Extensive collection of femtoliter pad secretion droplets in beetle *Leptinotarsa decemlineata* allows nanoliter microrheology

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Abstract

Pads of beetles are covered with long, deformable setae, each ending in a micrometric terminal plate coated with secretory fluid. It was recently shown that the layer of the pad secretion covering the terminal plates is responsible for the generation of strong attractive forces. However, less is known about the fluid itself because it is produced in extremely small quantity. We here present a first experimental investigation of the rheological properties of the pad secretion in the Colorado potato beetle *Leptinotarsa decemlineata*. Because the secretion is produced in an extremely small amount at the level of the terminal plate, we first develop a procedure based on capillary effects to collect the secretion. We then manage to incorporate micrometric beads, initially in the form of a dry powder, and record their thermal motion to determine the mechanical properties of the surrounding medium. We achieve such a quantitative measurement within the collected volume, much smaller than the 1 µl sample volume usually required for this technique. Surprisingly, the beetle secretion was found to behave as a purely viscous liquid, of high viscosity. This suggests that no specific complex fluid behaviour is needed during beetle locomotion. We build a scenario for the contact formation between the spatula at the setal tip and a substrate, during the insect walk. We show that the attachment dynamics of the insect pad computed from the high measured viscosity is in good agreement with observed insect pace. We finally discuss the consequences of the secretion viscosity on the insect adhesion.

1 Introduction

Among few other animal groups, insects possess a fascinating ability to walk on smooth vertical surfaces and even on ceilings. Such ability is robust, fault tolerant and resistant to contamination. Insects can stick well to both hydrophobic and hydrophilic surfaces and detach in a very fast manner. The comparative data show that the evolution of attachment mechanisms in insects has developed along two distinctly different mechanisms: smooth flexible pads and hairy (setose, fibrillar) surfaces [1] [2] [3]. In cockroaches, grasshoppers and bugs, pads are soft deformable structures with relatively smooth surface, whereas in flies, beetles and earwigs, they are covered with long deformable setae. Because of the flexibility of the material in both mechanisms – soft attachment pads or fine surface microstructures – the possible contact area with the wide range of substrate profiles is maximized despite the surface roughness of the substrates, and high proximity between contacting surfaces can ensure strong attachment forces between pad and substrata.

Hairy attachment systems are typical for evolutionary younger and successful insect groups, such as flies and beetles and have a huge diversity of forms. There are several geometrical effects, such as multiple contact formation, high aspect ratio of single contact structures, peeling prevention using spatula-like tips of single contact elements that are responsible for the generation of a
strong pull-off force. These effects found in attachment devices of insects are an important source of information for further development of biomimetic patterned adhesives. One may speculate about the different physical mechanisms that are able to generate sufficient adhesion despite variation in the physico-chemical properties of the surface (hydrophobic, hydrophilic), surface profile (rough, smooth) and environmental condition (dry, wet). The theoretical background pertaining to these physical effects has been intensively theoretically discussed in several recent publications [4, 5, 6, 7, 8].

Even though hairy attachment systems have been under investigation for more than 300 years [9], the attachment mechanism of animals walking on smooth walls or ceiling is still under debate. Different hypotheses for attachment, such as sticky fluid, microsuckers or the action of electrostatic forces, have been proposed [10, 11, 12]. It is now well known that hairy attachment pads of reduviid bugs [13], flies [14, 15] and beetles [16, 17, 18] secrete fluid into the contact area, whereas others, such as spiders or geckos, do not. Based on experimental data, adhesion has been recently attributed to molecular interactions and capillary attractive forces mediated by secretion (reduviid bugs, flies, beetles) [19] or Van der Waals interactions (spiders, geckos) [20, 21].

