

THE INHERENT WALKING DIRECTION DIFFERS FOR THE PROTHORACIC AND METATHORACIC LEGS OF STICK INSECTS

BY ULRICH BÄSSLER, EVA FOTH AND GERHARD BREUTEL

*Fachbereich Biologie der Universität Kaiserslautern, D-6750 Kaiserslautern,
Federal Republic of Germany*

Accepted 5 November 1984

SUMMARY

On a slippery surface the forelegs of a decapitated stick insect walk forwards and the hindlegs, backwards. Animals with only forelegs but that are otherwise intact walk forwards, whereas animals with only hindlegs walk mostly backwards. Usually when intact animals start to walk, their hindlegs exert a rearwards thrust on the substrate, but occasionally the starting forces are directed forwards.

A rampwise extension of the femoral chordotonal organ in the fixed foreleg of a walking animal first excites the flexor tibiae muscle (positive feedback). Towards the end of the ramp stimulus the activity of the flexor decreases, and the extensor tibiae motor neurones become strongly active.

All experiments indicated that the inherent direction of movement of the metathorax is rearwards. In intact animals there must be a coordinating pathway from the prothorax to the metathorax that, together with the sub-oesophageal ganglion, induces the hindlegs to walk forwards.

INTRODUCTION

It has long been known that stick insects without heads will not walk (von Buddenbrock, 1921). If such a decapitated insect is stimulated mechanically on its abdomen, its body may jerk forwards and backwards, but the insect does not execute any steps. However, in a chance observation in which decapitated stick insects were fixed over a slippery surface we found that when given a mechanical stimulus, they moved their legs in such a way that the forelegs appeared to walk forwards and the hindlegs backwards (unpublished data). This observation leads to the hypothesis that the inherent direction of movement of the forelegs is forwards and that of the hindlegs is backwards. Inherent direction refers here to the direction in which the leg pulls the body when the walking pattern generator of the leg is free of coordinating influences, i.e. when the direction of movement is determined by the pattern generator alone. According to this hypothesis, decapitation eliminates the coordination between the

Key words: Stick insect, walking direction, reflex reversal.

pro- and metathoracic legs so that their inherent directions of movement are expressed. When decapitated animals are stimulated to walk on a solid substrate (mechanical coupling of the legs), the forward pull of the forelegs is cancelled by the backward pull of the hindlegs, resulting in no net movement. The following experiments not only support this hypothesis but also suggest a possible explanation for the reflex reversal of the femur-tibia feedback loop in the active animal.

METHODS

All experiments were performed on adult female *Carausius morosus* from the stick insect colony of the University of Kaiserslautern.

The experiments dealing with walking on slippery surfaces were carried out as described by Epstein & Graham (1983), using low viscosity silicone oil ($10^{-4} \text{ m}^2 \text{ s}^{-1}$). With this method it was possible to determine accurately whether the tarsus was on or above the substrate. The experiments were filmed using a Beaulieu Super-8 camera at 18 frames s^{-1} , and analysis was performed using a Lafayette single-frame projector.

Walking was tested as described by Foth & Graham (1983a) on a pair of treadwheels with a friction of 1.2 mN, mounted on a common axle. Leg movement was recorded automatically with position detectors. Some experiments were also conducted using only one wheel of the pair.

To investigate reflex reversal in the foreleg, the insect was fastened to a narrow strip of 5 mm thick cork glued to a glass plate that was coated with silicone oil so that the unrestrained legs could walk on it (Fig. 1). The right foreleg was fixed at an angle of about 60° to the body and a plasticine well was built up around the basal part of its femur and filled with *Carausius* physiological saline (Bässler, 1977). The femur was opened dorsally, the receptor apodeme of the femoral chordotonal organ was cut and attached to the stimulation clamp, part of the extensor tibiae muscle and its tendon removed, and the extensor nerve F2 cut and its end inserted into a suction electrode. Stimulation of the chordotonal organ and electrophysiological recording were performed as described by Bässler (1976).

Since the extensor tibiae muscle was no longer functioning, the force generated by the flexor tibiae muscle could be measured by a force transducer (Swema, combined with a balanced bridge TF 19 from Hellige) positioned near the end of the tibia. The position of the receptor apodeme corresponded to a femur-tibia angle of about $100\text{--}120^\circ$. Ramp stimuli with rise times of from 0.1 to 3.3 s were delivered by pulling the receptor apodeme out by $400 \mu\text{m}$ and then pushing it back into its starting position.

