RESEARCH ARTICLE

Activity of the claw retractor muscle in stick insects in wall and ceiling situations

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SUMMARY

The activity of the middle part of the claw retractor muscle was examined in two species of stick insects (*Carausius morosus* and *Cuniculina impigra*). We performed electromyographic recordings while the animals were standing on a smooth or a rough surface of a platform in horizontal, vertical or inverted positions, as well as during rotations of the platform. We recorded tonic and phasic motor units. The tonic units were active all the time without significant differences in spike frequency, regardless of the position of the animals (although there was a tendency for higher discharge frequencies to occur during platform rotations). The phasic units were active almost exclusively during platform movement. In contrast to the tonic units, we detected significant differences in the activities of the phasic units; namely, higher spike frequencies during rotations compared with the stationary phases, especially for rotations into 'more awkward' positions. A comparison of the two species revealed no difference in muscle activity, despite differences in the animals' tarsal attachment structures. The same was true when comparing the muscle activity of the two species on both the smooth and the rough surfaces.

Key words: locomotion, retractor unguis, posture control, adhesion, attachment structures, electromyogram (EMG), phasmatodea, *Carausius morosus*, *Cuniculina impigra*.

INTRODUCTION

Stick insects have long been (e.g. Bässler, 1983; Bässler and Büschges, 1998) and still are (e.g. Ritzmann and Büschges, 2007) established model systems in neurobiology, biological cybernetics and biologically inspired areas of engineering of autonomous systems. This is especially true for the motor control of walking, and indeed some of the first walking robots were constructed along control principles discovered in stick insects (e.g. Cruse et al., 1995; Cruse et al., 2007; Dürr et al., 2004; Müller-Wilm et al., 1992). Similar to insects, walking machines should be able to deal with unpredictable and extremely uneven surfaces even when climbing (Abbott, 2007; Daltorio et al., 2005; Dürr et al., 2004; Ritzmann et al., 2000; Wile et al., 2008a). In this context, the control principles governing leg movement (e.g. Bässler and Büschges, 1998; Cruse et al., 2007; Laurent and Hustert, 1988), leg coordination (e.g. Espenschied et al., 1993), and the biomechanics and recruitment of leg muscles (e.g. Duch and Pflüger, 1995; Guschlbauer et al., 2007) have been studied in some detail.

Insect legs are controlled by several sets of muscles. One set is used for producing the swing phase (or protraction) in horizontal walking, such as the abductor muscles of the coxa, the second remotor and the anterior rotator of the coxa, and the flexor of the tibia in the locust (Duch and Pflüger, 1995). These muscles move the leg forward through the air until the tarsus touches down on the ground. Another set of muscles, usually the antagonists of the swing phase muscles, controls the stance phase. Among these are the adductor coxae, posterior rotator coxae and extensor tibiae muscles. During the stance phase the tarsus is in contact with the ground and the leg is moved backwards relative to the body, resulting in forward movement of the animal. However, one stance phase muscle of the insect leg, namely the claw retractor muscle (retractor unguis), has no antagonists.

In the stick insect, this muscle consists of three parts (RU I, RU II, RU III), two of which are situated in the tibia, and one in the femur (Fig. 1) (Radnikow and Bässler, 1991). The three muscle parts are connected *via* a common tendon attached to the unguitractor plate in the pretarsus. From the unguitractor a short tendon-like strand of cuticle leads to the claws. When the muscle contracts the pretarsus is flexed and the claws are curled and hooked into the substrate, where possible. Microtrichia on the unguitractor plate and on the opposing part of the inner pretarsus have an interlocking function and help to keep the claws flexed against external forces (Gorb, 1996). Depending on how strongly the claws need to be engaged, not all of the three muscle parts are activated simultaneously (Radnikow and Bässler, 1991). When the muscle is relaxed, the pretarsus and claws uncurl by means of elastic structures that produced a counterforce while the muscle was strained. Experiments demonstrating the retractor muscle function in ants (Endlein and Federle, 2008; Federle and Endlein, 2004; Federle et al., 2001), bees (Federle et al., 2001), flies (Niederegger and Gorb, 2003) and wasps (Frantsevich and Gorb, 2004) revealed the range of movements the muscle is responsible for. The function of the muscle includes not only recruitment of the claws but also control of attachment devices, such as arolium and pulvilli. Additionally, the muscle is responsible for bending and stiffening of the tarsal chain and tarso-pretarsal joint. It might also be used by flies in detachment movements where claws possibly act as a lever lifting the tarsus from the ground (Niederegger and Gorb, 2003).

The retractor unguis muscle thus plays an important role in securing ground contact, in horizontal walking and even more so on slopes and during climbing. Equally important, of course, are
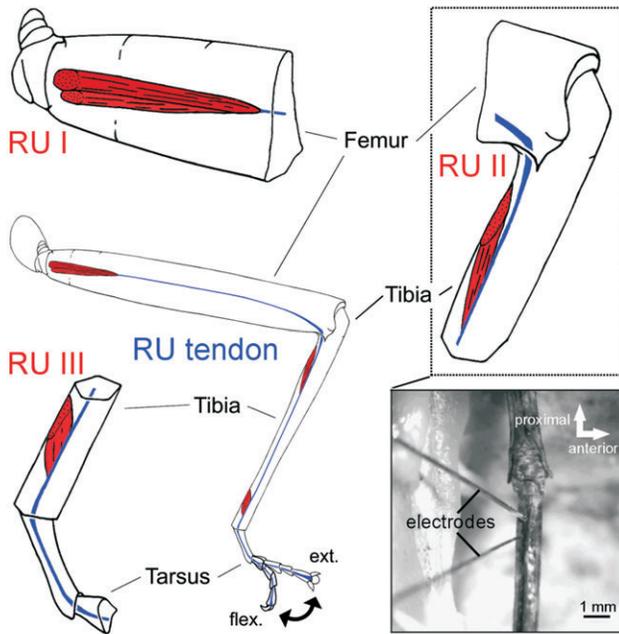
Fig. 1. Stick insect leg. RU I, RU II and RU III show the three parts of the retractor unguis muscle connected by a common tendon (shown in blue). We used the middle part of the muscle (RU II) for our experiments. The tarsus is shown in extended (ext.) and flexed (flex.) positions to illustrate the movement effected by the muscle. Inset shows a photograph of the electrode placement in ventral view, in the middle leg of *Cuniculina impigra*.