2 Motivation

In hairy wet attachment systems, the role of the secretion still remains unclear. It has already been shown that the presence of the fluid is required for generating adhesion in insect adhesive pads. For example, as shown in 1970, attachment was impaired when hairy pads of the bug *Rhodnius prolixus* were treated with organic solvents [13]. In 1980, experiments with beetles on various substrates have also strongly suggested that cohesive forces, surface tension and molecular adhesion, mediated by pad secretion, may be involved in the mechanism of attachment [19]. It was inferred from experiments [22, 1] that the secretion induces a viscous resistance to detachment of pad from substrate and a capillary attraction which applies both before and during detachment (static an dynamic process). Later, on the scale of individual seta terminal plates, experiments conducted on fly *Calliphora vicina* using multiple local force-volume AFM measurements have demonstrated that the adhesion between the AFM tip and the seta (used to estimate the local seta adhesive properties) is about two times stronger in the center of the terminal plate, where thickness of the fluid is higher, than on its border [23]: adhesion was shown to strongly decrease as the volume of the secretion decreases, indicating that a layer of pad secretion, covering the terminal plates, is crucial for generation of the strong attractive force.

However, by measuring attachment forces on a smooth glass using a centrifuge technique, recent experiments demonstrate two apparently contradictory results [24]: (i) both friction and adhesion of insects pads on smooth surface are greater when less secretion is present, and (ii) the adhesive force on rough substrates decreases when the pad secretion is depleted through multiple consecutive pull-off motions. From a physiological point of view, these results seem to suggest that the most important function of the secretion is to provide attachment on rough substrates. Indeed, most of the substrates encountered by insects are not smooth but rough. From a physical point of view, these results closely reproduce two complementary requirements well known for adhesive substances, whether natural or manufactured: they must (a) establish a good contact with the substrate even in the presence of surface roughness and (b) resist separation, in other words dissipate a significant amount of energy during separation [25].

Usual pressure-sensitive adhesive materials such as those included in adhesive tapes or stickers, which are solids, manage to establish a good contact (point (a)) as a result of their high deformability, as recognized by Dahlquist more than 40 years ago [26]. *A fortiori*, a liquid secretion is suitable for establishing an intimate contact with a rough substrate and helps maximizing the contact area.

Concerning the strong resistance during separation (point (b)), it implies that the secretion be a very dissipative material. In other words, the loss modulus $G''$ in the relevant frequency range corresponds to a significant amount of dissipation. This property may hinder the ability of the secretion to establish a good initial contact within a timescale compatible with the insect walk.
For this reason, it is crucial to determine the rheology of the secretory material. That issue is the central goal of the present work.

3 About known relevant secretion properties

Secretion is known to contain a non-volatile, lipid-like substance in diverse insects, but in some other groups, such as flies and ants, it is a two-phasic microemulsion presumably containing water-soluble and lipid-soluble fractions \([1, 27, 28]\). Concerning its mechanical properties, up to now, measurements were interpreted assuming that secretion was purely viscous. The secretion dissipation has been shown to be large, although a quantitative evaluation of its rheological properties could not be conducted because the exact geometry of droplets, capillary bridges, and contact areas were unknown \([22, 23]\). Observing the dewetting velocity of ants’ secretion droplets through Interference Reflection Microscopy were interpreted in terms of viscosity and provided an estimate of 40 to 150 mPa.s \([27]\). In this experiment, because the dewetting velocity is constant, the mechanical properties of the material are probed at a fixed frequency, and no inference can be made about the response at other frequencies and hence about the possibly visco-elastic properties.

4 Our mechanical measurements

In the present paper, we quantitatively evaluate the rheological properties of the attachment pad secretion in the beetle \(L. \text{decemlineata}\) for the first time and we relate them to the attachment dynamics of the insect.

