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How aphids lose their marbles

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Insects provide examples of many cunning stratagems to cope with the challenges of living in a world dominated by surface forces. Despite being the current masters of the land environment, they are at constant risk of being entrapped in liquids, which they prevent by having waxy and hairy surfaces. The problem is particularly acute in an enclosed space, such as a plant gall. Using secreted wax to efficiently parcel and transport their own excrement, aphids were able to solve this problem 200 Myr ago. Here, we report on the physical and physiological significance of this ingenious solution. The secreted powdery wax has three distinct roles: (i) it is hydrophobic, (ii) it creates a microscopically rough inner gall surface made of weakly compacted wax needles making the gall ultra-hydrophobic, and (iii) it coats the honeydew droplets converting them into liquid marbles, that can be rapidly and efficiently moved.

Keywords: aphid hygiene; non-wetting droplets; rolling drops

1. INTRODUCTION

Hygiene is of the utmost importance to the survival of aphids living in galls. The large volume of sugary, yet nitrogen-deficient liquid that they suck from the phloem to meet their nutritional requirements must, of course, subsequently be excreted. This excrement, honeydew, poses a double threat: (i) entrapment and eventual drowning is a virtual certainty once the liquid has wetted the insect, since surface forces dominate bulk forces on the small-scale preventing escape (Denny 1993), (ii) pathogens, such as fungi, thrive on the carbohydrate-rich liquid and substrate that is left behind when the water component evaporates (Fokkema *et al.* 1983). Free-living aphids can avoid these problems by administering honeydew to tending ants, by flicking globules of honeydew with their caudae, or by moving to new feeding sites (Broadbent 1951). Such solutions are unavailable to galling aphids, which may spend their entire lives inside the confined space of a plant gall (Benton & Foster 1992). Indeed, speculation that all aphids might originally have been gall dwellers (Eastop 1998) lends credence to the notion that ever since aphids existed, there have been liquid marbles. The necessity to reduce gall contamination would certainly act as a strong selective pressure on the evolution of wax glands, and it is probable that aphids engineered their technique of utilizing their secreted wax to parcel and transport liquid at least 200 Myr ago (Heie 1987*a*). The advantages of this technique, in which 'liquid marbles' are manufactured, are only now being investigated and appreciated by man (Aussillous & Quéré 2001; Mahadevan 2001).

The waxy tufts produced by insects were once thought to be merely a waste product consequent to the phloem-feeding habit (Pollister 1938). However, it is now clear that the wax is intentionally synthesized and that its chemical composition is unrelated to that of the host plant (Brown 1975; Jackson & Blomquist 1976). The wax of galling aphids, which is an, as yet, undescribed mixture of long-chained esters, alcohols, aldehydes and fatty acids (Brown 1975), is produced by specialized epidermal cells, which are particularly numerous on the abdominal tergites (Smith 1999). Each of these epidermal cells produces minute wax needles (diameter 0.05–0.15 μm) that fuse to form a hollow skein with a diameter of 10–20 μm (Pope 1983; Smith 1999). The skeins tangle to become tufts of wax that break off and disintegrate into a fine powder that coats the inner surface of the gall (Dunn 1959).

Honeydew makes contact with the wax the moment that it leaves an aphid's body. In *Eriosoma lanigerum*, and other species closely related to our study animal, *Pemphigus spyrothecae*, droplets of honeydew are coated as they move past the wax strands surrounding the anus (Smith 1999). Even after a droplet has fallen from the aphid, it continues to be coated with the powdered wax inside the gall and remains spheroidal and non-wetting (Dunn 1959; Benton & Foster 1992).

The social behaviour of galling aphid species makes them the object of special attention because this behaviour is very rarely found outside the Hymenoptera and Isoptera. Although the group first received attention because of its possession of soldiers, or morphologically and behaviourally specialized defensive morphs (Aoki 1977), it is now clear that soldiers also perform altruistic cleaning (Aoki 1980; Aoki & Kurosu 1989; Kurosu & Aoki 1991; Benton & Foster 1992). Even large liquid marbles can easily support the weight of aphids (Smith 1999) and, in *P. spyrothecae*, soldiers are responsible for manipulating the marbles out of the gall opening by kicking, pushing or walking on their surface in an effort to make them roll. In this way, *ca.* 10 mm^3 of honeydew is removed per day per gall at peak aphid populations, and if this removal is prevented by blocking the gall opening, 90% of the aphids inside are dead after 5 days (Benton & Foster 1992).

