J Comp Physiol A (2008) 194:49–60
DOI 10.1007/s00359-007-0287-x

ORIGINAL PAPER

Walking on smooth or rough ground: passive control of pretarsal attachment in ants

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Received: 29 July 2007 / Revised: 24 October 2007 / Accepted: 27 October 2007 / Published online: 28 November 2007

Abstract The hymenopteran tarsus is equipped with claws and a movable adhesive pad (arolium). Even though both organs are specialised for substrates of different roughness, they are moved by the same muscle, the claw flexor. Here we show that despite this seemingly unfavourable design, the use of arolium and claws can be adjusted according to surface roughness by mechanical control. Tendon pull experiments in ants (*Oecophylla smaragdina*) revealed that the claw flexor elicits rotary movements around several (pre-) tarsal joints. However, maximum angular change of claws, arolium and fifth tarsomere occurred at different pulling amplitudes, with arolium extension always being the last movement. This effect indicates that arolium use is regulated non-neuronally. Arolium unfolding can be suppressed on rough surfaces, when claw tips interlock and inhibit further contraction of the claw flexor or prevent legs from sliding towards the body. To test whether this hypothesised passive control operates in walking ants, we manipulated ants by clipping claw tips. Consistent with the proposed control mechanism, claw pruning resulted in stronger arolium extension on rough but not on smooth substrates. The control of attachment by the insect claw flexor system demonstrates how mechanical systems in the body periphery can simplify centralised, neuro-muscular feedback control.

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Keywords Passive control · Claws · Adhesion · Locomotion · Surface roughness

Introduction

When insects walk or run, their tarsi and pretarsi perform movements which help the legs transmit forces to the ground and attach to and detach from various substrates. The insect tarsus represents a complex mechanical system free of intrinsic muscles (Snodgrass 1935). All tarsal and pretarsal movements are mediated by leg movements and/or by muscles located in proximal parts of the leg. A central player in the control of (pre-) tarsal movement and attachment is the claw flexor (unguitractor muscle), a tripartite muscle located in femur and tibia connected to the pretarsus through a very long apodeme (Walther 1969; Radnikow and Bässler 1991). This muscle not only controls the flexion of the claws but it also elicits movements of the tarsal segments and the adhesive pad (Federle et al. 2001; Frantsevich and Gorb 2004). As a consequence of this morphological design, one may expect that movements of tarsus, claws and adhesive pads are strictly coupled.

However, the two types of pretarsal attachment organs serve very different functions. The claws are rigid, sickle-shaped structures that can interlock with protrusions of rough substrates (Dai et al. 2002). Adhesive pads, on the other hand, are soft cuticle organs used to adhere to relatively smooth substrates (Gorb 2001). As smooth adhesive pads have been shown to be vulnerable to abrasion on rough surfaces (Slifer 1950), restricting their use to smooth substrates may be important for preserving the insect's capacity to hold on to smooth plant surfaces. How is it possible that two attachment organs required in different situations are moved and controlled by the same muscle?

In Hymenopteran insects, the smooth adhesive pad (arolium) is a fluid-filled cuticle structure that can be unfolded and retracted (Snodgrass 1956; Federle et al. 2001; Frantsevich and Gorb 2002; Frantsevich and Gorb 2004). Its movement is mediated by the interaction of the arcus, a U-shaped endosclerite and the manubrium, a longitudinal sclerite on the dorsal side of the pretarsus. The arolium can be unfolded actively through contractions of the claw flexor muscle or passively when an arolium in surface contact is subject to a pull toward the body (Federle et al. 2001). Observations on ants and bees walking on smooth substrates show that arolium extension and increase of contact area coincide with a retraction of the claws (Federle et al. 2001; Frantsevich and Gorb 2002; Federle and Endlein 2004). It has been hypothesised that interlocking of the claws on rough ground will limit the further contraction of the claw flexor and will prevent the arolium from being unfolded (Snodgrass 1956; Federle et al. 2001). However, evidence in favour of this non-neuronal control mechanism has been weak, because it was unclear how arolia are moved on rough surfaces and because the detailed sequence of arolium, claw and tarsus movements was unclear. In this paper, we test the "passive control" hypothesis in weaver ants (*Oecophylla smaragdina*) by characterising the tarsal and pretarsal movements elicited by the claw flexor and by investigating how arolium unfolding is influenced by surface roughness and by experimental removal of the claw tips.

Materials and methods

Workers of Asian Weaver ants (*Oecophylla smaragdina*) were taken from two laboratory colonies that had been collected in Brunei. The colonies were kept in plastic boxes in the laboratory (12:12 h light-dark cycles) and fed with honey-water and dead insects ad libitum.

Morphological studies

To characterise the morphology of the tarsus and pretarsus of *O. smaragdina* ants, semithin sections were prepared of the tarsus by fixing tarsi in Carnoy's solution for 2 days and dehydrating them 2 × 1 h in 100% ethanol. Specimens were immersed 2 × 20 min in propylene oxide, stored for 12 h in a 1:1 solution of propylene oxide and epoxy embedding material (Epon)–Araldite mixture, and left in 100% Epon–Araldite mixture for 1 day. The resin was allowed to polymerize for 12 h at 60–75°C in a rubber mould. Blocks were sectioned serially at 1.5 µm by using glass knives and a microtome. Sections were attached to albuminised glass slides and stained with methylene blue at 60°C.

To examine UV autofluorescence characteristic of resilin (Andersen and Weis-Fogh 1964), whole-mounts of *O. smaragdina* pretarsi were bathed in distilled water. Specimens were mounted in a water-soluble medium (Moviol) and viewed with a Leica DMR HC microscope under UV fluorescence (excitation 340–380 nm, emission 425 nm).

For scanning electron microscopy (SEM), samples were sputtered with gold for 5 min (25 mA) and investigated by using a Zeiss DSM 962 scanning electron microscope (working voltage 5–15 kV).

Analysis of the claw flexor system

Experiments on the claw flexor tendon were conducted similar to the method described by Federle et al. (2001). Front, middle and hind legs of freshly killed ant workers were severed from the body and positioned on a glass coverslip mounted inside a transparent petri dish. Using UV-curing cyanacrylate adhesive (Henkel, Loctite), the tibia and the four proximal tarsal segments were glued firmly to the glass surface so that the fifth tarsomere and the pretarsus remained mobile and protruded above the cover slip edge. Legs were mounted for lateral and ventral views of the pretarsus.