RESULTS

Decapitated animals on a slippery surface

Twelve decapitated stick insects were suspended one at a time over a glass plate coated with silicone oil and filmed from above for a total of 318 s. Most of the insects

were active only when they were being tweaked lightly on the abdomen. Since the hypothesis being tested concerned only the fore- and hindlegs, analysis was limited to the behaviour of these legs. During the periods of activity, the legs showed the following types of behaviour.

- (1) *Walking movements.* The stance phase (movement of the tarsus on the substrate relative to the body) was clearly distinguishable from the swing phase in which the leg is returned to its starting position without the tarsus touching the substrate.
- (2) *Back and forth movements.* In contrast to walking movements the tarsus did not leave the substrate, but moved repeatedly back and forth approximately in the walking rhythm. Since the tarsus was registered as on the substrate as long as it touched the film of oil, it is possible that very shallow swing phases were also included in this category.
- (3) *Standing on the substrate.* The tarsus rested on the plate of glass.
- (4) *Standing on the holder.* The tarsus (of a middle or hindleg only) gripped the wooden stick supporting the animal or the fingers of the experimenter.
- (5) *Tarsus immobile in the air.* The femur-tibia joint was usually extended.

Table 1 shows how much time the fore- and hindlegs spent in each of the above types of behaviour. During walking, all foreleg swing phases contained an anteriorly-directed component, i.e. forelegs walked forwards. Some of these swing phases also had a strong lateral component. All the hindlegs walked backwards, with the start of

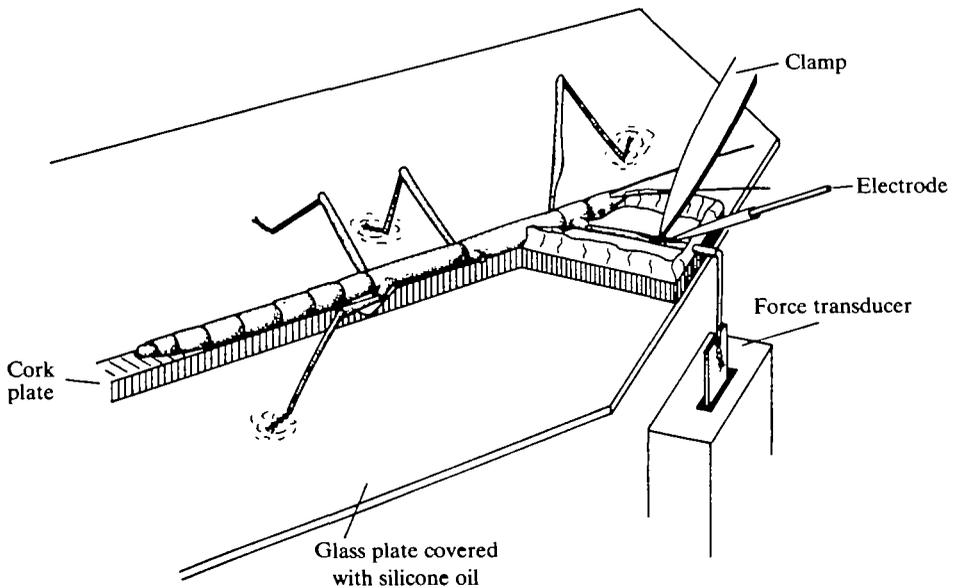


Fig. 1. Experimental set-up for recording in the walking insect the force generated by the flexor tibiae muscle and the activity of the extensor motor neurones in response to stimulation of the femoral chordotonal organ (see text for further description).

the swing phase being more anterior than its end. When the forelegs were walking, the hindlegs were usually engaged in one of the other types of behaviour and *vice versa*. Only for a total of 6 s (2%) did both forelegs walk forwards and both hindlegs backwards at the same time. Simultaneous walking of two forelegs and one hindleg was observed for 7 s (2%), and of two hindlegs and one foreleg, for 17 s (5%). Since the walks generally appeared to be very irregular, the coordination of leg movement was not analysed.

In a control experiment ten intact stick insects were studied under the same conditions. All but one insect walked forwards with all legs. The single exception, which walked relatively irregularly, showed occasional, brief periods during which its hindlegs walked backwards at the same time that its forelegs were walking forwards.

