THE SWIMMING OF *NYMPHON GRACILE* (PYCNOGONIDA)

THE SWIMMING GAIT

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INTRODUCTION

Most arthropods move by means of metachronal waves of activity passing over the paired appendages, and the resulting gaits have been studied extensively, with regard both to their locomotory effectiveness (Manton, 1953) and to co-ordination (Bethé, 1930; von Holst, 1935). Most attention has been focused on terrestrial or benthic arthropods and the mechanism of co-ordination of insect walking especially has aroused considerable interest (see reviews by Hughes, 1965; Wendler, 1966; Wilson, 1966). Here, as in all walking animals, the ambulatory appendages perform the dual function of support and propulsion, and gravity is of considerable importance in determining the gait employed. During swimming, however, it is frequently more important that the movements of the locomotory appendages be co-ordinated so as to ensure some degree of hydrodynamic stability if efficient progression is to be achieved. This would appear to be especially so in the pycnogonid *Nymphon gracile* where the four pairs of legs are arranged in almost radial symmetry about the body. During swimming these beat dorso-ventrally, and the animal moves through the water dorsal side first (Knight-Jones & Macfadyen, 1959). The forces generated by the leg during the swimming beat have been discussed earlier (Morgan, 1971). In this paper the swimming gait of *Nymphon* is described and some aspects of co-ordination between the swimming legs are considered.

Investigations into the gait patterns of invertebrates generally involve the amputation of one or more of the locomotory appendages. In insects this results in a marked change in phase between the remaining legs (Hughes, 1957; Wendler, 1966; Wilson, 1966; Delcomyn, 1971) and Wilson (1967) found that removal or immobilization of the third leg pair likewise effected a phase shift in the walking legs of tarantula spiders. Comparable results have been reported for opilionids (Bethé, 1930) and araneid spiders (Jacobi-Kleeman, 1953). The effect of similar amputations on the pattern of swimming leg movement in *Nymphon* are described here together with the results of experiments involving forced cyclical leg movements.

METHODS

The animals were collected at low water from Mumbles Pier, Swansea, and kept in a marine aquarium at Birmingham as described earlier (Morgan, 1971). The swimming gait of free-swimming animals was investigated cinematographically, and the start of the power stroke was taken as an arbitrary reference point for phase

analysis. When studying the effect of forced leg movements on the swimming gait
the animal was held with fine forceps between segments two and three. Leg four of
the right side (R4) was held by the tibia in a similar pair of forceps attached to a
probe and driven by a mechanical variable sine-wave generator similar to that used
by Lowenstein and Finlayson (1960).

As a result the femur of leg R4 was moved phasically through an arc of about 40°,
extending approximately 20° on each side of the vertical. This arc is considerably
smaller than that found during normal swimming, but is included in the range of
femur movement at the upper end (Morgan, 1971), and movements of as little as 30°
have been observed during slow swimming. Amputation of specified appendages was
carried out without anaesthetic, and the animals were filmed both immediately after
the operation and after a lapse of about 18–20 h. Filming was carried out at 18 frames/
sec as before.

<div align="center">RESULTS</div>

<div align="center">*The swimming gait of intact animals*</div>

The swimming gait of ovigerous male *Nymphon* was not noticeably different from
that of females or of sexually immature animals, and data obtained from two ovigerous
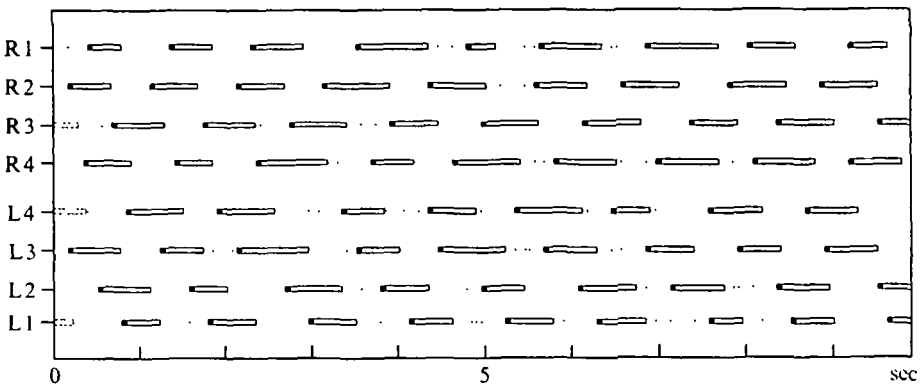males are included in the results of this section.