The essential difficulty of such a rheological measurement resides in the extremely tiny amount of secreted fluid: it totally excludes the use of standard rheology techniques which require at least a few milliliters of material. Microrheology, by contrast, typically requires less than 1 microliter of sample and is thus suitable for performing rheological measurements in situations where the available volume of material is a limiting factor. This issue is particularly vivid with the insect secretion: the typical volume of a secretory droplet of the Colorado potato beetle \(L. \text{decemlineata}\), collected on a glass slide and shown in Fig. 1 is 1 µm\(^3\), which corresponds to \(10^{-9}\) µl. At first sight, in order to obtain the required volume of 1 µl, it would be necessary to collect a discouragingly large number of droplets: typically \(10^9\) droplets! In the present work, as we shall see, we achieve a reliable microrheological measurement with a much smaller sample volume, namely \(10^{-4}\) µl (0.1 nl).

The microrheology technique involves using micrometric beads to measure the relation between stress (probe force) and deformation (probe position) in materials, at the microscopic scale. The driving force applied on the probes is thermal, with an energy scale corresponding to \(kT\). Measurements of the particles’ mean-squared displacement (MSD) give access to the possibly visco-elastic properties of the fluid material. Because the driving force is small, only the linear viscoelastic response of the material is probed. From the stress-deformation relation, the visco-elastic properties of the surrounding medium can be derived \([29, 30]\).

In the following, we first propose a procedure to collect the secretion, in a sufficiently large amount, by using capillary effects, taking place in a home-made microneedle. We then describe how micrometric probes were immersed in the collected volume, and their Brownian motion recorded. We then validate our measurements performed in the secretion (statistical accuracy and geometry) with additional tests in a calibrated Newtonian oil. We finally suggest a scenario for the contact formation between a single seta and the substrate in such a fluid. Using this scenario, together with the rheological data, we then estimate the attachment dynamics of the setae, and therefore of the insect pads.
5 Collecting the secretion using capillarity

A home-made microneedle, with a few microns tip, was used to draw up the secretion droplets (1 to 10 µm in diameter) spread on a glass slide (Figure 1). The procedure used to collect the secretion from insects is described in the Appendix. The microneedles were pulled from thin-wall borosilicate glass capillaries with 1 mm of the outer diameter and 0.78 mm of inner diameter (Harvard Apparatus, France) with a Narishige PB-7 double-stage puller (Narishige Instruments, Tokyo, Japan). By adjusting the puller settings, microneedles with tip of a few microns were produced. The microneedle was then mounted on a three axes piezo micro-manipulator Burleigh, and attached to an inverted microscope (Leica DMI3000).

Due to capillary effects, the rise of the wetting secretion takes place spontaneously in the tube as the tip is maintained in contact with the support and the droplet. The microneedle tip was moved onto the slide surface in order to collect the largest possible amount of secretions. Due to its high flexibility, it could be maintained in contact with the slide without breaking, thus optimizing the collection of the secretion. The microneedle tip was connected to a syringe, allowing us to apply a positive or negative pressure to the microneedle. Spontaneous suction – due to surface tension – could possibly be assisted by applying slight negative pressure to the microneedle. After an entire day (~ 8 hours) collecting the secretions, the final volume of fluid represented a drop which was about 100 µm in diameter, and 30 µm in height (Figure 2).

6 Brownian motion measurements

Dry powder of melamine beads (Acil, France) was deposited on a clean glass slide. The collected secretion volume was then ejected on the dry beads, which were 0.740 ± 0.005 µm in diameter, by applying a positive pressure to the microneedle (Figure 2). During the ejection, the microneedle was slightly pulled away, in order to avoid secretion to rise along the outer side of the microneedle tube. After ejection, the microneedle tip was moved along the glass slide to unstick beads from the surface. The secretory fluid was then drawn up again and ejected several times in order to mix beads with secretion. The final mixture was then ejected in a square chamber (10 × 10 mm²) made of a microscope plate and a cover-slip separated by a thin adhesive spacer (100 µm thickness). The container was sealed to avoid contamination or possible evaporation of the secretion drop (Figure 1).

