

SEVERAL PYGMY SUNDEW SPECIES POSSESS CATAPULT-FLYPAPER TRAPS WITH REPETITIVE FUNCTION, INDICATING A POSSIBLE EVOLUTIONARY CHANGE INTO AQUATIC SNAP TRAPS SIMILAR TO *ALDROVANDA*

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Abstract: Approximately 50 species of pygmy Sundews (genus *Drosera*, section *Bryastrum*) occur in the South of Australia and one each in New Zealand (*D. pygmaea*) and Venezuela (*D. meristocaulis*). They grow mainly as small stemless rosettes possessing minute trapping leaves of 1-2 mm diameter with prominent marginal tentacles, or have elongated erect stems. The caulescent species possess only mucus-producing tentacles that are most effective in capturing small flying insects. The acaulescent species in contrast are specialized on crawling prey (Verbeek & Boasson 1993) and have developed mucus-free snap-tentacles (Fig. 1), able to bend surprisingly rapidly towards the leaf center. They lift prey like, e.g. springtails (Collembola) from the ground and carry it with a 180°-movement from the periphery of the plant onto the sticky leaf. Our examinations brought to light that several small species of section *Bryastrum* are able to catapult small animals even within fractions of a second. If the whole leaf is touched, several or even all marginal tentacles perform such bending movements simultaneously. We documented this behavior on video, featured on our film “Catapults in Pygmyland” on YouTube (www.youtube.com/watch?v=5k7GYGibdjM). Our results prove that more than only one species in the genus *Drosera* possess rapidly moving catapult-flypaper traps and that the examined pygmy catapults show a further specialization and function repeatedly (in contrast to the one-shot snap tentacles of *D. glanduligera*). The mucus-free and rapid catapult-mechanism functions independent from the initially slow mucilage-based trapping. Furthermore, our study demonstrates that in contrast to *D. glanduligera*, each single pygmy catapult possesses a similar sensor system and hydraulically operated motion-sequences analogous to the lobes of snap-traps. Catapult-flypaper traps submerged in water, forming a kind of grid-cage when triggered simultaneously could be able to capture prey underwater even without any mucilage. This is a possible scenario for the development of aquatic snap traps similar to *Aldrovanda*.

Catapult-flypaper Traps

Very rapid catapulting tentacle movements have only previously been reported

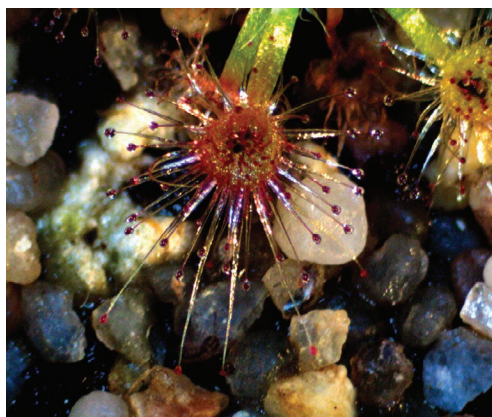


Figure 1: *Drosera callistos* with springtail below the front snap-tentacles.

in the annual *D. glanduligera* (section *Coelophylla*), a close relative of the pygmy Sundews (Hartmeyer & Hartmeyer 2005). These movements, which occur in fractions of a second, are comparable in speed with those of *Dionaea* and *Aldrovanda*. In 2012, we were able to prove in a common project with the Plant Biomechanics Group of the Botanic Garden of the University Freiburg, using a high-speed camera, that *D. glanduligera* utilizes its protruding snap-tentacles to catapult fruit flies (*Drosophila*) within 75 milliseconds (Fig. 2). Activated by the prey's impact, the hit glue-tentacles on the lamina lift the victim within 1-2 minutes into a particularly pronounced patelliform digestion cavity in the leaf center, able to contain 3-4 *Drosophila*. It is a hydraulically driven co-ordinated two-stage capture mechanism, for which we coined the designation catapult-flypaper trap (Poppinga *et al.* 2012). The catapults of *D. glanduligera* function only once because cells in the hinge-zone burst during the movement through compressive stressing. A slight touch of the tentacle head is sufficient here to trigger a complete bending with maximum speed after a response time of 400 milliseconds. The mucilage producing tentacles show a uniform response time of 8-12 seconds after touching or prey impact. A conspicuous feature of this trap type is that only stimulated tentacles move while the leaf itself remains immobile, it does not curl around the prey. The aim of this study was to determine if tentacles in the pygmy Sundews with a structure similar to *D. glanduligera* react with the same rapidity and exhibit the same behavior as this catapult-flypaper trap and to determine how wide spread this behavior is within this branch of the Droseraceae. For our experiments, we had approximately a dozen plants each of *D. glanduligera* (section *Coelophylla*) and 20 pygmy Sundew species (all section *Bryastrum*) available: *D. androsacea*, *D. callistos* "Brookton", *D. dichrosepala*, *D. helodes*, *D. echinoblasta*, *D. eneabba*, *D. enodes*, *D. lasiantha*, *D. leucoblasta*, *D. mannii*, *D. microscapa*, *D. miniata*, *D. occidentalis*, *D. platystigma*, *D. pulchella*, *D. pycnoblata*, *D. pygmaea* "Australia", *D. roseana*, *D. scorpioides*, and *D. walyunga*. In addition, Gideon Lim from Malaysia kindly provided us his video of the rapid catapulting action of the all green New Zealand variety of *D. pygmaea*.



Figure 2: *Drosera glanduligera* with just flung fruit fly.