Fig. 1. Leg-beat sequence recorded during the swimming of a single *Nymphon*. The blocked
regions indicate the duration of the power stroke, the onset of which has been emphasized
for each beat.

In freely swimming *Nymphon* the legs beat in a regular metachronal sequence, 4321,
as described by Prell (1910) and Knight-Jones & Macfadyen (1957). The swimming
gait of a single intact animal is shown in Fig. 1. When the leg-beat period for each
leg was averaged over 30–40 cycles, involving three different animals, all the legs
were found to beat equally often (Table 1) and there is no evidence that the front
legs may beat more often than the posterior pairs as reported for the closely related
genus *Anoplodactylus* (Cole, 1901).

The phase relationship of the contralateral leg pairs of seven intact animals is
shown in Fig. 2.

The contralateral legs of each segment beat alternatively. This antagonism is most
obvious in the posterior legs and becomes progressively less precise in the legs of the

more anterior segments, a phenomenon similar to that reported by Wilson (1967) for tarantula spiders. The ovigers, palps and chelicerae make no obvious contribution to the swimming effort of *Nymphon*.

Table 1. *Mean leg-beat period recorded for individual legs of three intact* Nymphon *during normal swimming*

	Mean leg-beat period (sec)	
Leg number	Left	Right
4	0·738 (±0·062)	0·738 (±0·062)
3	0·756 (±0·067)	0·743 (±0·051)
2	0·749 (±0·052)	0·741 (±0·062)
1	0·742 (±0·054)	0·741 (±0·059)

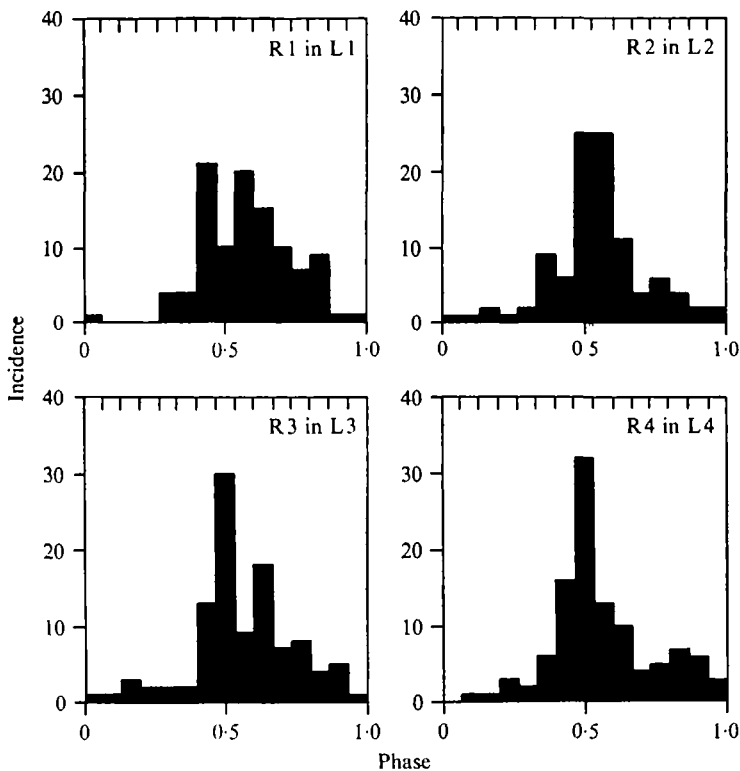Figures in parentheses are standard errors of the means.

Fig. 2. The phase relationship of contralateral leg pairs of seven intact *Nymphon* during normal swimming. In all histograms the phase measurement refers to the time interval between successive starts of the power stroke.

