

## Load compensation in targeted limb movements of an insect

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### Summary

The task of a multi-jointed limb making an aimed movement towards a target requires that the movement is regulated against external perturbations such as changing load. In particular, loading one part of a limb leads to altered static forces on all proximal segments, and to additional dynamic joint interaction forces when the limb moves. We have addressed the question of load compensation in an insect preparation in which a locust makes aimed scratching movements with a hind leg in response to tactile stimulation of a wing.

We show that loading the femur or tibia with the equivalent of 8.5 times the mass of the tibia (corresponding to an increase of up to 11.6 times the rotational moment of inertia at the femur–tibia joint) does not impair the animal's ability to make well-coordinated, aimed movements of that leg towards different targets. The kinematics of the movements are the same, and animals aim the same part of their distal tibia at the target, regardless of loading. The movements are carried

out with equal accuracy and at the same initial velocity under all load conditions. Because loading of the leg does not change the behavioural performance, there is no indication of a change in aiming strategy. This implies high leg joint stiffness and/or the existence of high gain proprioceptive control loops. We have previously shown that in the unloaded condition, movements elicited by stimuli to different places on the wing are driven by a single underlying movement pattern that shifts depending on stimulus location along the wing surface. Our present data show that leg proprioceptive inputs are also integrated into the leg motor networks, rendering hind limb targeting robust against large changes in moment of inertia.

Key words: reaching, load compensation, scratching, locust, *Schistocerca gregaria*, coordination, insect, motor control, sensory feedback.

### Introduction

Reaching movements made by primates and other vertebrates are compensated well against perturbations caused by external loads such as artificial force fields (e.g. Lackner and Dizio, 1994; Shadmehr and Mussa-Ivaldi, 1994) or by internal loads such as joint interaction torques (e.g. Gribble and Ostry, 1999; Koshland et al., 1991). There is considerable debate about whether muscle forces are encoded explicitly by the nervous system, or whether limb position is encoded and the movement arises as the result of changes in a neuronally specified 'equilibrium position' (Feldman, 1986). In insects, load compensation has been studied in the context of posture or gait control (e.g. Pearson, 1972; Bässler, 1977; Cruse, 1990; Dean, 1991; Noah et al., 2001), but not in the context of aimed limb movements where load sharing and other coordinating mechanisms between legs can be excluded. The goal of this paper is to determine whether such aimed movements in an insect are compensated against altered loading.

In walking stick insects the protraction (swing phase) movements of the rear legs are targeted at the position on the

ground where the foot of the next anterior leg already has a foothold. Perturbations of the position of the anterior foot lead to adjustments of the touch-down position of the posterior leg (Cruse, 1979), and when the swing movement is resisted by an external force the targeting also remains accurate (Dean, 1984). When the swing movement of a rear leg is assisted by an external force, the velocity is increased only slightly. Taken together, Dean's data show that position is controlled for the endpoint of swing, but that velocity is controlled during the movement itself (reviewed in Dean and Cruse, 1986). Nevertheless, in walking, the control of any given leg is influenced by the actions of the others. For example, when the animal drags a load the velocity of swing movements is increased, presumably to permit a relatively longer stance phase (Foth and Graham, 1983). Such interactions between limbs can be excluded in the present study because (1) the load is added directly to the leg that is observed, (2) the leg moves to a target location that is independent of the postures of the other legs and (3) the aimed movement is carried out while the other legs remain still.

To test whether loading affects the performance of a targeted leg movement we analysed scratching movements made by a hind leg of a locust in response to tactile stimuli on the ipsilateral wing (see Matheson, 1997, 1998; Dürr and Matheson, 2003). We have previously shown that in unloaded conditions the pattern of leg movements used by locusts to move the distal end of their tibia towards different targets forms a continuum that is modified (shifted) by changes in target location on the wing surface. The location of the target signalled by wing exteroceptors is monitored throughout the movement so that re-targeting can occur to track a moving object (Matheson, 1998), but it is not known whether proprioceptive signals from the moving leg are similarly used to maintain accuracy.

To examine the effects of loading different leg joints, we loaded either a basal or a distal position on the femur or a distal location on the tibia. Loading the leg so that the rotational moment of inertia was increased by up to 11.6-fold had no detectable effect on any of the measured kinematic variables. We conclude that both position and velocity are controlled in this targeted movement, and that proprioceptive signals from receptors on the leg must be integrated throughout the movement with exteroceptive signals from the wing that signal target location.

### Materials and methods

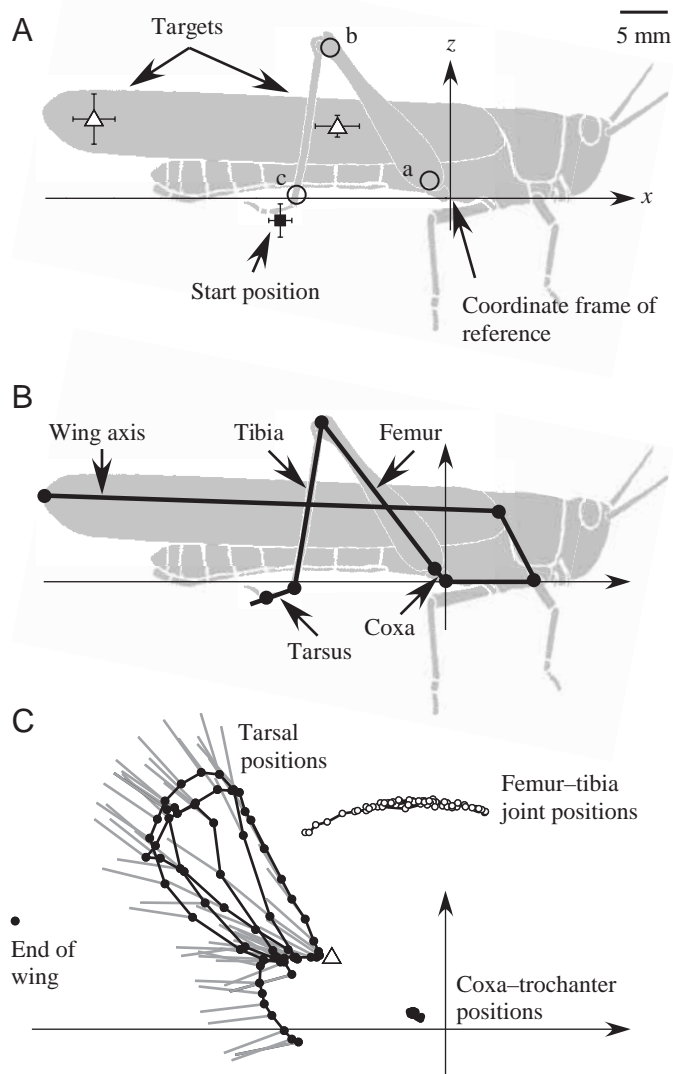
The recording and analysis methods follow the protocols set out in detail in Dürr and Matheson (2003) and are summarised only briefly here for completeness.

