaves the ground at the same time as L1 and R2 (see Fig. 7a). At first, the animal does not appear to make any coordination change in response to the restraint and the coordination changes only appear after the first few steps or when the leg is finally autotomised. It would appear that the coordination system adopts a new mode of operation in response to absence of afference but not necessarily to errors in afference.

As a further example of this observation consider what happens when a mesothoracic leg is immobilised or restrained. The movements of front and rear legs are no longer properly coordinated. It might be suggested that this reduction in coordination is entirely the result of vigorous attempts to release the leg but this suggestion is not supported by the relatively relaxed behaviour of I(R1) and I(R3) where the animal appears to be unaware of the restraint. The amputation experiments show that if middle legs are autotomised a new and effective leg coordination pattern is produced. Thus only autotomy appears to provide the necessary condition for a new coordinated pattern. It seems that restraint of the legs places the animal in a unique situation outside its normal range of behaviours and its only fully effective response is to autotomise the restrained leg.

The uncoordinating effect of restraint on middle legs was first observed by Wendler (1966). Pearson and Iles (1973) in their work on the cockroach suggested that hyperactivity of the campaniform sensilla at the trochanter could be the cause of this abnormal coordination by slowing the central oscillator of the restrained leg. This does not necessarily provide an explanation of the stick insect behaviour as the leg is held in the natural thanatotic position rather than being subject to the distortion present in the cockroach preparation. Bässler (1976) has examined the influence of these sensory receptors on the adult stick behaviour and found that stressing these receptors prevents the full sequence of movements in a normal step from occurring and the leg remains directed towards the rear until such time as the stress is removed. Similar effects can be produced by permanent stimulation of the hair fields signalling a protracted position of the limb.

At the present time it is not possible to provide even a plausible suggestion for the mechanism by which the coordination between front and rear legs is destroyed by the presence of the restrained middle leg. Nevertheless, these experiments strongly suggest that there is a direct pathway between receptors on the middle leg and the neural apparatus responsible for timing and coordinating the movement of the front leg on the same side. Only when the middle leg moves normally or has been autotomised can an effective coordination be maintained between the front and rear legs. If there is any major change in the afference from a middle leg the only action the insect can take in response to this disruptive influence is to autotomise the offending leg.

In conclusion, this study of amputee walking behaviour has shown that all the step patterns required to overcome possible leg deficits of up to one per side can be produced by an assembly of inhibitorily coupled relaxation oscillators. It is not necessary to make any major changes in the original model other than to add a direct forward pathway from rear to front legs. Such an additional pathway can account for the changes in the step pattern which result from mesothoracic autotomy and also reduce the occurrence of improperly timed steps during starting sequences in the intact animal.

The preferred asymmetry in all single leg amputees can be predicted by assuming that the input to the amputated side is increased in response to the operation and the loss of afference from an amputated leg lowers its own natural step frequency regardless of overall input changes and reduces the inhibitory delay postulated in the model for that leg. Finally, the experimental obervations on the relationship between asymmetry and the position of the legs removed suggest that the 1st instar insect uses a "front leg drive" or tractor walking system in which middle and rear legs are used for support rather than propulsion when walking on a smooth horizontal surface.

The symmetrical step patterns found in most two leg amputees cannot be quantitatively derived from the present model without a slight increase in complexity. The model parameters appropriate for the intact animal can give such patterns but they are unstable. This limitation can be overcome by the generation of longer delays across the body than along the body axis. Under this condition right and left step patterns can lock into a symmetrical relationship while retaining their normal ipsilateral timing.

Statistical Appendix

Examination of Table 1 shows that most of the ipsilateral distributions of phase are unimodal, symmetrical and strongly concentrated. However, in contralateral measurements bimodal histograms sometimes occur. The ideas described in an earlier paper (Graham, 1972) are used to interpret such histograms and it is assumed that they represent alternative modes of operation of a model walking system and arise solely from differences in input to right and left sides. In accordance with this assumption such histograms are divided into two separate distributions for statistical purposes. The low values of the two minima between the modes suggest that such a decomposition is justified and these minima are used as the points of separation.

