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Comparative physiology of the Droseraceae *sensu stricto*— How do tentacles bend and traps close?

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Abstract: The family Droseraceae, which currently includes only *Drosera*, *Dionaea* and *Aldrovanda*, is related to *Drosophyllum* and *Nepenthes*, and also to the Plumbaginaceae and Polygonaceae. *Plumbago* has *Drosophyllum*-like hairs on its sepals which catch insects and secret digestive enzymes upon stimulation. All members of the Droseraceae *s.s.* have active traps, which respond rapidly to mechanical stimuli with capture movements that are mediated by action potentials triggered by mechanical stimuli. In all of these plants the rapid bending and closure movements involve rapid acid growth but in *Aldrovanda* a turgor loss mechanism may play an important role as well. A slower set of secondary movements initiated and maintained by both mechanical and chemical stimuli act on closing the whole leaf in *Drosera*. In all members of the family these slower movements, which are mediated by auxin at least in *Drosera*, maintain the closure until the prey is completely digested and ceases to release chemical signals. The question of rapid acid growth in *Dionaea* has caused some controversy because most very rapid action potential mediated plant movements are due to turgor loss mechanisms that involve the loss of K^+. However, experiments with buffers in *Dionaea* indicate that acid growth is the most important cause of movements in the *Dionaea* trap. Neutral buffers can prevent movements in *Dionaea* and still allow the action potentials to occur in response to mechanical stimuli. *Aldrovanda*, *Dionaea* and *Drosera* also have a loss of ions from the cells during the action potential. This may assist in rapid early phase closure in *Dionaea* and may be the main force in early phase closing of *Aldrovanda*. All cells in both *Dionaea* and *Aldrovanda* traps have been shown to be excitable. It appears to be the anatomy of the sensory organ and the trap lobes that determines which cells have a sensory function and what the pathway of the action potential is rather than the membrane properties of the cells. In addition the anatomy of the trap lobes accounts for much of the rapid movement by allowing them to flip shut in a way that greatly amplifies cell size changes in the lobes of *Dionaea* and *Aldrovanda*.

It is probable that the original Droseraceous carnivorous plant was a sticky haired stationary trap, such as in *Drosophyllum*. These traps probably originally served another function such as defense against insect predation or pollen theft. Cladistic analysis of DNA from both the matK gene (Meimberg *et al.*, 2000) and the rbcL gene (Albert *et al.*, 1992; Williams *et al.*, 1994; Fay *et al.*, 1997) has revealed that the members of the Droseraceae are closely related to an as yet unnamed natural sister group of plants that includes the Nepenthaceae, Dioncophyllaceae, Ancistrocladaceae, and *Drosophyllum* (now Droserophyllaceae but formerly considered to be in the Droseraceae). Other closely related families include the Polygonaceae and Plumbaginaceae. In the Plumbaginaceae *Plumbago* has *Drosophyllum*-like glandular hairs on its sepals that capture prey and are capable of being induced to secrete proteolytic enzymes (Stoltzfus *et al.*, 2002). The pitfall trap of *Nepenthes* and the snap-trap of *Dionaea*/*Aldrovanda* are probably independent modifications of a trapping system that began as an adhesive trap similar to that of *Drosophyllum*. None of the plants in the sister group has rapid movements involved in prey capture but active traps characterize all of the Droseraceae *sensu stricto* (*Drosera*, *Dionaea* and *Aldrovanda*). It is the movements of the Droseraceae that will be compared here.