the adhesive and interlocking structures deployed by the muscle, namely the claws, as well as the adhesive/frictional pads associated with the tarsus, namely the arolium and euplantulae. The attachment devices in insects and other legged animals have consequently received much attention, not least in view of possible technical applications: for example, geckos (e.g. Autumn et al., 2002; Gao et al., 2005; Huber et al., 2005a; Huber et al., 2005b; Russell and Higham, 2009), beetles (e.g. Bullock and Federle, 2009; Gorb, 2001), flies (e.g. Gorb, 1998; Langer et al., 2004; Niederegger et al., 2002), stick insects (e.g. Bullock et al., 2008; Dirks et al., 2010; Drechsler and Federle, 2006), locusts (e.g. Gorb et al., 2000; Gorb and Scherge, 2000; Jiao et al., 2000; Perez Goodwyn et al., 2006; Vötsch et al., 2002) and cockroaches (e.g. Clemente and Federle, 2008; Frazier et al., 1999; Larsen et al., 1997). Studies of adhesive devices on animal legs have mainly focused on material properties, the generation of adhesive and frictional forces, or the role of adhesive fluids. In contrast, there are few studies on the activity of leg muscles during climbing or clinging to vertical or inverted substrates (Duch and Pflüger, 1995). Data on the activity of muscles that directly deploy attachment devices of the tarsus (retractor unguis, levator and depressor tarsi) when animals are clinging to walls and ceilings are absent from the literature.

Our study is the first approach towards understanding the role of muscle activity in attachment through examining the activity of the claw retractor muscle on different substrates and in various behavioural situations. We recorded retractor unguis muscle activity in two species of stick insects while the animals were attaching to a substrate in different positions to examine whether there are differences in the claw retractor activity when the animal is in different postures (see also Busshardt et al., 2010). Because the retractor unguis has no antagonist, the question arises of whether

the muscle activity persists while the animal is clinging to a substrate with claws, arolium or euplantulae. Both possibilities are imaginable, with different respective advantages. On the one hand, the claws may enable interlocking to a rough substrate without further need of muscle activity, once they are hooked into the ground, and provide enough grip to support the animal's weight. Lack of muscle contraction would certainly save energy, particularly during the long periods of inactivity the phasmids maintain in the context of twig mimesis (Bässler, 1983). On the other hand, it may be necessary to keep the muscle strained to balance the elastic counterforce that would disengage the claws from the ground. Tonic muscle contraction would further allow continuous fine adjustment of attachment to the substrate. One would further expect, in this case, that muscle activity increases during transition to 'more awkward' positions for the animal; that is, from standing on a horizontal platform to clinging in an upside-down position to a 'ceiling'. This is a relevant question in animal locomotion as stick insects often sit vertically or upside-down on leaves or twigs. Transition from one substrate to another is also a challenging situation for climbing robots (Wile et al., 2008b).

While the claws and pretarsal adhesive pads (arolia) of the two stick insect species examined are rather similar, there are clear differences in the structure of the tarsal attachment pads, the euplantulae (Fig. 2) (P.B., H.W. and S.G., submitted). Euplantulae are attachment pads found in many orthopteroid insects that can generate adhesive (Jiao et al., 2000) and frictional forces (Clemente and Federle, 2008; Gorb and Scherge, 2000). The two phasmid species selected for the present study, *Cuniculina impigra* and *Carausius morosus*, bear euplantulae of the same number and roughly the same size. However, they differ in their surface structure. In another study we examined the morphology of the euplantulae of both species and found that all pads of *C. impigra* are smooth down to the micrometre scale while all euplantulae of *C. morosus* bear small nubs all over their surface (P.B., H.W. and S.G., submitted). We used both species in our electrophysiological experiments to reveal possible differences that might be related to these two types of euplantulae surface.

The present study provides new information towards a better understanding of how insects control body posture in various behavioural situations and how they make use of their attachment devices. The following questions were asked. (1) Are the tonic and phasic activity of the retractor unguis different for different positions of the animals on the substrate (upright, wall, ceiling)? (2) Does the retractor unguis muscle activity change during rotation of the platform? (3) Is there any difference in retractor unguis activity pattern in the two different species of stick insects with different euplantulae microstructure? (4) Does substrate roughness influence muscle activity pattern?

MATERIALS AND METHODS
Animals
Experiments were carried out with adult female animals of two stick insect species from the family Phasmatidae: *Carausius morosus*, Sinéty, and *Cuniculina impigra* (syn. *Medauroidea extradentata* Brunner). Only the middle legs were used. The animals were taken from our laboratory colonies at the Institute for Neurobiology, University of Ulm. They were kept at room temperature and fed with bramble leaves.