A fairly regular phase relationship was also evident between the ipsilateral legs, but the legs of adjacent segments did not beat strictly in antiphase. Fig. 3 shows the phase relationship between various ipsilateral leg pairs. Each leg is phased approximately one-third of a beat after the posterior adjacent leg, with leg 3 following leg 4 most frequently at about 0·3 phase. Leg 2 followed leg 3 most often after a phase delay of 0·43, while leg 1 in leg 2 showed a dominant phase delay of 0·36. However, the phase delay of leg 2 in leg 4 shown in the histogram occurs at 0·6, a value lower
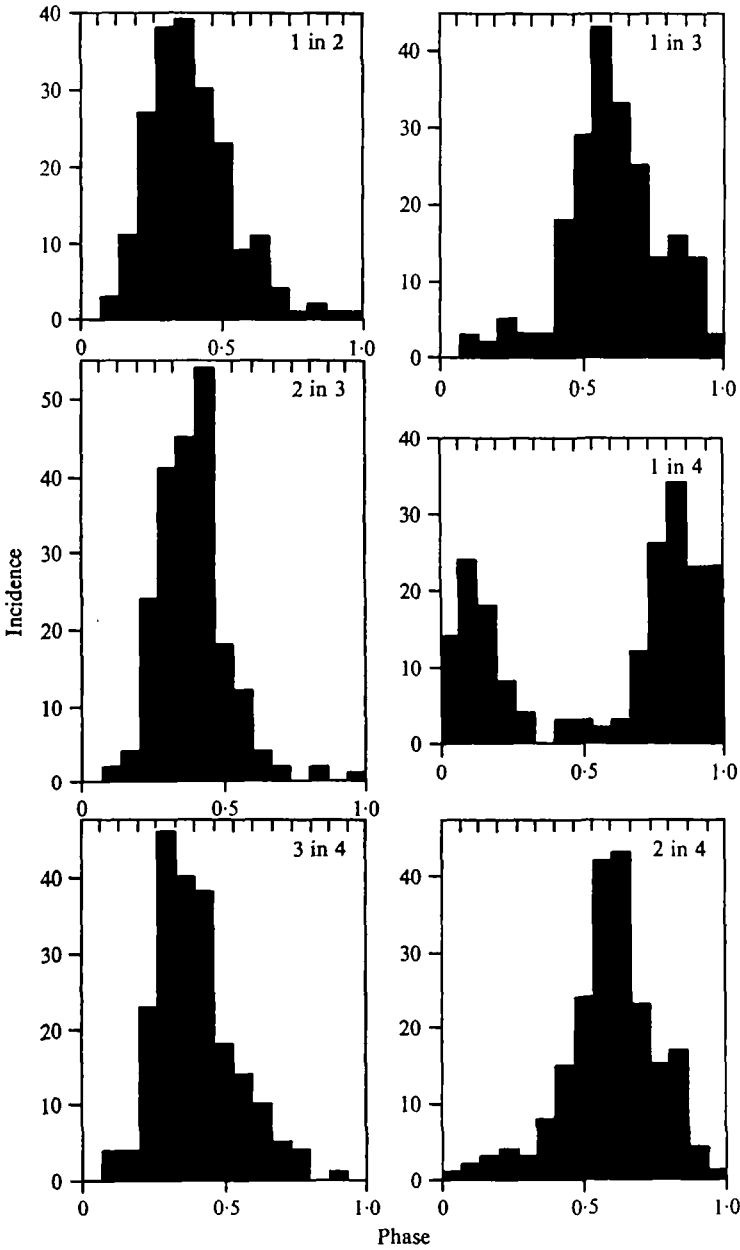
Fig. 3. The phase relationship of ipsilateral leg pairs of seven
intact *Nymphon* during normal swimming.

than would be expected since the mean leg-beat periods of legs 3 and 2 are of equal
duration. The film speed used (18 frames/sec) was often low relative to the leg-beat
frequency, but any inaccuracies resulting from the method of analysis would be small
and should average out over a series of observations. Thus this factor alone could
not account for the total discrepancy and so some other explanation must be sought.