The fluctuating motion of the tracer beads, immersed in the secretory fluid, was recorded with a fast CCD camera (Kodak motion) mounted on an inverted Leica microscope, with an oil immersion objective (63 X). The fast camera was typically sampling at 125 Hz, during 8 s. For reliable analysis of the Brownian motion, particular attention was brought to record the motion far from the rigid wall imposed by the glass slide and the drop edges. A home-made image analysis software allowed us to track the beads positions x(t) and y(t) close to the focus plane of the objective (see Appendix for more details). For each bead, the time-averaged mean-squared displacement \( \langle \Delta r^2(t) \rangle_{t'} \) = \( \langle [x(t' + t) - x(t')]^2 + [y(t' + t) - y(t')]^2 \rangle_{t'} = 2 \langle \Delta x^2(t) \rangle_{t'} \) is calculated, improving the statistical accuracy. To preserve a reliable statistics, the data of the mean-squared displacement were kept in the range below \( t < 2 \) s \([34]\). The quantity \( \langle \Delta r^2(t) \rangle_{t'} \) was then averaged over several beads (about 20), and identified as the ensemble-averaged mean-squared displacement. The resolution on the bead position, determined with a sub-pixel accuracy in the image analysis detection, was about 0.3 pixel corresponding to \( \delta = 30 \) nm. This resolution determines the lowest accessible MSD in these experiments, and corresponds to about \( 2.10^{-3} \mu m^2 \).

Figure 3 shows the ensemble-averaged mean-squared displacement \( \langle \Delta r^2(t) \rangle_E \) (MSD) of the tracer beads, as a function of the lag time \( t \). As can be seen, a diffusive behaviour of the tracers, characterized by a linear dependency of the MSD with time, is measured. This linear dependency implies that the beetles’ pad secretion simply behave in a purely viscous manner in the investigated time window. The secretory fluid viscosity corresponds to \( 110 \pm 5 \) mPa.s at the room temperature \( T = 21 \pm 1^\circ C \). This value corresponds to about one hundred times the water viscosity.

In order to test the reliability of the Brownian motion measurements in the 'drop' geometry
used for secretion, microrheological tests were also performed in a calibrated Newtonian fluid (100 BW, ZMK-ANALYTIC-GmbH), in two different geometries. The first one – bulk geometry – corresponds to the usual geometry used in microrheology (10mm × 10mm × 100µm), while the second one – drop geometry – corresponds to a drop, approximately 100µm in diameter and 30µm in height, spread on a glass slide (Figure 4). In both experiments, the Brownian motion of the tracers was recorded, far from the glass slide walls and the drop edges. Also, in both experiments, the MSD was averaged on about the same beads number, of the order of 20. The experimental results were found to be in good agreement in both geometries, in the time range investigated 7.10⁻² < t < 2.0 s (Figure 3). In both cases, the MSD increases linearly with time, indicating a purely viscous behaviour, characterized by a viscosity of the order of 100 mPa.s, in excellent agreement with standard rheological measurements in the calibrated oil. The ‘drop geometry’, as well as the statistical accuracy, were then confirmed to be reliable in our microrheological measurements.

7 Insect pad attachment dynamics

Let us now focus on the modelling of the attachment dynamics of the insect in the presence of secretion. The spatula at the setal tip is considered to be a portion of a hemisphere, with a radius of curvature $R$ and a diameter $2A$, as schemed in Fig. 4-I. Here, we assume that a thin film of secretion (thickness $H$) initially wets the spatula. The duration of the attachment process of the spatula onto the substrate, and hence of the insect pads, can then be estimated from the above rheological measurements.