Scanning electron microsopy
Morphological studies of the tarsi were conducted using a Hitachi S-4800 scanning electron microscope (Hitachi High-Technologies
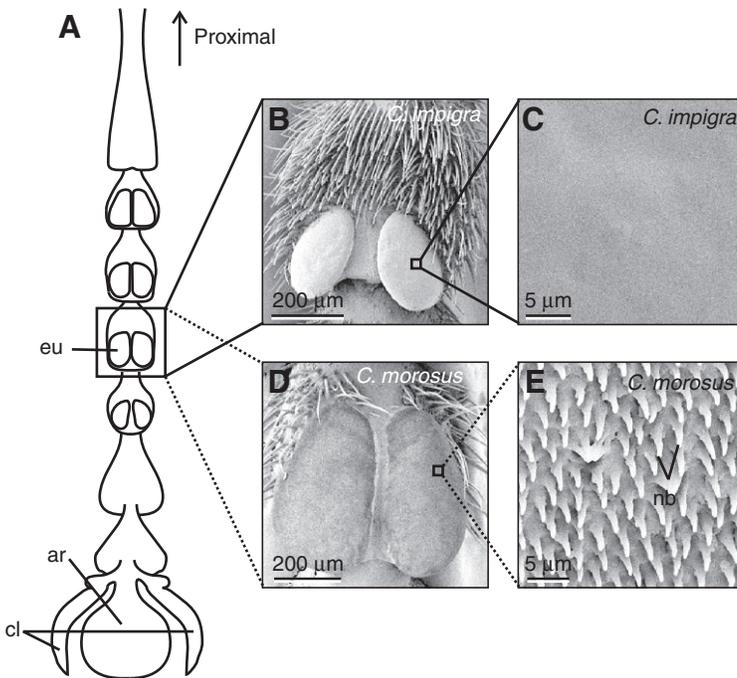
Fig. 2. Attachment devices of the two stick insect species studied. (A) Ventral aspect of the tarsus. (B) Euplantulae of *C. impigra*. (C) Euplantula surface of *C. impigra*. (D) Euplantula of *Carausius morosus*. (E) Euplantula surface of *C. morosus*. B–E, cryo-scanning electron microscopy (SEM) micrographs. Note that the pads of *C. impigra* are smooth, whereas those of *C. morosus* bear small nubs (nb). ar, arolium; cl, claws; eu, euplantulae.

Corp., Tokyo, Japan) equipped with a Gatan ALTO 2500 cryo-preparation system (Gatan Inc., Abingdon, UK) (cryo-SEM). Tarsi of living animals were severed with a razor blade, then attached to a metal holder using Tissue-Tek1 O.C.T. compound mounting fluid (Sakura Fine technical Co. Ltd, Tokyo, Japan). Mounted samples were frozen in the preparation chamber at –140°C, then sputter-coated with a 3 nm thick layer of gold–palladium and examined in the frozen condition in the cryo-SEM at 2 kV and –120°C.

Electromyographic recordings

The animals stood on a rotatable Perspex platform. The conditions of standing on a horizontal surface, as well as clinging to a vertical wall and to a ceiling, could be mimicked by rotating the platform accordingly around the longitudinal axis of the animal. As the platform was being turned in both directions during the recordings, the leg under investigation faced both upwards and downwards in the vertical state at different points in time of the same recording. Muscle activity was recorded when the platform was motionless as well as during rotations. Experiments were performed on a smooth and on a rough surface. The Perspex surface served as the smooth substrate. For simulating a rough surface, a piece of cotton fabric (2.9 threads per mm) was glued to one side of the platform.

Prior to experiments, the animals were chilled in the refrigerator at 8°C for about 30 min to immobilize them. The electrodes were then placed in the tibia near the femur–tibia joint (Fig. 1), where they recorded the middle part of the tripartite claw retractor muscle (Radnikow and Bässler, 1991). This recording site was chosen because this part of the retractor unguis is the most active during posture control (Radnikow and Bässler, 1991). The electrodes were connected to a custom-made preamplifier–amplifier combination (Peter Heinecke, Seewiesen, Germany) with adjustable high- and low-pass filters (mainly used to cut off 50 Hz pick-up from the power grid and high-frequency noise above 10 kHz). The amplified signals were digitized with a data acquisition instrument (model PCI-1200, National Instruments, Austin, TX, USA) and stored on the computer hard disk with the software WinEDR (V2.9.2, John Dempster,

University of Strathclyde, Glasgow, UK). We monitored muscle signals in the experimental situations noted below with a sampling rate of 1 kHz. Further analysis was done with the software Spike2 (version 6.05a, Cambridge Electronic Design, Cambridge, UK).

The rotating platform was attached to a 360 deg potentiometer (Novotechnik, Ostfildern, Germany), which was also connected to the PCI-1200 digitizer. This potentiometer coded the tilting angle of the platform as output voltage and was recorded along with the electromyograms (EMGs). Three stationary positions were taken into account for evaluation: horizontal, vertical and inverted, and the following rotations were considered: (1) 90 deg rotation from horizontal to vertical; (2) 90 deg rotation from vertical to horizontal; (3) 90 deg rotation from vertical to inverted; (4) 90 deg rotation from inverted to vertical; (5) 180 deg rotation from horizontal to inverted; (6) 180 deg rotation from inverted to horizontal; (7) 180 deg rotation from vertical to vertical; and (8) 360 deg rotation from inverted to inverted (indicated by the icons in Fig. 4, and on the abscissae in Fig. 4, Fig. 5B,C and Fig. 6). After each turn the platform was stationary for at least 3 s before the next turn was executed. The platform was rotated manually with a median angular velocity of 53 deg s^{-1} (25% quartile, 45 deg s^{-1}; 75% quartile, 60 deg s^{-1}).

Statistical analyses were performed with the software SigmaStat for Windows (version 3.11) and SigmaPlot 2004 for Windows (version 9.01, both from Systat Software, San José, CA, USA). Comparison of groups was made using Kruskal–Wallis ANOVA on ranks for many groups, and further optional pairwise comparisons were made with Dunn's test.

