

## THE EFFECTS OF SENSORY MANIPULATION UPON INTERLIMB COORDINATION DURING FAST WALKING IN THE COCKROACH

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### SUMMARY

1. A re-examination of fast stepping in free-walking cockroaches showed that there was considerable variation in the alternating tripod gait.
2. Small load increments applied to the whole animal significantly reduced this variability and concentrated the range and speed of movements used.
3. Animals suspended above oiled glass surfaces moved their legs in normal gait patterns indicating that mechanical coupling *via* the substrate was not a critical factor for normal high-speed coordination.
4. Increasing the viscosity of the oil produced the same reduction in variability found with load increment so the change cannot be due to increased mechanical coupling.
5. Increasing the viscosity of the oil under one leg alone resulted in concentration over the whole pattern so the result was not considered to be due to viscosity changes.
6. The changes seen in artificial loading situations were mimicked in natural loading situations such as walking up a slight incline.
7. The functional significance of the phenomenon for the stability of the rapidly moving animal is considered and the role of load afference in biasing central pattern generators to produce a continuous range of functional output is discussed.

### INTRODUCTION

Early work on insect stepping patterns suggested that insects use a relatively stereotyped alternating tripod gait except at very low stepping frequencies (Hughes, 1952, 1957; Wilson, 1966). Later studies showed that this does not apply to the stick insect *Carausius* (Wendler, 1964) and that there is considerable variation in gait even during steady walking in a straight line (Wendler, 1966; Graham, 1972, 1977).

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One factor which could be important in these differences is the extent to which sensory feedback is involved in determining the output. Several authors have suggested that this could underlie the finding that stepping patterns are more stereotyped in rapidly moving insects than in those that only move slowly (Wendler, 1966; Delcomyn, 1971). One of the most effective methods for examining the role of sensory input on coordination has been to alter the sensory input experimentally and then to observe the resulting output (Hoyle, 1976; Stein, 1976).

Another factor considered likely to bear on interlimb coordination is the mechanical coupling of limbs through substrate contact. While such coupling can be shown to have an effect (Cruse, 1976; Graham, 1981) it is not a prerequisite for normal coordination (Graham & Cruse, 1981).

Almost all the work examining these two factors has been carried out on the slow-moving stick insect *Carausius* (for a review see Bässler, 1983). Where insects capable of more rapid stepping have been studied, the observations have generally been limited to slow stepping (Greene & Spirito, 1979; Spirito & Mushrush, 1979).

The aim of the work described here was to examine the variation in stepping pattern in rapidly stepping cockroaches and then to observe the effect of manipulating sensory input and substrate coupling on this.

#### MATERIALS AND METHODS

Adult cockroaches of both sexes were selected at random from a permanent culture maintained at the University of Melbourne. The results cited here were taken from 25 animals.

The walking of normal, unrestrained animals was filmed by releasing them onto an enclosed, transparent, Perspex walkway 5 cm wide by 2 cm high by 1 m long. Each subject was ejected from a dark enclosure at one end of the walkway and made its way towards a darkened enclosure at the other end. A puff of air, loud noise or sudden vibration at the moment of release was usually sufficient to ensure rapid and direct progress towards the sanctuary. The walkway could be inclined or inverted for observations on the effects of tilt and upside-down walking on the stepping pattern.

To examine the stepping patterns of animals not subject to mechanical coupling between the legs by way of the ground, the pronotum was waxed to a glass rod and the legs of the restrained animal were allowed to contact smooth Perspex plates covered with a thin film of silicone oil with viscosity ranging from  $5 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$  to  $1 \times 10^{-3} \text{ m}^2 \text{ s}^{-1}$ .

When loading was required, low melting point wax was attached to various parts of the body and legs.

Films of the animals were made using either a Bolex 16 mm ciné camera at a speed of  $64 \text{ frames s}^{-1}$  or a Hycam rotating prism ciné camera operated at speeds of up to  $500 \text{ frames s}^{-1}$ . The camera ran on a track parallel to the walkway and filmed the side view and the dorsal aspect of the animal simultaneously by means of a dorsally positioned strip of mirror.

