COMMENTARY

The carnivorous bladderwort (*Utricularia*, Lentibulariaceae): a system inflates

Victor A. Albert[1,2,*], Richard W. Jobson[3], Todd P. Michael[4] and Derek J. Taylor[1]

[1] Department of Biological Sciences, University at Buffalo, Buffalo, New York 14260, USA

[2] New York State Center of Excellence in Bioinformatics and Life Sciences, 701 Ellicott Street, Buffalo, New York 14203, USA

[3] Institut des Sciences de l'Evolution, Université Montpellier 2, CNRS UMR 5554, Place E Bataillon, F-34095 Montpellier, France

[4] Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854, USA

Abstract

Carnivorous plants inhabit nutrient-poor environments, where prominent targets of prey capture are organic nitrogen and phosphorus.

Some carnivorous plants also acquire carbon from their victims. A new report focusing on *Utricularia*, the bladderwort, demonstrates that carbon assimilated from photosynthesis is paradoxically secreted into the trapping environment, where it may help to support a mutualistic bacterial community. This bacterial community may also secrete allelochemicals that attract microcrustaceans which bear a strong overt resemblance to bladderwort traps. Furthermore, *Utricularia* and its sister genus *Genlisea* share anomalous molecular evolutionary features, such as highly increased rates of nucleotide substitution and dynamic evolution of genome size, from approximately 60–1500 megabases depending on the species or even population. A mechanistic hypothesis, based on the mutagenic action of reactive oxygen species (ROS) is proposed to underlie these phenomena, involving error-prone repair at the level of DNA bases and double-strand breaks. It is argued that these plants are prime candidates for further research on the complexities of plant physiology associated with carnivory, metagenomic surveys of trap microbial communities, novel plant nitrogen/nutrient utilization pathways, the ecology of prey attraction, whole-plant and trap comparative development, and, finally, evolution of the minimal angiosperm genome.

> 'What a wonderful and long-continued series of variations must have led up to the perfect "trap" in *Utricularia*, while at any stage of the process the same end might have been gained by a little development of roots and leaves, as in 9999 plants out of 10 000!'
> Alfred Russel Wallace, from a letter to Charles Darwin dated 21 July 1875 (Marchant, 1916, p. 234)

The carnivorous bladderwort, (*Utricularia*, Lentibulariaceae), a favourite of Darwin's (Darwin, 1875), has proved to be an excellent system for studying biological processes related to plants trapping animals for nutritive purposes. Carnivorous plants are typically inhabitants of

* To whom correspondence should be addressed: E-mail: vaalbert@buffalo.edu

nutrient-poor environments, and it is well known that organic nitrogen and phosphorus are prominent targets of prey digestion (Ellison and Gotelli, 2009). It has also been shown that some carnivorous plants acquire carbon from their prey, in addition to that provided by photosynthesis (Juniper *et al.*, 1989; Ellison and Gotelli, 2009). On page **101** of this issue, Dagmara Sirová *et al.* report on the paradoxical secretion of photosynthetically absorbed carbon into the *Utricularia* bladder trap environment (Sirová *et al.*, 2009a). Given this capacity, it is easy to imagine that carbon acquired from *carnivory* may also make its way into the negatively-pressured, liquid-filled bladders. Sirová's team used ^{13}C labelling experiments to document that assimilated carbon is first allocated to apical growth, as expected for a plant in active development, but that as a function of shoot age, more and more soluble carbon is released into the bladder environment than into the vegetative structures that bear them. Still, the highest absolute rates of carbon release occur in the youngest traps, which is understandable, given that they will have the longest triggering/resetting lifetimes. Sirová *et al.* compare the phenomenon observed to carbon exudation by roots into the rhizosphere, which permits regulatory influence on local microbial communities. The intriguing issue with *Utricularia* is that the plant is rootless! Previous histochemical studies have detected the excretion of sugars from bladders, but the investigators were unable to uncover any reason for this phenomenon (Cheema *et al.*, 1992).