The box plots were created with SigmaPlot and further arranged with CorelDRAW (version 12, Corel Corporation, Ottawa, ON, Canada). The plots always show the median and the inter-quartile range, i.e. the 25th and 75th percentiles, and the whiskers denote the 10th and 90th percentile limits. Different letters above the plots indicate significant differences between samples ($P<0.05$). Numbers in or above the boxes denote the number of measurements. Statistical analyses were performed by comparing animals; that is, after averaging the data from any given animal.
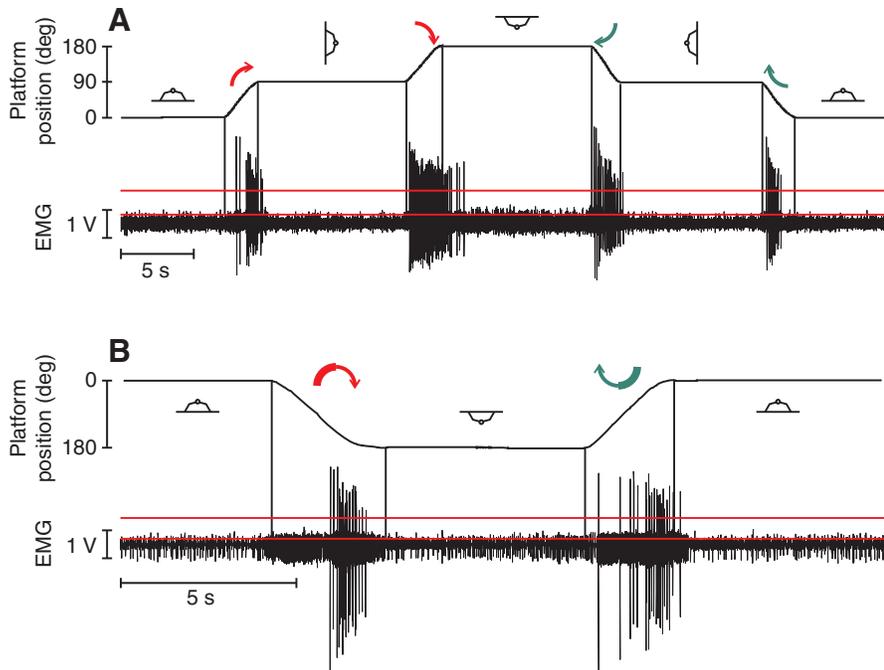
Fig. 3. Two electromyogram (EMG) recording samples from the retractor unguis in *C. morosus*. (A) Complete set of 90 deg rotations from horizontal *via* vertical to inverted, and back. (B) Sample of two 180 deg rotations from horizontal to inverted, and back. Bottom traces, retractor unguis EMGs; top traces, position of animal platform. Vertical lines indicate the beginning and end of rotation movements. Arrows show the direction of platform rotation; red arrows, rotations into 'more awkward' positions; green arrows, rotations into 'less awkward' positions. Icons above the stationary phases show the posture of the animal (horizontal, vertical, inverted) (cross-sections through stick insect; circle, body; angled lines, legs; and platform, straight base line). Red horizontal lines along the recording traces indicate the thresholds used to detect the spike events for small (lower line) and large (upper line) units. A is a representative sample with typical activity of the tonic and phasic units during rotations and stationary phases. B is a sample where tonic activity was clearly more pronounced during transitions than during stationary phases. This effect was not significant when pooling all examined animals.

RESULTS

We recorded EMGs from the claw retractor muscle, retractor unguis, while the animals were standing either on the smooth or on the rough side of the rotary platform (see Materials and methods). We were able to identify between one and three motor units, depending on the position of the electrodes and the quality of the recordings. Among the different motor units, tonic as well as phasic units were observed (sample recordings are shown in Fig. 3). This is in agreement with a previous report showing that the retractor unguis muscle parts are supplied by at least five excitatory motoneurons (Walther, 1980). In most of the recordings we could not differentiate between more than one tonic or phasic unit. Thus, we treated all detected tonic spikes as one group and all detected phasic spikes as another group. We distinguished these groups by their spike amplitudes and occurrence in time. Only in two recordings of *C. morosus* were we able to clearly identify two different tonic units. To test for possible different discharge behaviours, we checked the units' spike frequency characteristics separately with statistical tests (one-way ANOVA followed by Holm–Sidak pairwise comparisons of different stationary and rotatory phases; data not shown). The tests revealed that the two tonic units in one recording had the same qualitative manner of discharge in the different situations. This response was similar to that of the tonic units in the recordings where we could not distinguish between different units. For each experimental animal, the recordings were subdivided into the different stationary and rotatory phases, and the spike frequencies of the motor units were averaged for each phase. These averaged values of spike frequencies were then used as a measure of muscle activity. We further compared the spike frequencies between rough and smooth substrates, animal species and body positions or body rotations.

A comparison of the different stationary and rotatory phases is shown in Fig. 4. The results of the phasic units for *C. morosus* are shown in Fig. 4A,B, and those for *C. impigra* in Fig. 4D,E. In these figures the first three data points represent the mean spike frequencies during the tested stationary positions: horizontal, vertical and inverted

(see icons on abscissa). The following four data points represent the spike frequencies during the different rotations through 90 deg, followed by the three different rotations through 180 deg. The 360 deg rotation is shown as the rightmost data point. Such evaluations consistently revealed an increase in spike frequency during rotations of the platform (indicated in red, green or black) compared with the steady positions (horizontal, vertical and inverted; indicated in blue). Stationary positions are shown in blue, rotations into 'more awkward' positions in red, rotations into 'less awkward' positions in green, and other rotations in black. In addition, we observed higher spike frequencies for rotations into more awkward positions (red) compared with rotations into less awkward positions (green; this colour code is maintained for Figs 5 and 6). A more awkward position is defined here as one that would be expected to demand more effort to cling to the substrate (for example, rotation from a horizontal to a vertical position). This is clearly seen in Fig. 4C,F, where the phases are grouped into stationary, less awkward and more awkward rotations for both species. Here, we found significantly higher values for the rotations compared with the stationary phases, and also for *C. morosus* a significant difference between less awkward and more awkward rotations. For neither species did we observe differences concerning the body side examined; the upward and downward facing legs responded similarly.