Film of the walking animals was analysed frame by frame with an analytical projector (L-W Photo-optical) and the data processed using Fortran IV programs on a Cyber 73 computer.

#### RESULTS

There is general agreement in the literature that at stepping frequencies above about 5 Hz the cockroach uses the alternating tripod gait. We have examined how much variation occurs in this gait during normal stepping. The results quoted are for the mid-sections of sequences taken when the animals were progressing in a straight line. Three animals were examined. Fig. 1 shows a typical segment of stepping from such a sequence and shows that while the general pattern can be described as an alternating tripod gait there is considerable variation in the timing details for specific steps. A measure of variation can be obtained in several ways. One method is to consider the phase relationships between two limbs. The term 'phase' is used as defined by Wilson (1966): 'The phase of an event is the fraction of some cycle which has elapsed at the time of the events' occurrence'. In the case under consideration here, we chose an easily defined instant in the cycle of one leg, for example the start or end of the stance, and used this to derive the phase position of the same instant for a second leg (Fig. 2). The cycle derived from the leg supplying the basic rhythm (or denominator of the phase fraction) we term the base cycle, and that supplying the event (or numerator) we term the test cycle. A plot of the phase relationship between two legs, cycle by cycle, gives a good visual measure of the variability over time of the relationship between the two. The sequential phase plot in Fig. 3B uses the start of the stance as reference point and is derived from a sequence of unrestrained walking. The example shows the variability of the phase position of the right metathoracic leg in the right mesothoracic leg but the result is similar regardless of which pair of legs is considered. Fig. 3 shows an example of the phase relationship for a normal stepping sequence.

We noticed during preliminary experiments that any manipulation that increased the loading on the animal or on any of the legs resulted in a change in the stepping pattern. This observation is consistent with findings from a variety of arthropod

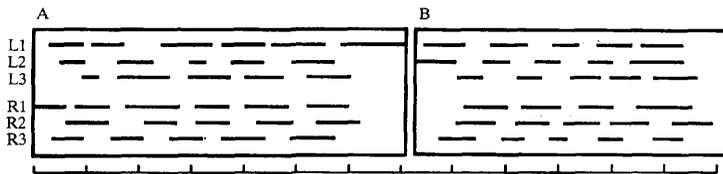


Fig. 1. Stepping pattern from a sequence of untethered walking in a straight line with a stepping frequency of (A) 8 Hz and (B) 10 Hz. Note that the powerstroke or stance phase is represented here by a line and the returnstroke by a space. Legs are labelled L1-L3 and R1-R3 from front to rear on the left and right side, respectively. Time marker, 100 ms intervals.

systems (Macmillan, 1975; Macmillan & Kien, 1983; Macmillan, Wales & Laverack, 1976). Systematic investigation of the changes in the cockroach stepping pattern showed them to be uniform and reproducible. Previous studies in several insects have shown that heavy loads alter the powerstroke/returnstroke ratio in a direction that augments the power output, but we found significant changes in the stepping pattern before we could detect any change in powerstroke/returnstroke ratio. The adult cockroaches in our culture had a mean weight of 1.3 g (s.d. 0.3). Additional load of as little as 5% of body weight resulted in a relatively stereotyped alternating tripod gait (Fig. 4A). Furthermore, the variability in the stepping pattern was significantly reduced (Fig. 4B,C). As one might predict from this result, the range of observed step durations and the angles swept out by the femur during each step were also narrowed (Fig. 5). Eight animals were subjected to various loading regimes.

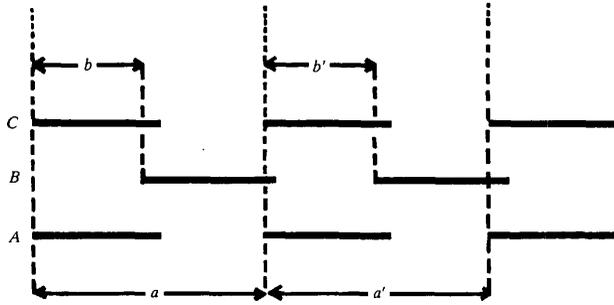


Fig. 2. Derivation of the measure of variation used in this study. 'Phase' derived according to Wilson's (1966) definition. Sequential phase of *B* in *A* where  $a$  = base cycle and  $b$  = test cycle. Cycle  $a'$ : phase =  $b'/a'$ .