Materials and Methods

We propagated the annual *D. glanduligera* from seeds. Most of the perennial pygmies were only some 8-10 weeks old and grown from gemmae. Some plants are part of our collection since several years (see Table 1). All plants thrived inside our cool greenhouse in Weil am Rhein (Germany) in a southwest location with night temperatures of 4-12°C and 12-26°C during the day. From October to April, we added a 400W HQI-lamp for ten hours daily to complement the low sun intensity during winter. As a reference, some plants thrived inside an adjacent tropical greenhouse with night temperatures of 14-18°C and 22-30°C during the day, applying two 400W HQI-lamps during the same months as mentioned above. Videos and photos: Sony Z5 HDV camera (PAL) with Sony G-Lens. Lumix MH DMC-TZ 10. Microscope: Wiloskop F Zoom (Hund Wetzlar), magnification 13.4-180

Table 1. *Drosera* species examined and trigger response.

Examined <i>Drosera</i> species (<i>D. glanduligera</i> = section <i>Coelophylla</i> . All pygmy <i>Drosera</i> = section <i>Bryastrum</i> .)	Catapult motion in relation to <i>Dionaea</i> (0.1 to 2 sec.)
G = grown from gemmae P = perennial plant S = grown from seeds	(<) slower than (3 to >30 sec.) (~) about equal (0.1 to 1 sec.) (>) faster than (max. 75 ms, recorded in 2012) (--) no snap-tentacles
<i>D. androsacea</i> (G)	<
<i>D. callistos</i> (G)	<
<i>D. dichrosepala</i> (P)	--
<i>D. helodes</i> (G)	<
<i>D. echinoblasta</i> (G)	<
<i>D. eneabba</i> (G)	<
<i>D. enodes</i> (P)	--
<i>D. glanduligera</i> (S)	>
<i>D. lasiantha</i> (G)	--
<i>D. leucoblasta</i> (G)	<
<i>D. mannii</i> (G)	<
<i>D. microscapa</i> (G)	~
<i>D. miniata</i> (G)	<
<i>D. occidentalis</i> (P)	~
<i>D. platystigma</i> (G)	<
<i>D. pulchella</i> (P)	<
<i>D. pycnoblasta</i> (G)	<
<i>D. pygmaea</i> AUS (P)	~
<i>D. pygmaea</i> NZL (?)	~
<i>D. roseana</i> (P)	--
<i>D. scorpioides</i> (P)	--
<i>D. walyunga</i> (G)	<

Remark: Triggered by touching, the initial rapid movement of plants slower than *Dionaea* stopped often after approximately 45° to 70°, species moving like *Dionaea* after about 120° to 140°. They needed further touching to complete the bending. With adding fish food, the bending was usually complete (~180°), but the speed differed even for identical species; however, was always the fastest during the first 45°. Due to this behavior and without a high-speed camera, it was impossible to achieve more precise data for maximum movements.

with iDS CMOS camera UI146xLE-C. Fiber light source FLQ 150 M with gooseneck light guide SHL 250.

In contrast to *D. glanduligera*, fruit flies are too large as prey for experiments with the minute snap-tentacles protruding from leaves with a diameter of only 0.8 to 2 mm. The test plants in our greenhouse spontaneously captured abundant springtails and several mites that occurred naturally in the growing media (Figs. 3 & 4). Photos of *D. glanduligera* *in situ* show quite a broad prey spectrum ranging from springtails and mites to ants and small centipedes of even larger size than fruit flies.

By examining the prey pattern of the co-occurring *D. erythrorhiza*, Watson *et al.* (1982) established that springtails also play a very important role at the natural growing sites. This glue trap with relatively extensive leaves captured mainly Collembola (76%). Moreover, this important nutrient source appears in abundance exactly at the right moment: when the returning rain opens a new growing season and the plants awake from dormancy. Considering 100,000 springtails in one square meter humid soil to be quite usual, Hopkin (1997) gives a measure of their relevance for all *Drosera* with suitable traps.

In addition, Collembola are detritus eaters and like rotting plant debris. Exactly such slowly putrefying leaves are common at the base of many *Drosera* plants. Even the annual and very fast growing *D. glanduligera* develops one new trapping leaf with about 12-18 catapulting tentacles every 3-4 days while the oldest leaves wither correspondingly, becoming a real temptation for detritus eaters. Attracted in such a manner, they touch the snap-tentacles that lie on the ground like the thread sensors of some spiders and are abruptly lifted onto the sticky leaf center.

We conducted an additional experiment inside our tropical greenhouse (now 18-20°C night, 28-32°C afternoon) to examine the behavior of submerged *Drosera* traps. Therefore, an 8-cm pot with green and red *D. capensis* was placed inside a 3-liter plastic tank and slowly submerged with deionized water. We applied two freshly caught houseflies to separate trapping leaves, taking photos after 15, 30, and 60 minutes to document the curling around the prey underwater, and once within 24 hours during the next four days. As the flies do not stick to submerged tentacles, their legs were “hooked” into the tentacles and the bodies were once squashed with a forceps to make them immobile as well as to release some body fluid. Both traps folded around the prey in approximately one hour and remained curled for two to four days, thus indicating that even though submerged, a certain amount of body fluid reached the traps. However, this is only possible without current in standing water.

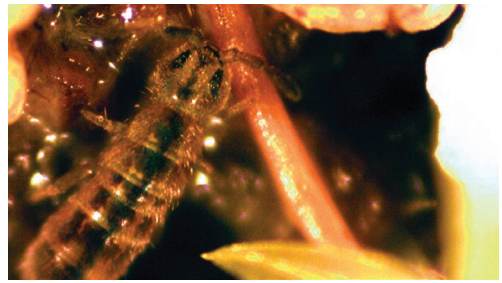


Figure 3: An important food source for small *Drosera*: Springtails.