Carausius morosus exhibited significant differences in the discharge frequency of phasic units in several situations on both the rough (Fig. 4A) and smooth surfaces (Fig. 4B). There were no differences in spike frequency between the stationary phases but these values did differ from the spike frequency associated with several of the rotations ($P<0.05$; number of animals $N=13$; number of examined legs per species and per stationary or transitory phase, n, is given for each surface in Fig. 4A,B inside or just above the plotted box; the number of measurements per stationary or transitory phase and per animal, m, varied from 0 to 57; these measurements were averaged before statistical analysis, see Materials and methods). In *C. impigra*, both the horizontal and the vertical stationary positions showed significantly lower discharge frequencies than the
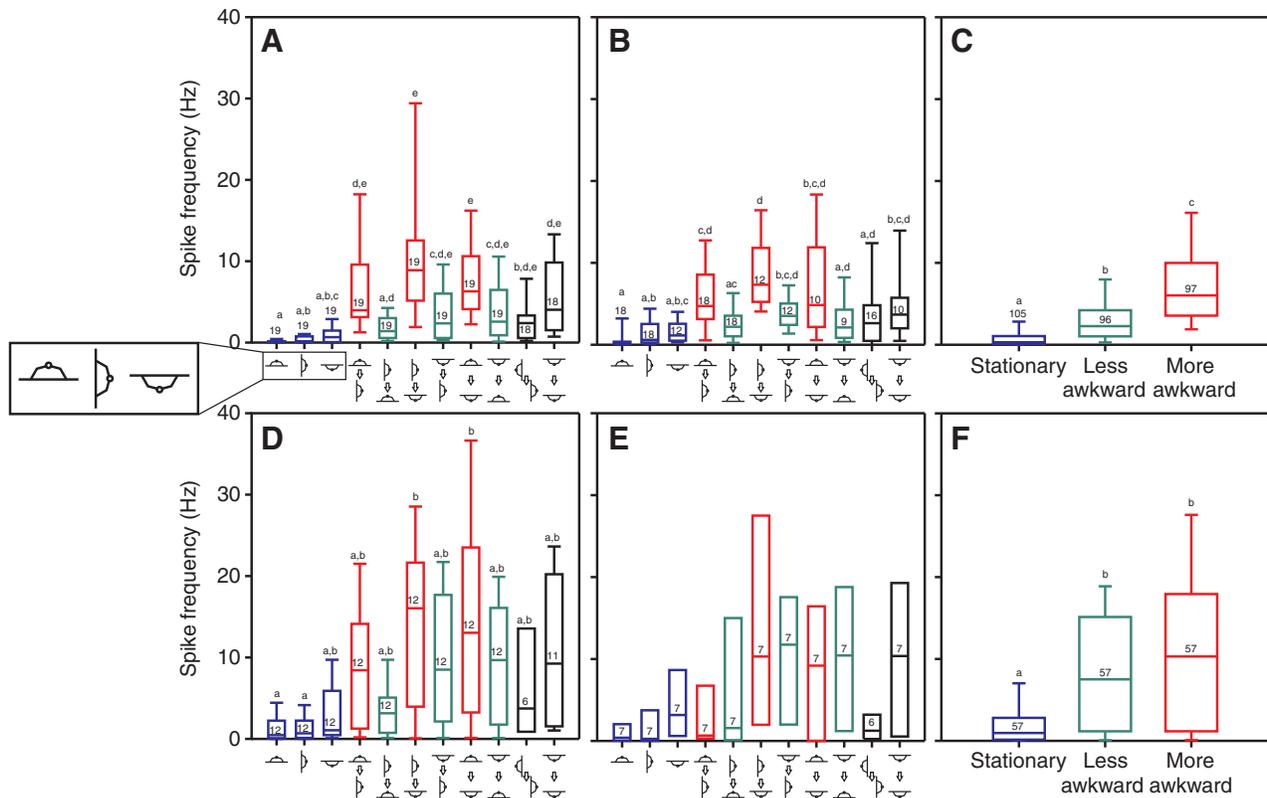
Fig. 4. Comparison of the different stationary and rotatory phases. Spike frequencies are shown for the phasic units of *C. morosus* (A–C) and *C. impigra* (D–F). For both species the results on the rough (A,D) and the smooth surface (B,E) are illustrated. C and F show the values of the phasic units for both surfaces grouped into stationary positions, less awkward rotations and more awkward rotations. Stationary positions are shown in blue, rotations into more awkward positions in red, rotations into less awkward positions in green, and other rotations in black. Box plots in this and the following figures show medians (centre lines) and interquartile ranges (boxes); whiskers indicate the 10th and 90th percentiles (see Materials and methods). The numbers in or above the boxes denote the number of examined legs per species and per stationary or transitory phase, *n*. Different letters indicate significant differences (*P*<0.05). The inset shows a magnification of the icons below the boxes: cross-sections through stick insect (circle, body; angled lines, legs) and platform (straight base line); from left to right: horizontal, vertical and inverted animal positions.

two rotations from the horizontal to the inverted position and from the vertical to the inverted position, though only on the rough surface (*P*<0.05; *N*=12; *n*, indicated next to data boxes in Fig. 4D; *m*, 0–29). No significant differences were detected between the positions and rotations in *C. impigra* standing on the smooth surface. This is probably due to the smaller number of measurements, as the tendency to higher spike frequencies during rotations is still visible (*N*=6; *n*, indicated next to data boxes in Fig. 4E; *m*, 0–25).

An analysis of the tonic units revealed no significant differences between any of the various positions and movements of the platform. Fig. 3B shows an exceptional case where the tonic units are clearly more active during the rotations compared with the stationary phases. Considering just this recording, differences in tonic unit discharge frequency are in fact significant between the stationary positions and two of the more awkward rotations on each surface (*P*<0.05). However, this was not statistically verified when the data from all recorded animals were pooled. Nevertheless, a non-significant tendency was observed in the whole data set of the tonic units in *C. morosus*; discharge frequencies tended to be higher during rotations than during stationary phases (*N*=11; *n*, indicated next to data boxes in Fig. 5B,C). When groups of stationary and rotatory phases, sorted by their awkwardness as for Fig. 4 for the phasic units, were compared the non-significant tendencies turned to significant differences (*P*<0.05). This is illustrated by the plot of the spike

frequency *versus* awkwardness of position or rotation in Fig. 5A. Awkwardness in this context is used as an index to group the transitions from one position to another. The term 'more awkward' indicates a transition into a position that is expected to require more tarsal grip than the initial position; 'less awkward' transitions started from a position that potentially demanded more grip, to a less demanding position. In *C. impigra*, we did not record a sufficient number of tonic units for meaningful statistical analysis.