With the tibia covered with drops of Ringer's solution, we removed parts of the cuticle of the tibia to uncover the claw flexor apodeme. The pretarsus remained in air (outside the solution). After transferring the preparation onto the stage of an upright microscope, we clamped the tendon with self-closing forceps attached to a micromanipulator. A solenoid device attached to the forceps arms was used to open and close the forceps with minimal vibration. Starting from the point where the first movements of the pretarsus became visible (defined as amplitude zero), the tendon was pulled in steps of 10 µm until maximum extension of the arolium was reached, and released back to amplitude zero. Pulling amplitude averaged 180 ± 33 µm (mean ± SD of $n = 48$ pull/ release cycles in 16 legs). Pretarsal movements were recorded with a CCD camera (QImaging, QIC-F-M-12) attached to the microscope.

In lateral recordings of the (pre-) tarsal movements the following joint angles were measured (see Fig. 1): (1) "Ta4–Ta5 angle": the angle between the fourth and fifth tarsal segments, (2) "claw angle": the angle between the claws and the fifth tarsal segment and (3) "arolium angle": the angle between the arolium and the claws. The arms of these angles were defined as follows: (a) the medial axis of the fourth tarsal segment, (b) the medial axis of the fifth tarsal segment, (c) the axis connecting unguifer and claw

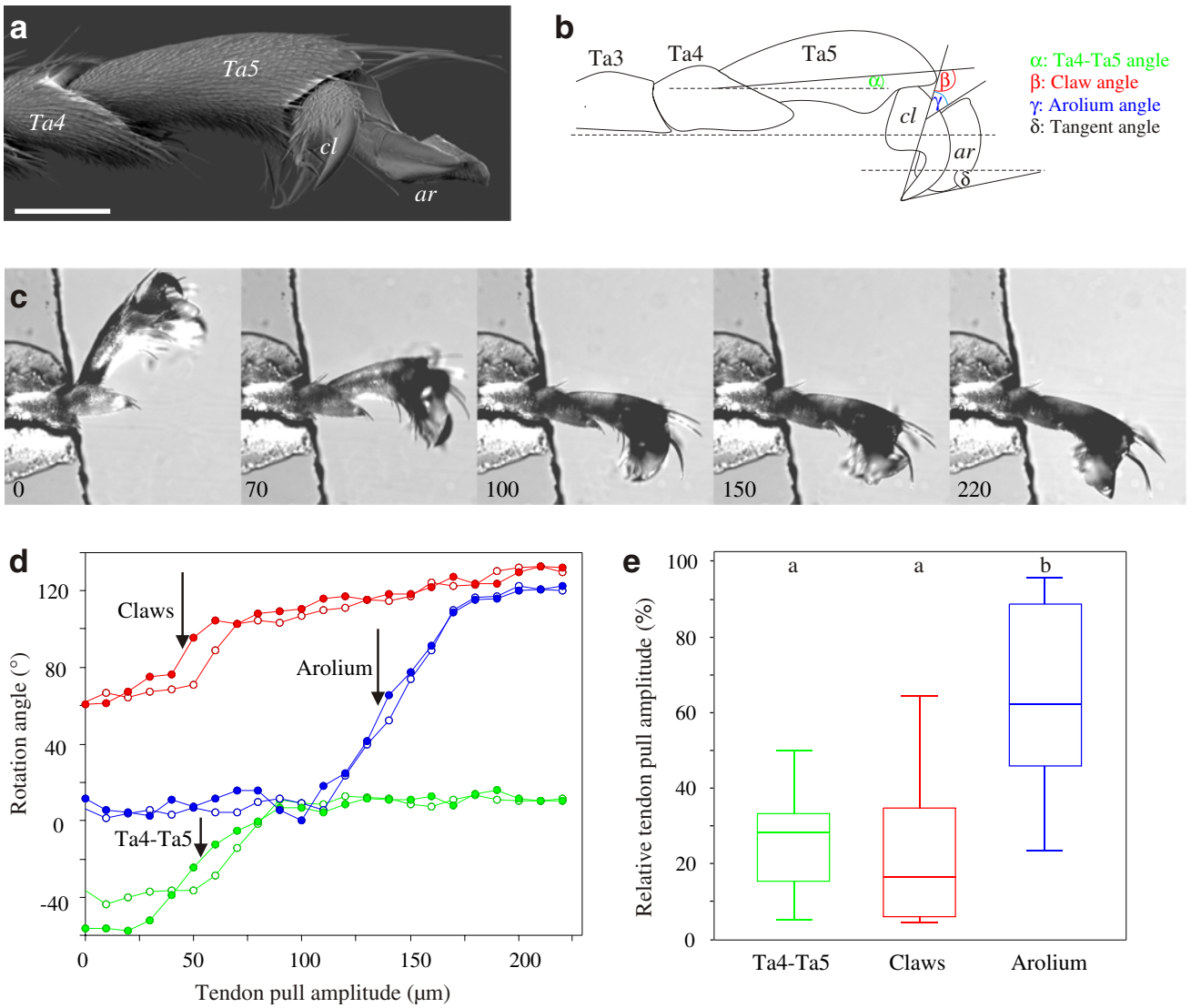

Fig. 1 **a** SEM of *O. smaragdina* tarsus in lateral view; scale bar: 100 µm. **b** Schematic of tarsus and pretarsus indicating the angles measured in lateral view. **c** Experimental pull of the unguitractor tendon in *O. smaragdina* hind leg. Numbers indicate tendon pull amplitude in µm ('0' is defined as the amplitude where the first pretarsus movement became visible). **d** Rotation of fifth tarsal segment (Ta5), claws and arolium during a tendon pull. *Arrows* indicate the pulling amplitudes where the change of rotation was maximal. *Filled markers* denote contraction, *open markers* release of the claw flexor. **e** Summarised result of 47 tendon pull experiments showing the pulling amplitude of maximum angular change for the fifth tarsomere, the claws and the arolium. Rotation of the arolium occurred significantly later, i.e. at higher amplitudes than the fifth tarsomere and the claws. *Boxes* denote medians and upper/lower quartiles, whiskers mark the 5 and 95% percentiles. Significant differences between groups are indicated by different letters. *ar* arolium, *cl* claws, *Ta3* third tarsomere, *Ta4* fourth tarsomere, *Ta5* fifth tarsomere

tip and (d) the dorsal edge of the lateral arolium wall. These lines were selected for good visibility. The points of intersection of these arms are very close but not identical to the actual pivot points.

To test whether our manually applied tendon excursions lay within the physiological range of the unguitractor muscle, tarsi of live ants were glued to the glass surface in the same way as described above for severed legs ($n = 8$ ants). The ant's thorax and abdomen were fixated using small droplets of melted wax. The joint angles reached during maximum flexion in live ants (elicited by anaesthetising with CO_2) were similar to the joint excursions in the experimental tendon pulls (spontaneous movements: Ta4–Ta5 angle: 8.4 ± 2.7°, claws: 113.7 ± 6.7°, arolium: 87.1 ± 12.6°; tendon pulls: Ta4–Ta5 angle: 8.9 ± 7.3°, claws: 121.9 ± 10.3°, arolium: 112.4 ± 18.3°). This indicates that our experimental tendon pull amplitudes lay within a physiological range.