We investigated the physical properties of aphid wax and the function it serves in facilitating the manufacture of liquid marbles. We hoped that the study of the synthesis

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and manipulation by aphids of non-wetting droplets might improve the possibility for the use of such droplets in human applications. We therefore drew on our newly gained knowledge of the unique properties of these non-wetting droplets (Mahadevan & Pomeau 1999; Richard & Quéré 1999; Aussillous & Quéré 2001), to determine if these properties might enhance aphid hygiene.

2. MATERIAL AND METHODS

(a) *Study animal*

Pemphigus spyrothecae Passerini (Hemiptera: Aphididae) forms spiral galls on the petioles of the leaves of its host plant, the black poplar, *Populus nigra*. The galling phase lasts for approximately six months from early spring until early winter. Several hundred galls were collected from Lombardy poplar trees (*P. nigra* var. *italica*) at a single site in Cambridge, UK to provide sufficient material for study.

(b) *Size of liquid marbles*

The radii of 1000 randomly chosen liquid marbles were measured with the aid of a dissecting microscope. The anal circumference of 30 aphids, spanning all instars, was also measured to determine the selection criteria for the size of the liquid marbles.

(c) *Properties of aphid honeydew*

Galls were broken open and liquid marbles harvested to provide honeydew. This honeydew was first purified by manual removal of any contaminating aphids or debris, and then by forcing the marbles to coalesce by centrifuging them at 2000 rpm for 1 min to enable clean honeydew to be pipetted from beneath the wax layer which forms on top.

The density, ρ, of honeydew was deduced from the weight of liquid slugs of different lengths inside a capillary tube of known radius, using a microbalance. The surface tension was inferred from the height, h, to which honeydew rises in a capillary tube using Jurin's law

$$h = \frac{2\,\sigma}{\rho\, g\, R_t}. \tag{2.1}$$

Here, g is the acceleration due to gravity, R_t is the inner radius of the tube, and σ is the surface tension, which may be deduced by knowing all the other parameters.

The viscosity was determined by measuring the steady velocity, V, of a honeydew slug as it falls through a narrow vertical tube. In this case, both the driving force (weight) and the viscous friction are proportional to the length of liquid. If enough liquid is used to make surface tension effects negligible (Bico & Quéré 2001), Poiseuille's law gives the value of viscosity in terms of known parameters:

$$\eta = \frac{\rho\, g\, R_t^2}{8\ \ V}. \tag{2.2}$$

All the experiments were carried out with capillary tubes of different radii (680, 480 and 266 μm) to improve the accuracy of the measurements.

(d) *Properties of aphid wax*

A horizontally mounted dissecting microscope, attached to a digital video camera, was used to measure the contact angle of a 10 μl drop of distilled water on aphid wax in two morphologies: (i) flat wax that had been collected by hand from the aphid caudae, rinsed to remove sugars or other impurities, and then squashed to adhere to double-sided sticky tape on a microscope slide; and (ii) rough wax as it is found on the inner surface of a gall of *P. spyrothecae*. This process was repeated to ensure reproducibility.

(e) *Structure of wax layer inside gall*

The arrangement of wax needles lining the inner surface of the gall was determined by cutting fine fresh sections of galls and examining the profile of the inner surface under a light microscope. The tip diameter of the protruding needles and the average distance between them was measured. This information characterizes the roughness of, and the effective contact area available in, the inner gall.