Walking of two-legged decapitated animals

If the starting hypothesis is correct, decapitated insects with only two legs must be capable of walking on a solid substrate. To test this, the middle and hindlegs were amputated at mid-coxa in five decapitated stick insects. In contrast to autotomy, this operation removes the campaniform sensilla on the trochanter. On an even surface these insects walked forwards readily, with their forelegs alternating regularly and the abdomen and most of the thorax dragging along the ground. It was, however, necessary to keep touching the animals on the abdomen. In contrast to two-legged insects with heads, the decapitated insects often fell on their sides, apparently being unable to maintain an upright body posture. Walking could be elicited as long as 8 h after the operation.

In five other decapitated insects the forelegs and middle legs were removed. After mechanical stimulation of the abdomen or the prothorax, these insects (without exception) walked backwards with the hindlegs. The leg movements were not noticeably coordinated and appeared rather hectic. These animals also frequently fell over sideways. Amputation of the prothorax of these animals 4 h later had no effect on the behaviour.

Often after a mechanical stimulus decapitated animals showed twitches very similar to those described for decerebrate stick insects (Graham, 1979). The twitches appeared in series, but were never followed by walking as occurs in decerebrate animals.

Table 1. *Time (in seconds with relative time in parenthesis) spent by fore- and hindlegs of twelve decapitated insects in different types of behaviour*

	Forwards walking	Backwards walking	Back and forth movements	Standing on substrate	Standing on holder	Tarsus in air
Forelegs	127 (20 %)	0	129 (20 %)	378 (60 %)	0	2 (0.3 %)
Hindlegs	0	205 (32 %)	130 (21 %)	283 (44 %)	7 (1 %)	11 (2 %)

For detailed explanation see text.

Walking on a treadwheel

These experiments were performed to determine whether in insects with intact central nervous systems the hindlegs also walked backwards.

Insects that had only the legs of one thoracic segment (five animals per leg pair) but were otherwise intact were fastened over a double treadwheel. Since each leg rested on its own wheel, there was no mechanical coupling between the legs. The wheels were slightly out of balance and the friction was not exactly the same for the two wheels. Thus, because the resistance that must be overcome affects the leg movement (Foth & Graham, 1983*a,b*), asymmetries could be expected to show up in one direction or the other depending on the relative positions of the two wheels. The insects were induced to walk by light mechanical stimulation of the abdomen. In the following the number of steps refers to the leg with the higher step number.

The insects with forelegs walked exclusively forwards with a fairly stable coordination between the two legs (Fig. 2A). The two legs alternated regularly in 542 steps. In 103 steps more right steps were performed and in 84 steps, more left steps. Only one leg moved in 16 steps. Step duration was between 0.8 and 2.0 s.

Only three of the five animals with middle legs could be induced to walk. Of a total of 177 steps, both legs stepped forwards in 99 steps (65%); both legs backwards in 7 steps (4%); and one leg forwards and one backwards in 71 steps (40%) (Fig. 2B). In 25 steps one leg stepped more than the other. The execution of the steps looked quite irregular.

Of the 216 steps analysed for animals with hindlegs, 49 steps (21%) were directed forwards and 147 (68%) backwards (Fig. 2C). In 26 steps (11%) one leg walked forwards and the other backwards. During backwards walking both legs took the same number of steps in 87 steps and a different number in 60 steps (Fig. 2C). This demonstrates clearly the irregular course of a step. Step duration was between 1.0 and 3.5 s.

In a further experiment four insects missing only the middle leg pair were fastened over one half of the treadwheel described above with the axle at a fixed height. Only the hindlegs stood on the wheel; the forelegs rested on a stationary platform, about 15 mm in front of the animal's head. These insects all walked forwards with their hindlegs; but 2 h after they were decapitated, their hindlegs walked exclusively backwards in the same experimental situation.

Forces exerted on the substrate at onset of walking movement

Inactive intact animals were fastened in a holder with their left fore- and hindlegs in the normal resting position on separate force transducers that registered the force exerted along the longitudinal axis of the body and with the other legs on a stationary rough wooden platform. The insects were activated by tweaking them lightly on the abdomen. In all 50 such activations from three animals the foreleg exerted a posteriorly-directed force. In 42 of these activations the hindleg also generated a posteriorly-directed force, after which it usually lifted off the force transducer and

reached far forwards. However, in eight activations in two animals an anteriorly-directed force was first produced, followed by a posteriorly-directed one (Fig. 3).