#### *Animals and stimulation protocol*

Experiments were carried out on adult female desert

locusts *Schistocerca gregaria* Forskål. They were tethered by a loop of fine wire that passed around their pronotum without obstructing movements of any of the legs and were suspended above a light (4.5 g) foam ball on which they stood or walked. The eyes were covered with solvent-free typists' correction fluid, and the tarsus of a hind leg was placed on a horizontal rod located at a fixed position (Fig. 1A) that corresponded to the anterior position used in Dürr and Matheson (2003). To elicit scratching movements of the hind leg, the ipsilateral forewing was touched gently with a fine paintbrush. Stimuli were applied to one of two different locations in pseudo-random order. These locations correspond to positions 2 (anterior) and 5 (posterior) used in Dürr and Matheson (2003). The exact location of the stimulus and the resulting movements were measured from videotape as described below. A small metal bead of mass 142 mg was fixed using sticky wax (Lactona Surgident, Philadelphia, USA) to the hind leg at one of three different locations (a–c in Fig. 1A) to provide a load (the hind leg mass was approximately 138 mg). The added mass was chosen so

Fig. 1. Location of digitised points and experimental setup. (A) Tactile stimulation of the wing at one of two stimulus sites (open triangles, means  $\pm 1$  S.D.) elicited scratching movements that began with the tarsus of the ipsilateral hind leg standing on a rod that defined the start position (filled square, means  $\pm 1$  S.D.,  $N=462$  trials in 3 animals). In 'loaded trials', a mass of 142 mg was added to the ipsilateral leg at one of three locations (a–c, open circles). In 'control trials' the leg was left unloaded. The coordinate frame of reference used in all of the analyses was centred on the metathoracic coxa, with the horizontal  $x$ -axis passing through the mesothoracic coxa. (B) To track movements of the body and limb, eight points (filled circles) were digitised manually in all video frames. Stimulus location and start position of the tarsus were digitised in the first frame. (C) In the simplest representation of a movement, we reconstructed the trajectories of the points representing the proximal and distal ends of the femur, the distal end of the tarsus, and the tip of the wing. For clarity the tarsus is represented as a grey line segment. This example shows a movement made in response to a stimulus at the anterior target (open triangle) in the unloaded condition. The distal end of the femur described an arc dorsal to the body (femur–tibia joint positions), while the distal end of the tibia and tarsus moved towards the target and then in three repeated loops (tarsal positions). In this case the wing did not move (end of wing), and the coxa rotated only a little (coxa–trochanter positions).



that it approximately matched the leg mass. By adding this mass to different sites on the leg, the moment of inertia of a single joint was altered in a range from 1% to 1160% of the normal value. Each set of scratches elicited under loaded conditions was interspersed with a set of control scratches elicited under unloaded conditions. There was no evidence of a temporal change in scratching behaviour (i.e. learning) throughout the course of the experiments, which lasted for 6–30 h in total. Each animal was tested for periods of up to 90 min at a time separated by intervals of at least 90 min to avoid fatigue. Experiments were carried out at 22–24°C.

#### Video acquisition and analysis

Animals were videotaped from the side using a calibrated CCD camera with a spatial resolution of 0.1 mm pixel<sup>-1</sup>, allowing manual digitising accuracy of 0.5 mm, as determined from the standard deviation of all digitised points in repeated analyses of three sequences. The images were recorded on video tape, captured on a personal computer and deinterlaced to yield AVI files with a frame size of 768×576 pixels at a frame rate of 50 s<sup>-1</sup>. A custom-written program (Borland Delphi) was used to access the AVI files and digitise the coordinates of up to 12 points per frame (Fig. 1B). The coordinates of the digitised points were output as a text file that was read into a second custom-written program for a variety of analyses (see below). The stimulus location and the position of the tarsus rod were digitised in the first frame of each trial. A further eight points were digitised in all frames to record movements of the stimulated wing and the ipsilateral hind leg. These were: (1) the front leg coxa, (2) the base of the wing on its midline, (3) the distal end of the wing on its midline, (4) the hind leg coxa, (5) the hind leg trochanter, (6) the distal end of the hind leg femur, (7) the distal end of the hind leg tibia and (8) the distal end of the second segment of the hind leg tarsus (Fig. 1A,B). Sequences were analysed from the first frame of tarsal movement until one of the following four events occurred: (1) the tarsus touched the ground, (2) the tarsus hit the stimulus brush, (3) the leg completed three complete cycles of movement or (4) the tarsus stopped moving for 120 ms.

#### Analysis

453 loaded or unloaded (control) scratches were analysed in three animals, all of which expressed qualitatively the same behaviour. Two components of each response were distinguished: a short (200 ms) initial component, in which the trajectory of the tarsus was relatively straight, and a second cyclic component, in which the tarsus moved in repeated loops near the target (see e.g. Fig. 2). The average velocities of the initial and cyclic components were calculated separately. The cyclic component was further quantified by means of probability distributions, which described the likelihood that a particular part of the leg moved across any given point in the leg's work space (for derivation, see Dürr and Matheson, 2003).

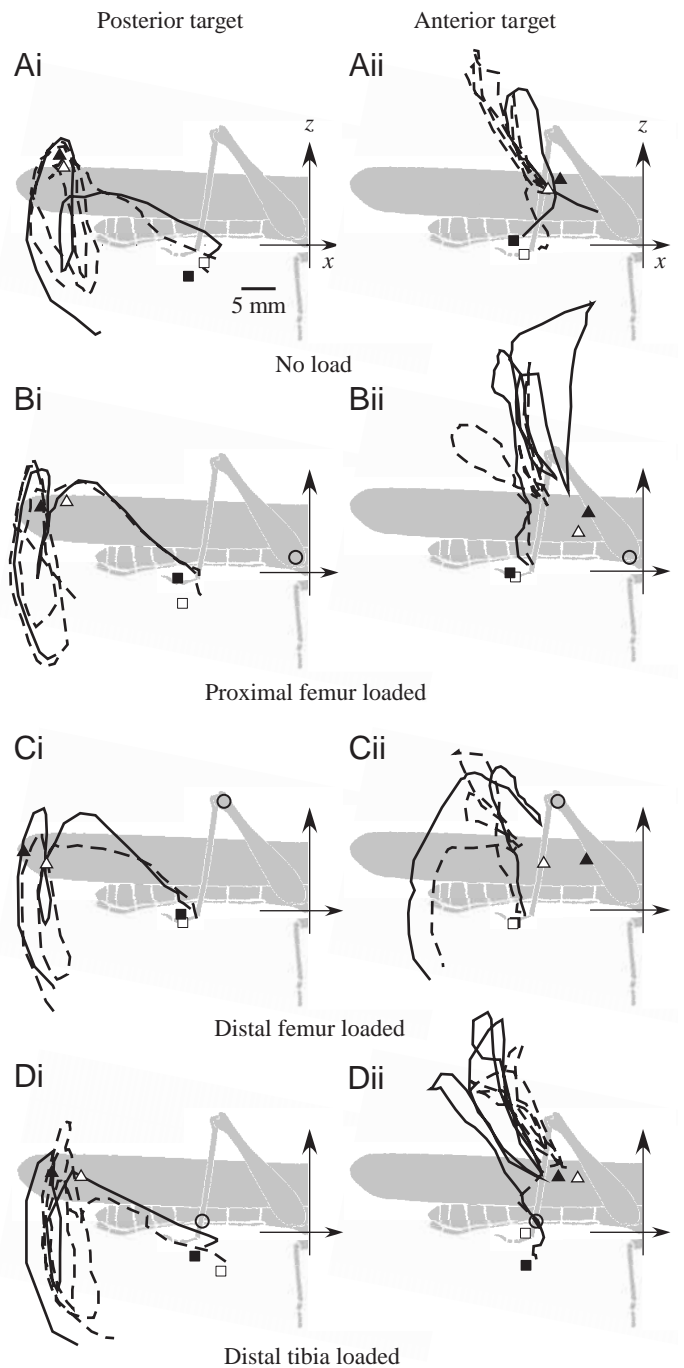


Fig. 2. Loading had little effect on the overall pattern of leg movement. Two example scratches are shown for each experimental situation (solid and broken lines respectively). (Ai–Di) Movements made in response to stimuli at the posterior target site (solid and open triangles are the targets corresponding to the scratches shown using solid and broken lines, respectively). The start positions are indicated by solid and open squares. (Aii–Dii) Movements made in response to stimuli at the anterior target. (Ai,ii) Unloaded condition; (Bi,ii) 142 mg load on the proximal femur (open circle); (Ci,ii) 142 mg load on the distal femur (open circle); (Di,ii) 142 mg load on the distal tibia (open circle).