Table 2. Statistical parameters for the bimodal distributions

Experiment	Phase	First mode	*n*	Second mode	*n*	Fig. No.
A(0) gait II	R3:L3	0.34, 0.7101	18	0.64, 0.8195	26	1b
A(R2)	R3:L3	0.36, 0.8610	20	0.66, 0.8329	15	5a
A(L2)	R3:L3	0.31, 0.8624	12	0.62, 0.8221	22	5a

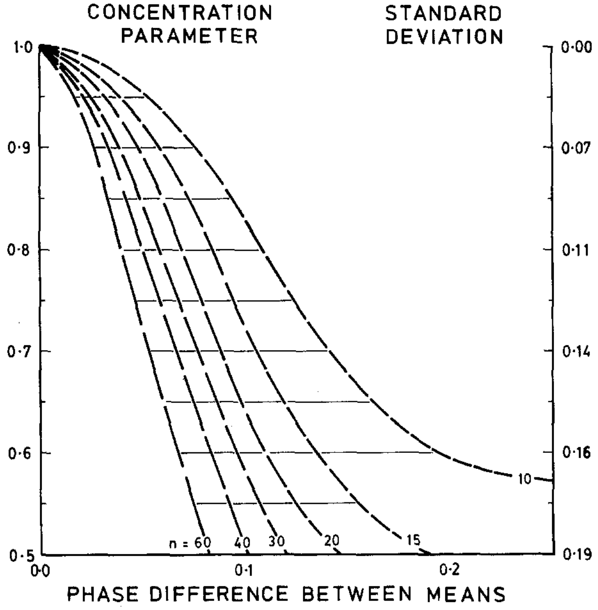

Fig. 11. Curves relating the differences between means to the concentration parameter for various values of sample size at a confidence level of 0.01. For application of these curves to the data in Table 1 see Appendix example. Left ordinate: concentration parameter. Right ordinate: approximate standard deviation measured by linear statistics. Abscissa: phase difference between the means; *n* is the sample size

The results of all experiments are given in Table 1 for selected phase relationships. The mean and a concentration parameter are given for unimodal results using circular statistics. Data for bimodal results are represented by modes and the statistical parameters of these modes are given in Table 2. The statistical parameters are derived by summing the sine and cosine components of a distributed set of unit vectors.

The mean $\theta = \tan^{-1} \dfrac{\sum_0^n \sin\alpha}{\sum_0^n \cos\alpha}$.

The product of the concentration parameter r and the number of vectors n,

$\mathrm{r}\,\mathrm{n} = [\sum_0^n \sin\alpha + \sum_0^n \cos\alpha]^{1/2} = \mathrm{R}.$

For highly concentrated distributions where all the data lie within a phase range of 0.25 it is possible to use linear statistics, but as methods of analysis are available for circular distributions and they are more exact they have been used as the basis for the data tables. Figure 11 shows the approximate relationship between the standard deviation and the concentration parameter (r) in the range of interest.

Two statistical tests have been used in examining the data presented in this paper. The first is a null test to decide whether or not a distribution differs significantly from a uniform distribution having no mean value. This test is appropriate for the leg restraint data where sample sizes are small and coordination is weak. The Rayleigh test (see Batchelet, 1965, Section 20) is used here as tabulated by Greenwood and Durand (1955) and the results are given in Table 3 for some of the restrained data where doubt exists. All other experiments are significant in this respect. A second test can be used to determine whether significant differences between means exist or conversely whether means said to be unchanged by an operation represent samples from the same distribution. For all the conclusions quoted in the text the differences or similarities between

Table 3. Test of null hypothesis for leg restrained animals

Experiment	Phase	*n*	a	Z expt.	Z (0.05)	Z (0.01)	Significant
I(R2)	R1:R3	13	0.3572	1.65	2.93		no
	L3:L2	13	0.6594	5.65		4.36	yes
	L3:R3	12	0.1746	0.57	2.93		no
I(2, 2)	R3:R1	22	0.0843	0.15	2.96		no
	L1:L3	26	0.4085	4.33		4.48	yes
	R3:L3	24	0.4081	3.99		4.47	yes

Z tables from Greenwood and Durand (1955)

means are so clear that they can be quite simply confirmed using a graph of critical differences based on a test developed by Stephans (1962) from the research of Watson and Williams (1956) (see Batchelet, 1965, Section 21). I have assumed that all the samples have a circular normal distribution. In the Stephans test the component of the given distribution in the direction of some test mean value is determined as $X = R \cos(\Delta\alpha)$ where R is the product of n the number in the sample and r the concentration coefficient. When R exceeds a critical value R_0 then the probability that the sample is not from the same distribution can be assessed. In order to simplify the use of this test on the data presented here I have constructed a set of curves relating the difference between an experimental mean and some test value, to the concentration coefficient r for the 0.01 confidence level over a suitable range of n (see Fig. 11).

As an example of the use of the graph, consider the hypothesis that the ipsilateral phase relation for the bilateral amputee A(1, 1) differs significantly from that for the unilateral amputee A(R1). Reference to Table 1 shows that for R3:R2 the phase difference Δ phase $= 0.71 - 0.64 = 0.08$. Examination of Figure 11 shows that for $n = 32$ and $r = 0.9$ a significant difference is 0.04. Thus a difference of 0.08 is highly significant.

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