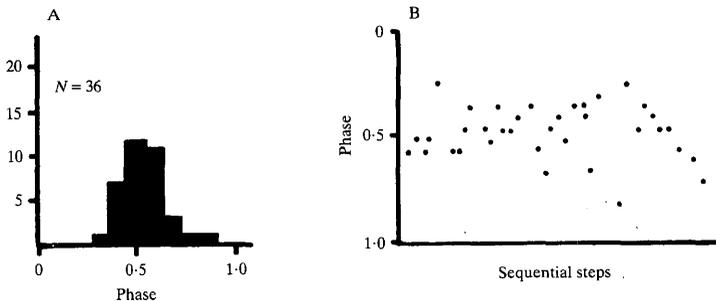


Fig. 3. Example of the measure of variation used in this study derived from a sequence of unrestrained free walking. (A) Phase frequency histogram of the phase of R3 in R2 using the end of the powerstroke as reference point. (B) Sequential phase plot showing phase of R3 in R2 in successive steps in sequence of unrestrained free walking using the end of the powerstroke as reference point.

F-tests comparing these parameters in the same animals before and after loading indicate that all show a significant ( $P < 0.05$ ) reduction in variability.

The observed reduction in variability which results in a stereotyped alternating tripod gait could be due to a change in the sensory input caused by the load or it could be due simply to an increase in mechanical coupling. To test whether mechanical coupling is an important factor for interlimb coordination in rapidly running cockroaches, we observed the stepping patterns of animals tethered and supported above oiled glass. Six animals were tested. We found, as have previous studies of other insects walking on low friction surfaces, that there was no marked alteration in the stepping pattern (Fig. 6A). The variability of low friction stepping samples was indistinguishable from that of free-walking animals. From this we concluded that mechanical coupling is not an important factor in determining the stepping pattern.

We then tested the effect of loading in the absence of coupling by increasing the viscosity of the oil. This treatment resulted in the same reduction in variability and the same stereotyped stepping pattern that was observed in freely walking but loaded animals (Fig. 6B).

To see whether these effects upon the walking movements could also be produced by a localized stimulus, an animal was supported over oiled glass and a load was applied to one leg by raising the viscosity of the oil for that leg alone. This reduced

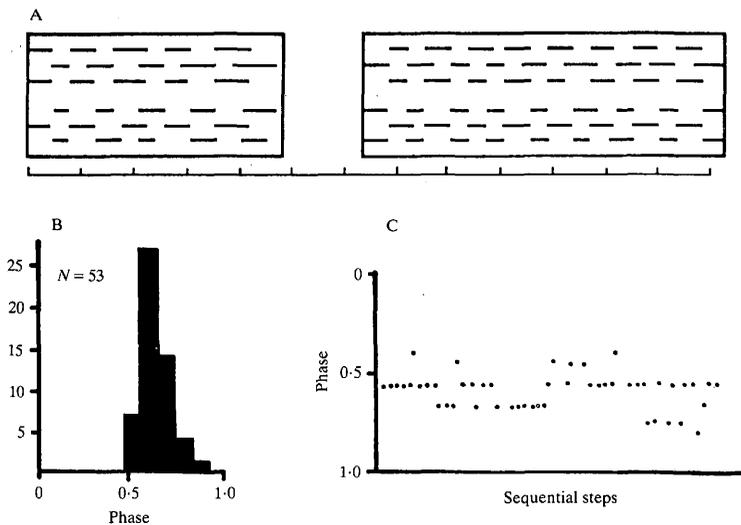


Fig. 4. Examples of the same measures used in Fig. 3 taken from sequences of walking at the same frequency in the same animal after loading at 6% body weight. (A) Stepping pattern at two different frequencies. Time marker, 100 ms intervals. (B) Phase frequency histogram. (C) Sequential phase plot.