Table 1. *Effect of load on rotational moment of inertia*

Joint	Unloaded	Site of load		
		Proximal femur	Distal femur	Distal tibia
Thorax–coxa	38962	43289 (1.1)	103265 (2.7)	247260 (6.4)
Coxa–trochanter	27176	27744 (1.0)	73184 (2.7)	197860 (7.3)
Femur–tibia	4846			56108 (11.6)

Values are mg mm<sup>2</sup> ( $N=5$  animals).

Numbers in parentheses indicate the factor of increase in rotational inertia caused by each load. The basis of the calculation is as follows: Coxa: mass 7.8 mg, length 3.6 mm, centre of mass 1.8 mm. Femur: 107.8 mg, 20.0 mm, 7.3 mm. Tibia: 19.0 mg, 21.0 mm, 12.6 mm. Tarsus: 3.3 mg, 6.8 mm, 3.4 mm. Load: 142 mg, centred 2 mm from end of limb segments. Leg posture: thorax–coxa joint angle 11.8°, coxa–trochanter joint angle 27.8°, femur–tibia joint angle 125.2°, tibia–tarsus joint angle 45.2° (all as required to reach the mean position of the posterior stimulus site).

## Results

To assess the effect of load on the ability of locusts to make aimed movements of their hind legs, a mass of 142 mg was attached to different locations on the leg (Fig. 1A,B). This effectively doubled the total mass of the leg and, in particular, when the mass was added to the distal end of the tibia, it increased the mass of this segment from 19 to 161 mg (an 8.5-fold increase). The rotational moment of inertia for a joint depends on the square of the distance of the centre of mass from the axis of rotation. Each load position that we used therefore resulted in different changes in rotational moment of inertia at each joint (Table 1.) For example, loading the tibia caused an 11.6-fold increase in rotational inertia for the femur–tibia joint, but a 6.4-fold increase for the thorax–coxa joint (Table 1).

### *The effect of load on limb trajectory*

Locusts made aimed scratching movements of a hind leg (e.g. Fig. 1C) in response to tactile stimuli at one of two target sites on the dorsal surface of the ipsilateral forewing (the outermost surface when the wings are folded into the normal resting posture, Fig. 1A). The leg always started from the same position, which was defined by a small rod on which was placed the animal's tarsus (Fig. 1A).

Stimulation of the distal (posterior) site elicited movements in which the tarsus was lifted and moved posteriorly towards the target before making a variable number of cyclical movements in the vicinity of the target (Fig. 2Ai). Stimulation of the proximal (anterior) site elicited movements in which the tarsus was lifted and moved approximately vertically towards the target before again making cyclic movements largely dorsal to the animal (Fig. 2Aii). Only the first three cycles were analysed. For a detailed analysis of unloaded scratching movements and their natural variability, see Matheson (1997, 1998) and Dürr and Matheson (2003).

Addition of a 142 mg load to the proximal femur, distal femur or distal tibia had no effect on the general form of scratching movements for either target site (compare Fig. 2Ai,ii with Bi,ii, Ci,ii and Di,ii). Slight variations in the movements illustrated in Fig. 2 fall well within the variance seen in unloaded scratches, as we go on to demonstrate in the following sections.

To examine the movements in more detail we analysed separately the initial 200 ms of movement that formed the outgoing trajectory and the remaining part of the movement during which the tarsus followed a cyclical path. The use of 200 ms as a cut-off criterion is justified quantitatively in Dürr and Matheson (2003). It is the mean duration of the outgoing trajectory. When the leg was unloaded the median direction of movement in the first 200 ms was 112° for scratches elicited by stimulation of the anterior site (black vector in Fig. 3A), and 134° for scratches elicited by stimulation of the posterior site (black vector in Fig. 3B). Loading the leg had no significant effect on initial movement direction (coloured vectors and curved lines in Fig. 3A,B: Dunnett's two sided *t*-test for each treatment *versus* control, 453 scratches from three animals pooled; all values of  $P>0.05$ ). Each animal was also analysed separately. For movements to the anterior site, loading the distal femur caused a significant increase in the angle of movement for one animal (Dunnett's *t*-test,  $P=0.005$ ,  $N=28$  unloaded, nine loaded scratches), but a decrease in the second ( $P=0.047$ ,  $N=36$ , 15), and had no effect in the third ( $P=0.089$ ,  $N=57$ , 10). For movements to the posterior site loading the distal femur caused a significant reduction in the angle of initial movement in one animal ( $P=0.005$ ,  $N=43$ , 12). No other load condition caused any significant difference in initial movement direction in any animal (all values of  $P>0.05$ ).

In the unloaded condition, 89% of scratches aimed at the anterior target site had three or more cyclical loops (Fig. 4A). Loading the proximal or the distal femur led to a small but non-significant reduction in the relative frequency of occurrence of scratches with three loops (black bars in Fig. 4A:  $\chi^2=14.7$ , 9 d.f.,  $P>0.05$ ). Only 40% of unloaded movements aimed at the posterior target had three loops, with most of the remainder having one or two loops (Fig. 4B). Once again, loading the leg caused a small but non-significant reduction in the proportion of scratches with three loops when compared to the unloaded condition (black bars in Fig. 4B:  $\chi^2=16.9$ , 9 d.f.,  $P>0.05$ ).

### *What part of the leg is aimed?*

When the leg is unloaded the part of the leg that is most reliably aimed at the stimulus, irrespective of stimulus location, is the distal end of the tibia (Dürr and Matheson, 2003). To test

whether locusts change this aiming strategy when the limb is loaded we determined the point on the leg that came closest

to the target under each loading condition. For unloaded movements made in response to stimulation of the posterior target site, the distal end of the tibia and the base of the tarsus were, on average, aimed most accurately (Fig. 5Ai). The minimum values of closest distance (i.e. highest accuracy) reached 0 mm only for the distal end of the tibia and tarsus, indicating that the proximal tibia and femur could not reach the target (white line in Fig. 5Ai). For movements made in response to stimulation of the anterior target the distal end of the tibia was aimed most accurately (Fig. 5Aii). The morphology of the hind leg means that when a locust rotates