Legs 4 and 1 beat almost together, but due to slight fluctuations in the leg-beat periods leg 1 sometimes starts to move early in the leg 4 cycle, as may be seen from the histogram of Fig. 3 so that the legs now beat in the sequence 4132. However, precise antagonism between legs of the same segment does not always occur, so that
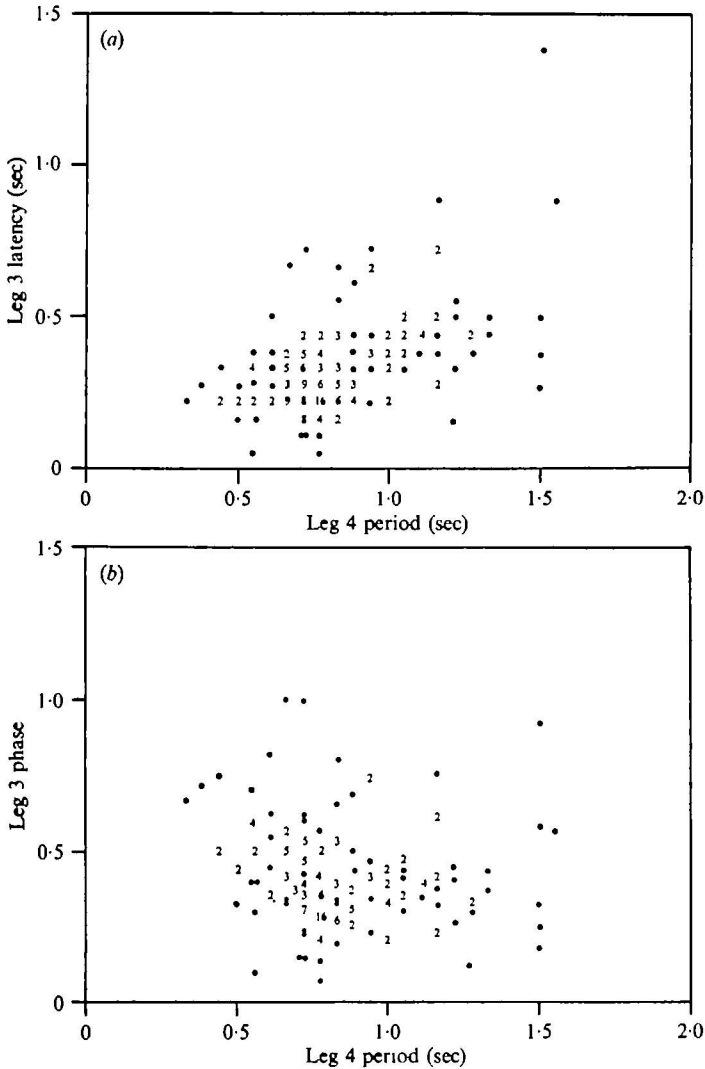

Fig. 4. The relationship between latency (*a*) and phase (*b*) of leg 3 beat and the stepping interval of ipsilateral leg 4.

the simultaneous beats of legs 4 and 1 tend to coincide with either leg 2 or 3 of the opposite side, and may often beat regularly in phase with one or the other, thus resulting in the mirror-image gaits reported by Prell (1910) and Rice (1962).

During normal swimming the mean leg-beat frequency was found to vary between 0·7 and 1·5 beats/sec (Morgan, 1971), and it seemed possible that the swimming gait might vary with the leg-beat frequency. Changes in frequency could be achieved without loss of the metachronal pattern by a change in the phase relationship of

adjacent legs, while keeping the leg-beat period constant, or conversely by varying the period at a constant phase delay. To determine the method employed by *Nymphon* the relationship between the phase and latency of the third leg beat and the stepping interval of the ipsilateral fourth leg was studied using the method of Wilson (1967). As may be seen from Fig. 4 the latency of leg 3 in leg 4 beat increases with the increase in period of the leg 4 cycle with a correlation coefficient of $+0.888$. Although there is some scatter, all leg movements are speeded up or slowed down in proportion. The phase delay of leg 3, on the other hand, shows no obvious dependence on the leg 4 period, and the apparent slight negative correlation is not significant ($r = -0.0894$). At leg-beat frequencies of below 0.6 beat/sec the animal sinks to the bottom and the swimming gait is often disrupted as the animal tries to grip the smooth glass bottom of the observation tank (see Morgan, 1971). The leg-beat frequency now varies considerably, both between legs and in the duration of successive beats of individual legs. At very low stepping frequencies individual legs were seen to beat more than once during the cycle of adjacent contralateral or ipsilateral legs, and the movement of one or more legs was frequently arrested apparently at random during the leg-beat cycle.