Reflex reversal in the foreleg

In the resting animal extension of the femoral chordotonal organ excites the excitatory extensor motor neurones of that leg (Bässler & Storrer, 1980). In middle and hindlegs the same stimulus briefly inhibits these motor neurones if the animal is

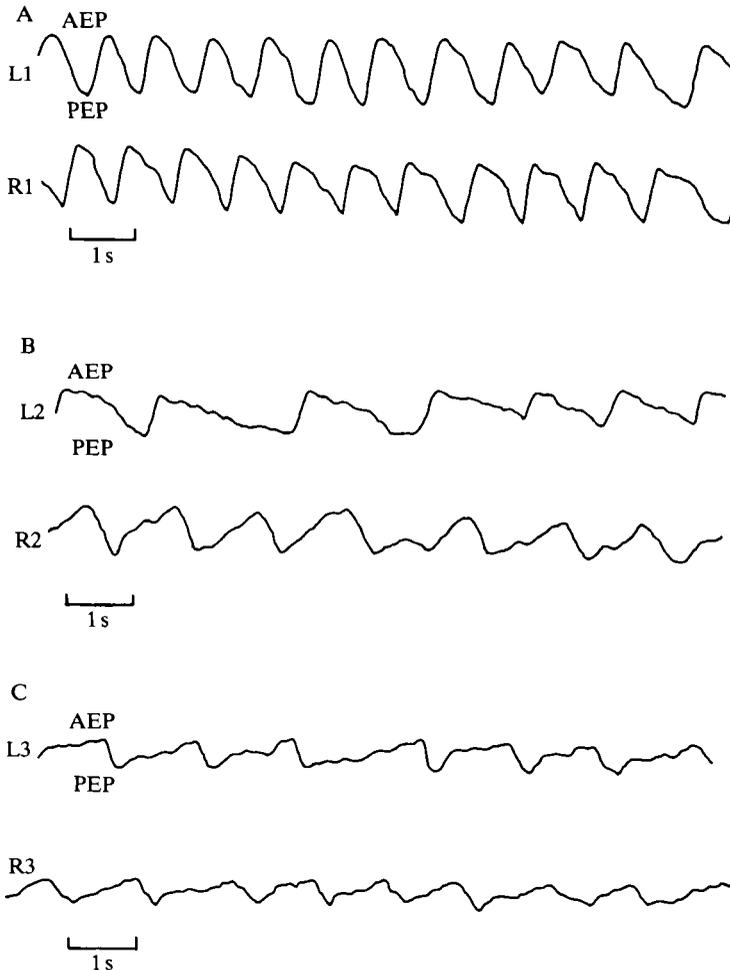


Fig. 2. Walking movements of animals that have only one remaining pair of legs but are otherwise intact. Each leg is walking on its own treadmill. AEP, anterior extreme position; PEP, posterior extreme position. (A) Forwards walk of the two forelegs. (B) Middle legs with the left leg (L2) walking forwards and the right one (R2), backwards. (C) Backwards walk of the two hindlegs with the right one (R3) taking an extra step.

active but unable to walk (reflex reversal: Bässler, 1973, 1974, 1976; Bässler & Wegner, 1983), and briefly excites them if the animal is walking (Cruse & Pflüger, 1981; Cruse & Schmitz, 1983; Bässler & Wegner, 1983). Until now the forelegs of active animals have been studied only in restrained insects (Bässler, 1974) but not in walking ones. To see whether reflex reversal in the forelegs differed from that in the hindlegs, stimulation was applied to the femoral chordotonal organ as described in Methods.