To visualise the surface contact area of the arolium elicited by the action of the claw flexor, severed legs were mounted upright so that a pull on the tendon brought the pretarsus into contact with the transparent petri dish supporting the cover slip. Ventral views of the pretarsus were recorded using an inverted microscope (Zeiss Axiovert 405 M). We used brightfield epi-illumination to image the pad contact area with high contrast (Federle and Endlein 2004). Arolium contact area was measured for each tendon excursion step using image analysis routines written in Matlab (The MathWorks, Inc.). In addition to pad contact area, we recorded claw movement by the angle between the claws and the medial axis of the tarsus. Observation of the claws required additional fibre optic illumination. Of each foot, the mean angle of left and right claws was used for further analysis. Raw data were filtered using a lowpass second-order Butterworth filter with the cutoff frequency being a quarter of the sampling frequency.

An attempt was made to evaluate which part of the tarsus would come into contact with the substrate if the foot was put down at a given claw flexor muscle contraction. In the lateral recordings, we measured the angle between the tarsus and the common tangent to the arolium and the claws ("tangent angle", Fig. 1b). If, in the moment of attachment, the angle of the tarsus with the substrate (which we estimated separately in running ants, see below) exceeds this tangent angle, the arolium would contact the surface before the claws. If it is smaller, the claws would make contact before the arolium.

Tarsus angle during attachment

To investigate which parts of the tarsus and pretarsus come into contact with the substrate first, we recorded front, middle and hind leg steps of *Oecophylla smaragdina* ants walking upside down. Ants were allowed to walk inside a cube-shaped plastic box (25 mm side length) covered on its top side with a glass cover slip. We filmed at 250 frames per second using two synchronised Redlake PCI 1000 B/W high-speed video cameras oriented at approx. 90° to each other.

When a foot was put down, we recorded whether claw tips or arolium touched the surface first. At the moment of attachment, we also measured the angle of the tarsus with the glass substrate. The angle was obtained from three-dimensional coordinates calculated by the direct linear transformation method (Abdel-Aziz and Karara 1971; Biewener and Full 1992) in Matlab. Prior to recording, a 3D calibration of the field of view was performed. We used four small points on the walking surface as markers (forming a square of approximately 0.5 mm side length). Instead of calibrating the field of view with an object of known dimensions, we moved the substrate by 0.75 mm with the fine axis of a micromanipulator (Narishige MLN-330, 10 μm resolution) which was oriented exactly perpendicular to the surface so that eight points spanning a cuboid of $0.5 \times 0.5 \times 0.75$ mm were digitised in both camera views. The angle of the tarsus with the substrate was measured by digitising two points on the distal edge of the first tarsomere and on the distal edge of the fourth tarsomere and three points on the substrate plane.

Test of the passive control of arolium use in live ants

We anaesthetised *Oecophylla smaragdina* ants with CO_2 and carefully clipped all claw tips on one body side using microscissors. After approx. 10 min of recovery, the ants were allowed to walk upright either on smooth glass, rough sandpaper (30 μm particle size polishing discs, UltraTec Inc.) or glass plates coated with slippery Fluon GP1 (Whitford, Diez). While walking on the surface, the ants were again anaesthetised, causing them to contract their claw flexor muscles and to "freeze" on the surface.

We compared arolium extension and claw flexion between ants standing on rough (sandpaper), smooth (glass) and slippery (Fluon) surfaces. As the non-transparent surfaces made a ventral recording of the pad contact area impossible, we quantified the width of the arolium by measuring in dorsal view the lateral distance between the arms of the U-shaped arcus, which support the lateral walls of the arolium (Fig. 5). To obtain a measure of the strength of unguitractor muscle contraction, we also measured claw flexion (in dorsal view) as the angle of the claws with the medial axis of the tarsus (see above).

Ants were tested on smooth and rough substrates. Another set of individuals were tested separately on the Fluon-coated surfaces. To allow paired comparisons between intact and manipulated tarsi, claw pruning was performed on one side of the body.

Results

Characterisation of claw flexor system

Consistent with previous studies on the (pre-) tarsus in the Hymenoptera (Snodgrass 1956; Federle et al. 2001; Frantsevich and Gorb 2004), we found that the claw flexor muscle controls several tarsal and pretarsal joints. A pull on the claw flexor apodeme, equivalent to a contraction of the muscle, elicited the following rotary movements (Fig. 1b–d):

(1) Flexion of the joint between the fourth and the fifth tarsal segments (termed "Ta4–Ta5 joint" in the

following). Contraction of the claw flexor muscle moves the last tarsal segment from a hyperextended "default" position in which it is bent away from the substrate at an angle of −43.3 ± 10.6° (mean ± SD) toward the ventral side. As a consequence, it becomes slightly flexed (angle 8.9 ± 7.3°) and the pretarsus (claws and arolium) can make contact with the substrate (Arnold 1974; Gorb 1996; Frazier et al. 1999).

(2) Retraction of the claws. Contraction of the claw flexor muscle mediates (through the movement of the unguitractor plate, see below) the retraction of the claws, which are hinged dorsally on a projection of the fifth tarsomere (unguifer). The claw tips move ventrally and proximally (the claw rotation axes are oblique, intermediate between the dorsoventral and the horizontal axis). In lateral view, the claw angle changes from 73.2 ± 19.6° (extended) to 121.9 ± 10.3° (retracted). The axis of this movement will be termed "claw joint" in the following.

(3) Extension of the arolium which brings its adhesive zone on the ventral side into contact with the surface, combined with a lateral expansion. In lateral view, the arolium angle changes from 14.3 ± 15.7° (retracted) to 112.4 ± 18.3° (extended). This rotation occurs around a joint-like structure formed by the pretarsal sclerites manubrium and arcus (termed "arolium joint" in the following).

If the proximal tarsal segments are not immobilised, the claw flexor also causes some flexion of the tibia-tarsus and the three more proximal tarsal joints (Frantsevich and Gorb 2004). As the detailed observation of the pretarsus required the proximal tarsus to be fixated, these movements were not considered in this study.

When the claw flexor muscle relaxes, the three joints return to their starting position not by the action of an antagonist muscle but by elastic recoil. This is achieved by several spring-like membranous and resilin-bearing cuticle elements (Gorb 1996; Frazier et al. 1999; Frantsevich and Gorb 2002).

