BUTTERFLY PROBOSCIS AS A BIOMICROFLUIDIC SYSTEM

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Abstract

It looks amazing how butterflies and moths with their thin feeding trunk are being able to sip very thick liquids like nectar or animal extractions. Their sucking ability goes beyond that: one can observe butterflies and moths probing liquids from porous materials like fruit flesh or wet soils. This suggests that the suction pressure produced by these insects is sufficiently high. The estimates based on engineering hydraulic formulas show that the pressure can be greater than one atmosphere, i.e. it can be greater than that any vacuum pump could supply. In this experimental study, the principles of interfacial flows are used to carefully analyze the feeding mechanism of butterflies and moths. We document the feeding rates and proboscis behavior of Monarch butterflies (Danaus plexippus) in different situations: when butterfly feeds from droplets, from vials modeling floral cavities, and from porous materials modeling fruits, wet soils, or dung. Using high speed imaging and simple models, we propose a scenario of butterfly feeding which is based on capillary action. According to the proposed mechanism, the trunk of butterflies and moths works like a fountain pen where the air bubbles play a significant role in controlling fluid flow.

Introduction

Butterflies and moths have unique trunk called proboscis. Proboscis is coiled under the head at rest and it is extended when butterflies and moths probe different food. Proboscis morphology and functions are interconnected, hence its design is complex to offer different modes of operation. The main proboscis function is to probe and deliver liquids to the sucking pump where it can be further transported to the digestion canal (1-4). Butterflies and moths feed at sugary fluids like nectar and honey, some of them imbibe eye and nasal-fluids and blood (5, 6). The viscosity of these liquids can be sufficiently high (4, 7) therefore the transportation of liquids through the proboscis requires high pressure drop (6, 8). Numerous observations on butterfly puddling and visits of butterflies and moths to wet and damp substrates suggest that they have efficient pumping power to extract nutrients from porous materials (9-11). It was proposed that the needed suction pressure was created by a suction pump – a compartment inside animal's head equipped with specific muscles. When these muscles contract, they expand the compartment like piston does in syringe. This expansion reduces the pressure relative to atmospheric pressure, forcing liquid flow up the proboscis (1, 4, 5, 12).

Kingsolver and Daniel modeled the feeding process using an analogy with sipping juice with a straw (2, 5, 12). According to this model, the proboscis was considered as a rigid tube with impermeable walls and the pump pressure was assumed constant for the species in question. Kingsolver and Daniel employed the Hagen-Poiseuille formula from hydraulic engineering to relate the intake rate, liquid viscosity and the suction pressure (2, 5, 12). The

results of this model provided very useful insight on the rate of energy gain for butterflies consuming different liquids. On the other hand, this model posed a challenge for biologists: it reveals that the suction pump of the butterflies and moths feeding on very viscous liquids should be able to produce the pressure of the order of one atmosphere (6, 8). As known from hydraulic engineering, so-called suction cavitation occurs when the pump suction is under a low-pressure/high-vacuum condition where the liquid turns into a vapor. The bubbles should expand to the proboscis tip and the pump will lose its suction. In recent elegant experiments on orchid bees (Euglossia imperialis) which also have proboscis similar to that of butterflies and moths, Borrell reduced the ambient pressure from 1 atmosphere (101 KPa) to 0.43 atmosphere (43 KPa) thus reducing the equilibrium vapor pressure of water (8). Aqueous solutions with different Sucrose concentration where used for feeding of orchid bees. The thicker solutions (~ 45% Sucrose) are much harder to suck up, but Borrell found that the incremental reduction in intake rate is the same as that for the thinner solutions (8). These findings question the applicability of the Kingsolver-Daniel model which predicts that the bee ability to suck up nectar depends on its muscular strength, i.e. on how much force the muscles in suction pump produce to reduce the air pressure in the proboscis.