3. RESULTS

While the trick of coating honeydew in powdered wax is widespread in aphids and has been known for over 100 years (Buckton 1876), its physical significance seems to have gone unnoticed. The wax, which is excreted in filamentous tufts by the aphids, breaks up into small needles that cover the entire inner gall. Chemically, the wax is composed of long-chain esters similar to candle wax and is strongly hydrophobic. The secreted powdery wax has three distinct roles in the packaging and removal of the honeydew: (i) its physico-chemical hydrophobicity prevents even a smooth coating from being wetted, (ii) its mechanical layout as a microscopically rough surface enhances the hydrophobicity still further, and (iii) for good measure the wax coats the honeydew as it is excreted, forming coated liquid marbles that do not wet the surface and do not coalesce easily. We now quantify these effects.

The contact angle θ between water and a smooth layer of aphid wax was measured as 115° (figure 1*a*), close to the contact angle between water and candle wax (110°). This high value is made even higher if contact occurs with a rough surface made of hydrophobic material (Shibuichi *et al.* 1996). This effect stems from the simple observation that for water to enter the microscopic depressions (*ca.* 10 μm for the inner gall surface) associated with the roughness would require much larger capillary pressures than associated with drops. Therefore, a water drop deposited on this kind of surface lies on the roughness, as the mythical Indian fakir on a bed of nails. Consequently, the actual liquid–solid contact is but a small fraction, φ, of the actual area of the flattened part of the drop. Hence, the observed contact angle should be close to 180°, because the liquid is primarily in contact with its own vapour almost everywhere. An analysis of such a configuration (Bico *et al.* 1999) leads to the following formula for the apparent contact angle θ^* on a rough surface:

$$\cos\theta^* = -1 + \varphi\,(1 + \cos\theta), \tag{3.1}$$

where φ is the areal fraction of liquid–solid contact and θ is the contact angle on a smooth substrate of the same material. If $\varphi \ll 1$ and the substrate is hydrophobic ($\theta > 90°$), the effect of roughness is to increase the contact angle. In the inner gall, the wax needles have a typical diameter of 12 μm with an average spacing of 55 μm. If one assumes that the area of contact between solid and liquid is limited to the area of the needle tips, $\varphi = 0.04$.

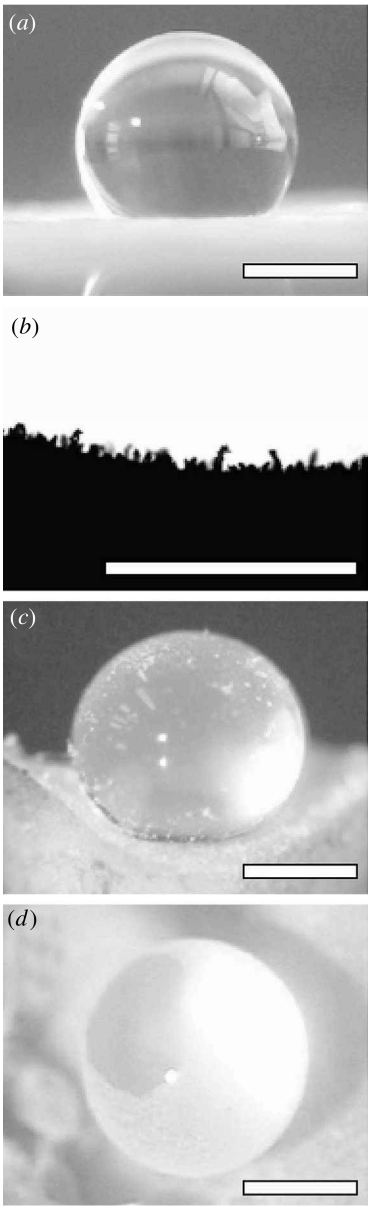

Figure 1. The physical properties of aphid wax. (*a*) The hydrophobic properties of the wax are already evident when it is presented as a smooth surface—this droplet of water has a contact angle of 115° (scale bar, 1 mm). (*b*) The microscopically rough architecture of the wax on the inner surface of a gall of *Pemphigus spyrothecae* decreases the effective contact area (scale bar, 500 μm) to just 4% of the actual area of the flattened interface. (*c*) On the gall surface, a drop of water the same size as that in (*a*) has a contact angle of *ca*. 160°. (*d*) A newly formed (man-made) liquid marble has an even coating of microscopic wax particles which adhere to its surface. Blowing on the marble shows that the wax particles are easily dispersed and do not form an impermeable coat (scale bar, 1 mm).