Sirová and collaborators (Sirová *et al.*, 2009b) have previously demonstrated that a large microbial community is associated with living bladderwort traps, and their hypothesis was that these bacteria play a mutualistic role in a complex food web that exists in and around the bladders. They compared bladderworts with corals, which 'cultivate' food from a microecosystem they create themselves by local water filtration (Ferrier-Pagès *et al.*, 1998). In addition to bacteria, *Utricularia* traps collect a diverse flora and fauna of microplankton, as well as detritus. Dissolution of this 'periphyton' into vital nutrients in the enclosed bladder environment would certainly be aided by bacterial enzymes. In the work presented in this issue, the suggestion is made that carbon secretion and enhanced periphyton utilization in the sealed bladder environment, (i.e. after prey stimulation causes water engulfing and trap closure) permits bladderworts to live relatively competition-free in habitats otherwise occupied by rooted macrophytes. Regardless of the strength of such an effect, Sirová *et al.*'s carbon allocation experiments break new ground in pointing attention to the complexity of *Utricularia*'s amazing bladders (Sirová *et al.*, 2009a).

The nutrition of *Utricularia* still holds many mysteries. Do these trap-dwelling bacteria secrete allelochemicals in addition to serving the roles discussed by Sirová *et al.*? Cohn (1875) first proposed a chemical prey attractant for *Utricularia*, and bladder trichome secretions have since been shown to attract epibiotic rotifers (Wallace, 1978).

Darwin (1875), noted yet another insight: aquatic *Utricularia* bladders bear a striking resemblance to microcrustaceans. The bladder shape, surface reticulations, stalk, and especially the antennae and bristles resemble microcrustacean anatomy. Interestingly, the bladders most closely resemble the littoral zone cladocerans (bosminids and chydorids) that are frequently found or overrepresented in bladders (Guiral and Rougier, 2007; Alkhalaf *et al.*, 2009); see the silhouettes of clade-specific trap forms in Fig. 1. Moreover, experiments reveal that the cladoceran-like structures of bladders significantly improve the capture rates of cladocerans (Meyers and Strickler, 1979; Harms, 1999; Jobson and Morris, 2001). Some fish apparently selectively graze bladders (Walker, 2004). *Utricularia* has even been reported to capture and kill small fish, but there is a dearth of evidence for predation
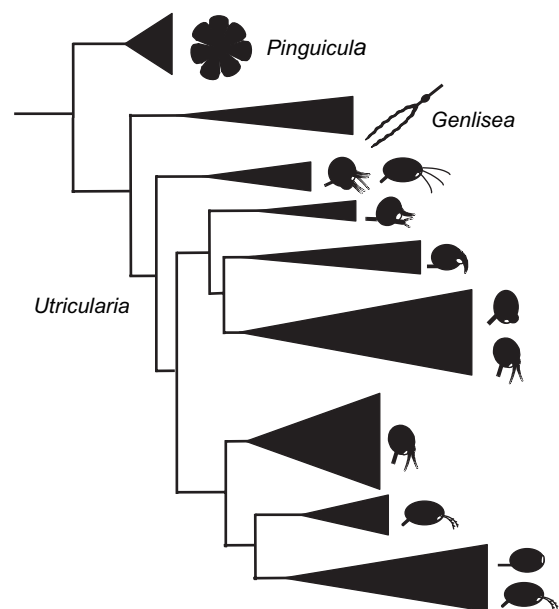
Fig. 1. Diagrammatic representation of Lentibulariaceae phylogeny, including sillhouettes of general trap types per lineage. The tree is modified from Jobson and Albert's 2002 research (Jobson and Albert, 2002). *Pinguicula*, the butterworts, form rosettes of sticky leaves that capture both animals and detritus. *Genlisea*, the corkscrew plants, have specialized vegetative organs that include an eye-dropper-like bulb above bifurcate, twisting, and partly open traps. *Utricularia* species have active suction bladders that are triggered by prey movements. General trap forms among bladderworts are strikingly lineage-specific, with a few exceptions. Branch lengths, (inferred nucleotide substitutions) on the tree are approximately relative to each other, with stem lineages leading to generic and species groups indicated by single lines, and minimum–maximum branch lengths within these clades represented by the starts and ends of each triangle. Triangle size represents the taxonomic sampling in the Jobson and Albert study. Note that branch lengths are considerably greater among the bladderworts and corkscrew plants than they are within *Pinguicula*, despite the fact that the stem lineages leading to *Pinguicula* on the one hand and *Utricularia* + *Genlisea* one the other are quite comparable.