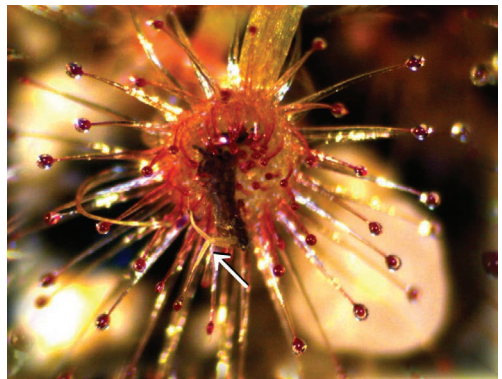


Figure 4: *Drosera callistos* captured a springtail, which is unable to escape using its furcula (arrow).

Tentacle Movement Experiments

Using a zoom-microscope, we comparatively examined the catapult-flypaper trap *D. glanduligera* and 20 species of pygmy *Drosera* for their tentacle movement. Five species possess only glue-tentacles and grow erect in their course of development. They are obviously specialized to capture small flying insects. Their often far protruding marginal tentacles are also able to bend in the range of several seconds; however, not within fractions of a second (example *D. scorpioides*). Therefore, we limited ourselves to the basal rosettes with glue-free snap-tentacles. To trigger the bending we touched the tentacle heads with a needle.

We carried out the experiments inside a sun-shaded room at a temperature of 22-24°C and artificial 150 Watt LED workplace lighting. Temporarily, when we needed light that is more intense, the temperature underneath the microscope could rise up to 30°C. The test plants came directly from our greenhouse with an afternoon temperature of 20-26°C (see material and methods). As expected, only a slight touch was necessary to trigger a complete and very fast movement with *D. glanduligera*. Its raised tentacle head is unique in the genus (Fig. 5) and works like a foot-switch (Hartmeyer *et al.* 2012). Nevertheless, the compressive stressing destroys the hinge-zone; therefore, each catapult bends only once. In contrast, the snap-tentacles of the pygmy Sundews extend again within a day and function repeatedly. However, it was more difficult to trigger them; especially the 12 larger species often needed multiple hits. Several tentacles did not react, or an initial bending stopped after just a short time. Touching the tentacle head only once was apparently not sufficient for complete bending. With further touching, the movement continues. Presumably, the repeatable functioning catapults, especially in the larger species examined, need quite a few action potentials for a complete 180°-bending. Triggered by a receptor potential that occurs when the sensitive head is irritated, such action potentials are electric impulses, which flow through the plant tissue (Fig. 6). In this particular case, triggered in the tentacle head it actuates a hydraulically powered bending (calculated by Poppinga *et al.* 2012) of the underneath hinge-zone.

To achieve an uninterrupted bending, we decided to add chemical stimuli together with the mechanical. Lichtner *et al.* (1977) refer to Darwin's experiments and mention a response to sodium ion, ammonium ion, and urea. Therefore, we applied minute pieces of crushed fish food flakes (salty protein with traces of ammonium from decomposition) on the tentacle head. The presence of fish food turned out to be a smart move: With very few exceptions, all tentacles reacted after some seconds with a complete bending to the leaf center. Obviously, the chemical stimulation produced a cascade of action potentials causing a complete bending, unlike single mechanical stimulation. However, the speed of the catapults varied even within the same species, but now it was possible to determine the response time between the application of fish food and the start of bending relatively exactly. It is 1-2 seconds for the three smallest and fastest species *D. microscapa*, *D. occidentalis*, *D. pygmaea* and 3-12 seconds for the larger ones.

To apply the minute pieces of fish food, adhering to a needle tip, onto the less than



Figure 5: *Drosera glanduligera* tentacle with raised head (SEM).

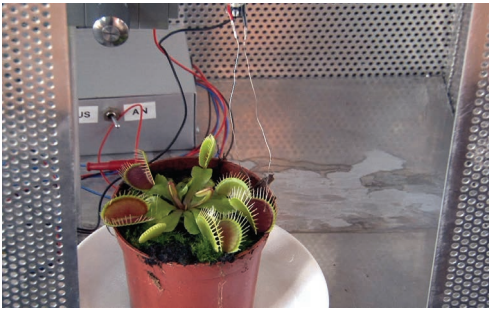


Figure 6: Measurement of action potentials on *Dionaea* lobes in 2009 (a rewarded experiment by students of the Friedrich-König-Gymnasium in Würzburg, Germany).

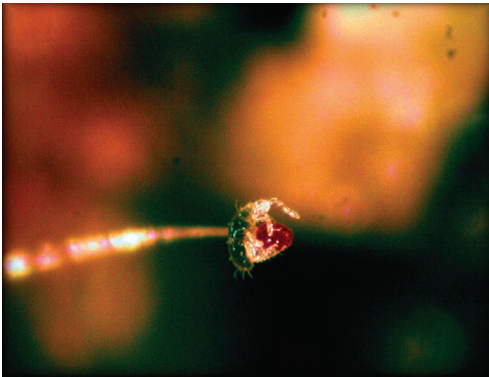


Figure 7: Juvenile springtail sticks to the glue-free snap-tentacle head of *Drosera miniata* in spite of the use of its furcula to escape.

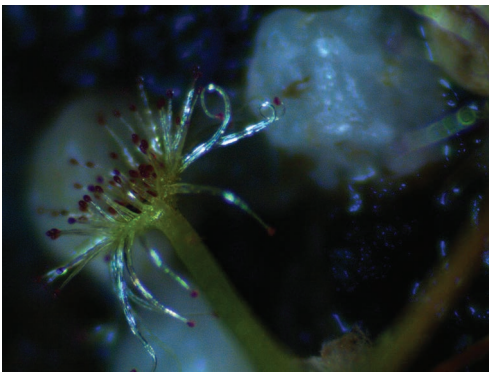


Figure 8: *Drosera enaebba* with snap-tentacles curled by warmth.