The effect of third leg amputation

Removal of one or more swimming legs usually results in the remaining appendages being shifted in position towards the gap. This lateral movement would at best only partially redress the ensuing hydrodynamic instability, however, and it seems likely that some change in the swimming gait might also occur. However, there was no measurable change in the frequency of beat of any of the remaining legs. The phase values of both ipsilateral and contralateral leg pairs of three *Nymphon* following amputation of the third leg pair is shown in Fig. 5. The contralateral legs again beat in antiphase with the last pair of legs showing the strongest coupling as in normal swimming. Of the ipsilateral legs the phase relationship of leg 1 in leg 2 also corresponds to that shown by intact animals, but the beat of leg 2 in the leg 4 cycle is noticeably advanced, and the histogram is nearly symmetrically distributed on either side of the 0.5 phase value. This mean value does not necessarily reflect the preferred gait of individuals, however, and when the phase relationship of leg 2 in leg 4 is re-plotted independently for the right and left legs (Fig. 6) it is evident that leg 2 follows leg 4 on the left side after a shorter delay than on the right. The second contralateral legs no longer beat exactly in antiphase. Instead the beat of R2 in L2 is a little delayed relative to that of intact animals and the first and last legs of the left side do not show such close synchrony. The swimming gait may thus be represented (R4 R1 L2) (L1) (L4 R2) and this pattern may alternate with its mirror-image sequence as in intact animals.

In another series of experiments the third left leg alone was removed from a further three *Nymphon* and the pattern of swimming leg movements was investigated as before. The phase value of R2 in R4 now corresponded to that recorded for intact animals and the phase distribution of R2 in R4 and R1 in R2 was no longer bimodal as when both third legs were removed, suggesting that the tendency to drift between mirror-image gaits was not as marked. The gait may thus be represented (R4 R1 L2) (R3 L1) (L4) (R2) in contrast to the situation resulting from the removal of both
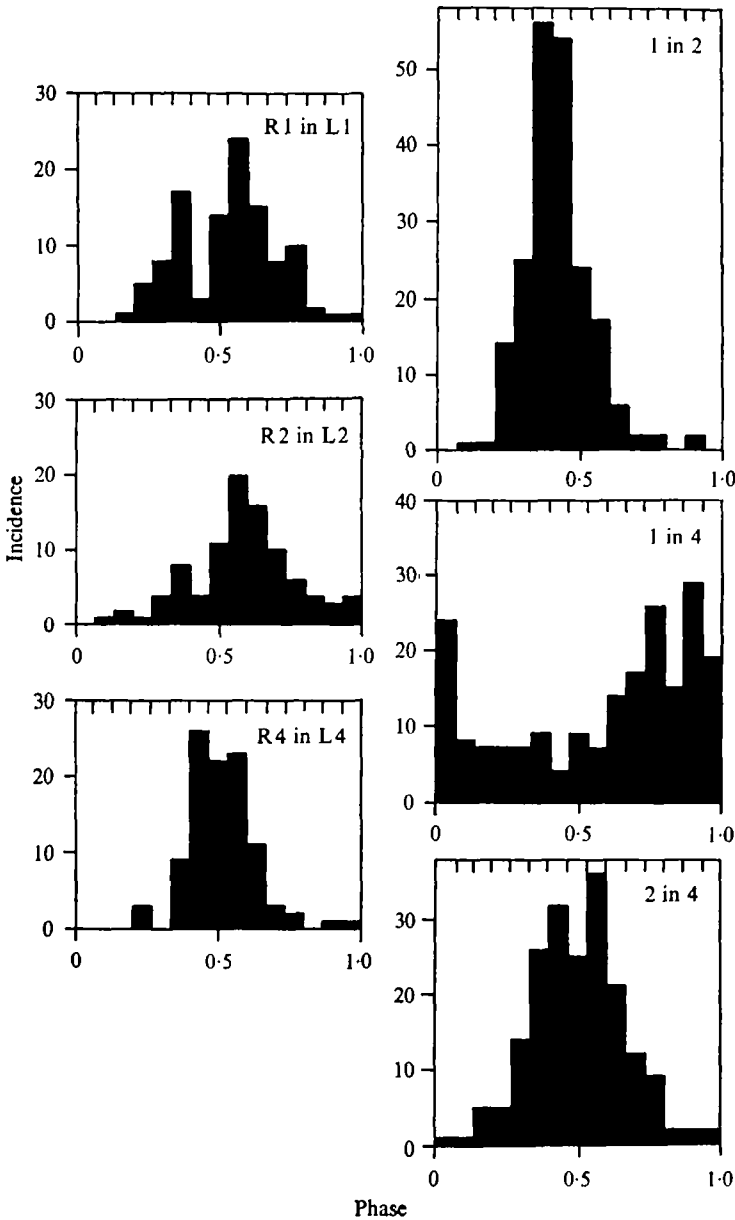
Fig. 5. Phase relationship of both ipsilateral and contralateral leg pairs recorded during the swimming of three *Nymphon* following amputation of the third leg pair.

third legs when L4 happens to coincide with R2, and L1 occurs approximately midway between R2 and R4.