As a spatula approaches the substrate, the contact region of radius $a$ rapidly expands laterally. We shall now estimate the capillary force between the spatula at the setal tip and a smooth substrate in the case of partial contact ($a < A$). Immediately outside the contact region of radius $a$, the surface of the spatula is expected to depart from the substrate with a weak slope $\theta$, with $\theta \approx a/R$ (Figure 4-II). Considering that there is very little secretion at the tip, the meniscus width $\Delta$ is much smaller than $a$. The meniscus height (which is also twice its radius of curvature $r$) is then typically equal to $h \approx \theta \Delta$. Integrating the capillary (Laplace) pressure $-\gamma/r = -2\gamma/h$ over the contact surface area between the meniscus and the spatula, which is approximately $2\pi a \Delta$, provides the capillary force $F_{\text{capil}}^a < A \approx 4\pi R \gamma$. Interestingly, this capillary force turns out to be independent of both the radius $a$ of the contact and of the amount of liquid in the meniscus. Note that depending on the spatula curvature and rigidity, the secretion surface tension and the force applied by the insect, the contact – of final radius $a_{\text{fin}}$ – remains either partial ($a_{\text{fin}} < A$) or total ($a_{\text{fin}} = A$). In both cases, the dynamics of the attachment/detachment process remains identical.

Let us then focus on the attachment dynamics. The viscosity $\eta$ extracted from the above Brownian motion measurements now allows us to derive a prediction for the duration of the attachment/detachment process of a spatula on a substrate. As the spatula approaches the substrate, the contact radius widens at velocity $\dot{a}$. The intersection of the film of thickness $H$ deposited on the tip radius of curvature $R$ and the planar substrate is a disk (radius $a_{\text{in}} \approx \sqrt{HR}$) which approximately represents the initial contact area. The capillary force thus pulls the surfaces together at velocity $v_z = a\dot{\theta}$, yielding a power $P_{\text{capil}} = F_{\text{capil}} v_z$, and causes the fluid in the meniscus to move outwards with an average velocity $\dot{a} > 0$, as schemed in Figure 4-III. The zero-velocity condition at the substrate and the spatula surfaces implies a dissipation of order $\eta (a/\theta x)^2$ per unit volume, where the local gap reads $h(x) = \theta x$, with $x$ the horizontal position along the meniscus width. The total power dissipated in the meniscus is thus approximately $P_{\text{viscous}} \approx \int \eta (a/\theta x)^2 2\pi a \theta x \, dx \approx \eta \dot{a}^2 a/\theta$. Note that the above calculation of the dissipation in the meniscus overlooks the detailed shape of the spatula as deformed dynamically by the meniscus (to the best of our knowledge, such a calculation is not currently available). It is likely that the spatula surface does not locally make a sharp (although small) angle with the substrate, and that the true dissipated power is in fact larger than the above estimate.

Equating $P_{\text{viscous}}$ and $P_{\text{capil}}$, we find that the contact widens at velocity $\dot{a} \approx a^2/Re\eta$. The corresponding time needed to establish the equilibrium contact reads $T = \int_{a_{\text{in}}}^{a_{\text{fin}}} (1/\dot{a}) \, da = \eta R/\gamma \log(a_{\text{fin}}/a_{\text{in}})$. 

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Considering that the quantity \( \log(a_{\text{fin}}/a_{\text{in}}) \) remains of order unity \((a_{\text{fin}}\) not so different from \(a_{\text{in}}\)), the duration of the attachment process can be estimated to scale as \( T \approx \eta R/\gamma \). Using our rheological measurements \( \eta = 10^{-1} \) Pa.s, and assuming \( \gamma \approx 10^{-2} \) N.m\(^{-1}\) and \( R \approx 1 \) \(\mu\)m, we obtain \( T \approx 10 \) ms.

This represents the duration of spontaneous contact formation, without any active effort from the insect: \( F_{\text{attach}}^{\text{insect}} = 0 = F_{\text{capil}} - F_{\text{attach}}^{\text{viscous}} \), where \( F_{\text{viscous}} \) is the viscous resistance related to the dissipated power through \( P_{\text{viscous}} = v_z F_{\text{viscous}} \). To achieve detachment, the insect must exert a tensile force greater than the capillary force: \( F_{\text{detach}}^{\text{insect}} = F_{\text{capil}} + F_{\text{detach}}^{\text{viscous}} > F_{\text{capil}} \). For detachment to occur at the same rate as attachment, which seems reasonable, it is required that \( F_{\text{detach}}^{\text{viscous}} \approx F_{\text{attach}}^{\text{viscous}} \), hence \( F_{\text{detach}}^{\text{viscous}} \approx F_{\text{capil}} \). As a result, during detachment, the insect must exert a tensile force typically equal to \( 2 F_{\text{capil}} \).