We further tested for possible differences between the two phasmid species and between the two surfaces. The only difference we observed was between the two species; namely, for the phasic units in the horizontal position on the rough surface. This may be due to the difference in frictional performance of the two species' tarsal pads (P.B., H.W. and S.G., submitted), which has to be compensated for by muscle activity. Another explanation for this difference in muscle activity could simply be occasional small leg or tarsus movements in the standing position, rather than an effort to establish and maintain good attachment.

Finally, we compared the activity of the retractor unguis in all positions and during all rotations with the situation of standing on the horizontal platform. We first normalized the spike frequency values of all other situations with regard to that in the horizontal position. With this evaluation procedure, we observed significant differences for the phasic unit discharge frequency in different

Fig. 5. Comparison of stationary and rotatory phases. Spike frequencies of the tonic units in *C. morosus* are shown for both surfaces. (A) Data for both surfaces are grouped into stationary positions, less awkward rotations and more awkward rotations. (B) Data for the rough surface shown separately for the different stationary and rotatory phases. (C) Corresponding representation for the smooth surface. Numbers in the boxes denote the number of examined legs per species and per stationary or transitory phase, *n*. Different letters show significant differences in A (*P*<0.05). No differences reached significance level in B and C.

situations (*P*<0.05; Fig. 6A–D), in agreement with the evaluation of the absolute values, as shown above. In addition, though, there were significant differences in the discharge frequency of tonic units in certain situations. In particular, this was true for *C. morosus* on both surfaces (Fig. 6E,F). On the smooth surface, there were significant differences between the horizontal position and four rotations, including all three more awkward rotations (Fig. 6F; *P*<0.05). On the rough surface, there were a number of statistically significant differences (Fig. 6E; *P*<0.05). Again, we did not analyse the values of the tonic units in *C. impigra* as we did not have a sufficient number of recordings.

DISCUSSION
The central question of this study was to what extent do the two phasmid species employ their claw retractor muscles while clinging to a substrate? Our assumption was that insects more or less passively hold on to the substrate once they have found a suitable attachment for their claws and tarsal pads. This would certainly appear more economical than constant muscle activity. However, insects have been shown to employ tonic activity in several muscles during a number of behavioural situations, in particular during posture control. Examples are the flexor and the extensor muscles of the tibia (Bässler and Stein, 1996) or the abductors and adductors of the coxa (Duch and Pflüger, 1995). These muscles may even co-contract to maintain body posture or to stabilize catalepsy. A similar function might be realized by the retractor unguis (Radnikow and Bässler, 1991) and its elastic cuticular counterparts (Frantsevich and Gorb, 2004; Frazier et al., 1999; Neff et al., 2000). These two components may work together to stabilize the posture of the claws and ensure a reliable grip for the animal. In this case, the muscle would be active almost all the time, whenever the claws or tarsal pads are in active use. In addition to such basal activity, the retractor unguis would accomplish adjustments in muscle strength as needed by the behavioural situation at the time.

We found that there are tonic muscle units which are permanently active, during upright posture and while clinging to vertical and inverted substrates, as well as during platform rotations. We did not detect any significant differences in the activity of the tonic muscle units in different situations when analysing the data from all animals. However, during substrate movements there was a tendency

towards higher spike frequencies compared with the steady states (see Fig. 5B,C). In four individuals, we found significant differences for the tonic units between stationary positions and more awkward rotations (*P*<0.05; data not shown). These tonic muscle units are probably responsible for the maintenance of a stable and safe adhesion of the claws to the ground, and they may be required for small adjustments in the tarsal and claw positions. It has been shown previously that insects continuously optimize the grip of their attachment devices to the substrate (Duch and Pflüger, 1995; Pearson and Franklin, 1984; Wigglesworth, 1987). This continuous adjustment presumably involves the observed tonic muscle activity, which is increased during substrate rotations (Fig. 5). The normalized data of the tonic units reveal large variances at least on the smooth surface (Fig. 6F). This may be a sign of recruitment of additional motor units on the smooth surface where the animals are not able to find a secure hold easily. The recruitment of other motor units may only occur from time to time, e.g. when the animal detects slipping, thus increasing the variance. The large variance could then lead to non-significant results in our statistical tests. However, as we do not see such large variance in the non-normalized data on the smooth surface compared with the rough one (see Fig. 5), this interpretation has to be treated with caution. It appears more likely that the large variance is due to one or two individual recordings where the tonic units showed unusually low discharge frequencies while the animal is standing on the horizontal platform. After normalization this leads to high values for the rotations while the absolute discharge frequencies of rotations reached the same level as in the other recordings. This interpretation is supported by close examination in at least one recording of the tonic units of *C. morosus*. In this recording the spike frequencies of all positions and rotations lie within the range of the other recordings, except for the horizontal position, where the discharge frequency deviates from the average value for all recorded animals by a factor of 40.

