THE ULTRAFAST VALVE OF AN AQUATIC CARNIVOROUS PLANT

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ABSTRACT

A process difficult to perform within the channels of a Lab-on-a-chip is the sudden transfer of a small sample of fluid in a closed container. This operation can be found in the plant kingdom in the aquatic carnivorous Utricularia (whose common name is bladderwort). These plants are gifted with tiny suction traps: a slight contact opens their door, the trap sucks in liquid and the prey hereby. This motion is due to the release of elastic energy stored in the trap body. We present an experimental study, imaging the fast fluid motions under a high-speed camera. We use a laser sheet for intense illumination in a slice that is few micrometers thick, in order to record fluorescence images at high speed. We found that the door buckles inside after triggering, then the suction lasts only half a millisecond, and finally the door closes hermetically. All this open-and-close sequence is performed within a few milliseconds, thus barely visible with the naked eye. The liquid reaches impressive accelerations up to 600 g, preventing any escape of prey animals. We finally discuss how this model will prove useful for the design of a biomimetic reproduction of the trap, and its implementation in a microfluidic circuit.

1. INTRODUCTION

1.1 Carnivorous plants

There exist more than 600 species of carnivorous plants, resulting from an adaptation to nutrient-poor habitats [3]. These plants present different kind of traps to catch animals. It is possible to classify these traps into two groups:

- Passive traps, without a trapping movement, such those from Nepenthes (fig. 1a)
- Active traps, such as those from the Dionaea (fig 1b).

A complementary approach between physicists and biologists has recently lead to elucidate the mechanisms of certain traps, such as the use of an elastic instability to maximize the speed of capture in Dionaea [1] or the presence of a visco-elastic fluid in the pitchers of Nepenthes [2].

1.2 Bladderwort (Utricularia spp.)

The bladderworts are a genus of aquatic carnivorous plant that comprises of more than 220 species, including the aquatic ones we studied, Utricularia inata, U. vulgaris and U. australis. Numerous biologists have contributed to their description and to the elucidation of their mechanism. First studied in the 19th century, their carnivorous character has been discovered in 1876 [11]. A first detailed study is due to Lloyd [6], who described

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the plant constitution and proposed an electromechanical analogy to describe its behaviour. This model was later abandoned by the author himself, because of its extreme complexity.

The aquatic plants consist of a long submerse shoot with ramified leaves where the traps are situated. The traps, called utricules or bladders, have a size in the order of 1mm (see figure 2).

It is possible to distinguish two main parts:
- the wall consists of only two layers of cells. Its thickness is sensibly constant all over the surface (we measured 60 micrometers), and increases near the door to form a quasi-circular (ring) rigid structure, whose thicker bottom part is called the threshold.
- the door is attached to the upper part of this ring. The lower free edge of the door is in compression against a threshold called the velum whose role is to avoid any leaks when the door is closed. The door consists of only two cell layers, but here thinner than in the wall. The precise structure is complex. Several trigger hairs are located on the door wall, and are responsible for the triggering of the door. The thickness of the door is around 15 micrometers.

1.3 Summary of the literature on the trap mechanism

We now sum up a few results that generally admitted concerning the trap mechanism of the bladderwort.

1.3.1 Trap setting

The plant is able to pump water from the inside to the outside of the trap, probably through the action of glands situated in the wall (internal glands, see [7]). The utricle is waterproof, and this deflates the trap, with a
depression of the water inside as low as 0.17 bars [7]. The time needed to set the trap varies from 30 minutes to 4 hours. The plants resist the pressure due to its waterproof door and velum [4].

1.3.2 Trap activation

When a prey triggers the trap by touching one of the trigger hairs situated at the bottom of the door, it opens and water and prey are sucked in. The door then re-closes, which impedes the prey from escaping and renders the utricle waterproof again. The capture time is evaluated to be 30 milliseconds approximately [6, 9]. The door opening and closing mechanism remains poorly understood, and two main hypothesis are generally put forward:

- Mechanical hypothesis: the trigger hairs act as levers, and un-block the door, which entails the inflow of water, until the pressures are equilibrated again [5].
- Active hypothesis: touching the hair triggers a stimulus that propagates in neighbouring cells, and provokes a softening of the door, that is therefore not able to resist the applied pressure anymore [8]. Measurements have shown a change of electrical potential [10], which would corroborate this hypothesis.

The originality and complexity of the trap arise from the fact that, contrary to the other active carnivorous plants that have only a closing phase (such as Dionaea), the trapping action of bladderworts consists of an opening and closing within a fraction of a second.

The comprehension of the trap triggering is limited by several aspects: the observation of ultra-fast phenomena, and a modelisation of the physical phenomena at play.