The present picture thus predicts, on the basis of the measured viscosity, a total duration \( T \) of the contact (attachment and detachment) and thus a pace rate, as well as a detachment force \( F_{\text{detach}}^{\text{insect}} \approx 2 F_{\text{capil}} \). It turns out that the estimate \( T \approx 10 \) ms obtained for the contact duration is compatible with the duration observed in videorecordings of walking beetles (personal observations).

Concerning detachment, moderate viscous dissipation is preferable for the insect, but perhaps not crucial: we speculate that beetles, like geckoes [20] and flies [31], adopt a leg motion suitable for easing detachment significantly. This may imply bending or rolling of the spatulae in such a way that the local curvature becomes stronger at the contact with the substrate.

### Discussion and conclusion

Our passive microrheology nanoliter procedure is innovative in two respects: (i) the material under investigation is initially available as an assembly of 1 femtoliter droplets deposited on a glass slide, (ii) the 0.1 nl collected volume used to perform the microrheological measurement was significantly less than the usual 1 \(\mu\)l.

These measurements show that the secretion behaves as a purely viscous fluid of high viscosity over the range of frequencies investigated. This the first experimental indication that beetle locomotion might not need a specifically visco-elastic behaviour. Let us emphasize, however, that due to the resolution (30nm) of our particle tracking setup, we could not explore frequencies above 15 Hz, which is slightly below the beetle pace rate (around 100 Hz). It is therefore not to be excluded that the secretion response should depart from a purely viscous behaviour at higher frequencies. In future studies, we intend to extend our measurements to the secretions of other animals over a wider range of frequencies.

Our model provides expression \( T \approx \eta R/\gamma \) for the duration of the pad/substrate contact formation. It implies that the secretion viscosity \( \eta \) sets an upper bound of the order of \( 1/T \) for the insect’s pace rate. This formula is only an estimate since (i) the exact spatula geometry and dimensions are not precisely known and vary within one single animal [32], (ii) numerical prefactors were omitted. Nevertheless, using this expression with the measured viscosity provides a timescale for attachment which is compatible with live observations. The high secretion viscosity should therefore not prevent the insect from forming good contacts.

One might wonder, however, why the secretion viscosity is so high (100 times the water viscosity). A lower viscosity would ease the insect locomotion and speed up its pace. It is not unreasonable to imagine that high viscosity, and correspondingly high molecular weight, ensures slow evaporation — a crucial issue at such small length scales. Additionally, a high viscosity ensures that the adhesion is robust under unexpected and fast varying conditions.
Appendix

Insects and preparation of the footprints

Adult beetles *L. decemlineata* Say (Chrysomelidae) were collected on various species of annual plants from the family Solanaceae in the Botanical Garden of Dresden University of Technology, Germany. The tarsi were cut off the body using small scissors and used to prepare footprints on the substrate by pressing ventral side of tarsi against clean glass slide. A pressure was applied on the foot in order to have a strong contact with the glass slide and to make the secretion go out from the pore channels. At the same time, the leg is rubbed against the slide, which results in a strong shear that is able to deposit the secretion on the surface. It produces a collection of small droplets of various sizes, from a few microns to ten microns for the largest ones (Figure 1). In all cases, the droplets were spread, their height never exceeding a few microns. Interestingly, evaporation of the secretions was not observed within the experiment time.