The effect of fourth leg manipulation

In another series of experiments the animal was restrained and leg 4 on the right side was manipulated mechanically by means of a probe attached to tibia 1. In the

control experiments the probe was detached and moved through the water inde-
pendently of R4. All movements of the probe were sinusoidal.

The effect of an imposed cycle of 1·2 sec period on the phase delay of L4 and R3
is shown in Fig. 7. This is of slightly longer period than the leg-beat cycle of free-
swimming animals. L4 shows no constant phase relationship with R4 and frequently
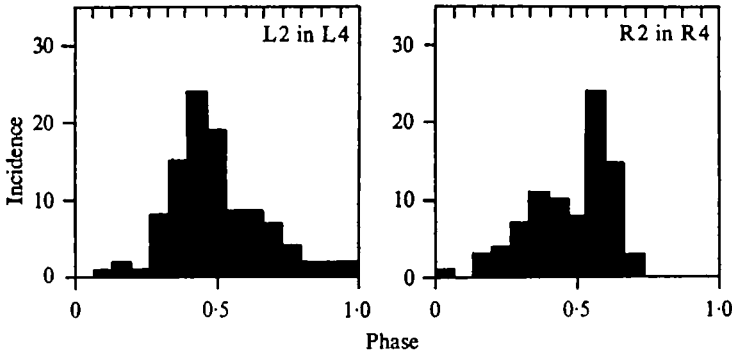

Fig. 6. Phase relationship of ipsilateral leg pairs 2 in 4 following amputation of the third leg
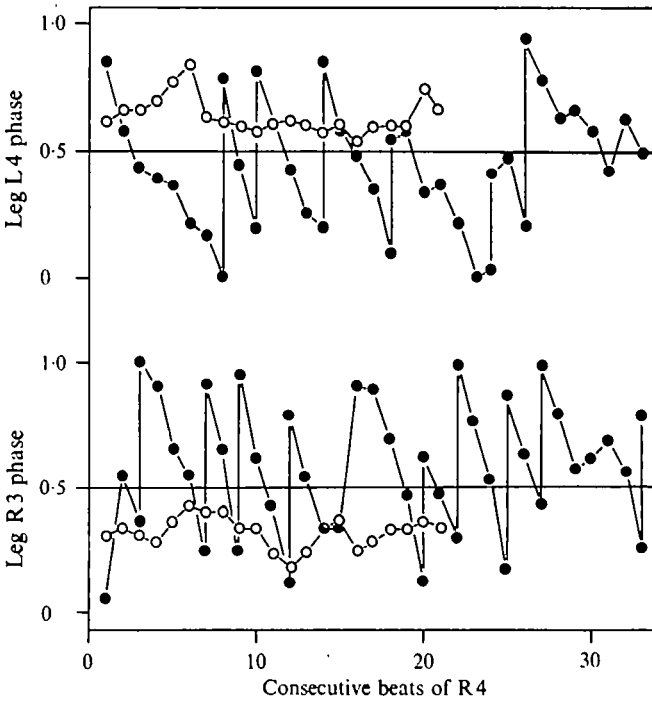pair, plotted separately for the right and left sides.

Fig. 7. Phase delay of the contralateral leg L4, and of the ipsilateral leg R3 in consecutive beats
of leg R4; ●, R4 moved by a probe through cycles of 1·2 sec period; ○, control in which
R4 moved independently of probe.