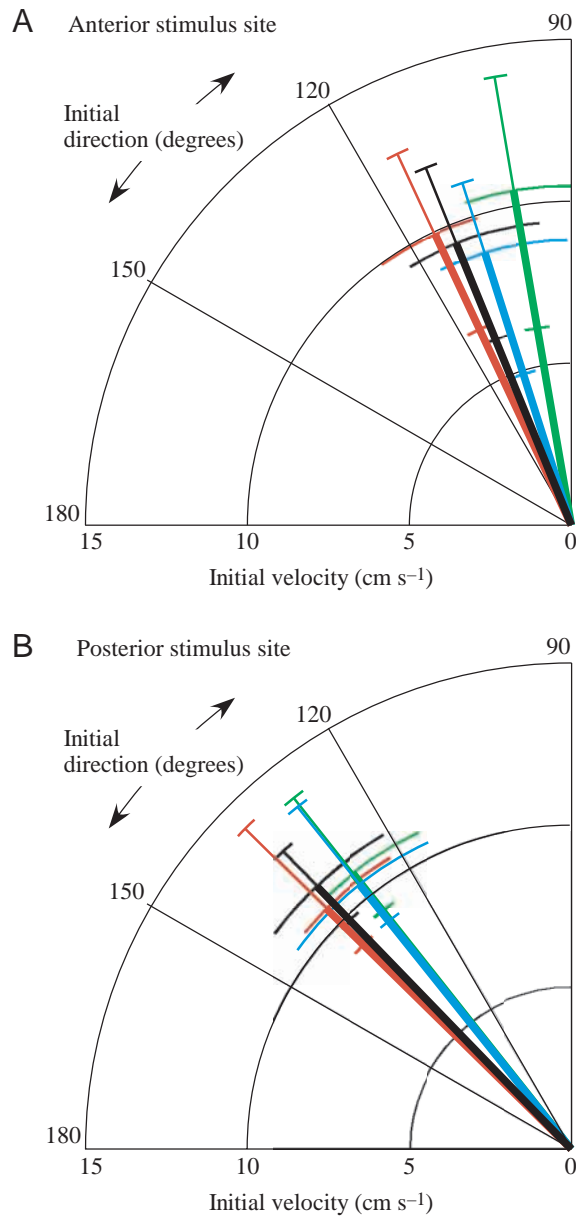


Fig. 3. Loading had no systematic effect on the initial direction of movement of the tarsus measured over the first 200 ms for scratches aimed at either the anterior (A) or posterior target (B). Median movement directions are represented by thick coloured vectors originating at the starting point of movement (black, unloaded; red, 142 mg load on the proximal femur; green, 142 mg load on the distal femur; blue, 142 mg load on the distal tibia). The correspondingly coloured long curved lines indicate the interquartile ranges of movement direction. The median velocity of movement over the first 200 ms is indicated by the length of each thick vector, and the interquartile range of velocity is indicated by the thin error bars (see Fig. 6 for additional analysis of movement velocity). Values of *N* are as follows. Anterior site, unloaded, 111; load on the proximal femur, 36; load on the distal femur, 33; load on the distal tibia, 40. Posterior site, *N*=121, 31, 34, 47 respectively.

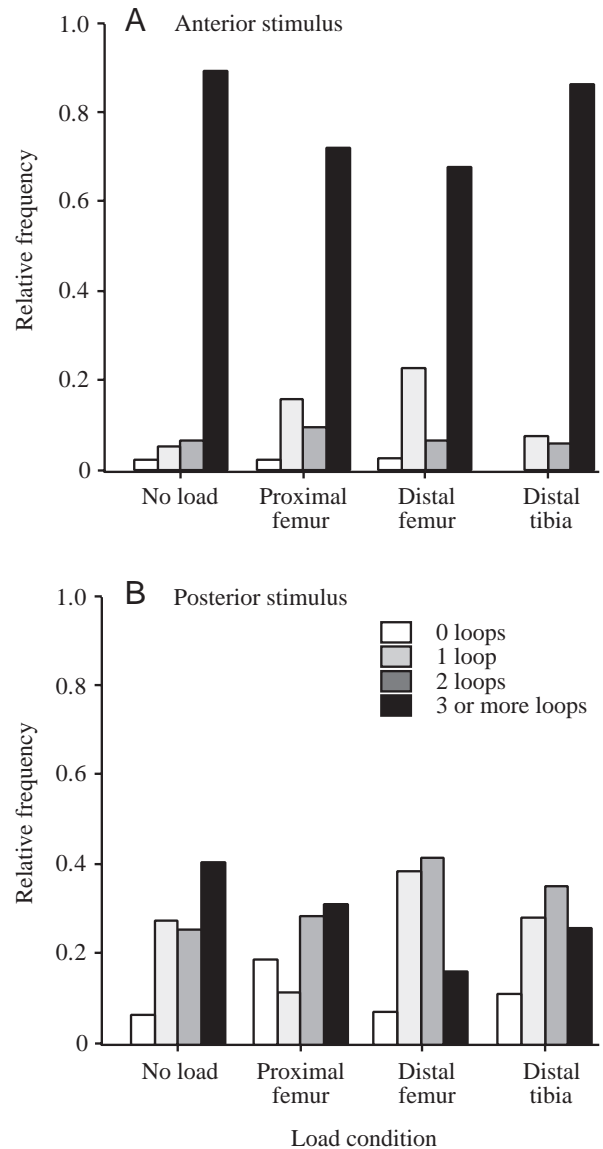
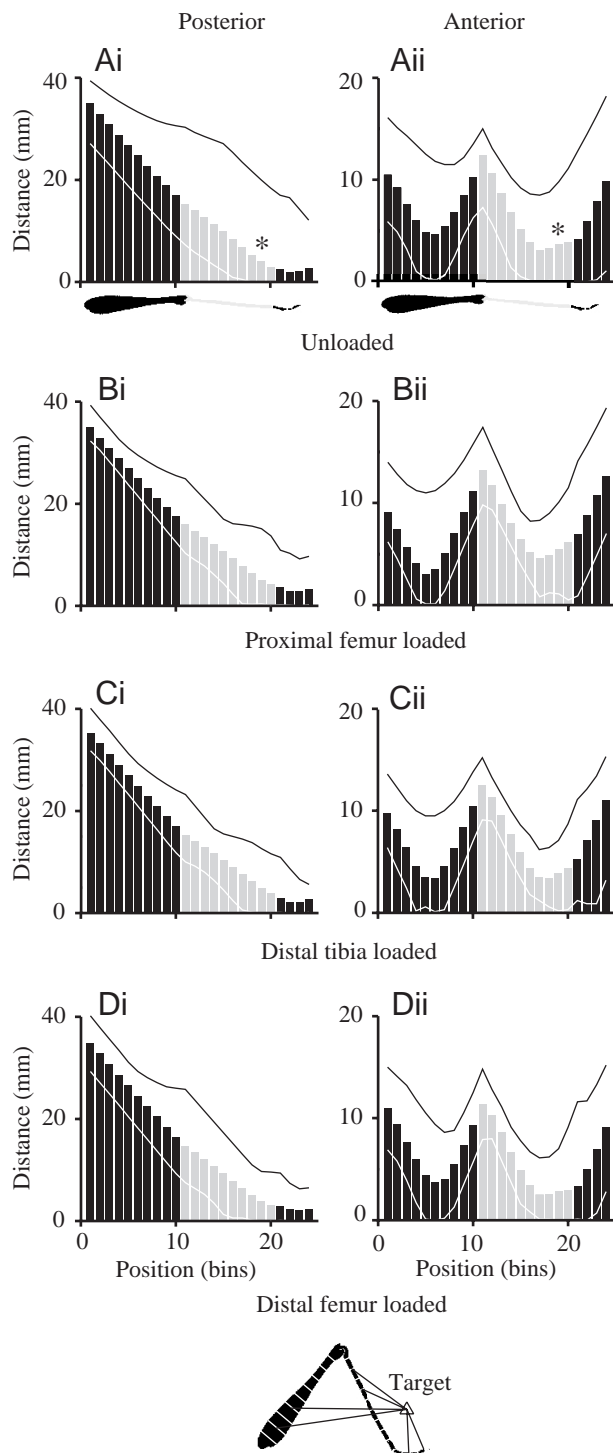