100 μm sized tentacle heads under the microscope turned out to be easier than expected. Mostly a slight touch was sufficient and it adhered easily to the mucus-free dry tentacle head. However, in some cases the pieces flipped away rapidly, like being repelled. Such a behavior suggests that electrostatic effects may be involved. If the fish food (crushed inside a plastic lid) had an opposite charge, the tentacle head attracted it and application was easy, while an identical static charge rejected the pieces. During our experiments, we could just coincidentally film a quite small springtail that jumped on a tentacle head of *D. miniata* (Fig. 7). The impact was sufficient to trigger the bending and to lift the prey rapidly from the ground, but then the movement stopped. The action potentials were probably insufficient for a complete bending because the victim appeared to be too small. Surprisingly, even now, the little springtail was not able to release itself from the tentacle. Circling around the head it adhered although it desperately used its catapulting furcula to escape (demonstrated on “Catapults in Pygmyland”). This observation suggests that electrostatic attraction could be involved for prey capture with snap-tentacles. Their speed depends strongly on the condition of the plant and the temperature. Generally, cool nights and moderate day temperatures up to 25°C seem to stimulate a good function. If the temperature is too high, for instance caused by the lighting during the examination, the thin snap-tentacles tend to curl (Fig. 8) and do not bend anymore or only very slowly. Unfortunately, we had no high-speed camera, and the growing and plant conditions in spring 2015 were not optimal. In addition, the 13 species propagated from gemmae (see Table 1) were very young, only about 8-10 weeks old. Bending caused by touching was mostly not complete (180°) and when triggered with fish food we observed varying speed even in the same species. Therefore, it was impossible to de-

termine realistic maximum bending speeds of individual species. However, our experiments allow assessing if a catapult moves faster, in a similar range, or slower than a snap-shutting *Dionaea* trap (about 0.1 to 2 seconds). With catapults achieving the amazing speed of 75 milliseconds (Poppinga *et al.* 2012) for a 180°-movement, so far *D. glanduligera* remains the fastest and largest catapult-flypaper trap in the genus. Its high-performance catapults are clearly faster than *Dionaea*. Speeds similar to the flytrap, with bending in fractions of a second, are achieved by the catapults of *D. microscapa*, *D. occidentalis*, as well as by the Australian and New Zealand variety of *D. pygmaea*. The larger species showed a movement in the range of approximately 3 to more than 30 seconds; these data are, however, most likely unsuitable to establish maximum speed. *D. pulchella* for instance often moved in 10-25 seconds, but once achieved complete movement within approximately 3-4 seconds. *D. enaebba*, *D. mannii*, and *D. miniata* certainly warrant further experiments as they were not in best condition. To determine the fastest movement of pygmy catapults correctly will most likely need observations at their natural habitat. It would be only logical if electrostatic effects between tentacle head and prey affected the frequency of action potentials and thereby the movement pattern. Many species grow on silica sand, diatomaceous earth, or between laterite pebbles. Certainly, such soils charge electrostatically by friction and thereby the soil-dwelling organisms become charged. Silica sand is quartz, well known for its strong piezoelectric effects generated by friction. However, regarding pygmy *Drosera* we found no publications on such phenomena so far.

Results and Discussion

The terrestrial and larger *Dionaea*, which snaps-shut rapidly by a combination of turgor changes that take place in the trap lobes and an elastic instability, is presumably different from the catapulting tentacles of *D. glanduligera* that are small enough to fling prey in fractions of a second solely actuated by hydraulic power (as calculated by Poppinga *et al.* 2012). Remark: Direct measurements on the rate of hydraulic actuation (in case that fast tentacle movement relies additional on a release of elastic energy stored in pre-stressed cells) still have to be undertaken (Poppinga, 2015, pers. comm.).

The features of the catapult-flypaper trap of *D. glanduligera* encouraged us to keep a closer eye at the minute tentacles of the considerably smaller pygmy *Drosera*, focusing at the basal rosettes with mucus-free snap-tentacles. Their rapidly moving structures are only hard to notice with the naked eye; therefore, we examined the cultivated plants with a zoom-microscope.

Our experiments show that in contrast to the erect species in section *Bryastrum*, the acaulescent species do not bend their laminae during prey trapping. Only tentacles are active, exactly as in the closely related catapult-flypaper trap of *D. glanduligera*. Of course, prey-trapping works for all these species often with mucilage only, in this case the catapults remain inactive. Interestingly both mechanisms function independently. Isolated snap-tentacles, which we dissected at the base of the lamina for high-speed filming in 2012, continued to operate properly without the lamina. The action potentials affect only the tentacles and have no connection with the lamina in the species reported by Williams (1976) and very likely these species as well. The tentacles are physically connected just not electrically. Nevertheless, when the catapults are involved, they start a two-stage capture mechanism. The independently acting glue-tentacles perform the second step, no matter if triggered by the impact of the flung prey or a direct touching of an insect. They provide the fixation and correct positioning for digestion like a conveyor belt. This two-stage mechanism is a potential advantage, apparently increasing the availability of nutrients by a larger trapping area in comparison to plants without catapults.