Drosera traps look deceptively simple. Although the trap of *Drosera* is often referred to as passive, Lloyd (1942) correctly characterized it as an active trap. Its movements, which bring a fly captured on the marginal tentacles to the center of the leaf and then envelop the prey and digest it, resemble those of a very slow hydra (Williams, 1976). The individual tentacles respond to direct stimuli by producing action potentials that terminate near the base of the tentacle (Williams and Pickard, 1972; Williams and Pickard, 1980). Individual tentacles respond to the action potential with a rapid growth movement directed toward the center of the leaf that carries the prey in that direction. The stimuli that cause these responses are mechanical but are greatly enhanced by chemicals such as Na^+ and NH_4^+ (Williams and Pickard, 1980; Suda *et al.*, 2002). These responses begin 15 seconds after an action potential and are completed in a few minutes. The bending movement is due to differential growth since the outer cells of the tentacle expand more than the inner ones during bending (Hooker, 1916, 1917). A slower response begins in as soon as a few minutes and can take from an hour to several hours to complete (Williams and Pickard, 1980; Suda *et al.*, 2002). This secondary bending response involves the bending of tentacles not directly stimulated by the prey and also the blade of the leaf itself and requires that both chemical and mechanical stimuli be present. It is mediated by auxin as many growth movements are (Bopp and Weber, 1981). Here again the chemical stimuli include Na^+ and NH_4^+. Secretion of mucilage and digestive enzymes is also stimulated and the captured prey is digested. Unbending seems to occur when chemical and mechanical stimuli are no longer present. The slow unbending movements are also due to differential growth since the inner cells expand relatively more during unbending (Hooker, 1916, 1917).

Dionaea is closely related to *Drosera* but has a trapping mechanism that superficially appears to be very different. In reality it is a modification of the *Drosera* trap. The physiology of the *Dionaea* trap resembles the *Drosera* trap in many ways. Both have rapid action potential mediated movements that begin in a sensory hair and spread. Unlike that of *Drosera*, the action potential of *Dionaea* spreads throughout the trap and quickly triggers a rapid movement in the entire leaf blade. Chemical stimuli are not involved in this response. The mechanically stimulated signal begins in a trigger hair that is probably a highly modified tentacle (Williams, 1976). It even has an endodermis, a layer found in secretory structures of plants, such as the *Drosera* tentacle, although it does not secrete anything. If the trap has captured a visitor, chemical stimuli from the digested prey provide an additional stimulus which keeps the trap closed until the stimulus ceases (Lichtner and Williams, 1977). Just as in *Drosera*, the *Dionaea* trap shows a net increase in size during closure and during reopening. This is the plant physiologist's definition of growth. But the speed of closure conceals this mechanism. Rapid movements in plants that are triggered by action potentials are often caused by rapid loss of turgor in "motor cells" and growth is traditionally thought of as a slow process. Hooker (1916, 1917) called the relatively slow movement of the *Drosera* tentacle a growth movement. Brown (1916), on the basis of evidence identical to Hooker's, called *Dionaea*'s movements a turgor movement. Turgor movements are often fast and triggered by action potentials. Growth movements were not known to be fast or triggered by action potentials. The description of *Drosera*'s movements as growth movements and *Dionaea*'s movements as turgor movements meets expectations but does not explain the experimental data.

Is *Dionaea* closure a turgor movement or a growth movement? Solute and turgor pressure changes are involved in both growth and turgor movements. The difference is in what happens in the cell walls. In turgor movements the cell walls remain elastic acting as a girdle that presses inward on the cells. During growth the wall's fibers loosen and the turgor pressure within the cells then causes them to expand. Finally solutes taken up by the cells restore the osmotic pressure and thus restore the turgor pressure. In slow growth these two processes overlap and a relatively smooth expansion occurs.

Turgor movements are caused by the gain or loss of water from cells that is driven by the gain or loss of ions or sugars from the cells. All known rapid turgor movements, such

as those in *Mimosa*, are caused by a turgor loss due to a loss of K^+ with associated anions and the subsequent loss of water from the cell (Satter, 1979).