beats twice during the imposed cycle whereas in the control experiment L4 followed
R4 after a much more constant phase delay of about o·6. The phase relationship of
L4 in the imposed R4 cycle is irregular and this is undoubtedly due in part to
apparently random changes as seen in the results of the control experiment. There is

however, some evidence of phase coupling between the two legs, especially during the early cycles where L_4 follows R_4 with a preferred phase delay of about 0·4. The phase delay of R_3 in R_4 also shows a tendency to drift, but follows a more regular pattern than that of L_4 (Fig. 7). During the control sequence R_3 followed R_4 after a preferred delay of about 0·33 phase.
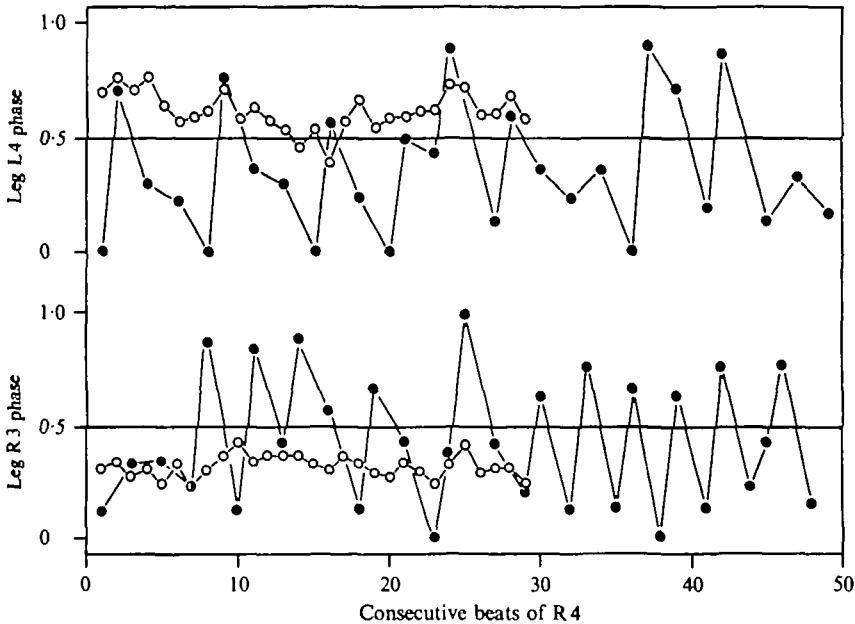

Fig. 8. Phase delay of legs L_4 and R_3 in consecutive beats of leg R_4. ●, R_4 moved mechanically through cycles of 0·44 sec period; O, control in which R_4 moved independently of the probe.

Similar results were obtained in another experiment in which the period of the cycle of movement imposed upon R_4 was about 0·4 sec. This was about half the leg-beat period shown by R_4 during the control sequence when it moved independently, and thus L_4 was frequently observed to beat in alternate cycles of R_4 as may be seen in Fig. 8. The drift is not regular, and the L_4 beat cycle appears to be periodically slowed down and accelerated in a manner reminiscent of the gliding co-ordination described by von Holst (1939) and Wendler (1966). However, closer analysis indicated no clear relationship between the duration of L_4 beat and the phase delay of L_4 in the manipulated R_4 cycle.

In comparison the phase delay of R_3 in R_4 shows a more regular pattern of drift corresponding approximately to that expected if R_3 was beating independently of R_4, and with a period of about 0·7 sec. It is thus unlikely that forced movements of the fourth leg have any marked effect on the pacemaking centre for leg movement.

<div align="center">DISCUSSION</div>

Wilson (1967) has proposed two models to explain the ambulatory gait of arthropods. The first, derived mainly from earlier studies on insect walking, considers the gait sequence to be a function of the metachronal wavelength and assumes that successive legs beat in metachronal sequence from back to front, with a constant leg interval,

and that contralateral legs of the same segment beat in antiphase. Although predicting many gait patterns this model fails to account for one of the most common patterns (4312) employed by both tarantulas and crayfish and hence was rejected in favour of an alternative, more flexible system based on reciprocal inhibitory coupling between pacemakers controlling the stepping of each leg. The swimming gait of *Nymphon* violates none of the assumptions fundamental to either model and each may adequately account for the swimming sequence observed in intact animals (allowing a phase delay of approximately 0·3 between adjacent ipsilateral legs as opposed to the 0·5 phase delay recorded for tarantulas). The apparent synchrony of different legs in various combinations may thus result incidentally from nearly identical leg-beat frequencies. Exact synchrony between legs is rare, and a slight phase shift (< 0·1) would be adequate to produce the mirror-image gaits observed. Isolated phase shifts of this magnitude would not be revealed in the phase histograms. Such slight deviations in phase are unlikely to upset greatly the pattern of support and movement of perambulatory arthropods as Wilson (1967) has pointed out, but they may well be of consequence during the swimming of *Nymphon* when hydrodynamic stability is of prime importance.