One more important step toward understanding of the feeding mechanism of butterflies has been recently provided in a series of papers (13, 14). Using real-time phase-contrast x-ray imaging, the authors showed that cabbage butterfly (Pieris rapae) sucks up liquids in discrete portions – boluses looking like slender droplets. Using the frame dimensions, 1 mm×1.3 mm, we estimated the bolus diameter as d ~ 100 μm. From the movie, the authors measured the bolus speed v =1.5 mm/s. Using this speed we infer that the bolus length is not greater than 3 mm. The diameter of the food channel in the proboscis of cabbage butterfly is about 50 μm (3), i.e., it is twice smaller than the bolus diameter. The length of the proboscis is about 12 mm (3), hence the bolus is presumably formed by the liquid column filling the proboscis.

Hypothesis

Taking into account the results of recent experiments, we propose a hypothesis that the capillary forces play the key role in feeding process. We conducted a series of experiments on butterfly feeding from sessile droplets, from vials resembling floral cavities, and from porous materials. Analyzing the morphology of food channel, movies and images, we found that the dorsal zipper-like linkage connecting two tubular muscles of the proboscis (Fig.1) is important feature of the animal's fluidic system. In particular, these pores might be a source of bubbles in the food channel.

Butterfly proboscis consists of two galeas – tubular organs which accommodate some elements of the respiratory system, nerves, gland cells and muscles. These muscles are responsible for coiling and uncoiling of proboscis during the feeding process. Two galeas are joined together to form a food channel in between. The upper dorsal part of the joining linkage is represented by the overlapping lance-shaped plates. This linkage looks like a zipper joining two edges of fabric. The lower ventral linkage consists of tightly packed hooks. The exocuticular hooks in the ventral part are rigid and they are placed so tightly, that there are no visible gaps between them on SEM micrographs. The dorsal linkage is much more flexible because of the lance-shaped plates which form visible gaps-pores (Fig. 1 (b)) (1, 15).

The pores formed by the lance-shaped plates in the zipper-like linkage are open and allows air to invade the food channel. We hypothesize that every time the butterfly opens up

the sucking pump compartment, the liquid moves in caused by the pressure drop. Due to the pressure reduction, menisci are formed between lance-shaped plates in the zip-like linkage. These menisci evolve to form the bubbles. The bubbles expand as the flow progresses and eventually fill up the food channel in the proboscis. When the sucking pump shuts the valves to close the compartment, the food channel in the proboscis becomes filled again due to capillary action of menisci. The process of filling/empting of the food channel in the proboscis is therefore very similar to that observed in the fountain pens. The proboscis works as a self-regulating system in which the capillary forces control the size of the bolus and its intake rate.

(a)   (b)
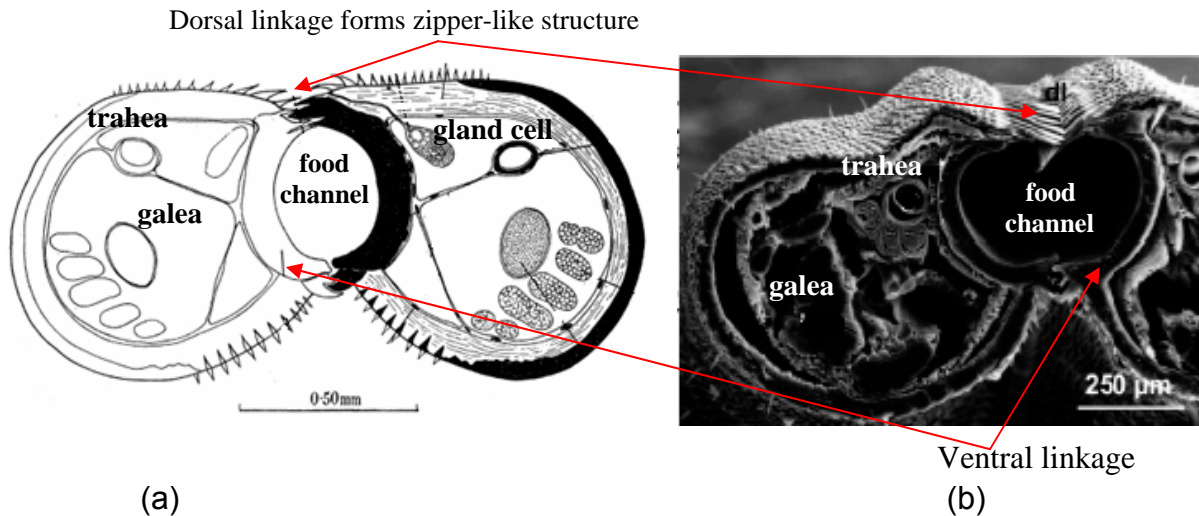
Figure 1. (a) Schematic representation of the proboscis cross-section perpendicular to the food channel (1), (b) SEM micrograph of the proboscis cross-section (15).