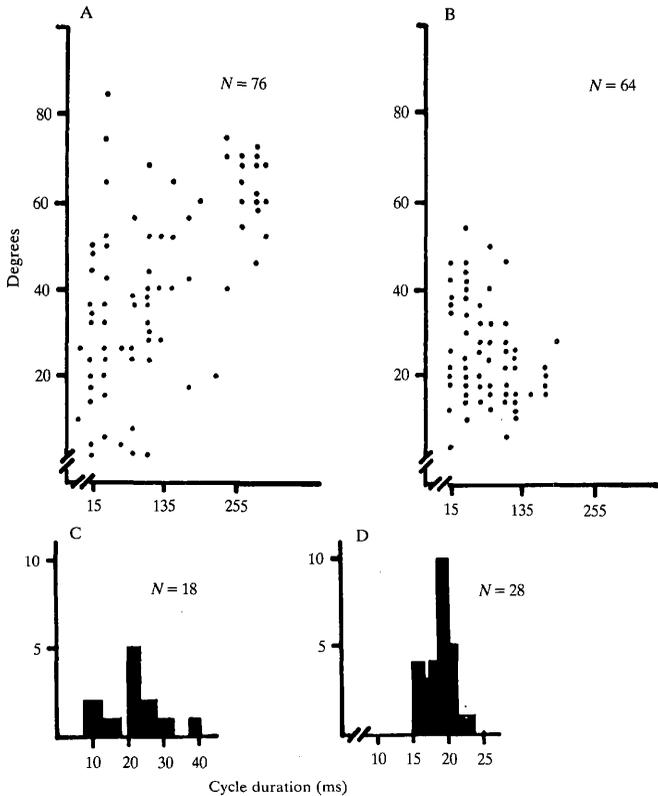


Fig. 5. Graph of angle swept out by femur of metathoracic leg as a function of cycle duration. (A) Freely walking animal. (B) Same animal walking in same velocity and step frequency range but carrying a load of 8% body weight. Frequency histograms of cycle durations (ms) from samples of unloaded and loaded walking. (C) Free walking. (D) 8% loading.

the variability of the entire stepping pattern (Fig. 6C). All legs were effective in producing this result but we did not search for fine differences in the level or quality of effectiveness.

The effects of a more natural loading situation were examined by running four animals up an inclined plane. The concentrating effect was observed at angles above 10–15° from the horizontal (Fig. 7A). At 25–30° tilt there were additional changes in the walking pattern of a type described previously (Spirito & Mushrush, 1979). As the angle of the tilt increased, we also noted a steady increase in the duration of the overlap period when the weight is transferred from one supporting triangle to the

other. The extreme of this change is encountered when the animal reaches the upside-down position (Fig. 7B).

It is difficult to make strict comparisons between the results from extreme-inclined-surface and upside-down animals with those from our other experiments because the stepping frequency was always lower in the more unusual situations. The concentrating effect was still present, however, as even the slower walking becomes more stereotyped in these cases. The gradual onset of the changes observed as the tilt was increased deserves further investigation because a detailed analysis could reveal so much about the mechanisms involved.

#### DISCUSSION

It is known that changes in load alter the powerstroke/returnstroke ratio in walking insects (Foth & Graham, 1983; Pearson, 1972). Our experiments show that the precision with which the movements of the walking cockroach are executed is altered by small loads applied to the body of the animal or to only one of the limbs.