The resting animal showed the usual negative feedback responses, i.e. stretching the chordotonal organ excited the slow extensor tibiae motor neurone (SETi) and usually also the fast extensor tibiae motor neurone (FETi), whereas relaxation of the chordotonal organ led to a force build-up in the flexor muscle (Bässler & Storrer, 1980). The active animal showed reflex reversal, which was the same no matter if all four free legs were walking forwards or if some or all the legs were holding on to the cork platform. Typically any activity of the FETi and SETi that might have been present ceased immediately after the onset of the stretch stimulus, and often the common inhibitor (CI) was activated (Fig. 4). At the same time the flexor generated a very large force (positive feedback). The reaction cannot be distinguished from that of the hindleg in an active, but not walking animal described by Bässler & Wegner (1983). For stimulus rise times of 0.3 s and longer this response was regular but not always of the same intensity insofar as the animal was active (moving at least one unrestrained body part). With shorter rise times this response was observed only occasionally in the active animal. After the end of the receptor apodeme movement and even before the end of a slow stimulus, flexor force started to decline, and the FETi and SETi started firing again at a high discharge rate. Little can be said about the activity of the CI after the onset of FETi and SETi discharge because any CI

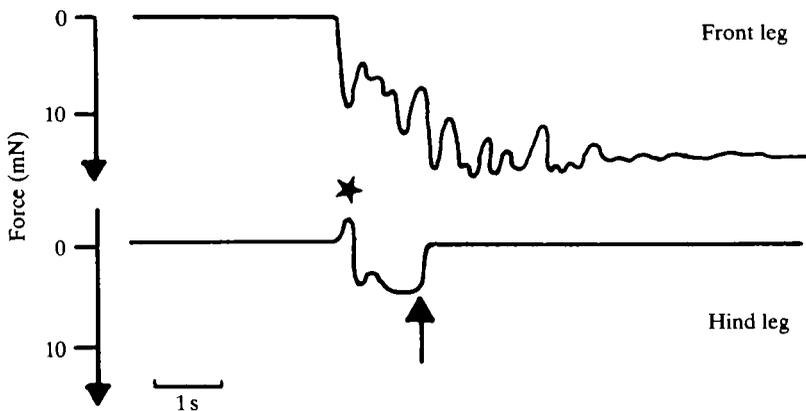


Fig. 3. Force exerted on the substrate parallel to the longitudinal body axis by the left foreleg (upper trace) and hindleg (lower trace) of an insect as it becomes active. Down corresponds to a posteriorly-directed force. The asterisk indicates an anteriorly-directed force. At the arrow the hindleg was lifted off the force transducer.

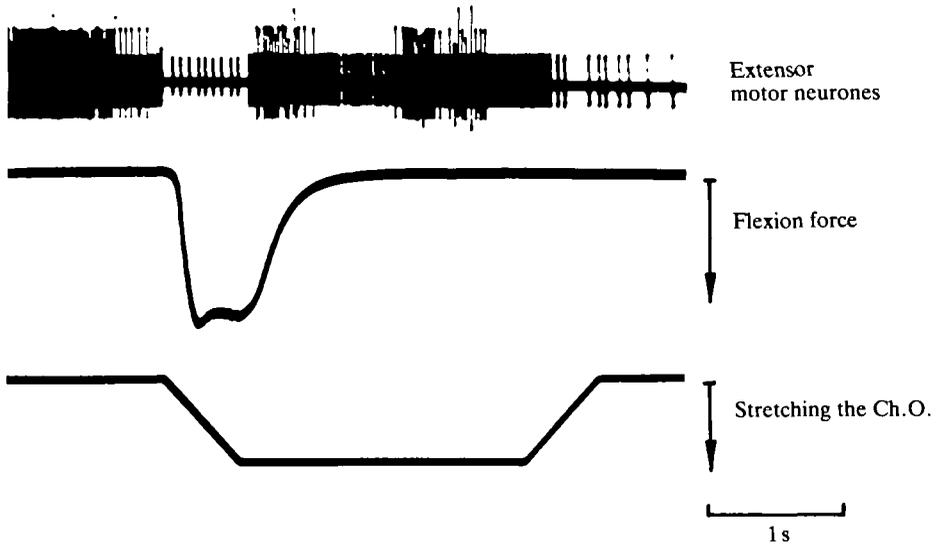


Fig. 4. Response to stretch and relaxation (amplitude $400\ \mu\text{m}$) of the chordotonal organ (Ch.O.) of a foreleg when the other legs are walking. The flexor force is not calibrated. In the recording of the extensor motor neurones, the large spikes are from the FETi, the middle-sized spikes from the SETi, and the small spikes from the CI.

spikes that might have been present would have been masked by the larger spikes of the FETi and SETi. The responses will be described in more detail in a following paper.