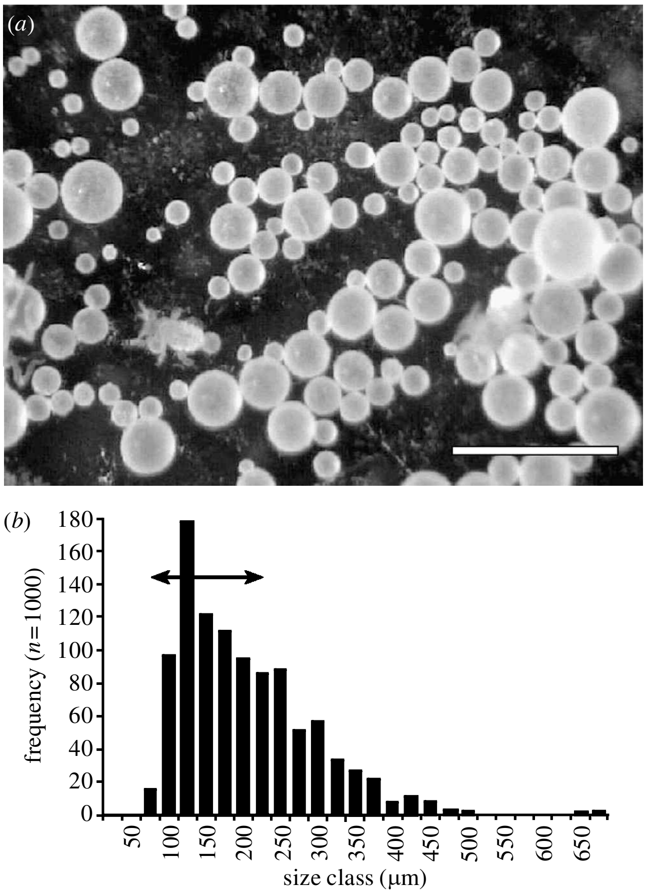

Figure 2. (*a*) The typical appearance and range of sizes of the wax-coated liquid marbles (scale bar, 1 mm), (*b*) frequency distribution of the various size classes of marbles, plotted as length of radius. The horizontal line with arrows shows the range of aphid anus perimeters.

Using the measured value of θ in equation (2.1) leads to $\theta^* = 167°$, close to the measured value of *ca*. 160° (figure 1*c*). Contact angle hysteresis has been observed to decrease dramatically for these very rough and hydrophobic surfaces (Johnson & Dettre 1964), as the contact lines are not well pinned. No measurable hysteresis was detected for the coating of the inner surface of the gall. This allows honeydew droplets to move easily around the gall; as it does so, loose wax needles progressively coat its entire surface. The encapsulation of the droplets makes them ultra-hydrophobic, and prevents them from coalescing easily.

We now consider the size distribution of the aphid liquid marbles. The capillary length arises naturally by balancing gravitational and capillary forces and sets the ruler for comparison. For honeydew, the capillary length is $\kappa^{-1} = \sqrt{\sigma/\rho g} \cong 2.3$ mm. On scales smaller than this, gravity is dominated by capillarity, as is the case for honeydew droplets whose radii are much smaller than the capillary length. More precisely, the Bond number $Bo = \rho g R^2/\sigma = 10^{-3}$ (density of honeydew, $\rho = 1$ g cm^{-3}; gravity, $g = 10$ m s^{-2}; marble radius, $R \cong 100$ μm; surface tension, $\sigma = 0.05$ N m^{-1}), so the drops resemble weakly flattened spheres (figure 2*a*). As shown in figure 2*b*, the distribution of the radii of the marbles has an average value of 100 μm, more than an order of magnitude smaller than κ^{-1}. The average radius of the aphid anus is $D \approx 20$ μm. Then a rough estimate of the drop radius R, given by the balance between gravitational and capillary forces at the anus, leads to $R \cong (\sigma D/\rho g)^{1/3} \approx 450$ μm which is slightly larger than the observed radii. However, since droplet expulsion is actively controlled by the opening and closing of the anus, the drop size will be typically smaller than 450 μm, consistent with observation. The width of the distribution arises from a multitude of causes that include the distribution of aphid anus sizes, the coalescence of incompletely coated marbles and losses due to evaporation (Kurosu & Aoki 1991; Smith 1999).