The response time of 8-12 seconds and the 1-2 minutes lasting conveyor belt motion of the sticky apparatus moved at the same level for all examined species. Only the mucus-free catapults

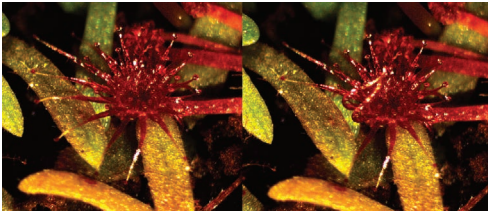


Figure 9: Two catapults of *Drosera occidentalis* moved synchronically.

ing the still remaining big question in the Sundew family (Droseraceae): How could initial passive or slow flypaper traps develop into mucus-free fast snap-traps?

The surviving stages of trap development are well known. Simple straight glue traps like the phylogenetically oldest known Sundews *D. regia* and *D. arcturi*, the discussed hydraulic catapults combined with an initial flypaper lamina and eventually the rapid hydraulically powered mucus-free snap traps of *Aldrovanda* and the rapid lobes of *Dionaea* actuated by a combination of hydraulic movement and snap-buckling (Poppinga *et al.* 2013). The thrilling ability of all three Droseraceae genera to capture prey in fractions of a second, started most likely with the development of broad based marginal tentacles in the plane of the leaf. Different from the erect tentacles on the lamina they are additionally equipped with a hinge-zone that contains the necessary motor cells to perform the fast movement. Possibly, they initially still had sticky heads, because all known catapult-flypaper traps still carry a combination of marginal tentacles with and without mucus producing heads. While the erect glue-tentacles on the leaf surface are able to move slowly in all directions, marginal tentacles are restricted to bending up or down due to the broad hinge-zone, but they are very powerful, and rapidly achieve direct hits.

In this respect, it is noteworthy that *D. glanduligera* and the smallest examined species of section *Bryastrum* were able to move two or even almost all catapults nearly synchronically at once (Fig. 9) like a gripping whole hand. When touching a tentacle more intensely, so that the small leaf totters just like touched by larger prey, several catapults are triggered almost at once (documented on our film “Catapults in Pygmyland”). This behavior is certainly effective to capture struggling prey that is too large for one snap-tentacle only. Simultaneously bending catapults can even be able to fix prey without any mucilage because they form first a circular kind of grid cage and act like securing straps after the described gradual narrowing (Fig. 10). If rapid enough, they even capture prey without any glue and push it onto the sessile digestion glands. That is an important advantage in areas with heavy rain, as well as for temporarily submerged plants. Water washes off the mucilage, so the sticky part of the trapping mechanism becomes obsolete. Only the independent rapid capture mechanism remains active to supply animal nutrition. Our study shows that apart from the sessile digestion glands on the lamina, each single repeatedly catapulting tentacle has all properties known

were able to respond in 400 milliseconds (*D. glanduligera*) up to two seconds, and moved significantly faster. We never found glue-tentacles that bend in fractions of a second. Our observations revealed two distinct and independent acting capture mechanisms in one trap. One is fundamental, mucilage-based and relatively slow; the second appears derived, mucus-free and based on rapid movement. This is of interest regard-



Figure 10: Several snap-tentacles acting like securing straps on *Drosera burmannii*.

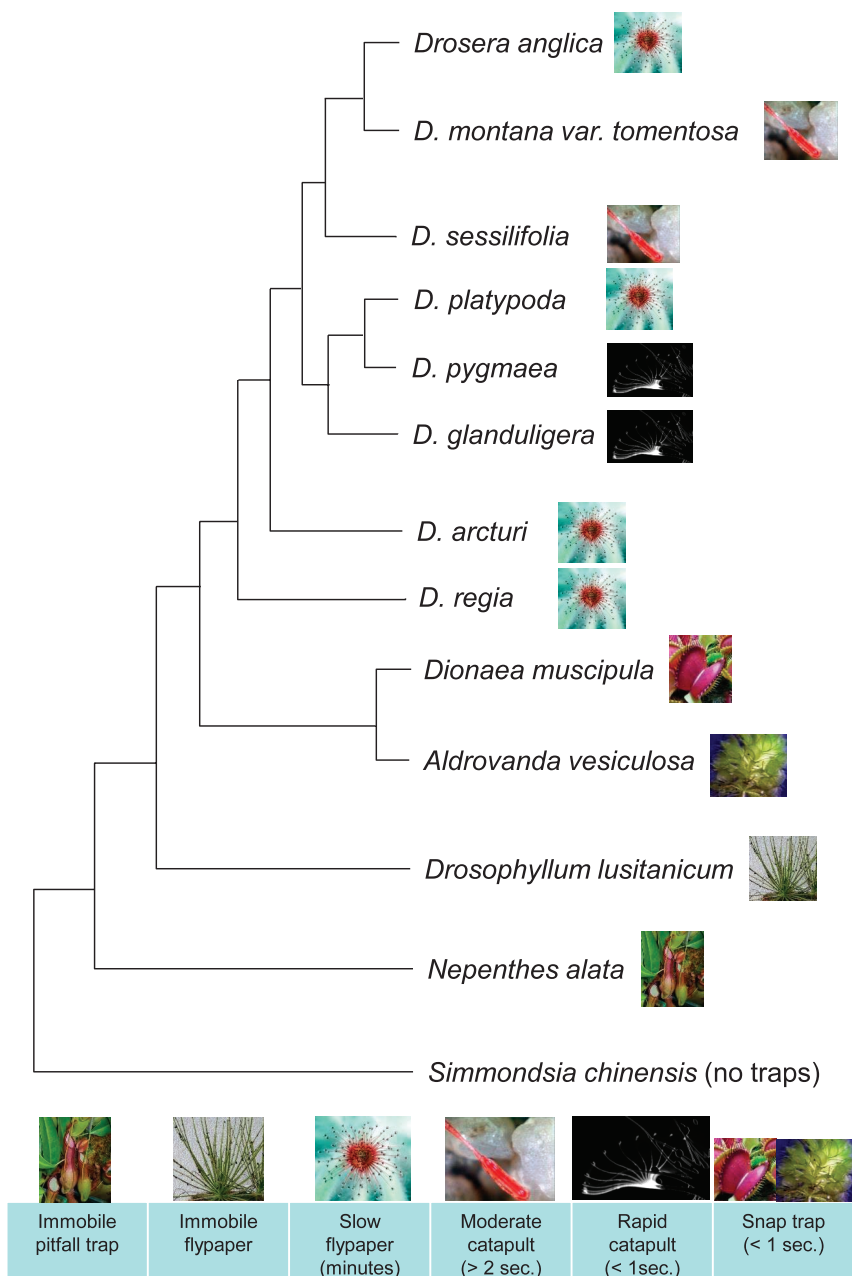
from the trap of the Waterwheel Plant, *Aldrovanda*. Both are touch-sensitive and able to recognize chemical stimuli like ammonium ions or sodium chloride (Williams 1976). Both are able to move initially rapidly as well as gradually for a narrowing by a cascade of action potentials to perform a cost-benefit calculation, deciding to continue/close or to bend back/reopen. *Aldrovanda* lobes show a phase of narrowing after the initial closing: “After the initial rapid closure, the lobes continue moving through a number of phases. After a period of additional slow closure lasting some 30-60 seconds, in which the outer zones of the two lobes press together completely, the free-side lobe becomes concave” (Cross 2012, p. 51). Finally, yet importantly, the existence of *Aldrovanda*, the observations on submerged *Dionaea*, and our own experiments with submerged *D. capensis* (see below) prove clearly that Droseraceae traps function underwater. These facts raise the question: Did an essential change from flypaper traps to snap traps go through an aquatic stage?