Relaxation of the chordotonal organ elicited very diverse responses or none at all. Fig. 4 shows the most frequently observed behaviour; however, there was also sometimes a further increase in the flexor force.

DISCUSSION

In the following it is assumed that the pattern generator for the movements of a leg is localized completely to the corresponding ganglion. This is supported by the observation that the forelegs show walking movements not only after an animal has been decapitated but also after the connectives between pro- and mesothoracic ganglia have been cut (U. Bässler, in preparation). This pattern generator, however, must be activated by other parts of the CNS.

When the hindleg of an intact animal is in the stance phase of a forwards walk, it can exert an anteriorly-directed braking force on the substrate even though it is moving posteriorly in this situation. This behaviour apparently helps to hold the body off the ground (Cruse, 1976). Thus, an anteriorly-directed force measured during the stance phase of the hindlegs in decapitated insects is not necessarily the result of a change in

walking direction. It could be merely an amplification of this body posture component.

In decapitated animals the hindleg starts its swing phase in the anterior extreme position (AEP), continues backwards through the air, and ends in the posterior extreme position (PEP). This differs in onset and direction from the swing phase of a forwards walk by an intact insect but corresponds to the swing phase in its backwards walk.

All experiments either confirmed, or at least did not contradict, our starting hypothesis that the inherent walking direction of the forelegs is forwards and that of the hindlegs, backwards. In order for mechanical stimulation of the abdomen to release backwards walking of the hindlegs the insect must either have been decapitated (decerebrate insects, which walk normally, differ from decapitated ones in that they still possess the suboesophageal ganglion), or the fore- and middle legs must have been amputated. Thus, the forelegs (and probably the middle legs), in cooperation with the suboesophageal ganglion, appear responsible for making the hindlegs walk forwards during normal walking. It is not necessary for the forelegs to be moving to have this effect (see Results: walking on a treadmill). Since the hindlegs of intact insects will sometimes walk backwards on a glass plate coated with silicone oil, it may be the tension receptors in the muscle or cuticle (Bässler, 1977) of the forelegs that cause the hindlegs to walk forwards. Signals from these, and perhaps other sense organs, could reach the metathoracic ganglion from the prothoracic ganglion either directly or *via* the suboesophageal ganglion. In the first case, however, they would still have to be accorded 'free passage' by the suboesophageal ganglion. These signals would then induce the metathoracic ganglion to produce the motor output pattern appropriate for forward walking.

The neural channel required here is a new kind of coordinating pathway, never before described in this form. It coordinates the direction of movement of different legs with different inherent walking directions. Whether it also adjusts the timing of the leg movement is not known. For backwards walking there may be an equivalent channel in the opposite direction with signals originating in the metathoracic ganglion that induce the forelegs and middle legs to walk backwards. Usually the walking direction of the hindlegs of intact insects is forwards (posteriorly directed force) from the very beginning of a walk. The few cases in which the posteriorly-directed force was preceded by an anteriorly-directed one indicate that the movement command might arrive at the metathoracic ganglion before the signal of the direction-coordinating channel and release first the inherent backwards walk. On the other hand the anteriorly-directed force may be a component of a postural reflex keeping the body raised above the ground. If so, it is possible that the movement command arrives at the metathoracic ganglion only in conjunction with the signal of the direction-coordinating channel.

In the restrained foreleg of an active animal, extension of the chordotonal organ always first inhibited the extensor motor neurones and excited the flexor motor neurones. The extensor motor neurones could then be excited by a further extension. This reflex reversal occurred no matter whether the other legs were walking or holding

fast to the substrate (see Results: reflex reversal in the foreleg). Except for the fact that reflex reversal in middle and hindlegs occurs only when an animal is active but not walking, reflex reversal in the forelegs has the same characteristics as in middle and hindlegs. (Stretching the chordotonal organ inhibits the extensor and excites the flexor motor neurones; there is a strong extensor burst towards the end of stimulation; responses to a fast stretch are rare; and relaxation of the chordotonal organ leads to diverse responses. For a summary see Bässler, 1983.) In contrast to middle and hindlegs, reflex reversal in the forelegs makes sense for normal forwards walking. A beginning stance phase in which the femur-tibia joint is starting to bend is first reinforced by the excitation of the flexor tibiae muscle. As the knee becomes more strongly flexed, which under natural conditions indicates that the leg is approaching the posterior extreme position, the flexor force decreases, and the extensor tibiae muscle becomes active. This could be interpreted as a component of the transition from stance to swing phase. Experiments on the stick insect *Cuniculina* (U. Bässler, in preparation) show that the transition from stance to swing phase can indeed be triggered by such stimulation of the chordotonal organ.