As we were unable to discriminate between different tonic motor units in most of the recordings, our indication of muscle activity was spike frequency, regardless of the origin of the spikes. As described above (see Results), we found two recordings with two distinguishable tonic units each. These units showed the same behaviour in terms of their discharge frequencies in the different positions or rotations the animal was subjected to. This corroborated
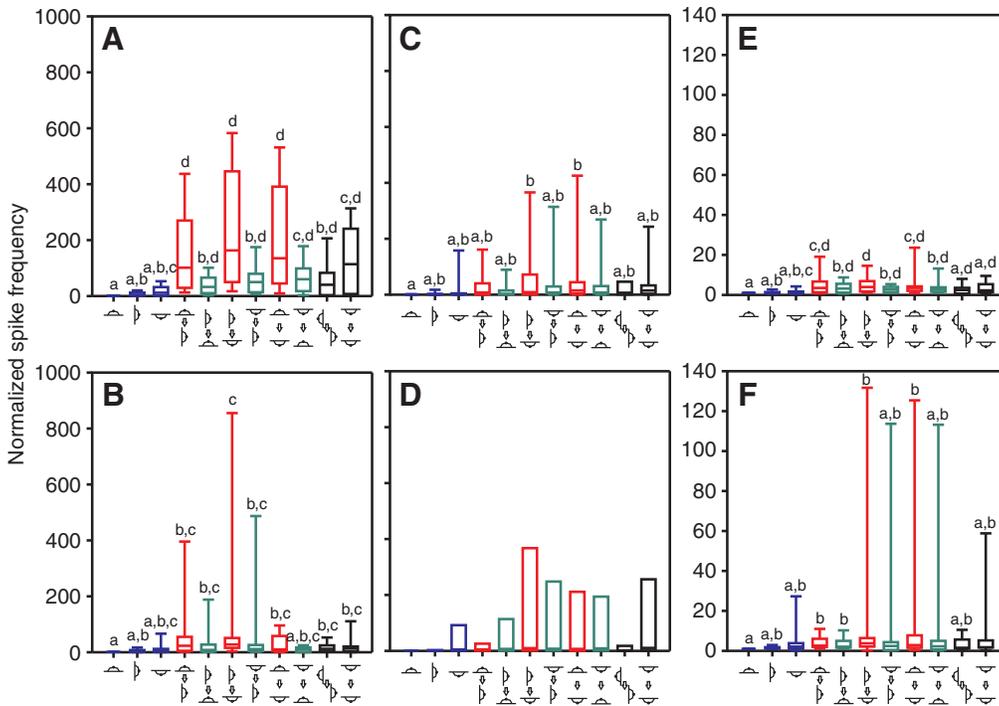
Fig. 6. Comparison of the different stationary and rotatory phases. Spike frequencies of phasic units for both species (*C. morosus* A,B; *C. impigra* C,D) and of tonic units for *C. morosus* (E,F) on both surfaces are normalized to the data obtained for the horizontal standing position (first value in the diagram=1). Same data sets as in the Figs 4 and 5.

our procedure of combining the tonic spike data and treating them as one group without losing qualitative information. It is conceivable that there are different tonic motor units which are recruited in different animal positions, e.g. one unit for horizontal standing, another for clinging to a vertical wall, and another for clinging to the ceiling. Alternatively, tonic units may be recruited gradually, one after the other, as the animal detects slippage on the substrate during tilting. Thus, we may have recorded different tonic units without distinguishing their activity patterns. This is no issue for our main question of whether muscle activity increases when the animal is clinging to a wall or ceiling compared with standing on the ground? To answer this problem, spike frequency is a good measure of net muscle activation, even without exact knowledge of unit composition.

The first of the above interpretations, at least – dedicated tonic motor unit recruitment in different situations – would further appear unlikely in view of previous reports in the literature. There is usually an orderly recruitment of the motor units in a given muscle, where motoneuron axon diameter, muscle fibre size and number, and force output are correlated (Henneman et al., 1974) (for a review, see Mendell, 2005). This is true even for rather complex invertebrate muscles that are composed of – probably functionally different – subunits (Hill and Cattaert, 2008). The flexor tibiae of the locust and stick insect is the only clear example of this, where differential recruitment of muscle portions was observed among the nine excitatory motor units (Page et al., 2008). Even here, the summed activity of different units provides a good measure of muscle performance (Gabriel et al., 2003; Page et al., 2008).

Our recordings demonstrate that the phasic muscle units, in particular, are much more active during substrate movement. This response probably represents a pronounced reaction to perturbations in the habitat that exceed a particular strength, perhaps a gust of wind or disturbance by other animals. A comparable increase in muscle tonus is generally observed in postural muscles in response to disturbance (Sauer and Stein, 1999). Action of the phasic units

in the retractor unguis muscle most likely provides fast and strong grip of the pretarsus and the claws to obtain a safe attachment – at least when the attachment requirements exceed a certain level. In our experiments, phasic muscle activity was always prominent during substrate rotation. As is evident in the first platform movement (0 to 90 deg) in Fig. 3A, the phasic unit was active as soon as the tilting angle exceeded ~30 deg. In the subsequent rotations (90 to 180 deg and 180 to 90 deg) the unit started to burst immediately the rotation began and continued until the end of platform movement (180 to 90 deg) or slightly beyond (90 to 180 deg). In the last rotation (90 to 0 deg) the phasic unit was active until the platform angle decreased below the value that presumably initiated phasic muscle discharge in the first rotation (~30 deg). This points towards a strong and fast phasic muscle response when the animal detects a critical degree of slippage or, more generally, disturbance.

The responses of the retractor unguis turned out to be even more pronounced when the rotations led to more awkward positions. This indicates that the animals readily notice to which position their body is moved and are able to react to the new situation quickly. Such responses to gravity and load are well known (e.g. Bässler, 1971; Markl, 1962; Jander et al., 1970) and appear to be mediated by sense organs in the legs and leg–thorax joints, such as the campaniform sensillae, hair fields and proprioceptors, which play a major role in controlling joint positions and body posture in insects (Bässler, 1977; Bässler, 1988; Bässler and Büschges, 1998; Delcomyn et al., 1996; Larsen et al., 1997; Laurent and Hustert, 1988; Stein et al., 2006; Stein and Sauer, 1998; Weiland and Koch, 1987).