Even temporarily submerged *Drosera* able to capture prey with a simultaneously rapid snapping of their catapults have to avoid a loss of nutrition by water flow. Therefore, there is a selective advantage in closing the gaps between the single catapults by merging the bars of the arising grid formed by the tentacles to achieve a sealed digestion cavity to withhold enzymes and nutrition. That would at the same time lead to a perfect simultaneous bending of the now connected catapults, improving the capture of larger prey. Particularly noteworthy in this context is that catapult-flypaper traps like *D. glanduligera* possess a pronounced patelliform digestion cavity in the leaf center, able to contain prey with the volume of 3-4 *Drosophila*, vanishing totally inside. Our experiments conducted in 2012 show that the activated overlying tentacles often notably close the opening after the deposition of prey. Therefore nutrients can be effectively withheld in case of heavy rain or when temporarily submerged. Our experiments in standing water show that even the leaves of *D. capensis* are able to roll in (applied immobile) prey underwater and make use of parts of the nutrients. The leaves remained curled for 2-4 days before they reopened, indicating that a significant quantity of nutrients reached the digestion glands even submerged. Optimizing the closure of the existing large digestion cavity and using the rapid catapults for prey capture would change the former terrestrial or amphibian catapult-flypaper like trap into an underwater working mucus-free snap-trap with lobes. It is that way roughly comparable with a primitive *Aldrovanda* trap. In this perspective, the development of straight snap traps from a *Drosera*-like extinct ancestor of all meanwhile phylogenetically independent Droseraceae clades, possessing simultaneously rapid bending catapults in temporarily submerged areas could be a possible and even plausible event. From an evolutionary point of view, a submerged useless flypaper apparatus became obsolete while the independent acting rapid and mucus-free capture mechanism prevailed successfully (see Table 2).

We do not speculate that initial snap-traps developed from pygmy *Drosera* or looked and functioned like *A. vesiculosa*, which is already highly adapted to straight aquatic conditions. Multifold aquatic snap traps appeared in the past. The surprisingly found trap of the about six million years old fossil of the extinct *A. inopinata* differs in parts. It is for instance lacking the trigger hairs (Schlauer 1997). About 20 other meanwhile extinct species of Waterwheel Plants left only seeds or pollen, so their trap morphology remains unknown. Modern molecular analyses of the chloroplast *rbcL* gene, 18S rDNA, ORF2280 (Williams *et al.* 1994; Fay *et al.* 1997; Lledo *et al.* 1998; Rivadavia *et al.* 2003), and the chloroplast *matK* gene (Meimberg *et al.* 1999) meanwhile provided widely accepted phylogenetic trees, which correspondingly show that the snap-traps appeared in the early phylogeny of the Droseraceae. Surprisingly, these cladograms show a reversal of development, placing the emergence of *Dionaea* and *Aldrovanda* before that of simple flypaper traps like *D. regia* and *D. arcturi*. However, Hosam *et al.* (2009) state that the estimation of genetic distances based on six chloroplast intergenic regions led to the conclusion that the chloroplast genome of *A. vesiculosa*

Table 2.