Sequence of tarsal and pretarsal movements

Figure 1d shows the angular movements of the Ta4–Ta5, claw and arolium joints as a function of pulling amplitude for the contraction and subsequent release of the claw flexor tendon. It can be seen that, even though all the three joints undergo considerable rotation, the maximal angular changes occur at different tendon pull amplitudes. The angular changes of the Ta4–Ta5, claw and arolium joints were maximal (efficiencies: Ta5 0.69 ± 0.20°/µm, claws 0.57 ± 0.26°/µm, arolium 1.21 ± 0.31°/µm) at tendon pull amplitudes of 54 ± 26, 51 ± 34 and 117 ± 45 µm (means ± SD), respectively. The pulling amplitude of maximum angular change differed highly significantly between the three joints (Friedman test: $\chi^2 = 42.4$, $df = 2$, $P < 0.001$). In all the experiments ($n = 47$ from 15 ants), extension of the arolium occurred after the depression of the fifth tarsomere and the flexion of the claws (Schaich-Hamerle post-hoc tests, arolium vs. claws: $D = -1.43$, $P < 0.001$; arolium vs. Ta4–Ta5: $D = -1{,}19$, $P < 0.001$; see Fig. 1e).

The ventral recording of pad contact area elicited by the tendon pull confirmed that the claw flexor muscle brings the arolium into contact with the substrate. The initial contact area was always circular and very small (i.e. the arolium was not yet unfolded) and increased steadily with a further contraction of the claw flexor (Fig. 2). Consistent with the lateral views, we found that the maximum increase of contact area occurred after the maximal angular change of the claws (117 ± 10 vs. 37 ± 28 µm; Wilcoxon matched pair test: $N = 6$, $Z = 2.20$, $P < 0.05$).

The coordinated "sequence" of movements elicited by the claw flexor muscle has two important consequences: (1) Because of the earlier flexion of the Ta4–Ta5 and claw joint, the claw tips make contact with the substrate before the arolium (we will justify this statement in more detail below). (2) Effectively before the arolium movement, the claws are flexed proximally so that their tips can interlock

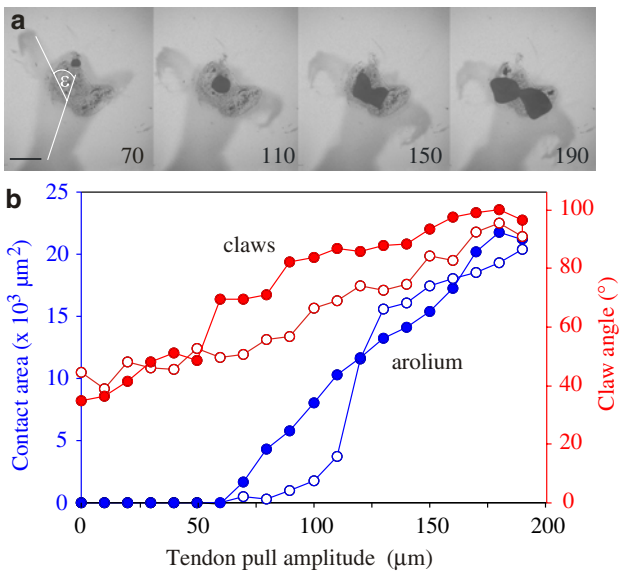

Fig. 2 **a** Ventral view of *O. smaragdina* hind leg tarsus during experimental tendon pull. In reflected light, the adhesive contact area is visible as a *dark zone*. Claw flexion was measured from the angle (ε) between the claws and the tarsus axis. Numbers denote the pulling amplitude (µm). *Scale bar* 100 µm. **b** Changes in contact area (blue or black in B/W image) and claw flexion (red or grey in B/W image) elicited by the tendon pull. *Filled markers* indicate contraction, *open markers* release of the claw flexor

with protrusions of the substrate. We hypothesise that both factors in combination represent a biologically meaningful, passive control mechanism. On a rough surface, the claws interlock with irregularities of the surface, which may stop the movement of the claw flexor apodeme and thus prevent the arolium from being further extended. On a smooth substrate, however, the claws will find no resistance so that the claw flexor can contract further. Thus, the arolium will be engaged and unfolded only when it is needed, i.e. when the claws fail to interlock. To test whether this hypothetical, passive control is indeed responsible for the regulation of arolium use on rough vs. smooth surfaces, we measured the effect of claw pruning on arolium extension (see below: "Test of passive control of arolium use in live ants").

Proximate mechanism of delayed arolium extension

What design properties of the claw flexor system cause the arolium movement to occur later than the movement of the claws and the fifth tarsomere? We have proposed (Federle et al. 2001) that the delayed arolium extension is a consequence of the two-phasic movement of the unguitractor plate. The unguitractor plate is a strongly tanned sclerite on the ventral side of the pretarsus (Fig. 3a, b). It transmits the pull of the claw flexor tendon to the pretarsus by being flexibly linked to the claws and, via the pretarsal planta, to the arolium (Fig. 3a–c). Due to the mechanical arrangement of the system, the unguitractor plate movement consists of two different phases. In the first phase (small claw flexor contraction), the entire pretarsus (claws, arolium, planta, and unguitractor plate) rotates around the unguifer joint. This phase continues until the unguitractor plate makes contact with the ventral, anterior margin of the fifth tarsal segment. At that moment, the unguitractor plate is usually sloped relative to the axis of the tarsus. In the second phase (large claw flexor contraction), further pull of the tendon aligns the unguitractor plate to the pulling direction (i.e. it rotates around the ventral anterior end of the last tarsomere). As a consequence, the unguitractor plate pushes the planta and attached arcus upward against the manubrium. Due to a resilin-containing elastic connection between planta and unguitractor plate (Fig. 3b), the planta is now no longer aligned to the unguitractor plate but slightly bent ventrally (Fig. 3a, c). The arcus movement in turn triggers the extension of the arolium.

Observations using light shining through the translucent tarsus cuticle (Fig. 3d) generally confirmed our earlier explanation of delayed arolium extension. However, we found that the arolium extension does not exactly start with the transition to the second phase of the unguitractor plate movement but slightly later. This can be explained by the effective moments acting at the arolium joint in phase 2, i.e. when the unguitractor plate is in contact with the ventral, anterior margin of the fifth tarsal segment.

We use a simplified, 2D mechanical model (an extension of the model by Frantsevich (Fig. 10c in Frantsevich and Gorb 2004)) to illustrate the mechanism (see Fig. 3c and Appendix). The model represents a system of limbs connected by three torsion springs, which are deflected by the pull of the claw flexor tendon. We consider the anterior ventral margin of the last tarsomere as a pivot with either high or no friction, resulting in either a pure rotation model (termed "rotation only" in Fig. 3e) or in a model allowing for simultaneous translation of the unguitractor plate ("translation and rotation" in Fig. 3e), respectively. We measured the positions of the unguitractor plate, planta, manubrium and arcus in three tendon pull experiments performed with light shining through the tarsus cuticle and used these to calculate the effective lever arms around the manubrium and arolium joints, as explained in the Appendix. The effective lever arm (M/F, where $M = H_2 \times F^*$ is the moment around the joint and F the force of the claw flexor contraction) of the arolium is plotted as a function of tendon pull amplitude in Fig. 3e. Both the "rotation" and the "translation-rotation" models predict the effective lever arm to be negative or very small at the beginning of the claw flexor contraction but positive and increasing for further contraction. Thus, the arolium will remain stationary for smaller contractions and will only begin to move once the lever arm has increased sufficiently. Figure 3e, f show that the effective lever arm is still small when the unguitractor plate makes contact with the fifth tarsomere, which explains our finding that arolium extension starts slightly later than this event. Our model shows that the delayed arolium extension can be explained without requiring a non-linear behaviour of the torsion spring at the arolium joint.