Materials

Twenty one female butterflies bought from Mr. Butterflies Co, FL were fed twice a day in the morning 11-12 am and in the evening 17-18 pm during three weeks. Butterflies were fed from sessile droplets, from vials, and from Mardi-Gras paper towels to imitate puddling. We used five different solutions: 25% Sucrose, 40% Sucrose, 55.56% Sucrose, 40% Sucrose + 0.1% Tylose, and 40% Sucrose + 0.3% Tylose. Tylose does not change the solution sweetness, but does change the solution viscosity (4, 7, 8). The feeding process was filmed with high speed camera (Motion ProX3, Princeton Instruments, NJ).

Experimental

Feeding from vials
The first series of experiments was designed to observe butterfly feeding from a circular vessel. This experiment most closely represents butterflies feeding from flowers. Data collected from these experiments was used to calculate the pressure drop produced by the butterfly during the nectar intake process.

Viscosity and Sucrose presence were two limiting factors observed during butterfly feeding. The insects refused to drink both 10% Sucrose solutions and 40% + 0.5% Tylose solutions despite multiple efforts to feed them from these solutions. It can be concluded that

the butterflies did not find the 10% Sucrose solution attractive. The butterflies failure to drink the 40% Sucrose + 0.5% Tylose solution can be explained by the butterflies inability to produce enough pressure drop to intake this very viscous solution.

Butterflies were different in size, and they showed significant difference in intake rates. To estimate the produced suction pressure, we did not measure the radius of the food channel and the proboscis length for each butterfly. In the Hagen-Poiseuille formula relating the flow rate and pressure drop, we used the average radius for the food channel R = 35.6 μm and the average length for the proboscis L =1.3 cm. Therefore, the formula reads

$$Q = \frac{\pi \rho \Delta P R^4}{8 \eta L} \tag{1}$$

where Q is the flow rate, ρ is the density, η is viscosity, and ΔP is pressure drop. Measuring the flow rate, density, and viscosity, we observed that the pressure drop can be as high as one atmosphere for 55.56 % Sucrose and for 40 % Sucrose + 0.3% Tylose.

One more important observation is that the butterflies are able to sip nectar through the side pores in their proboscis. As seen from the movies, the meniscus climbing up the proboscis changes its shape and the visible surface deformations suggest that the liquid is going in. Hence the proboscis cannot be modeled as an impermeable tube and it becomes evident that there is a fluid exchange between the interior and exterior of the food channel.

Feeding from paper towels

This series of experiments was conducted with porous materials of different saturation level. For our experiment we used untreated paper towel. Several cut stripes were hung from four different heights (Fig.2 (a)). The ends of each strip were immersed in 25% Sucrose solution and each strip was wrapped in polyethylene film to prevent evaporation. After twenty four hours, the cover was removed and the presence of liquid at each height was checked. Then the pore size distribution was estimated using the Jurin model. That is the paper towel is modeled as a system of interconnected capillaries, Fig. 2 (b). The equilibrium height of the liquid column H or the Jurin height in the capillary of the radius r is $H = \frac{2\sigma}{\rho r g}$, where, σ - the surface tension, ρ - the liquid density, g – acceleration due to gravity.

Therefore, changing the length of the hanging strips of paper towels we can scan the pore size distribution. At the height H, the pores larger than $r = \frac{2\sigma}{\rho H g}$ will be empty, while the pores of smaller radius will be full of solution. Using this technique, one can estimate the critical suction pressure which butterfly is able to produce. Indeed, beyond a certain height, the butterflies refused to sip from the surface being unable to extract liquid held by capillary forces in small pores. The result was significant to us as it proved our theory. Experiments showed that the butterflies cannot produce the suction pressure higher than ΔP ~ 2σ/R, where R is the radius of the food channel in the proboscis.