on fish in nature (Moseley, 1884; Gudger, 1947). The study of the ecomorphology of *Utricularia* is still immature, but the simple 'mousetrap' analogy for *Utricularia* is clearly too simple.

Other new lines of evidence put *Utricularia* biology in the spotlight. In 2006, Lubomír Adamec, a coauthor of the featured Sirová *et al.* (2009a) article, found that *Utricularia* bladders have immensely greater respiration, while exhibiting far lower photosynthetic rates than vegetative tissues (Adamec, 2006). The respiratory chain of mitochondria, normally coupled to electron transport, is one of the principal means that cells gain their energy for performing various activities. Electron transport drives a chemiosmotic pump that causes sequestration of protons in the mitochondrial intermembrane space, whereafter these positive charges enter the mitochondrial lumen to catalyse the phosphorylation of adenosine diphosphate into the cellular 'currency' of energy, adenosine triphosphate, (ATP). The 'gatekeeper' of the rate of oxidative phosphorylation is the enzyme cytochrome *c* oxidase (COX), positioned one step before ATP synthase. Two years before Adamec's report, Jobson and colleagues reported on the possibility that, due to specific amino acid positions under positive Darwinian selection, COX structure and function may be altered in *Utricularia* and some species of its sister genus, *Genlisea* (the corkscrew plant), to elevate energy production during times of need (Jobson *et al.*, 2004). Specific hypotheses were that the uniquely changed residues [two contiguous cysteines (C) found only in one other organism in the entire National Center for Biological Information database] could alter the dissociation kinetics between COX and cytochrome *c*, and this hypothesis was later extended by Liisa Laakkonen and

colleagues with a model whereby a redox switch at a vicinal C-C disulphide could actually cause a conformational change that would interfere with proton pumping, thus decoupling that process with electron transport (Laakkonen *et al.*, 2006). In this way, the intermembrane space was envisioned as a capacitor holding massive positive charge until ATP was needed, i.e. to pump water out of traps after their firing.

But sequestering protons has its cellular consequences. Oxidative phosphorylation is an imperfect process; electrons sometimes leak into the mitochondrial lumen to reduce oxygen only partially, forming reactive oxygen species (ROS) instead of the normal and harmless water molecule. Moreover, the leakiness of the electron transport chain will increase when a greater charge potential exists between the intermembrane space and the lumen, as with the capacitor hypothesis above. ROS can be extremely harmful to cells, producing damage to DNA at both the nucleotide and whole-helix levels. Repair is inherently an error-prone process, and an expensive one for cells. Damage at the nucleotide level must either be repaired, or the mutations fixed. ROS can also lead to double-strand breaks, which, in plants, can lead either to deletions or to insertions of DNA via homologous or non-homologous end-joining (Puchta, 2005; Pacher *et al.*, 2007). Intriguingly, and fitting quite well with the ROS hypothesis above, data suggest that both processes may be occurring rampantly in bladderworts.