Fig. 4. Loading had no effect on the number of loops made by the leg for either the anterior stimulus site (A, *N*=220 trials pooled across three animals) or the posterior site (B, *N*=233 trials pooled across three animals). Bars show the relative frequency of occurrence of scratches with either 1, 2, 3 or more cyclic loops under each load condition. See text for details. Open bar, 0 loops; Light grey bar, 1 loop; mid-grey bar, 2 loops; black bar, 3 loops.

the femur forward to move its tarsus near the anterior target (but not the posterior one), a point on the femur may also cross the target (see e.g. Figs 1, 2Cii). This results in a local minimum in the measures of closest distance for the femur in Fig. 5Aii. It is important to note that the femoral minimum is the inevitable result of forward rotation of the femur that is required to bring the distal tibia and tarsus close to the target. The tibia crossed the stimulus site repeatedly during the cyclic part of grooming, whereas the femur was typically rotated

forwards so that it passed the target only once and was then held anterior to the target throughout the response (see Fig. 1C). This indicates that, although the femur can pass over proximal targets, it is not specifically aimed at them.

Loading the leg had no effect on the aimed point for any of the three load sites or either of the targets (Fig. 5Bi,ii–Di,ii). The absolute measures of accuracy for each point along the leg were similar to the corresponding values for unloaded movements (compare Fig. 5Bi,ii–Di,ii with corresponding Fig. 5Ai,ii). It is important to note that the femoral minimum is the inevitable result of forward rotation of the femur that is required to bring the distal tibia and tarsus close to the target. The tibia crossed the stimulus site repeatedly during the cyclic part of grooming, whereas the femur was typically rotated



#### The effect of load on limb velocity

There was a weak but significant positive correlation between the initial velocity (over the first 200 ms) and the velocity during the cyclical part of the movement in all three animals analysed separately and in the pooled data (Pearson correlation coefficient  $r=0.285$ ,  $P<0.001$ ,  $N=414$ ; note that not all responses included a cyclical component). The median velocity of movement of the distal end of the tibia during the first 200 ms was unaffected by load for either the anterior stimulus (open bars in Fig. 6A) or the posterior stimulus (grey bars in Fig. 6A). This was also the case for each animal analysed separately (data not shown).

The velocity of movement in the cyclical part of the response was unaffected by load on the proximal femur for scratches aimed at either stimulus site (Fig. 6B). In contrast, loading the distal femur or tibia caused an increase in the median velocities for movements made in response to the posterior stimulus (two extreme right grey bars in Fig. 6B; 43% and 48% increases, respectively).

#### Load does not affect the cyclic component of scratching

Two measures were used to investigate the effect of loading on the cyclical part of the movement. First, the point of closest approach to the target was determined to obtain a measure

Fig. 5. The part of the leg that is aimed most accurately at the stimulus site does not change with load. The leg of the locust (bottom) was subdivided into units (white lines), and the closest distance by which each unit approached the target (e.g. open triangle) was assessed for all scratches. (Ai–Di) Mean closest distances for each unit of the leg (cf. leg outlines below Ai,ii) for movements made in response to stimuli at the posterior target site; (Aii–Dii) corresponding data for movements to the anterior site. Black bars correspond to units on the femur and tarsus, grey bars to units on the tibia. The solid white and black lines indicate the range. (Ai,ii) Unloaded condition; (Bi,ii) 142 mg load on the proximal femur; (Ci,ii) 142 mg load on the distal femur; (Di,ii) 142 mg load on the distal tibia. The asterisks in A mark the part of the leg that was most reliably aimed, and which was used in the following analyses. Number of trials as in Fig. 6.

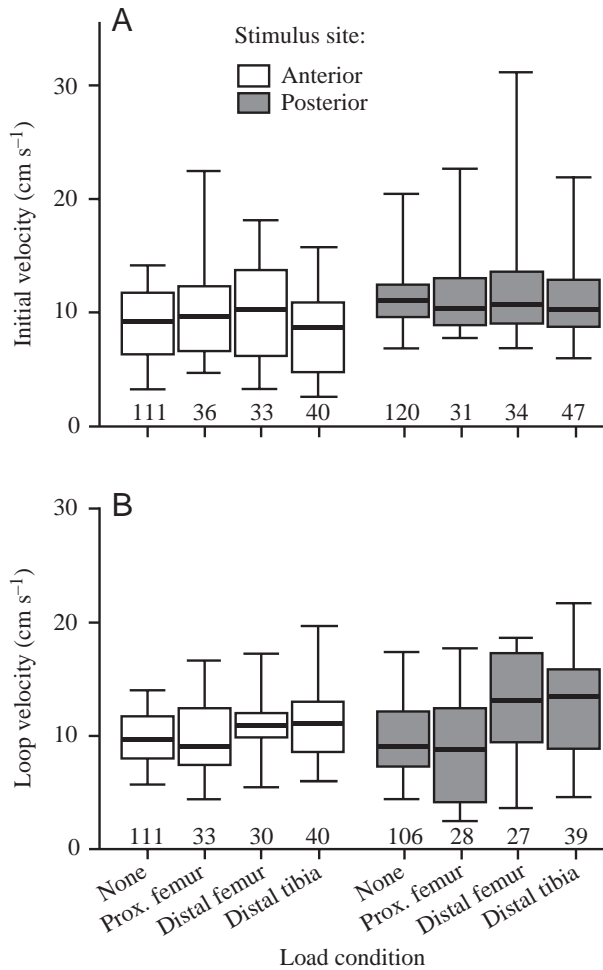


Fig. 6. Loading had no effect on the initial velocity of movement. (A) Over the first 200 ms of movement, the median velocity was between 8.7 and 10.3 cm s<sup>-1</sup> for all movements to the anterior stimulus site (horizontal lines in open boxes) and between 10.2 and 11.1 cm s<sup>-1</sup> for movements to the posterior site (horizontal lines in grey boxes), irrespective of load condition. The variances were large so the interquartile ranges overlapped almost completely (boxes). The whiskers indicate the range containing 90% of values. (B) Movement velocity during the cyclic part of each scratch ('Loop velocity') was similarly unaffected by load on the proximal femur (8.7–9.6 cm s<sup>-1</sup>), but was faster when the leg was loaded on the distal femur or tibia, at least for movements to the posterior stimulus site (12.9–13.3 cm s<sup>-1</sup>; medians fall outside the interquartile range for unloaded movements). Numbers indicate the number of trials at each site and load condition.

of performance accuracy. Second, the average 'movement distribution' was calculated to describe the region of the leg's workspace covered during the responses.

For unloaded movements to the posterior target, the mean point of closest approach was  $5.0 \pm 2.7$  mm (mean  $\pm$  S.D.) anterior to the target (Fig. 7). For unloaded movements to the anterior target, the point of closest approach was  $1.9 \pm 2.5$  mm posterior to the target. Loading the leg had no effect on the point of closest approach in any experimental situation.