Cladogram based on the most parsimonious tree resulting from parsimony analysis of the combined *rbcL* and 18S rDNA sequences by Rivadavia *et al.* (2002), supplemented by S. Hartmeyer (2015).



matches more closely to that of *Drosera regia* than its sister genus *Dionaea*. They suspect that the inconsistency between genetic distance estimates based on nuclear and cytoplasmic markers may reflect a chloroplast capture (e.g., by hybridization) because his result is inconsistent with Rivadavia *et al.* (2003) who conclude that the sister relationship of *Aldrovanda* and *Dionaea* indicates a single evolutionary origin of the snap trap system in plants. Nevertheless, Rivadavia states that it was not possible to elucidate which trap system the common ancestor of these lineages had or whether the two systems evolved independently from non-carnivorous plants. Phylogenetic analyses alone provide without doubt acknowledged cladograms; they are, however, not sufficient to establish the correct position of single organisms unambiguously, especially if they are closely related and have only few mutations in the analyzed genes, just like in the case of *Dionaea* and the particular relation of *Aldrovanda* with *Drosera*. A confirmation of the determined position inside the phylogenetic tree by other methods like physiological, morphological, and functional characteristics is necessary. Therefore, the existing cladograms do not definitely clarify whether the aquatic snap trap, the terrestrial snap trap, and the catapult-flypaper traps, all assigned to separate clades, developed independently from one another or not. Considering that, the hypothesis based on our experiments that the aquatic snap trap could have arisen from submerged simultaneously snapping catapult-flypaper-like traps in the early Tertiary or even in the late Cretaceous, can still be considered possible.

Our experiments prove first the existence of several *Drosera* species with rapidly moving catapults, which appear on all cladograms among the phylogenetically oldest Sundews (*D. glanduligera* and *D. pygmaea*) following the simple glue traps *D. regia* and *D. arcturi* (most parsimonious tree, Rivadavia *et al.* 2003). It is evident that each catapult shows the same hydraulically powered movement, identical tactile and chemical sensitivity and even a similar narrowing behavior that occur in *Aldrovanda* and *Dionaea*. Therefore, the current cladogram induced impression of an independent convergent evolution of the three Droseraceae genera from an unknown initial flypaper trap appears in a relative perspective. All Droseraceae genera possess a fast moving apparatus; rapid snapping is not a unique function of snap traps. Nevertheless, the development of rapid catapults from slow mucus-tentacles in *Drosera* is obvious. *Drosera* is the type genus of its family, and it has all structures present in the stalked glands of any of the other members (Williams 1976). A scenario that initial and slow flypaper traps like *D. regia* or *D. arcturi* emerged from *Aldrovanda* or *Dionaea* related snap traps is very unlikely. Considering this, we miss a common ancestor in the early Tertiary or late Cretaceous connecting the initial flypaper Sundews with the rapid catapulting *Drosera*. In this regard, the unique ontogeny of the *D. glanduligera* catapults provides an inside view how evolution acts. Other than the seedlings of more derived *Drosera* species that possess mucus-free snap-tentacles directly after the cotyledons, *D. glanduligera* starts with marginal glue-tentacles. Within about 4-6 weeks, the consecutive new leaves show through intermediate forms an ongoing development until functioning mucus-free catapults result (Hartmeyer & Hartmeyer 2010). That indicates an ancestor with straight flypaper traps in the early Tertiary or late Cretaceous. Unfortunately, it is impossible to complete the existing phylogenetic trees by adding that unknown initial DNA. However, assumed as unknown ancestor for the carnivorous genera in Drosophyllaceae, Dioncophyllaceae, and Nepenthaceae, which divided earlier on the cladograms is a plant that most likely had flypaper traps. It is certainly related with the Droseraceae, as all these genera are members of the order Caryophyllales (Meimberg *et al.* 2000).

Another Possible Area for Future Experiments

Our study shows that the importance of function and interplay of tentacles, in particular *Drosera* traps, is still underestimated and demands further examination. Through our experiments we could

prove that pygmy *Drosera* possess rapid catapult-flypaper traps, but there may be even more species in the genus and highly likely in section *Bryastrum*. To look out for more rapid traps is one field for future experiments, as there are still species with prominent snap-tentacles waiting for a closer inspection from a functional morphological point of view. In addition, the question why fish food and springtails adhere to the mucus-free dry tentacle heads is worth further examination. The receptor and action potentials that trigger the rapid movements result mainly from Ca^{++} -ions stored in the tentacle heads and lobes, thus turning those structures into a kind of electrode. How important are electrostatic effects for prey capture? What role do charged soils like silica sand play? That requires eventually a detailed observation of the traps in their natural environment. Another attempt could be to find the responsible genes for the rapid hydraulic movements for a comparative analysis of all rapidly moving Droseraceae traps. Their phylogeny still raises a number of questions, which demand further experiments.

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References

- Bopp, M., and Weiler, E.W. 1985. Leaf blade movement of *Drosera* and auxin distribution. *Naturwissenschaften* 72: 434. (doi:10.1007/BF00404889)
- Cross, A. 2012. *Aldrovanda*. Redfern Natural History Productions. Poole, Dorset, England.
- Darwin, C. 1875. *Insectivorous Plants*. London: John Murray.
- Elansary, H.O.M., Adamec, L., and Storchova, H. 2010. Uniformity of organellar DNA in *Aldrovanda vesiculosa*, an endangered aquatic carnivorous species, distributed across four continents. *Aquatic Bot.* 92: 214-220.
- Fay, M.F., Cameron, K.M., Prance, G.T., Lledo, M.D., and Chase, M.W. 1997. Familial relationships of *Rhabdodendron* (Rhabdodendraceae). Plastid *rbcL* sequences indicate a caryophyllid placement. *Kew Bulletin* 52 (4): 923-932.
- Gibson, T.C., and Waller, D.M. 2009. Evolving Darwin's most wonderful plant: ecological steps to a snap-trap. *New Phytol.* 2009; 183: 575-87; PMID:19573135; <http://dx.doi.org/10.1111/j.1469-8137.2009.02935.x>.
- Hartmeyer, I., and Hartmeyer, S.R.H. 2005. *Drosera glanduligera* – Der Sonnentau mit “Klapp-Tentakeln”. *Das Taublatt* 2005/2: 34-38.
- Hartmeyer, I., and Hartmeyer, S.R.H. 2010. Snap-tentacles and runway lights. *Carniv. Pl. Newslett.* 39: 101-113.
- Hartmeyer, I., Hartmeyer, S.R.H., Masselter, T., Seidel, R., Speck, T., and Poppinga, S. 2013. Catapults into a deadly trap: The unique prey-capture mechanism of *Drosera glanduligera*. *Carniv. Pl. Newslett.* 42: 4-14.
- Hartmeyer, S.R.H., and Hartmeyer, I. (2015). Catapults in Pygmyland. Accompanying documentary film on YouTube: <https://www.youtube.com/watch?v=5k7GYGibdjM>.
- Heubl, G., Bringmann, G. and Meimberg, H. 2006. Molecular phylogeny and character evolution of carnivorous plant families in Caryophyllales – Revisited. *Pl. Biol.* 8: 821-830.