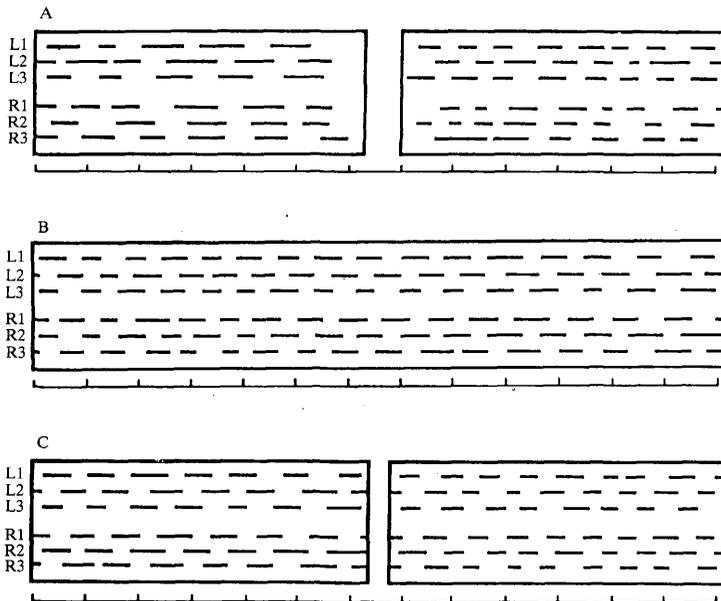


Fig. 6. (A) Gait of animal tethered and suspended above a glass plate covered with a film of oil of viscosity  $5 \times 10^{-5} \text{ m}^2 \text{ s}^{-1}$ . (B) Gait of animal suspended above glass plate with oil of viscosity approximately  $5 \times 10^{-4} \text{ m}^2 \text{ s}^{-1}$ . (C) Gait of animal with right metathoracic leg on a glass plate with oil of viscosity approximately  $1 \times 10^{-4} \text{ m}^2 \text{ s}^{-1}$  and the other five legs as in (A) above. Time marker, 100 ms intervals.

The changes can be observed with load increments that are too small to produce statistically detectable changes in the powerstroke/returnstroke ratio but which could be expected within the natural range of the animal's activities.

The loading produces a marked reduction in the variability of interlimb coordination, as a result of concentration of both the angles swept out and the timing of the movements. The magnitude of the parameters of loaded stepping fall within a narrow band in the range observed in free walking.

Previous studies of the effects of loading and changes in resistance on motor output patterning have identified several factors that may be important: mechanical coupling between legs, constant viscosity load, proprioceptive feedback and load-sensitive feedback. We will deal with each of these in turn.

A problem with most loading experiments is that any increase in load will also alter the mechanical coupling in any system doing work against that load. In the stick insect, substrate coupling can affect the patterning (Cruse, 1976; Graham, 1981) but is not primarily responsible for normal coordination (Graham & Cruse, 1981; Epstein & Graham, 1983).

Our result is in agreement with these findings. Since none of the parameters examined was altered during low-resistance walking on glass, which effectively removed the mechanical coupling, it is unlikely that mechanical coupling plays a primary role in normal locomotion in the cockroach. There may, of course, be some redundancy in the control system so that relatively minor changes in one input can be catered for, and the possibility remains that the mechanical coupling factor becomes more important as load increases. This appears to be a reasonable assumption, particularly with heavy loads. We do not feel that these considerations adequately

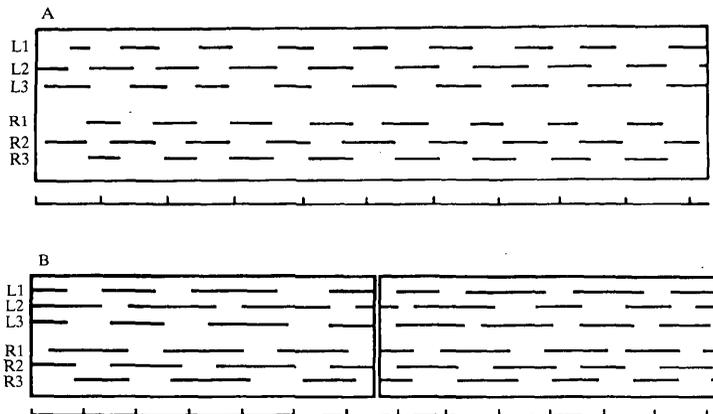


Fig. 7. The effect of substrate tilt on gait. (A) Gait of an animal walking up an inclined plane of  $12^\circ$  from the horizontal. (B) Gait of animal walking upside-down. Time marker, 100 ms intervals.

explain our results because the loads we used were deliberately kept very small and because the effects we describe occur in all legs in response to loading of one leg even though the mechanical coupling arrangements do not alter. It could be argued that our experiment exposes the insect to a situation which it might never normally encounter. Nevertheless, this experiment demonstrates that the walking system does receive input capable of initiating the changes in the absence of mechanical coupling or before the mechanical coupling factor has become significant. If the mechanical coupling factor does ultimately become important, then it must be interacting with the input that we have unmasked with our manipulations.