As it was impossible to measure l_2 (see Fig. 3c) with sufficient accuracy from our video recordings, we made the conservative assumption that this length remained constant during the claw flexor contraction. The increase of effective lever arm with contraction of the claw flexor may be even more pronounced than Fig. 3e suggests, because l_2 is likely to increase for larger tendon pulls due to the flexible attachment of the arcus to the distal end of the planta (Fig. 3a).

Which attachment structure comes into contact first?

To investigate whether claws or arolium come into contact with the substrate first, we video-recorded steps of ants walking upright and upside down. In 68% of all steps (n = 38 steps from nine ants), the claws touched the surface before the arolium. In the few cases where the arolium touched the substrate first, it was only partly unfolded. This

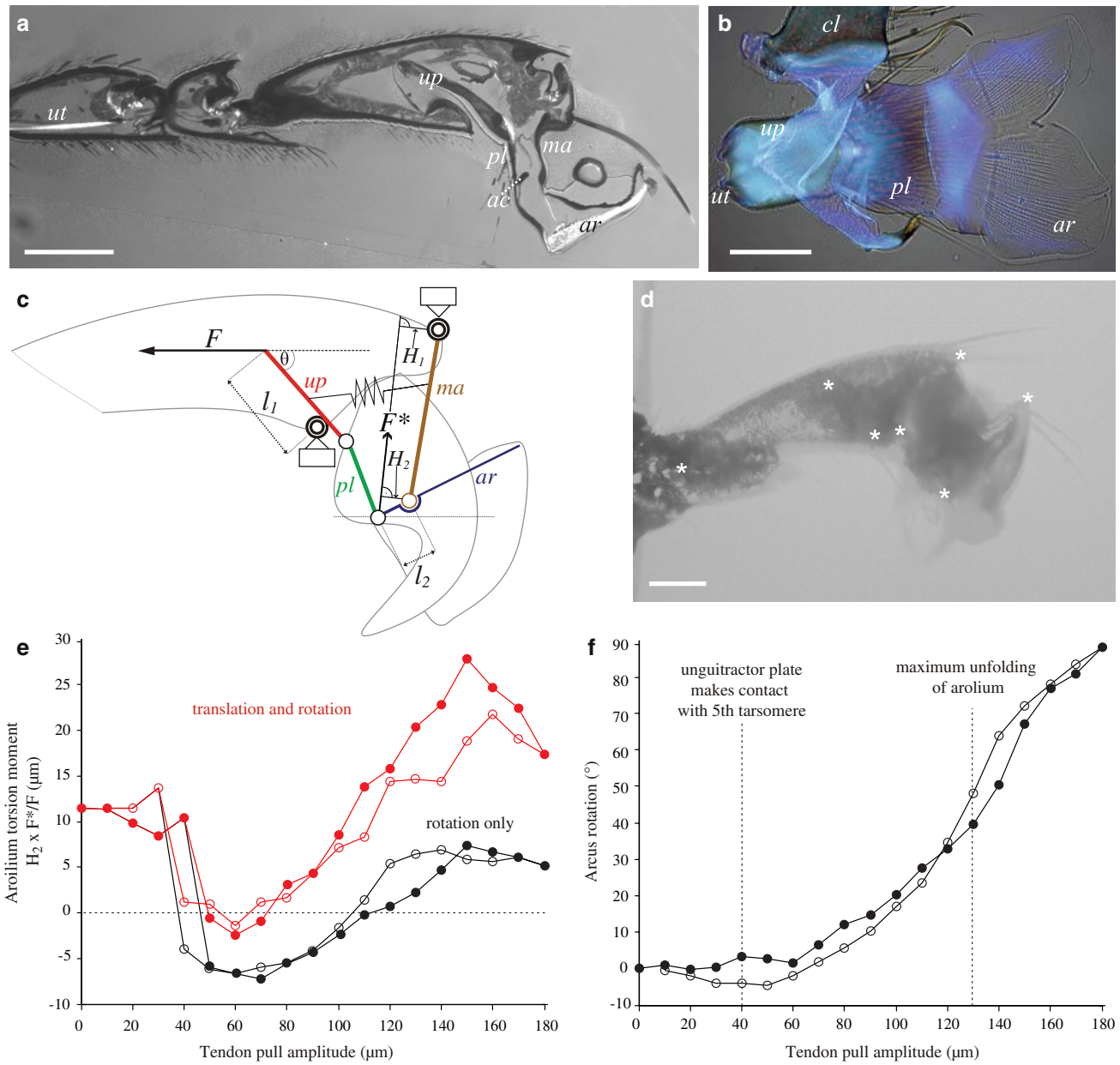

Fig. 3 **a** Sagittal section of *O. smaragdina* hind-leg tarsus. **b** Whole mount of *O. smaragdina* pretarsus; combined transmitted-light/UV-fluorescence image. Blue fluorescence (*bright* in B/W image) indicates the presence of resilin-containing elastic cuticle surrounding the unguitractor plate and at the base of the arolium. **c** Spring model of arolium unfolding. The unguitractor plate, the arcus and the manubrium are considered as stiff limbs connected by springy joints. The anterior ventral margin of the fifth tarsomere is considered a slide roller, on which the unguitractor plate can both slide and rotate (see text). **d** *O. smaragdina* hind leg tarsus during experimental pull; lateral view with bright back light to visualise position of the unguitractor plate. Digitised points shown by *asterisks*. **e** Calculated torsion moments around arolium joint $\frac{H_2 \cdot F^*}{F}$, normalised for the tendon force *F*. **f** Measured rotation of the arolium joint during claw flexor tendon pull. *Filled markers* indicate contraction, *open markers* release of the claw flexor. (*ac* arcus; *ar* arolium; *cl* claw; *ma* manubrium; *pl* planta; *up* unguitractor plate; *ut* unguitractor tendon; scale bars (**a**, **b**, **d**) 100 µm)

indicates that during walking, the arolium is being unfolded while the claw tips are already in contact with the substrate.