To complete the picture, we calculated the amount of liquid taken by the butterfly from each level H. We assumed that the butterfly can suck nectar only from the area underneath the proboscis (Fig. 3). The volume of absorbed liquid can be estimated for each height if the area under attached proboscis is known. The thickness of the paper towel was h = 0.3 mm, the width of the widest part of zip-like structure was estimated from the SEM micrographs as W =

0.11 mm, the length of area of contact of proboscis with the substrate, l, was estimated from images, and the saturation level at the given height S(H) was measured.


Pore radius vs capillary pressure

(a)   (b)   (c)
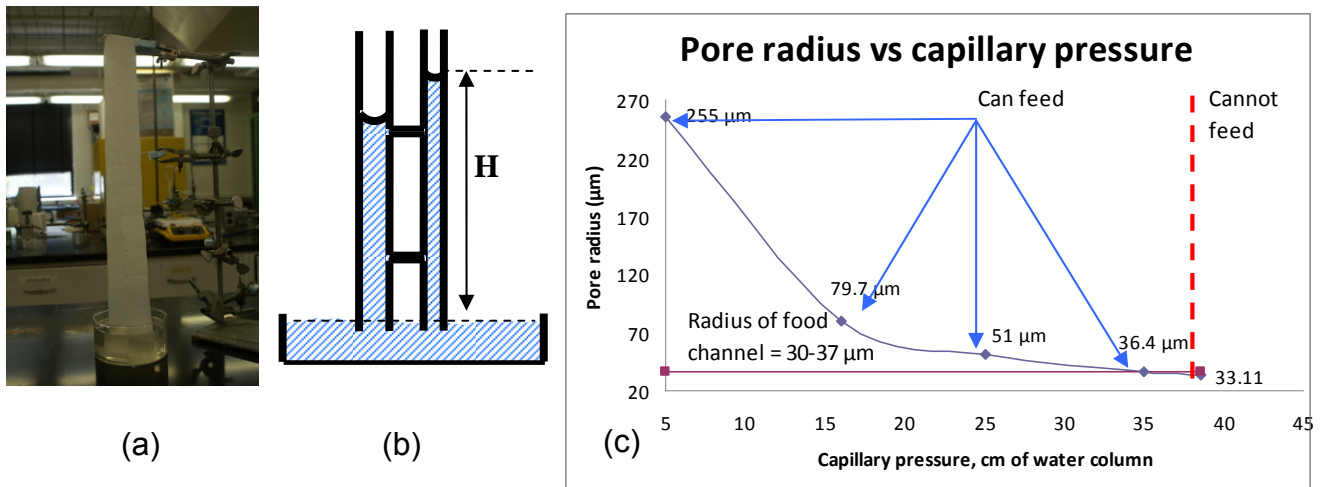
Figure 2. (a) Strips of paper towels overthrown through a horizontal stage with the ends soaked in solution, (b) The principle of pore size analysis. Porous material is modeled as a system of capillaries with a larger radius connected to capillaries with a smaller radius. H is the Jurin length for the smaller capillary, (c) Graph showing the pore radius corresponding to the height where the butterfly was fed. The smallest pore from which the butterfly was able to suck nectar was estimated as 36.42 μm, which is about the radius of the food channel.

Therefore, the volume was estimated as

$$V = \varepsilon \cdot h \cdot l \cdot W \cdot S(H) \tag{2}$$

where, ε = 0.74 is the porosity. The measurements of sample saturation are summarized in Table 1. Table 2 lists the liquid volumes absorbed at different levels H and calculated from Eq. (2).