For the hindleg the corresponding reaction is meaningful only for backwards walking where its movement is approximately a mirror image of that of the foreleg during forwards walking and thereby fulfils an analogous function. Hence reflex reversal in the hindleg can be viewed as a component of its inherent tendency to walk backwards. This would explain why it is not observed in an insect that is walking forwards but is instead replaced by a normal negative feedback (Cruse & Pflüger, 1981; Cruse & Schmitz, 1983). The hindleg response to chordotonal organ extension switches from the backwards walking condition (reflex reversal) to the forwards walking condition (negative feedback) only when the other legs are actually walking (Bässler & Wegner, 1983) and not merely exerting a force on the substrate. On the other hand, for a change in direction of hindleg movement the forelegs must only build up force and need not be walking. Hence, the change in the direction of hindleg movement from backwards to forwards appears to be a process that can be divided into at least two parts.

REFERENCES

- BÄSSLER, U. (1973). Zur Steuerung aktiver Bewegungen des Femur-Tibia-Gelenkes der Stabheuschrecke *Carausius morosus*. *Kybernetik* **13**, 38–53.
- BÄSSLER, U. (1974). Vom femoralen Chordotonalorgan gesteuerte Reaktionen bei der Stabheuschrecke *Carausius morosus*: Messung der von der Tibia erzeugten Kraft im aktiven und inaktiven Tier. *Kybernetik* **16**, 213–226.
- BÄSSLER, U. (1976). Reversal of a reflex to a single motoneuron in the stick insect *Carausius morosus*. *Biol. Cybernetics* **24**, 47–49.
- BÄSSLER, U. (1977). Sense organs in the femur of the stick insect and their relevance to the control of position of the femur-tibia-joint. *J. comp. Physiol.* **121**, 99–113.
- BÄSSLER, U. (1983). *Neural Basis of Elementary Behavior in Stick Insects*. Heidelberg, New York: Springer-Verlag.
- BÄSSLER, U. & STORRER, J. (1980). The neural basis of the femur-tibia-control-system in the stick insect *Carausius morosus*. I. Motoneurons of the extensor tibiae muscle. *Biol. Cybernetics* **38**, 107–114.
- BÄSSLER, U. & WEGNER, U. (1983). Motor output of the denervated thoracic ventral nerve cord in the stick insect *Carausius morosus*. *J. exp. Biol.* **105**, 127–145.

- CRUSE, H. (1976). The function of the legs in the free walking stick insect, *Carausius morosus*. *J. comp. Physiol.* **112**, 235–262.
- CRUSE, H. & PFLÜGER, H.-J. (1981). Is the position of the femur-tibia-joint under feedback control in the walking stick insect? II. Electrophysiological recordings. *J. exp. Biol.* **92**, 97–107.
- CRUSE, H. & SCHMITZ, J. (1983). The control system of the femur-tibia joint in the standing leg of a walking stick insect *Carausius morosus*. *J. exp. Biol.* **102**, 175–185.
- EPSTEIN, S. & GRAHAM, D. (1983). Behaviour and motor output of stick insects walking on a slippery surface. I. Forward walking. *J. exp. Biol.* **105**, 215–229.
- FOTH, E. & GRAHAM, D. (1983a). Influence of loading parallel to the body axis on the walking coordination of an insect. I. Ipsilateral effects. *Biol. Cybernetics* **47**, 17–23.
- FOTH, E. & GRAHAM, D. (1983b). Influence of loading parallel to the body axis on the walking coordination of an insect. II. Contralateral changes. *Biol. Cybernetics* **48**, 149–157.
- GRAHAM, D. (1979). Effects of circum-oesophageal lesion on the behaviour of the stick insect *Carausius morosus*. I. Cyclic behaviour patterns. *Biol. Cybernetics* **32**, 139–149.
- VON BUDDENBROCK, W. (1921). Der Rhythmus der Schreitbewegungen der Stabheuschrecke *Dyxippus*. *Biol. Zbl.* **41**, 41–48.