We used the results of the tendon pull experiments (see above) to predict which part of the pretarsus would make contact first, for a given contraction of the claw flexor and for a given tarsus-substrate angle in the moment of attachment. In the lateral views of the pretarsus during the tendon-pull experiments, we measured the angle between the tarsus and the common tangent to the arolium and the claws (see Fig. 1b). If the tarsus-substrate angle in the moment of attachment exceeds this tangent angle, the arolium will contact the surface before the claws, and vice

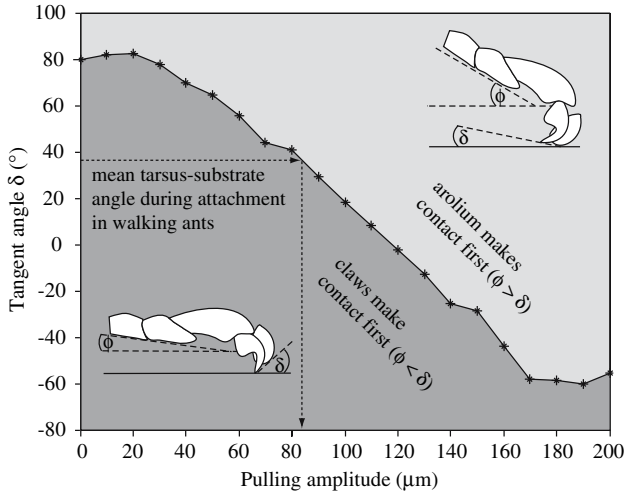

Fig. 4 Relationship between tarsus angle during attachment and claw-arolium tangent angle (see inset). The *line plot* shows the claw-arolium tangent angle during a tendon pull experiment. If during attachment, the tarsus angle (ϕ) is equal to this tangent angle (δ), both claws and arolium would touch the substrate simultaneously. If the tarsus angle is smaller (i.e. at smaller claw flexor amplitudes, *dark area*), the claws will make contact first. If the tarsus angle is greater, it will be the arolium (i.e. at higher flexor amplitudes, *light area*). The *dotted line* indicates the mean tarsus angle (36°) during attachment in walking ants, which corresponds here to a tendon pull amplitude of 84 µm (see text)

versa. We found the mean tarsus-substrate angle in the moment of attachment to be 36° ± 10° (SD). The tangent angle typically decreased steadily with tendon pull amplitude (Fig. 4). It equalled the mean tarsus-substrate angle (36°) at a mean tendon amplitude of 68 ± 35 µm (n = 39 pulls in 14 legs). Thus, it is the claws which would first contact the ground if the foot is put down with a weakly contracted claw flexor; for stronger contractions, however, the arolium would go first.

Of course, this analysis does not imply that a simultaneous substrate contact of claws and arolium is only possible for one particular tendon pull amplitude. Because of the elasticity of the pretarsus, both claws and arolium will yield when touching the ground so that a simultaneous contact of both organs is possible for a wider range of tendon pull amplitudes.

Test of passive control of arolium use in live ants

To test whether the extension of the arolium is indeed controlled mechanically by the interlocking of the claws with the substrate, we quantified arolium unfolding and claw flexion on different substrates and investigated the effect of claw tip removal. The result for arolium extension is shown in Fig. 5b. Consistent with the hypothesised control mechanism, arolium width was significantly smaller on rough sandpaper than on smooth glass (paired t-test for comparison of intact claws: $t = 6.91$, $df = 31$, $P < 0.001$). On the rough substrate, arolia were significantly more extended in the manipulated tarsi (t-test: $t = -4.41$, $df = 31$, $P < 0.001$), whereas no such effect occurred on smooth glass (t-test: $t = -0.20$, $df = 28$, $P = 0.84$). These results clearly indicate that claw-substrate interlocking limits the extension of the arolium.

This conclusion is confirmed by the very similar results obtained for claw flexion (Fig. 5c). Again, the flexion of intact claws was significantly smaller on rough than on smooth surfaces (t-test: $t = 8.29$, $df = 31$, $P < 0.001$). Also, the effect of claw tip removal was only significant on the rough sandpaper surface (t-test: $t = -2.80$, $df = 31$, $P < 0.01$).

It is likely that arolium extension is not only determined by claw interlocking and the contraction state of the claw flexor muscle but also by movements of the leg toward the body which can passively unfold the arolium (Federle and Endlein 2004). As the passive arolium extension involves a short proximal sliding of the leg, interlocking of the claw tips with the substrate could also limit this passive mechanism (Federle et al. 2001). In order to test whether this is indeed the case, we investigated arolium unfolding on slippery Fluon GP1 substrates, which are strongly anti-adhesive and where neither claws nor arolium can make significant contact. Since the passive extension mechanism requires that the arolium adheres well to the surface so that a significant shear force can be transmitted (Federle et al. 2001), passive unfolding may be impossible on the Fluon substrate.

Our findings confirm that arolium unfolding is partly passive. Even though the claws slipped and reached a similar retraction on Fluon as on smooth glass (Fig. 5c), the arolia were clearly less unfolded (Fig. 5b, comparison with smooth glass: t-test for independent groups: $t = 5.00$, $df = 52$, $P < 0.001$, with rough sandpaper: $t = 0.54$, $df = 52$, $P = 0.58$). Therefore we conclude that claw interlocking reduces arolium unfolding both by limiting the contraction of the claw flexor and by inhibiting the passive extension mechanism.

Discussion

The mechanisms of how animals manage to adhere and climb rapidly on various substrates have long fascinated biologists (e.g., Hooke 1665; von Wittich 1854; West 1862; Dellit 1934; Scherge and Gorb 2001) and have recently attracted the interest of engineers and roboticists (Sitti and Fearing 2003; Menon et al. 2004; Autumn et al. 2005; Daltorio et al. 2005; Greuter et al. 2005; Autumn et al. 2006). From a robotics perspective, adhesion-based