The results of the amputation experiments, however, are more easily explained in terms of the coupled oscillator hypothesis. If no coupling occurred, i.e. if each leg beats independently but at the same fixed frequency then the gait sequence expected following amputation would be that observed prior to the operation but without the removed appendages. This would appear to be so in *Nymphon*, but more detailed analysis shows that the movement of leg 2 in leg 4 is often noticeably advanced following third pair amputation (see Fig. 6) suggesting that the legs are not entirely independent. This change in gait is not as extensive as that reported for the insects (Hughes, 1957; Wendler, 1966; Wilson, 1966; Delcomyn, 1971) or araneid spiders (Jacobi-Kleeman, 1953) while in tarantulas (Wilson, 1967) erstwhile antagonistic legs were observed to step synergically after a similar operation.

However, as von Holst (1935) has pointed out, swimming arthropods are confronted with rather different problems during locomotion. In the giant water-beetle *Dytiscus*, for example, Hughes (1958) found that the remaining hind leg continued to alternate with the contralateral middle leg following amputation of a single metathoracic leg, and the only change in the rhythm of leg movements was a delay in the retraction of the other middle leg. In spite of this the animal continued to swim along a relatively straight path, mainly as a result of changes in the amplitude of movement and in rotation of the remaining swimming legs. In *Nymphon* the effect of amputation on the swimming gait is partly mitigated by the lateral movement of the adjacent legs towards the gap, but no observable change in amplitude of heat was recorded for the remaining legs.

The concepts of central and peripheral control of animal locomotion do not appear to be mutually exclusive, and it is reasonable to expect the relative importance of the latter to vary with the functional morphology of the organism. Thus, as Wilson (1967) has suggested, proprioceptive reflexes may be quite important in determining the gait of the tarantula but in *Nymphon* the alteration of afferent feedback by direct manipulation of the fourth leg affects the overall gait only slightly.

When a cycle of period slightly longer than that normally employed during swim-

ming was imposed on R4 the duration of the contralateral leg L4 cycle varied periodically, and the observed phase drift (Fig. 7) did not conform exactly to the pattern expected for two independent oscillators drifting one relative to the other. A more regular drift pattern is shown by the phase of R3 in the R4 cycle, but the leg-beat period which agrees best with this sequence is longer than that observed during the control experiment. This is also true for successive beats of L4 when these show a fairly regular phase drift and suggests that although complete coupling obviously does not occur some degree of co-ordination may exist between the adjacent legs and the cycle of movement imposed on R4.

Wilson (1965) has been able to demonstrate what he called reflexive correlation between muscle potentials of both contralateral and ipsilateral legs and the phase of movement of a forced leg in cockroaches, but found the intersegmental reflexes to be weak and often only evident after averaging the results of many cycles. It is of note therefore that in *Nymphon* the phase relationship of the ipsilateral adjacent leg R3 shows a more regular drift pattern than that of the contralateral leg L4, both in this experiment and in that involving an imposed cycle of shorter duration (see Fig. 8), suggesting that here too coupling is stronger across the segment than intersegmentally. Whether such coupling results from sensory input from the leg influencing oscillators of adjacent legs directly, or via its own oscillator as Wendler (1966) has demonstrated in the stick insect *Carausius*, is difficult to ascertain here.

SUMMARY

1. During the swimming of intact *Nymphon gracile* the legs beat ventrally in a metachronal sequence starting from the rear, and with a phase delay of approximately one-third between successive appendages. The first and fourth ipsilateral legs thus beat together, usually in synchrony with either the second or the third contralateral leg, rather than halfway between them.

2. The pattern of leg movement in swimming involves a succession of such combinations, e.g. (R4 R1 L2) (R3 L4 L1) (R2 L3), and the preferred gait alternates between this sequence and its mirror-image combination.

3. Amputation of one or both of the third leg pair was followed by a lateral movement of the adjacent legs, but the rhythm of movement was little impaired, to the extent of reducing slightly the phase delay of leg 2 in leg 4 on the same side.

4. The effect of an imposed cycle of movement on the phase relationship of adjacent legs suggests that proprioceptive feedback plays only a little part in entraining the rhythm, but there is some evidence for reflex coupling between adjacent legs. This appears to be strongest within the segment.

5. The results are best explained in terms of negative coupling between oscillators controlling each leg.

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