To assess the part of the leg's workspace that is most likely

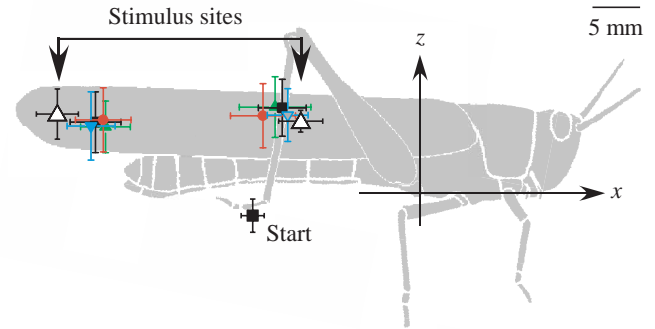


Fig. 7. Loading the leg with a mass of 142 mg had no effect on the mean closest point of approach of the tibia to either stimulus site (open triangles). Closest points of approach were determined for each trajectory. Small black square, unloaded condition; red circle, load on the proximal femur; green triangle, load on the distal femur; blue triangle, load on the distal tibia. The large black square indicates the mean starting position. The mean closest points of approach for movements aimed at the posterior stimulus site were, on average, anterior to the target. Those for movements aimed at the anterior stimulus site were, on average, posterior to the target. All values are the mean  $\pm$  1 S.D. of data pooled from three animals. Number of trials as in Fig. 6.

to be scratched, we calculated the likelihood that the distal part of the tibia crossed each point in space between two subsequent video frames, given a particular stimulus:load condition. This was done by counting the number of times the distal tibia crossed a given location in the leg's workspace during a single trial, and standardising the resulting distribution to a volume of 1. Averaging these two-dimensional distributions resulted in distributions of the average likelihood of observing a movement across any point in the workspace. Fig. 8 illustrates this as grey level maps in body coordinates (for details of the calculation, see Dürr and Matheson, 2003).

In the unloaded condition, the cyclical parts of scratching movements aimed towards the posterior target (Fig. 8Ai) differed from those aimed at the anterior target (Fig. 8Aii). The probability distribution for posterior scratches was centred anterior to the target site, extending both ventrally and dorsally above and below the wing (Fig. 8Ai). The centre of each distribution was characterised by the 'centre of gravity' (white circles in Fig. 8) and by the 'most likely point' (white squares in Fig. 8). For unloaded movements aimed at the posterior target, both measures lay approximately 8 mm anterior to the target near the ventral (leading) edge of the wing. The probability distribution for anterior scratches peaked approximately 7 mm posterior and dorsal to the corresponding target (Fig. 8Aii). The centre of gravity was a further 4 mm posterior and dorsal, reflecting the 'tail' of the distribution.

Bayes' theorem (see e.g. Quinn and Keough, 2002) was used to test whether the probability distributions resulting from movements to different targets or made under different load conditions could be distinguished from each other. This was done by estimating the minimum number of video frames in a scratching sequence that was required to determine statistically

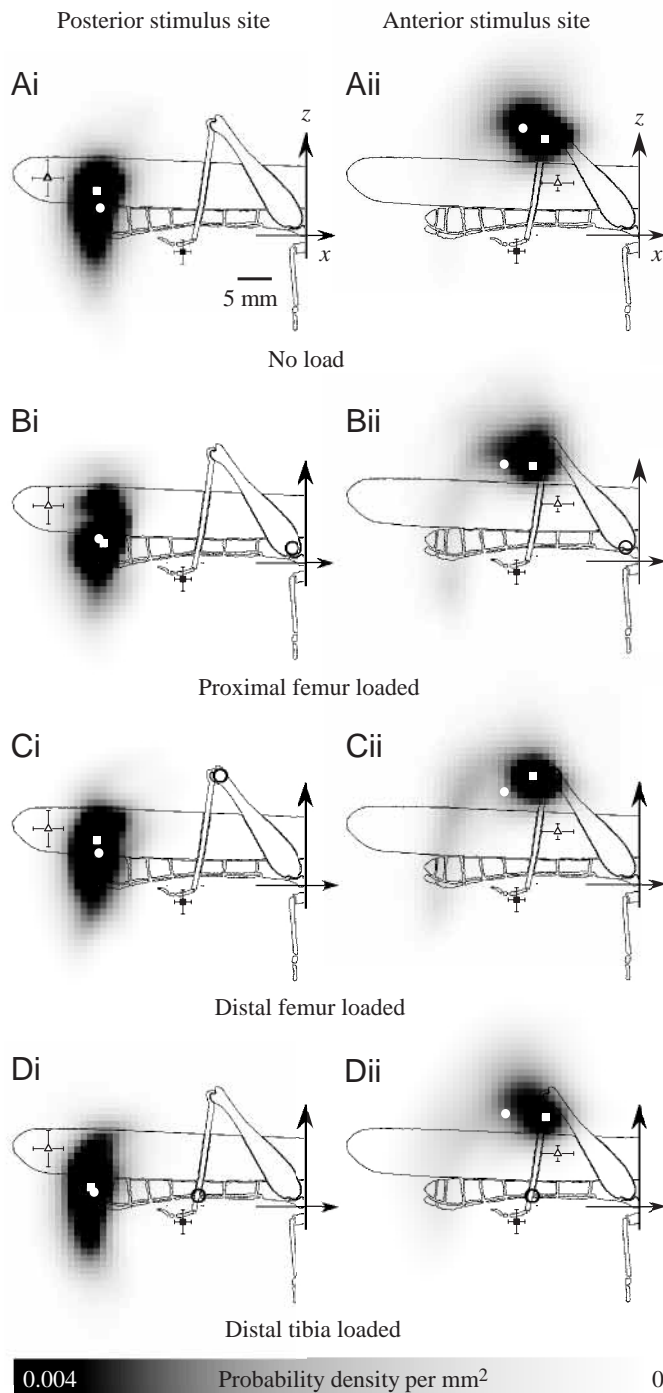


Fig. 8. Load had no effect on the area crossed by the distal end of the tibia for scratches aimed at either the posterior stimulus site (Ai–Di, open triangles) or the anterior stimulus site (Aii–Dii, open triangles). Shading indicates the likelihood of a particular position within the leg's workspace to be scratched. The greyscale represents probability density between 0 (white) and 0.004 (black) at 1 mm spatial resolution (see bar at bottom). White circle, the centre of density; white square, the maximum. (Ai,ii) unloaded condition; (Bi,ii) 142 mg load on the proximal femur; (Ci,ii) 142 mg load on the distal femur; (Di,ii) 142 mg load on the distal tibia. Number of trials as in Fig. 6.

Table 2. *Overlap and discriminability of probability distributions for movements to anterior and posterior target sites under each load condition*

Anterior versus posterior	No load	Load proximal femur	Load distal femur	Load distal tibia
Overlap%	15.7	24.3	31.4	6.5
Probability	0.120	0.177	0.226	0.189
No. required	$N=3$	$N=7$	$N=11$	$N=7$

Pairs of distributions obtained under the same loading condition but for movements to different target sites (anterior *versus* posterior) could always be discriminated reliably from one another within the average number of observations per response (i.e. 42 video frames).

Overlap% indicates the percentage volume overlap of the two distributions.

Probability is a measure of discriminability of the two distributions. It is the likelihood of predicting the wrong target condition from a single observation (one video frame).

No. required indicates the minimum number of observations needed to obtain a 95% chance of making a correct decision.

which experimental condition had given rise to the observed behaviour. For any given load condition, the probability distributions for movements made in response to stimulation of the anterior target site (Fig. 8Aii–Dii) could be distinguished easily from those made in response to stimulation of the posterior target (Fig. 8Ai–Di; see Table 2).