2. Methods

Plants were cultivated indoors, in deionized water, in order to reproduce their natural inhabitat. Traps were placed in a Petri dish and were observed under the microscope (up to 10x). The dynamics of the trap were studied with two kinds of imaging techniques: time-lapse, with a slow camera operated at 1 frame every 100s (0.01 Hz), and high-speed, with a camera that could operate up to 8000 frames per seconds (8000 Hz). Image analysis was performed with ImageJ (freeware) and Matlab (Mathworks, Inc.). More details can be found in the master thesis by Olivier Vincent [12].

3. Results

3.1 Trap setting

We present results obtained from the analysis of image sequences. Seen from the top, it is convenient to evaluate the volume diminution of the trap measured by its thickness $e$, at the waist of the trap profile (figure 3). The shape evolves with time, with an exponential decrease of the thickness (figure 4), with a characteristic time of 52 minutes on this measurement.

![Figure 3: Evolution of the trap profile, monitored by the thickness $e$.](image_url)
3.2 Trap suction

The door is triggered manually with the tip of a needle, by touching one of the trigger hairs. We could show that a very small bending of the trigger hair is enough to trigger the response. The duration of the suction is extremely short: around 2 ms (Fig. 5), fast enough to catch swimming animals.

![Graph](a) e vs time in linear units, (b) ln(e-e₀)/e vs time in logarithmic units.

**Figure 4:** Slow deflation of the trap, with an exponential decrease of the trap thickness $e$ towards a plateau value $e_0$, represented in linear units (a) and in logarithmic units (b).

The aspiration is monitored using tracer particles (hollow glass beads), placed near the door. The maximum fluid velocity measured is extremely high: 1.5 m/s at the largest. The acceleration is also impressive: up to 600 g. The fluid motion is therefore dominated transiently by inertia: the Reynolds number computed with this velocity and the size of the door (600 micrometers) gives a value of 900. This means we are in the conditions of a special realm of hydrodynamic flows: high-Reynolds number microfluidics!

We distinguish two zones in front of the door:
1. an aspiration zone, where the particles are completely trapped,
2. the outer zone, where particles are move due to the suction, but do not enter.

The extent of the zones of aspiration around the door as a radius of around 500 micrometers.

To record the progression of the median door axis during suction we developed a special set-up to image a living trap. A thin laser illuminates the median door axis as observed in the focused field of view of a microscope objective (10x). To obtain the laser sheet, the cylindrical beam from a solid state laser operating at 523nm is expanded by 10x, its diameter then reaching around 1cm, and then focused in one direction by a cylindrical lens to a few micrometers. Only the orange fluorescence is imaged. Because the laser intensity is high (200 mW) and the trap dyed by infusion in a rhodamin solution for a few minutes, we obtain high-speed fluorescence images up to 3000 images per second.

The trap is manually activated by touching the trigger hairs with a fine needle. We then see a complete inversion of the curvature of the trap, from convex to concave (Fig. 6a-d), then the door violently opens (Fig. 6e). The
strong influx of water equilibrates the pressures and the door inverts its curvature (Fig 6f).

We interpret this ultrafast open-and-close mechanism, as a buckling of the spherical shape of the door under pressure, then its unbuckling when the pressure difference vanishes.

![Figure 6](image)

**Figure 6**: Recording of the opening and closure of the valve of *Utricularia australis*. (a) \( t = 0 \) ms, initially closed door (b) 5.9 ms, inversion of curvature starts near the threshold (c) 7.6 ms, this inversion spreads (d) 7.9 ms, until the whole valve is completely inverted (e) 8.6 ms, the door suddenly opens wide (f) 9.7 ms, and resets to its initial position.

4. Conclusion and perspectives

A low-speed and high-speed video study revealed the huge dissymmetry of time scales at play during the trap setting and activation. The trap setting can be interpreted as a storage of elastic energy, and is similar to the bending of an bow. The release of the energy is violent and occurs within a few milliseconds, suggesting large amplitude bifurcation of the behaviour, triggered by touching the hair. A mechanical lever effect could explain the triggering of the metastable trap, but we cannot exclude an electrochemical/physiological process (as in Dionaea) that would account for the extreme sensitivity that we observed. However the door opening and closing after triggering can be explained by a purely mechanical phenomenon: buckling and unbuckling.

The door opens and closes in a very short time interval of a few milliseconds. In our presentation we will play high-speed recordings, that help seeing the progression of the door opening and closing. We will show that even in the absence of manual trigger, small actuations (from instance by tiny swimming paramecium present in the medium, or maybe just thermal actuations noise) were enough to trigger the trap, on average after a delay of 5 hours, with a lot of variations in the delay . The spontaneous activation of the trap was also recorded.

As a perspective, this work is also at the source of a biomimetic inspiration to design a miniature suction apparatus, helpful to isolate very small quantities of liquid. Indeed, the traps perform an action equivalent to that of a pipette. The principle of the very fast valve, based on buckling, could be copied with soft elastic materials such as the ones used in microuidic devices. The valve is naturally maintained close by the difference of pressure, and without any external actuation. This biomimetic design of this pipette would thus find its place within the realm of Lab-on-chip devices, that need to treat very small amounts of liquid.
REFERENCES