Acid Growth, a concept first used in the 1970s to describe the action of auxin, now has a much more general explanation. Plant cells grow because cell wall proteins known as expansins loosen the wall by allowing slippage between the hemicelluloses that form the matrix that binds cellulose microfibrils together. This loosens the wall fibers and lowers the inward force the wall exerts on the cell. The turgor pressure of the cell then causes it to expand. (Turgor pressure drives both growth movements and turgor movements.) Expansins have an acid pH optimum. Decreasing the pH of a cell increases wall loosening. Auxin stimulates growth by stimulating the pumping of H^+ into the cell wall compartment from the cytoplasm. Any process that causes the cell wall compartment to become acid is capable of stimulating acid growth in any growing plant cell (Cosgrove, 2000). Addition of expansins to cell walls results in expansion of cell walls within seconds (Cosgrove, 2001). The slippage caused by expansins performs the role that cleavage of hemicelluloses by hydrolytic enzymes in the cell wall was once though to have.

***Dionaea* Trap Closure** is the result of a flipping of the trap lobes from a position where the exterior of the trap is concave to one where the exterior is convex. This movement can begin as soon as 0.4 sec after stimulation and be completed in a second (Sibaoka, 1980). A slower movement follows which narrows the trap opening, ultimately resulting in the tight appression of the lobes. Both processes result in a net increase in trap cell volume (Fagerberg and Allain, 1991) but the cellular changes during the first fractions of a second have not been adequately measured. However, a greater increase in the volume of the outer layers of the trap lobes relative to the inner lobes supports the acid growth model of trap closure* as does a range of other evidence:

- Trap lobes show an irreversible increase in volume during closure (Fagerberg and Allain, 1991).
- Traps with walls perfused with neutral buffers are paralyzed but will still produce action potentials when the trigger hair is stimulated (Williams and Bennett, 1982).
- Traps perfused with lower concentrations of neutral buffers can be closed by multiple stimuli (Williams and Bennett, 1982).
- Traps with walls perfused with acid buffers will close without stimulation (Williams and Bennett, 1982; Hodick and Sievers, 1988).
- Significant ATP is used during trap closure. It is needed to move H^+ from the cytoplasm to the walls (Williams and Bennett, 1982).
- The surface of the outer lobes of the trap is more flaccid just after trap closure.
- Plasticity in the outer layers of the trap lobes increases dramatically during closure while that of the inner lobes remains unchanged (Hodick and Sievers, 1989).
- Ca^{2+} decreases in the cell wall and increases in the cytoplasm during the action potential (Hodick and Sievers, 1988). This decreases pectin cross-linking in the wall and increases the production of wall material in the cell.

The ability of neutral buffers to prevent trap closure without affecting the stimulation of action potentials in the trap is strong evidence that all phases of closure of *Dionaea* traps are caused by acid growth (Williams and Bennett, 1982). The three-fold increase in electrogenic H^+ pumping that is estimated to occur during the *Chara* action potential (Thiel, et al., 1997) demonstrates the feasibility of the same process in *Dionaea* traps. By contrast, Hodick and Sievers (1989) have criticized the acid growth model of trap closure based on the their determination that the amplitude of extracellularly measured action potentials was the same before and after closure in traps while intracellularly recorded action potentials from traps perfused with acid buffer show a decreased amplitude. The comparison of extracellular measurements to intracellular ones is problematic as is the comparison of cells

* Brown (1916) and Williams and Bennett (1982) assumed that the epidermis was responsible for trap movement. Fagerberg and Allain (1991) have demonstrated that the expansion takes place primarily in outer mesophyll cells. The acid growth model is supported by the new and more accurate data.

exposed to acid conditions for periods of minutes in one solution to those exposed for seconds or fractions of seconds in another. The conclusion that cell wall acidification does not occur based on this data is not strong evidence. However, Hodick and Sievers (1989) also demonstrated a differential plasticity increase in the outer cell walls of the trap lobes relative to the inner ones during trap closure. This is strong evidence in favor of a growth model of trap closure. The decrease in turgor of the outer walls, expected if the trap expanded by rapid wall loosening, also supports this model (Williams and Bennett, 1982).