(a)   (b)   (c)
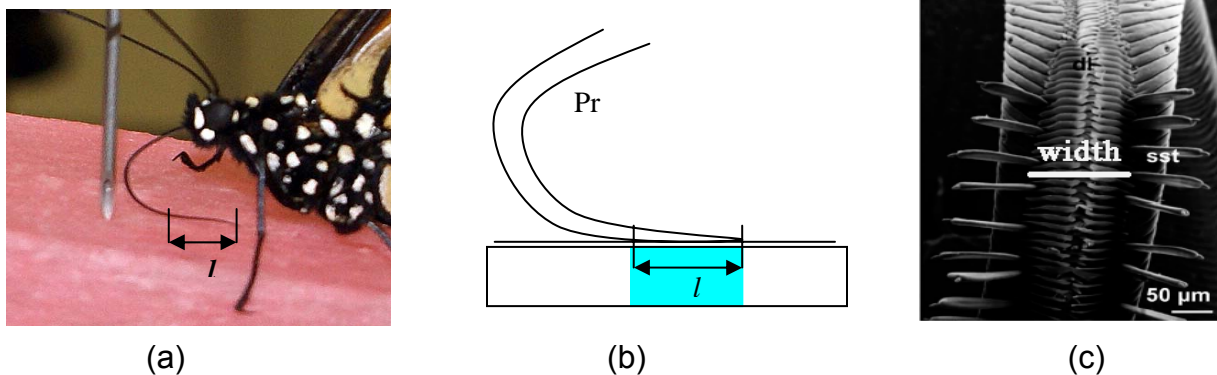
Figure 3. (a) The imitation of butterfly puddling on paper towel saturated with Sucrose solution. The needle is used as a reference, (b) Schematic of the proposed model, (c) The width of the zipper-like structure used in calculations. SEM micrograph adopted from Ref. (15).

Table 1. Saturation level of the sample at different heights

Height, cm	Sample length/width/height, cm	Sample volume, cm^3	Mass, g	Saturation level, S
5	4 x 2.5 x 0.03	0.3	0.1978	0.762
	2 x 2.5 x 0.03	0.15	0.0958	0.731
16	4 x 2.5 x 0.03	0.3	0.1595	0.579
	2 x 2.5 x 0.03	0.15	0.0789	0.568
25	4 x 2.5 x 0.03	0.3	0.1105	0.346
	2 x 2.5 x 0.03	0.15	0.0544	0.334
35	4 x 2.5 x 0.03	0.3	0.1043	0.316
	2 x 2.5 x 0.03	0.15	0.0493	0.285
Dry	4 x 2.5 x 0.03	0.3	0.0379	0
	2 x 2.5 x 0.03	0.15	0.0196	0
Wet	4 x 2.5 x 0.03	0.3	0.2479	1
	2 x 2.5 x 0.03	0.15	0.1239	1

Table 2. Absorbed volumes at different heights

Height, cm	length (l), mm	Volume under proboscis (v), mm^3	Absorbed volume (V), mm^3
5	3.3	0.11	0.061
16	4.667	0.154	0.066
25	5.664	0.187	0.047
35	6.996	0.231	0.052

Assuming that the food channel is a circular cylinder of the length L=13 mm (which is equal to the proboscis length) and the radius equal to the radius of food channel r = 0.036 mm, we have V$_{PR}$ = 0.052 mm^3. Therefore the amount of liquid, which the butterfly can actually sip, is enough to fill up the food channel completely.

According to our hypothesis, each portion of liquid sucked by the butterfly should be replaced by the new one which is filled by the capillary action. The Lucas-Washborn law (16, 17) predicts the time of filling of the food channel as

$$t = \left(\frac{2\eta}{\sigma r}\right) L^2 \qquad (3)$$

where, σ = 70·10^{-3} N/m - surface tension, r = 35 μm – the channel radius, η ≈ 0.00282 Pa·s - viscosity for 25% solution, L=13 mm is the average proboscis length. The time is estimated as t ≈ 0.4 s, which is sufficient to fill the proboscis between two pump cycles.

Conclusion

We suggested new feeding mechanism for butterflies and moths which resembles the mechanism of feeding of the nib of fountain pens. The experiments support this hypothesis and simple analysis based on the basic laws of capillarity provides reasonable estimates of the maximum suction pressure and feeling time for the proboscis between pumping cycles.

Acknowledgements

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