Particle tracking algorithm

Two dimensional analysis of the particle motion was performed using a home-made algorithm, implemented as an ImageJ plugin [33]. A classical cross-correlation method was used to determine the beads displacements. In order to achieve a sub-pixel spatial resolution, the computed correlation was interpolated using a paraboloid approximation. With this algorithm, several beads could be tracked at the same time.

Mean-squared displacement analysis

Passive, or thermally driven, microrheology is based upon the Generalized Einstein Relation (GER), valid in a visco-elastic stationary medium in thermal equilibrium at temperature $T$. It relates the frequency-dependent mean-squared displacement of the diffusing particle to its frequency-dependent mobility, according to the relation: $s^2 \langle \Delta \hat{x}^2 (s) \rangle = 2kT \hat{\mu}(s)$, where $k$ is the Boltzmann constant, and $s$ the frequency in the Laplace domain [29, 30, 34]. As can be seen, measuring the particles mean-squared displacement at equilibrium leads to the indirect measurement of the particle mobility. The bulk frequency-dependent viscosity of the fluid can then be deduced assuming that the Stokes relation $\hat{\mu}(s) = 1/6\pi R \hat{\eta}(s)$ remains valid in the visco-elastic material. In the particular case of a purely viscous fluid, the mean-squared displacement increases linearly with time and the GER writes $\langle \Delta r^2(t) \rangle = 4Dt$, where $D$ is the diffusion coefficient. The viscosity of the material is directly deduced from the diffusion coefficient according to the Stokes-Einstein relation $D = \frac{kT}{6\pi R \eta_0}$, where $R$ is the bead radius and $T$ the bath temperature.

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Figure 1: Secretion droplets from adults beetles *L. decemlineata* on a glass slide. The beetles’ legs are rubbed against the slide, which results in a strong shear that is able to deposit the secretion on the surface. The typical size of the droplets is a few microns. A home-made microneedle, mounted on a three-axes micromanipulator, was used to draw up the secretion droplets spread on the glass slide. The tip was moved onto the slide surface to collect the largest amount of secretion. Due to capillary effects, the rise of the liquid takes place spontaneously inside the capillary tube.

Figure 2: The collected volume is ejected on dry 0.74 µm in diameter Melamine beads (Top) before the ejection (the dry beads have been deposited on a glass slide) and (Bottom) during the ejection. The final volume of collected secretion is a drop of 100 µm in diameter, and 30 µm in height.
Figure 3: Mean-squared displacement of 0.74 \( \mu \)m Melamine beads immersed in: (*) the beetle secretion, (o) the calibrated 100 BW Newtonian fluid in the bulk geometry, and (x) 100 BW in the drop geometry, as a function of time. In all cases, the fluctuating motion of the tracers is purely diffusive, characterized by a linear dependency of the MSD with time. The secretion was found to behave as a purely viscous fluid on the time scales investigated, of viscosity about one hundred time the water viscosity.

Figure 4: Scheme of 'bulk' and 'drop' geometries used to perform microrheological measurements. The bulk geometry (1cm \( \times \) 1cm \( \times \) 100 \( \mu \)m) is the usual geometry to perform microrheological measurements. The 'drop' geometry was used with the secretion in the present work, due to the extremely small amount of fluid available. The drop size was 100 \( \mu \)m in diameter and 30 \( \mu \)m in height. The 'drop' geometry was shown to provide the same viscosity results as the usual bulk geometry, which shows that it is reliable.
Figure 5: Geometry of the setal tip. The seta consists of a tip of diameter $2A$ and curvature $1/R$ located at the end of a long fiber (I). The tip of the seta is covered by a thin film of viscous secretion. When the seta contacts substrate, the contact region expands laterally. (II). The meniscus (outer diameter $2(a + \Delta)$) still represents a small portion of the tip surface ($a \ll A$). (III). As the contact broadens ($\dot{a} > 0$), the tip surface approaches the substrate at velocity $\dot{a} \theta$. The viscous dissipation in the meniscus then determines the velocity $\dot{a} > 0$. 