There is almost certainly a loss of turgor due to a loss of ions during the action potentials during the earliest phase of trap closure. Freshwater and land plants have relatively low ion concentrations in their cell walls. If *Dionaea* and *Aldrovanda* work by the same mechanism as giant Characean algal cells (Thiel, et al., 1997), and all evidence that exists indicates that they do, the cells would loose both Cl^- and K^+ during the action potential. This would result in a loss of turgor during these ion fluxes. The movement of Ca^{2+} into cells would have the opposite effect but a smaller one since its flux is lower and since Ca^{2+} carries twice the charge per ion. Evidence in favor of involvement of turgor movements during early closure is:

- K^+ and probably Cl^- are lost during the action potential—these would result in a loss of turgor (Iijima and Sibaoka, 1983, 1984; Hodick and Sievers, 1988).
- Many rapid plant movements are turgor loss mechanisms.

There is certainly turgor loss resulting from the loss of ions during the closure of the *Dionaea* trap but it is unclear how much of a role it plays in causing the rapid movement. The fact that traps can be paralyzed by neutral buffers that do not prevent action potentials indicates that its effect is minimal in *Dionaea*.

Aldrovanda is a third genus in the family that resembles *Dionaea*. Its trap is a smaller faster aquatic version of the *Dionaea* snap trap. *Aldrovanda* had a great deal of excellent work done on it in the 1980s by Iijima and Sibaoka. The results parallel those of *Dionaea* very closely with some important differences:

- A single stimulus will close a trap but two are required in *Dionaea* (Iijima and Sibaoka, 1981).
- Trap closure is about ten times faster than in *Dionaea* (Iijima and Sibaoka, 1981).
- The trigger hair bends at what appears to be the vestigial endodermis rather than below it (Iijima and Sibaoka, 1982).
- There are only three cell layers. The epidermis must play a larger role in trap movement because it is a larger part of the trap (Iijima and Sibaoka, 1982).

Iijima and Sibaoka (1983, 1984) first provided the information on ion fluxes during the *Aldrovanda* action potential. Similar experiments on *Dionaea* were performed later by Hodick and Sievers (1988) with nearly identical results. The following results were found in *Aldrovanda* and *Dionaea* studies:

- All cells in the *Aldrovanda* and *Dionaea* traps are excitable (Iijima and Sibaoka, 1981; Hodick and Sievers, 1988).
- The action potentials in all cells of both *Aldrovanda* and *Dionaea* traps are similar (Iijima and Sibaoka, 1981; Hodick and Sievers, 1988).
- There is a low resistance between cells of the trap of *Aldrovanda* through which the action potential spreads (Iijima and Sibaoka, 1982).
- The loss of ions in *Aldrovanda* may contribute significantly to the early rapid flip of the trap (Iijima and Sibaoka, 1983).

Some general conclusions can be drawn from a comparison of *Aldrovanda* and *Dionaea*.

- Since all cells of the trap are excitable the sensory cells in the trigger hair are probably not physiologically unique. They are simply placed in a situation where they can be mechanically stimulated. Their uniqueness is more anatomical than physiological.
- Since all cells of the trap are excitable the pathway of the action potential and therefore the responsive area of the trap is determined not by which cells are excitable but by which

cells are excited by electrical current from their neighbors. Again the anatomy seems to determine the function.

The distribution of plasmodesmata in trap cells deserves further study and the anatomy of these traps in general needs more attention to detail but we have an emerging picture of how they work. The uniformity of trap cell function contrasts with the diversity of structures of trap components. It is likely that the rather complex picture of cell expansions and contractions seen by Fagerberg and Allain (1991) are more due to the pressures that the two lobes and the various cell layers place on each other than to differences in membrane physiology in the various cells. Families of expansins exist and the genes for them are turned on only in specific tissues. This is a way plant cell growth is controlled. It is also probably how Venus flytraps close.

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