- Hopkin, S.P. 1997. Biology of the Springtails (Insecta: Collembola). Oxford: Oxford University Press.
- Juniper, B.E., Robins, R.J., and Joel, D.M. 1989. The Carnivorous Plants. Academic Press, London.
- Lichtner, F.T., and Williams, S.E. 1977. Prey capture and factors controlling trap narrowing in *Dionaea* (Droseraceae). Amer. J. Bot. 64: 881-886.
- Lledo, M.D., Crespo, M.B., Cameron, K.M., Fay, M.F., and Chase, M.W. 1998 Systematics of Plumaginaceae based upon analysis of *rbcL* sequence data. Syst. Bot. 23(1): 21-29.
- Lowrie, A. 2013. Carnivorous Plants of Australia Magnum Opus Vol. 1-3. Redfern Natural History Productions. Poole, Dorset, England.
- McPherson, S. 2008. Glistening Carnivores - The Sticky-Leaved Insect-Eating Plants. Redfern Natural History Productions. Poole, Dorset, England.
- Meimberg, H., Dittrich, P., Bringmann, G., Schlauer, J., and Heubl, G. 2000. Molecular phylogeny of Caryophyllidae s.l. based on *MatK* sequences with special emphasis on carnivorous taxa. Pl. Biol. 2(2): 218-228.
- Nakamura, Y., Reichelt, M., Mayer, V.E., and Mithöfer, A. 2013. Jasmonates trigger prey-induced formation of 'outerstomach' in carnivorous sundew plants. Proc. R. Soc. B 280: 20130228. <http://dx.doi.org/10.1098/rspb.2013.0228>.
- Poppinga, S., and Joyeux, M. 2011. Different mechanics of snap-trapping in the two closely related carnivorous plants *Dionaea muscipula* and *Aldrovanda vesiculosa*. Phys. Rev. E 84: 041928. doi: 10.1103/physreve.84.041928
- Poppinga, S., Hartmeyer, S.R.H., Seidel, R., Masselter, T., Hartmeyer, I., and Speck, T. 2012. Cataapulting tentacles in a sticky carnivorous plant. PLoS ONE; 7:e45735; PMID:23049849; <http://dx.doi.org/10.1371/journal.pone.0045735>.
- Poppinga, S., Hartmeyer, S.R.H., Masselter, T., Hartmeyer, I., and Speck, T. 2013. Trap diversity and evolution in the family Droseraceae. Landes Bioscience: Plant Signal. Behav. 2013 Jul;8(7):e24685. doi: 10.4161/psb.24685. Epub 2013 Apr 18.
- Rivadavia, F., de Miranda, V.F.O., Hoogenstrijd, G., Pinheiro, F., Heubl, G., and Fleischmann, A. 2012. Is *Drosera meristocaulis* a pygmy sundew? Evidence of a long-distance dispersal between Western Australia and northern South America. Ann. Bot. 110: 11-21; PMID:22641141; <http://dx.doi.org/10.1093/aob/mcs096>.
- Rivadavia, F., Kondo, K., Kato, M., and Hasebe, M. 2002. Phylogeny of the sundews, *Drosera* (Droseraceae) based on chloroplast *rbcL* and nuclear 18S ribosomal DNA Sequences. Proc. 4th International Carnivorous Plant Conference, Tokyo, Japan: 9-13.
- Schlauer, J. 1997. Fossil *Aldrovanda* - Additions. Carniv. Pl. Newslett. 26(3): 98
- Seine, R., and Barthlott, W. 1993. On the morphology of trichomes and tentacles of Droseraceae Salisb. Beitr. Biol. Pflanzen 67: 345-366
- Verbeek, N.A.M., and Boasson, R. 1993. Relationship between types of prey captured and growth form in *Drosera* in southwestern Australia. Aust. J. Ecol. 18: 203-207.
- Watson, A.P., Matthiessen, J.N., and Springett, B.P. 1982. Arthropod associates and macronutrient status of the red-ink sundew (*Drosera erythrorhiza* Lindl). Aust. J. Ecol. 7: 13-22.
- Williams, S.E. 1976. Comparative sensory physiology of the Droseraceae - The evolution of a plant sensory system. Proc. Am. Philos. Soc. 120: 187-204.
- Williams, S.E. 2002. Comparative physiology of the Droseraceae *sensu stricto* - How do tentacles bend and traps close? Proc. 4th International Carnivorous Plant Conference, Tokyo, Japan: 77-81.
- Williams, S.E., Albert, V.A., and Chase, M.W. 1994. Relationship of Droseraceae: A cladistics analysis of *rbcL* sequence and morphological data. Am. J. Bot. 81(8): 1027-1037.