We did not observe differences in the activities of the phasic retractor unguis units between the three stationary phases (horizontal, vertical and inverted), a fact that may appear surprising on first sight. In particular, the demands on muscle force should be greater when the insects have to hold their weight against gravity in an inverted position. While this is true from a mechanical perspective, the claws and tarsal attachment pads probably already had a secure hold when

a new position was reached after turning the platform, and it is the proper engagement of attachment structures that holds the animal, rather than muscle force itself. After all, rotation of the platform took on average 1.7 s for a 90 deg rotation, more than sufficient time to make the necessary adjustments. And persistent adjustments of claw and tarsus attitude are probably affected by tonic muscle units, as is posture control in general. Further, as mentioned in the Introduction, the claw retractor muscle consists of three parts that are connected by a common tendon. Depending on the demands on the muscle, the three parts are activated in different combinations (Radnikow and Bässler, 1991). Because we recorded only the middle part, we probably did not observe the contributions of all muscle units. However, as reported by Radnikow and Bässler, it is primarily the middle part of the muscle that is active when the muscle is in normal use, and the other parts come into play only when a stronger force output is needed (Radnikow and Bässler, 1991). Thus, we may assume that the activity of RU II reflects much of the whole muscle's activity, at least in the temporal domain.

In summary, then, the act of clinging to a wall or a ceiling is not just a passive one, once the claws and tarsal pads have found a proper attachment site. The claw retractor muscle, at least, shows tonic activity throughout. One would expect, therefore, that the other leg muscles involved in positioning the tarsus and the leg are also tonically active, contributing to the attachment of tarsus and claws to the substrate. This is true, in particular, for the levator and depressor tarsi muscles, which lift and lower the tarsus as a whole and thus may influence the position and the engagement of claws and other tarsal attachment devices, such as the arolium or euplantulae. These antagonistic muscles would therefore be the obvious candidates for future electrophysiological study.

It was another goal of the present study to reveal possible differences in muscle recruitment between the two phasmid species. Such differences might result from the different structures of their tarsal euplantulae (P.B., H.W. and S.G., submitted). *Carausius morosus* has nubby tarsal pads, which show stronger frictional forces on a rough surface compared with the smooth euplantulae of *C. impigra*. One might expect that the larger friction of *C. morosus* results in less claw engagement on the rough surface of the platform, in our case a piece of fabric, while *C. impigra* might require more pronounced action of the tarsal claws. Our results show, however, that there is no notable difference between the two species in the activities of their claw retractor muscles. This is true for the smooth as well as for the rough surfaces. It would thus appear from our inter-species comparison that the structure of the tarsal attachment pads and the resulting adhesive and frictional forces do not influence the use of the retractor unguis muscle and the tarsal claws.

Finally, one may have expected differences in retractor unguis recruitment, and by inference claw engagement, on the smooth and the rough surfaces. Muscle activity might have been notably lower on a smooth surface than on a rough one, as animals cannot use their claws on a smooth surface because of the lack of structures that allow interlocking. It is known for some insects that arolia are deployed only if the claws do not find enough grip; that is, if the surface asperities are too small for the claw tips to engage (Bullock and Federle, 2009; Dai et al., 2002; Endlein and Federle, 2008; Frantsevich and Cruse, 1997; Frantsevich and Gorb, 2004; Snodgrass, 1956). It was assumed that the transition from a gripping to an adhering situation is controlled by claw retractor muscle and by a proximal pull of the legs towards the body (Endlein and Federle, 2008; Federle et al., 2001; Federle and Endlein, 2004; Frantsevich and Gorb, 2004; Snodgrass, 1956). In our experiments, we indeed

recorded similar activity on the two types of surface, meaning that certain preload/shear is necessary, independent of which type of attachment device is momentarily in contact.

There are three interpretations for these observations. First, (tonic) muscle activity, particularly on the smooth surface, might be due to the fact that the pretarsus keeps searching for a hold on the platform, for nearby places with suitable roughness for the claws. As natural surfaces normally exhibit a wide range of roughness this could be normal behaviour for finding a foothold. No noticeable tarsus movement was observed, however, no matter what surface a phasmid was on. Second, tonic contraction of the retractor unguis may be necessary to maintain appropriate stiffness of the tarsus, and of the tarso-pretarsal joint in particular. As stated above, tonic contractions and co-contractions of antagonistic muscles are not unusual for insects to stabilize the body posture. Also, a certain stiffness in the tarsus and the pretarsus segments is necessary for the tarsal chain to work properly, and avoid curling and detaching from the ground (Clemente and Federle, 2008; Frantsevich and Cruse, 1997; Frantsevich and Gorb, 2004). Third, the use of the claw retractor muscle on a smooth surface may be due to the fact that the arolium, as a pretarsal attachment pad, is also driven by this muscle (Federle et al., 2001; Frantsevich and Gorb, 2002). On smooth surfaces the activity of the retractor unguis may thus not indicate use of the claws but rather deployment and preload/shear of the arolium as an attachment pad for smooth surfaces. This would be electrophysiological evidence for the fact that one muscle controls different attachment structures depending on the requirements of a particular behavioural situation. However, it still remains unsolved how the retractor unguis is used in the context of arolium deployment in representatives of Phasmatodea.

Conclusions and outlook

In this study we examined the activity pattern of the middle portion of the claw retractor muscle during posture control in two stick insect species. We compared the activity of the muscle for different positions of the animals and for various types of movement of the support platform. During movement of the platform, we recorded pronounced phasic EMG discharge, presumably reflecting a disturbance response aimed at securing the grip of the animal to the substrate. We observed tonic activity of the muscle in all body positions and, unexpectedly, muscle activity was not greater when the animals had to cling to the vertical or inverted platform compared with standing on the horizontal surface.

The adaptation of natural systems to locomotion on various substrates is very efficient and that is why the biomechanics of attachment devices remains an important topic of research. A more detailed understanding of the attachment mechanism may be of use for the construction of economical artificial walking systems. The present data demonstrate how insects control body posture by using their attachment devices. In the future, recordings from other leg muscles will be essential to obtain a more exact idea of how the different leg muscles work together for secure substrate attachment.

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