A similar line of reasoning can be applied to the viscosity factor. Epstein & Graham (1983) found that in the stick insect some of the reduction in variability which occurred when the animals walked on oiled surfaces was related to the viscosity of the medium. This conclusion suggested that the concentrating effect was a by-product of placing the animal in an unnatural, constant-load situation and not of primary functional significance. While the viscosity of the medium may be important, particularly at high viscosity, we obtained a similar effect after loading free-walking animals and did not observe any change in variability with animals walking on oil of low viscosity. These different results suggest that as the medium becomes more viscous the viscosity factor becomes increasingly important and even dominant. As before, the effect we have discovered comes into play before the viscosity factor and must be interacting with it.

The range and timing of the movements in the loaded animal fall within the range seen in normal walking and this argues strongly against position-afference changes being the primary cause of the changes in motor output. It seems probable that the changes in position-afference input arising from the increased precision would serve to reinforce the change.

In view of the foregoing considerations, it appears to us most likely that the concentration observed is a result of load-sensitive afference acting directly on the motor patterning centres. Complex interactions between load afference and positional control have been found in several systems (Cruse, 1980*a,b*; Macmillan, 1973, 1975; Macmillan *et al.* 1976) but the actual mechanisms are not known. It has been suggested that in the locust, load afference from campaniform sensilla may be able to regulate the gain of the input from proprioceptors (Macmillan & Kien, 1983). Some such mechanism could well be involved here because a parallel for the distributed effects of loading one leg is also found in the locust.

The type of loading used in these experiments did not influence the effects upon step timing and its variability, although adding weight to the body or legs produces a mainly perpendicular loading whereas increasing viscosity produces a primarily horizontal load increase. We are aware, however, that we may be dealing with two different effects or that the results may only be similar under certain conditions or when measured by limited criteria.

The most likely source of the load-sensitive afference is the campaniform sensilla which have been shown to have sensitivity in planes suitable for this role (Zill & Moran, 1981*a*; Zill, Moran & Varela, 1981). There is a rapid motor response to

sudden load increments during slow stepping (Pearson, 1972) but Zill & Moran (1981*b*) concluded that the reflexes mediated by the campaniform sensilla were too slow to have a cycle-by-cycle adjusting effect at stepping frequencies such as those used in the present study. The reflexes might, however, influence the walking pattern by having a temporally distributed effect under the influence of the load. Such a system could be functionally important in promoting mechanical stability at stepping frequencies beyond the reach of step-by-step adjustment.

The gradual changes seen in response to slowly increasing tilt in both the concentrating factor and in other ways described in previous reports (Greene & Spirito, 1979; Spirito & Mushrush, 1979) contribute to a smooth transition from the free, horizontal walking gait to the upside-down gait. It seems probable, as suggested by other walking studies (Macmillan & Kien, 1983), that load afference is responsible for biasing the rhythm generator to produce this continuous, yet precisely adjusted, range of output.

It is perhaps surprising that the variation we have found in the alternating tripod gait of the cockroach has not been described previously. The main reason for this is probably that our analysis focuses at a different level of precision. Examination of published records from earlier reports indicates that the information was there but was simply not relevant to the problem being investigated (Delcomyn, 1971; Wilson, 1966). Other investigations have been limited to slower stepping sequences but even in some of these there is a suggestion that the concentrating mechanism might be present (Spirito & Mushrush, 1979).

This report shows yet another way in which a complex locomotory system can respond to changing environmental conditions. The findings emphasize once more the care that must be taken in defining the experimental situation and conditions if useful comparisons are to be made with other studies or if functionally sound inferences are to be drawn.

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