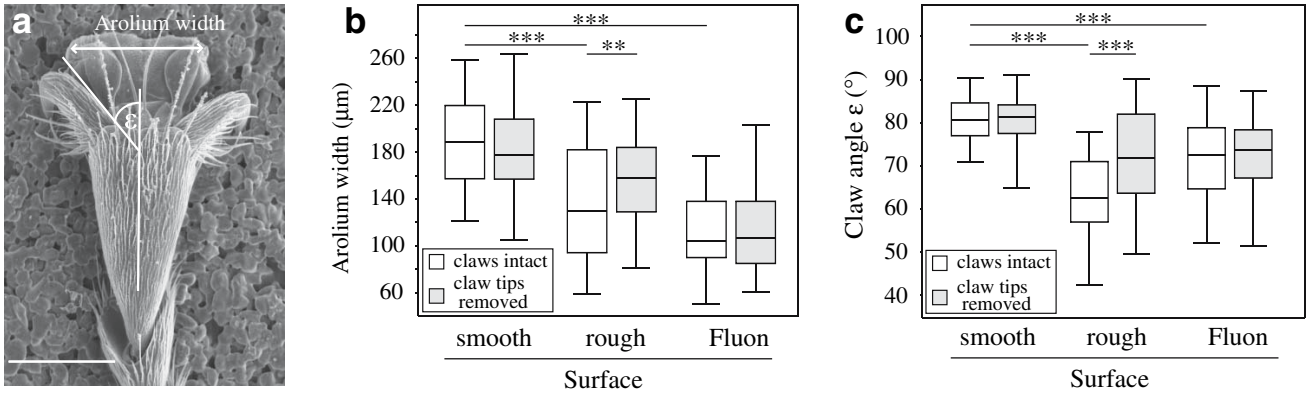

Fig. 5 **a** Dorsal view of *O. smaragdina* tarsus clinging to a rough sandpaper substrate (Scale bar: 200 µm). Arolium width was quantified as the lateral width between the arcus arms. Claw flexion was quantified by the angle (ε) between claws and tarsus (see Fig. 2a). **b** and **c** Arolium width and claw angle of ants with clipped claw tips or unmanipulated tarsi anaesthetised on different substrates. *Boxes* denote medians and upper/lower quartiles, whiskers mark the 5 and 95% percentiles; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

climbing is challenging in several respects. First, legs have to generate sufficient adhesive and frictional forces to keep stable contact. Second, these contacts have to be released effortlessly and rapidly during running. Third, when different attachment structures are present for substrates of different surface roughness, their use must be adjusted depending on substrate texture.

We have previously shown that ants and bees (Hymenoptera) control adhesive forces not only actively by the claw flexor muscle but also passively (Federle et al. 2001; Federle and Endlein 2004). Passive control of adhesive contact area is accomplished by a well-tuned mechanical system, which reacts to shear forces and unfolds the arolium when the foot is pulled toward the body. When ants walking upside down on a smooth substrate were loaded with additional weights, their adhesive contact area increased by a combination of active and passive control (Federle and Endlein 2004).

In this study we demonstrate that a similar passive mechanism controls the use of tarsal attachment structures depending on surface roughness. This control is inherent in the design of the claw flexor apparatus. Even though the movements of the tarsus, the claws and the arolium are all controlled by the same muscle, the use of the arolium can be regulated independently depending on surface roughness. The arolium of Weaver ants unfolded significantly less on a rough sandpaper substrate where the claws could interlock. When we prevented the claws from interlocking with the rough substrate by cutting their tips, arolia were significantly more unfolded. The experimental tendon pulls in severed legs show that the reduced arolium unfolding caused by interlocking claws is a consequence of the mechanical arrangement of the claw flexor system. When the claw flexor contracts, the arolium unfolds after the depression of the tarsus and the flexion of the claws. As a consequence, the claws typically attach before the adhesive pad and the arolium movement occurs while the claws are in contact with the substrate. On a rough surface, the movement of the arolium can therefore be suppressed, because the claw tips interlock and inhibit a further contraction of the claw flexor. Only if the claws find no grip (i.e. on a smooth surface), the flexor will contract further and the arolium will unfold. This is also the condition in which adhesive pads are needed, because they can achieve stable contact on smooth substrates where the claws fail to interlock. The separation of claw and adhesive pad movement provides the possibility of employing the pad only if required. This may protect the soft and vulnerable arolium cuticle against abrasion on rough surfaces (Slifer 1950). Our findings show that the interlocking of the claws not only suppresses the contraction of the claw flexor muscle but it also inhibits the passive arolium unfolding when legs slide toward the body. Arolia were less unfolded on the slippery Fluon substrates, where claws slide as well but the passive arolium extension mechanism does not operate because of insufficient shear forces (Federle et al. 2001). We conclude that claw interlocking reduces arolium unfolding not only via the active but also via the passive mechanism of arolium extension.

We cannot completely exclude that neuronal control contributes to the reduced arolium unfolding on rough surfaces. Tarsi of arthropods possess a variety of very sensitive mechanoreceptors (Gnatzy and Hustert 1989; Albert et al. 2001), and mechanoreception of surface topography has been reported from bee antennae (Kevan and Lane 1985) or from the butterfly proboscis (Goyret and Raguso 2006). However, it is unclear whether and how tarsal mechanosensors in ground contact are capable of detecting surface roughness (Seidl and Wehner 2006). Even if they are, an active reduction of arolium extension would only be possible through a weaker claw flexor contraction or a weaker pull of the legs toward the body.

Our observations show that the claws' flexion did not end prematurely but was indeed stopped by interlocking with the rough substrate. On the other hand, it is likely that stretch receptors provide neuronal feedback to adjust a certain maximum force acting on the tendon and that campaniform sensilla at several positions on the leg sense the force acting on claws and arolium to adjust the proximal pull of the whole leg (Larsen et al. 1997; Basibuyuk et al. 2000; Betz 2003). These neuronal control mechanisms, however, would be independent of surface topography and would not result in a surface-dependent reaction as observed in our study.

The delayed extension of the arolium probably results from the mechanical arrangement of joints in the (pre-) tarsus. Our observations shows that arolium extension is mainly triggered by the contact of the unguitractor plate with the anterior ventral margin of the last tarsal segment as we had hypothesised previously (Federle et al. 2001). The simple 2D model in Fig. 3c suggests that the effective lever arm for the arolium movement increases non-linearly, leading to a delayed and less gradual movement. Similar

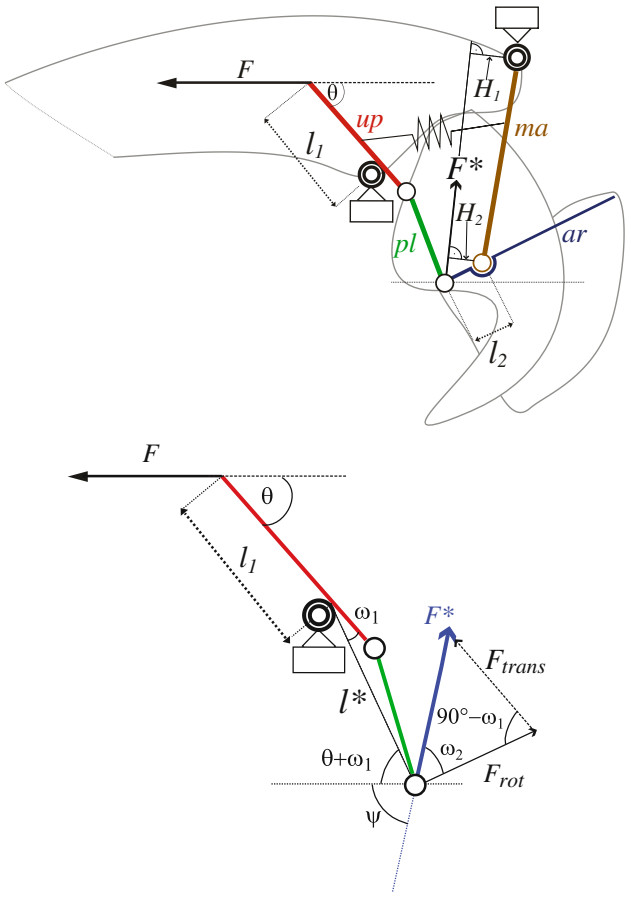

Fig. 6 Spring model of arolium unfolding, see Fig. 3c. The lengths and angles used for the calculation of *F** are shown in the *lower panel*. See Fig. 3 for explanation of abbreviations

triggering mechanisms have been described for various snapping and jumping movements in arthropods, where tendons are moved over a pivot point (fleas: Bennet-Clark and Lucey 1967; snapping shrimps: Ritzmann 1974; froghoppers: Gorb 2004).