Loading the leg had no significant effect on the probability distributions for movements made to the posterior target (Table 3, Fig. 8Bi–Di). For movements aimed at the anterior target, loading the leg increased slightly the density of the tail of the distribution, reflecting the terminal downward movement of the trajectory as the tarsus returned to the ground. Accordingly the centre of gravity (but not the most likely point) shifted ventrally (Fig. 8Bii–Dii). Despite this subtle change, the distributions for movements to the anterior target were also statistically indistinguishable (Table 3). Observation of more than 450 video frames (which is approximately equivalent to the duration of 10 scratches), would have been required to reliably detect the different loading conditions. In addition to these analyses of the pooled data from three animals, we examined each animal separately. None of the load conditions had any significant effect on the probability distributions in any of the animals (all  $P>0.15$ ), with the single exception that loading the distal femur caused a marginally significant shift in one of the animals ( $P\leq 0.04$ ).

The marked similarity of the probability distributions obtained under different load conditions becomes even more apparent when examining the pattern of iso-density contours that delimit different proportions of the total volume (Fig. 9). Contours that contained only the top 10% of volume (i.e. delimited the peaks of the distributions) overlapped each other almost completely, especially for anterior scratches (Fig. 9, top row). Contours for successively greater proportions of the total volume (25, 50, 75 and 90%) revealed an approximately



Table 3. *Overlap and discriminability of probability distributions for movements to a given target under each load condition*

No load <i>versus</i> load on:	Anterior target site			Posterior target site		
	Proximal femur	Distal femur	Distal tibia	Proximal femur	Distal femur	Distal tibia
Overlap%	82.0	79.5	83.1	88.2	92.2	85.5
Probability	0.463	0.461	0.469	0.482	0.488	0.479
No. required	<i>N</i> =495	<i>N</i> =443	<i>N</i> >500	<i>N</i> >500	<i>N</i> >500	<i>N</i> >500

Pairs of distributions derived from movements elicited by stimulation of the same target were tested for an effect of loading. In no case could load condition be determined reliably from observations of the grooming response because the required number of observations always exceeded by more than tenfold the mean number of video frames per scratch (42).

Other details as for Table 2.

symmetrical pattern of expansion for posterior scratches, irrespective of load condition (Fig. 9, left column). For anterior scratches the 50% contours revealed the posterior–ventral tail of the distributions, which was more pronounced for loaded trials (e.g. Fig. 9, 50%, right). The 90% contour revealed a slight anterior dorsal shift and a less pronounced tail when the proximal femur was loaded (Fig. 9, 90%, right; red line).

## Discussion

### *Insects compensate for external load on a scratching leg*

We have shown that applying a substantial load to one hind leg of an insect does not impair the animal's ability to make well coordinated, aimed movements of that leg towards targets on its wing. The general form of the movements is the same under all conditions (Figs 2–4), as is the initial velocity of movement (Fig. 6). Animals aim the same part of their distal tibia at the target regardless of loading (Fig. 5), and they remain equally accurate (Fig. 7). Loading has no effect on the region of the workspace that is scratched (Figs 8, 9). The mass on the distal femur or tibia applied a load to the coxal levator muscles during scratches aimed at both stimulus locations on the wing, but also loaded depressor muscles for movements to the anterior site as soon as the load passed through the vertical. The mass on the tibia loaded the tibial extensor muscle during the outward trajectory aimed at the distal target, but loaded the flexor muscle for the initial movement to the proximal target. The same mass alternately loaded flexors and extensors during the cyclical part of scratching. The load was substantial, representing an 11.6-fold increase in the rotational moment of inertia of the tibial segment, so its lack of effect on the velocity, accuracy and trajectory of leg movement indicates clearly that leg proprioceptive inputs are integrated in the motor networks that generate aimed scratching. In the following discussion we rule out the alternative possibility that leg stiffness is sufficiently high that an unchanging equilibrium point control mechanism could produce the same trajectory irrespective of loading.

### *Equilibrium point control and joint stiffness*

To move the tarsus along a particular trajectory to a target requires computation of motor commands that will act to

rotate the individual leg joints against their specific internal and external loads. This requires that the motor signals are represented in a coordinate system that encodes the magnitude of joint rotations, torques or muscle actions – i.e. it must be an intrinsic coordinate system, as opposed to the extrinsic system used by sensory receptors to encode the spatial location of the tarsus and target. In theory, an equilibrium point mechanism could permit the planning and execution of the trajectory of a coordinated reaching movement, even for a redundant manipulator like the locust leg (Feldman and Levin, 1995). Such a control mechanism requires high joint stiffness, however, particularly if the trajectory is to remain similar after changing the moments of inertia. Passive rotations of the locust femur–tibia joint that are observed during ongoing leg movements (Berkowitz and Laurent, 1996) indicate that stiffness of this joint is low in the absence of external load. During scratching the femur–tibia joint can extend with considerable force in the absence of extensor motor activity, at least in part as a result of energy transfer from the movements of more proximal joints (Berkowitz and Laurent, 1996). In other words, the stiffness of the unloaded femur–tibia joint is insufficient to stabilise the joint against the tibia's intrinsic moment of inertia. Only if proprioceptive information was to adapt joint stiffness to the additional load could joint stiffness explain the load compensation that we describe.

Joint stiffness is affected by three active mechanisms: co-contraction of antagonist muscles, neuromuscular inhibition and neuromodulation. The timing of activity of common inhibitory neurones during active movements must contribute to the low leg joint stiffness by downregulating tonic contraction forces of antagonist muscles at each joint (Wolf, 1990). Insect leg muscles are controlled not only by their excitatory and inhibitory motor neurones, but also by the activity of specific neuromodulatory neurones ['dorsal unpaired median' (DUM) neurones; reviewed in Burrows, 1996]. These leg DUM neurones can affect muscle tonus and force production (Hoyle, 1978) so, if their activity is modified as a result of loading, they might also contribute to load compensation. Nevertheless, none of these mechanisms could participate in load compensation in the absence of proprioceptive feedback, so we rule out the possibility that a

pure equilibrium point control mechanism underlies the load compensation we observed.

Our conclusion concurs with Dean's claim (Dean, 1992) that if stick insects use an equilibrium point mechanism to control targeted stepping movements, then they must also use proprioceptive signals. Indeed, most current versions of the

equilibrium point hypothesis accept that joint interaction torques are not automatically compensated for by joint stiffness, but that internal models of the limb's properties must be calibrated by sensory feedback (e.g. Hollerbach and Flash, 1982; Shadmehr and Mussa-Ivaldi, 1994; Ghez and Sainberg, 1995). Human arm stiffness is too low to permit pure equilibrium point control (Gomi and Kawato, 1996, 1997), and subjects who lack proprioception due to large-fibre neuropathy show marked errors in limb trajectory (Gordon et al., 1995). In spinal frogs, where loading of a hind leg has little or no effect on targeting accuracy (Schotland and Rymer, 1993), proprioception has similarly marked influences on initial trajectory direction, path straightness, knee joint velocity and overall accuracy (Kargo and Giszter, 2000).

#### *Proprioceptive feedback in insect leg motor control*

Proprioceptive feedback is clearly essential for the generation of normal motor patterns in most rhythmic motor systems (reviewed in Pearson and Ramirez, 1997). In insects the angle, velocity and acceleration of each leg joint are monitored by internal chordotonal organs (Hofmann et al., 1985; Matheson and Field, 1990; reviewed in Field and Matheson, 1998), the receptors of which have powerful reflex effects on motor neurones of the same joint (Field and Burrows, 1982) and of other joints of the same leg (Hess and Büschges, 1999). In the locust, information about hind leg joint angles excites particular spiking local interneurons that are also excited by exteroceptive inputs that signal a touch on the wings (Matheson, 2002). These interneurons are likely to be key elements in the generation of aimed leg movements and in compensation against load perturbations.