Finally, we turn to the dynamics of the marbles, which the aphids move around at typical velocities no larger than a body length per second, i.e. 0.5 mm s^{-1}. The typical Reynolds number ($Re = \rho VR/\eta$; velocity, $V \cong 10^{-3}$ m s^{-1}; viscosity of honeydew, $\eta \cong 3 \times 10^{-3}$ Pa s) is approximately 10^{-1}–10^{-2} so that the droplet motion is viscously dominated. In this regime, the ultra-hydrophobic marbles move primarily by rolling, with a small shearing zone that is limited to the vicinity of the region of contact. Furthermore, for small rolling velocities, the ratio of viscous forces to surface tension forces, given by the capillary number, $Ca = \eta V/\sigma = 10^{-5}$. Therefore, the shape of the droplets does not change much from that of a weakly flattened sphere. We are thus in the very situation predicted theoretically (Mahadevan & Pomeau 1999) and confirmed experimentally (Richard & Quéré 1999; Aussillous & Quéré 2001) of a viscous non-wetting spheroidal drop, rolling slowly on a solid, at a velocity V. The rate of energy dissipation $\dot{E}$ due to viscous friction inside such a droplet is (Mahadevan & Pomeau 1999):

$$\dot{E} \cong \eta V^2 R^4/\kappa^{-3}. \tag{3.2}$$

For a prescribed force F, the velocity of the droplet on a horizontal surface is therefore $V \cong F\kappa^{-3}/\eta R^4$. Since the soldier aphids work on the undulating inner surface of the gall, they have to fight gravity, which already provide a rationale for parcelling. But even on a featureless horizontal surface, parcelling a given volume of liquid into marbles of radii R increases their number n by a factor of R^{-3}. Since a smaller drop rolls faster than a larger one, with $V \propto R^{-4}$, the time needed to push all these marbles a given distance scales as $n/V \propto R$ thus, the total time taken is less than what it would be to move the entire volume at once. Thus, in addition to packaging and parcelling, the waxy coating proffers a dynamical advantage to the efficient transport of aphid honeydew.

4. DISCUSSION

Aphids have developed an efficient way of handling their excreted honeydew. To do so they use a very hydrophobic wax that coats every element inside the gall: the gall itself, the aphids and the liquid, leading to a virtually non-stick environment which is of vital importance in this enclosed space. Fossil evidence indicates that wax-gland plates were present in the hypothetical ancestor of the aphids (Heie 1987*b*), which indicates that aphids invented this solution to their surface tension problems very early in their evolutionary history. Although wax-gland plates have been lost in the more derived aphid groups, wax production is extremely common in aphids generally, and has clearly been evolved independently several times (Miyazaki 1987). The surface tension advantages conferred by wax are not just confined to those aphids that live in galls. Prehistoric aphids probably evolved wax-gland plates before they took to life in galls (Heie 1987*a*) and wax production is common in modern aphids that live in open situations.

Thus, the aphids' wax-coated honeydew droplets are the natural analogues of liquid marbles insofar as they are non-wetting and will roll freely on a smooth solid surface. As already emphasized, the waxy coating of the droplet and the gall surface serve to amplify the hydrophobic nature of the interfaces, using a combination of physico-chemical and mechanical effects. The unusual static and dynamic behaviour of such objects, which has only recently been elucidated, shows yet again that nature has not only already realized a clever design, but also seems to have almost perfected it. Perhaps we can poach from the micro-engineering solutions of aphids to improve our own attempts at efficiently manipulating small volumes of liquid on a solid surface?

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