Controlling tarsal attachment using a mechanical system instead of additional muscles offers a number of advantages. First, reducing the musculature controlling the tarsus conserves metabolic energy and enables a lighter weight construction, which minimises inertia and torsional moment of the legs. Second, a passive, mechanical reaction is always faster than an active, neuro-muscular one, because it does not have the temporal limitations of skeletal muscle contraction. Third, "delegating" simple control tasks to mechanical systems strongly simplifies the neuronal control needed for the coordination of muscles.

Locomotion control in insects is hierarchical (Delcomyn 1999) and combines centralised input from the brain with local, "distributed" sensing and control of leg movements in ganglia (Cruse et al. 1995). Passive, mechanical control in the body periphery adds an additional level to this hierarchy (Koditschek et al. 2004). It is increasingly recognised that mechanical, visco-elastic properties of the musculoskeletal system are essential for the dynamic stability of animal locomotion. Passive, mechanical reactions to disturbances have been termed "preflexes" (Brown and Loeb 2000) and are ubiquitous in animal locomotion (Dickinson et al. 2000). The passive control of arolium use represents a striking example of a mechanical system that is not only capable of stabilising locomotion against rapid perturbations (Kubow and Full 1999; Jindrich and Full 2002), but also to react to an environmental condition by adopting an "adaptive" state without any neuronal feedback. Many simple mechanical structures may be considered analogous in that they can react to environmental factors by switching between different biologically meaningful positions, such as many sensory cells or organs, body surfaces with anisotropic friction or structures passively controlling fluid flow around the body such as fins and feathers.

The use of good morphological design to facilitate control appears to be wide-spread in natural organisms. The implementation of this idea into robotics has only recently begun and is an area of active research (Matsushita et al. 2005; Paul 2006). The sophisticated mechanical systems involved in arthropod locomotion are particularly promising as inspiration for new robot designs.

Acknowledgments This study was financially supported by a postgraduate scholarship from the University of Wuerzburg to TE and by research grants of the Deutsche Forschungsgemeinschaft (SFB 567/C6 and Emmy-Noether Fellowship FE 547/1 to WF) as well as the UK Biotechnology and Biological Sciences Research Council. We thank two anonymous referees for helpful comments.

Appendix

We model the pretarsus as a system of limbs connected with joints containing torsion springs (Figs. 3c, 6). The torsion springs are located (1) at the unguifer, where the manubrium is hinged, (2) at the distal end of the manubrium, around which the arcus rotates; and (3) at the flexible connection between unguitractor plate and planta.

Direction of force relative to tarsus axis

The claw flexor muscle produces a force F along its tendon, which acts to move the unguitractor plate proximally into the last tarsal segment. This force can be divided into a translational and a rotational component:

$$F_{\text{trans}} = F\cos\theta \quad \text{and} \quad F_{\text{rot}} = F\sin\theta$$

We model the anterior ventral margin of the last tarsomere as a frictionless pivot so that the combination of F_{rot} and F_{trans} gives rise to a force F^* which deflects torsion spring (3) at the flexible connection between unguitractor plate and planta and pushes upward (dorsally) at the end of the planta, where the arcus is hinged. The lever arm l^* on the distal side of the pivot is l^* and its angle to the other side of the pivot is ω_1.

The ratio between the lever arms on both sides of the pivot is l_1/l^*. The magnitude of F^* can be obtained by adding $F_{\text{rot}} \cdot l_1/l^*$ and F_{trans} vectorially:

$$\begin{aligned}\overline{F^*} &= \sqrt{(F_{\text{rot}}\cdot l_1/l^*)^2 + F_{\text{trans}}^2 - 2(F_{\text{rot}}\cdot l_1/l^*)F_{\text{trans}}\cos(90^\circ-\omega_1)} \\ &= F\cdot\sqrt{(\sin\theta\cdot l_1/l^*)^2+\cos^2\theta-2(\sin\theta\cdot l_1/l^*)\cos\theta\sin\omega_1}\end{aligned}$$

To find the direction of $\overrightarrow{F^*}$ relative to the tarsus, the angle β_2 is calculated from:

$$\begin{aligned}\cos\omega_2 &= \frac{F\sin\theta\cdot l_1/l^* - F\cos\theta\sin\omega_1}{\overline{F^*}} \\ &= \frac{\tan\theta\cdot l_1/l^* - \sin\omega_1}{\sqrt{(\tan\theta\cdot l_1/l^*)^2 + 1 - 2l_1/l^*\tan\theta\sin\omega_1}}\end{aligned}$$

and the angle γ from:

$$\psi = 90^\circ - \theta - \omega_1 + \omega_2$$

Effective lever arm

The force F^* elicits both a proximal rotation of the manubrium (joint 1) and an extension of the arolium (joint 2). We determined the lever arms H_1 and H_2 around both joints (i.e. the distance of vector $\overrightarrow{F^*}$ to the unguifer and the arcus base, respectively). The "effective lever arms" of the arolium and manubrium joints can be calculated as the torsional moment $F^* \cdot H_{\text{i}}$ divided by the tendon force F.

Insufficiency of the model

Our simple model does not correctly predict the rotation of the manubrium. During the claw flexor contraction, the predicted force vector $\overrightarrow{F^*}$ did not consistently point in the proximal direction but it was sometimes oriented distally (i.e. H_1 was negative), suggesting that the manubrium undergoes proximal and distal movements while the claw flexor is contracting. However, our observations show that the manubrium only moves proximally when the claw flexor contracts. We assume that this behaviour is due to a spring-like connection between the unguitractor plate and the manubrium (indicated by the dotted spring in Figs. 3c and 6). Morphologically, this spring represents the elastic cuticle connection of the unguitractor plate via the claws to the claws and the unguifer. This spring may also be responsible for the fact that at the beginning of the claw flexor contraction (when the unguitractor plate is still not in contact with the anterior ventral margin of the fifth tarsomere), the plate rotates away from the direction of the tendon pull.

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