Increased load affects muscle forces that are monitored by multipolar tension receptors, which also have intra- and interjoint effects (Theophilidis and Burns, 1979; Matheson and Field, 1995). Load-induced strain in the exoskeleton is sensed by campaniform sensilla (Spinola and Chapman, 1975), which are generally located near joints (Pringle, 1938). Those on the coxal and trochanteral segments near the base of the leg are key elements in load-compensating reactions during walking, when the leg is in contact with the ground (e.g. Zill et al., 1999; Noah et al., 2001). These campaniform sensilla are generally inactive when the leg is off the ground (as during scratching). Nevertheless, loading the leg as we did is likely to have caused activation of these receptors, when muscles were acting against increased rotational inertias to accelerate and decelerate each

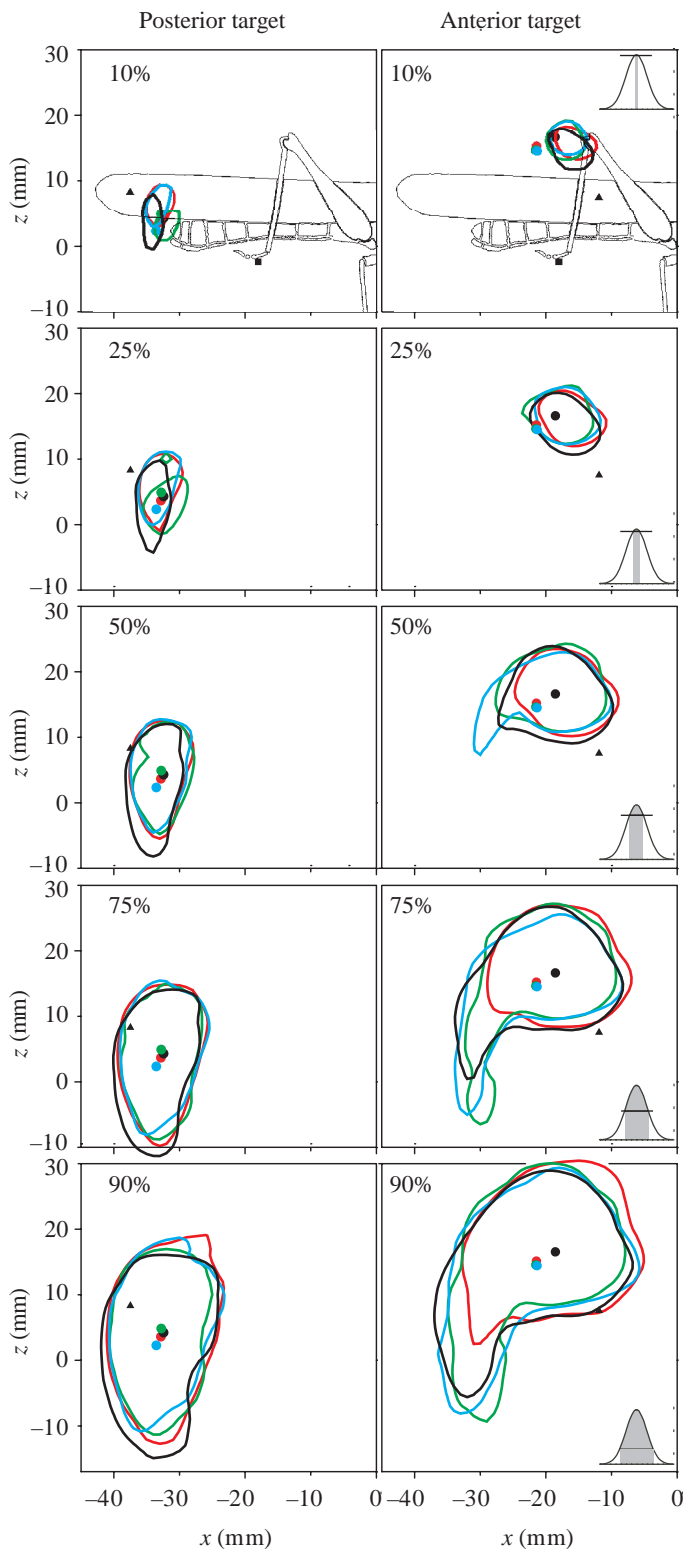


Fig. 9. Iso-probability contours, derived from the probability distributions in Fig. 8, emphasise the remarkable similarity of scratching movements made under different loading conditions (black, unloaded; red, 142 mg load on the proximal femur; green, 142 mg load on the distal femur; blue, 142 mg load on the distal tibia). The insets show normal distributions to indicate the region of the distribution enclosed by the corresponding iso-probability line for 10, 20, 50, 75 and 90% levels. Black triangles mark the target sites; black squares mark the start postures, and coloured circles mark the corresponding centres of probability density. Number of trials as in Fig. 6.

leg segment throughout the scratch. Whether the resultant feedback pathways alone (e.g. Newland and Emptage, 1996) could provide the complete control of movement velocity and accuracy that we see is unknown. Signals from campaniform sensilla converge onto leg motor neurones along with position-dependent signals from other leg sense organs (Schmitz and Stein, 2000), and the leg motor neurone output is modulated by non-linear interactions between the two modalities.

#### *Proprioceptive mechanisms underlying load compensation*

In humans, loading a limb results in complex EMG responses, the gain of which depends on the simultaneous angular motion of other limb joints (Lacquaniti and Soechting, 1986). This implies that the spinal cord integrates proprioceptive inputs from several muscles. Some neurones in the motor cortex are sensitive to loading at either the shoulder or the elbow, or respond to loads at both locations (Cabel et al., 2001). In insects, sensory influences on leg movements have been demonstrated in a wide range of experiments (for reviews, see Burrows, 1996; Cruse et al., 1984; Bässler and Büschges, 1998), and there is widespread convergence of proprioceptive information from different leg segments onto leg motor neurones. This holds for joint position- and movement-sensitive chordotonal organs (Hess and Büschges, 1997, 1999), load-sensitive campaniform sensilla (Zill et al., 1981), and muscle tension receptors (Matheson and Field, 1995). In a walking stick insect, the protraction movement of a posterior leg is aimed at the position where the next most anterior leg already has a foothold (Cruse, 1979). Loading the posterior leg has little effect on either the accuracy or velocity of its movement (Dean, 1984). In walking, however, the control system driving these movements is constrained by the need to coordinate the posterior leg with the weight-bearing legs so as to maintain a stable gait. Our data show complete compensation for loading during a limb-targeting behaviour in which the aimed limb is not subject to such coordinating influences. The control of movement velocity is therefore not a consequence of the requirement for interleg coordination, but instead appears to be a general feature of insect leg movements across species and in different behaviours. In compensating for increased leg loading, locusts did not trade-off accuracy for velocity, nor did they switch to an alternative movement strategy. Because trajectories do not change when the leg is loaded, and targeting is strongly dependent on the exteroceptive cue of a tactile stimulus (Dürr and Matheson, 2003), we conclude that load compensation in locusts must be due to a high-gain position control loop.

Our previous work shows that in locusts, unloaded scratching movements elicited by stimuli to different places on the wing form a fine-grained continuum (Dürr and Matheson, 2003), suggesting that a single underlying motor pattern is modulated in a continuous way by a somatosensory representation of the wing surface. The present results show that locusts, like vertebrates, also integrate leg proprioceptive information into an internal body model that is used to control aimed limb movements.

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