Richard Jobson and Victor Albert first reported in 2002 that nucleotide substitution rates in *Utricularia* and *Genlisea* were radically sped up relative to their sister group, the carnivorous butterworts (*Pinguicula*, a sticky-leaf
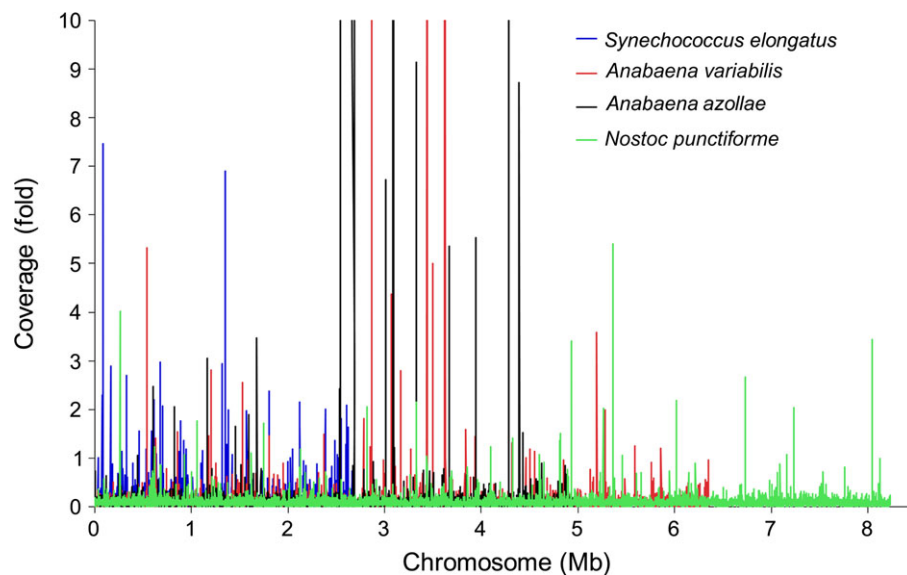
Fig. 2. Next generation high-throughput sequencing reveals periphyton at the nucleotide resolution. Whole *Genlisea aurea* plants, including traps and leaves, were sequenced with the SOLiD high-throughput sequencing platform. Resulting reads were mapped to a panel of complete bacterial and cyanobacterial genomes. Presented is the coverage per base (*y*-axis) annotated over four cyanobacterial genomes: *Synechococcus elongatus*, *Anabaena variabilis*, *Anabaena azollae*, and *Nostoc punctiforme* (*x*-axis, in megabase-pairs, Mb). It can be concluded that close relatives of these nitrogen-fixing bacteria species were associated with the *Genlisea* samples. Sirová *et al.*, (2009b) noted the presence of cyanobacteria in their analyses of *Uticularia* periphyton contents.

trapper), across the genomes of all three cellular compartments: the mitochondrial, plastid, and nucleus (Jobson and Albert, 2002; see also Müller *et al.*, 2005). This systemic effect was, at the time, attributed to enhanced speciation rates among the bladderworts, with their incredible diversity of body plans (Taylor, 1989), but the ROS mutation hypothesis presented here is a simpler causative explanation.

Now to wax ecstatic, an amazing twist on *Utricularia* and *Genlisea* was recently uncovered by Johann Greilhuber and colleagues: some species possess the smallest haploid angiosperm genomes known, at *c*. 60 megabases (Mb), less than one-half that of *Arabidopsis*, with many bacterial-size chromosomes that vary widely in number between species (Greilhuber *et al.*, 2006). There are still two more punch lines: (i) *Utricularia* and *Genlisea* species range in genome size from approximately 60 Mb to 1500 Mb, and (ii), as studied by Todd Michael, one of the species with the very smallest genomes, (*Genlisea aurea*) ranges from 60 Mb to 90 Mb to 120 Mb among three different geographically separated populations! Some preliminary work also suggests some populations of bladderworts have smaller than 60 Mb genomes. The dynamism of bladderwort and corkscrew-plant genome size suggests a minimal genome hypothesis whereby the downstream mechanisms that may be leading to DNA double-strand breaks, as indicated by the COX C-C amino acid motif, drive the turnover of non-essential genome space. This hypothesis could explain the wide span of genome sizes in the *G. aurea* populations and provide a platform to understand the evolution and specialization of genome structure in relation to nutrient acquisition. Despite the rather enormous genome size differences among such closely related genera, species, and populations, all Lentibulariaceae manage to make inflorescences and flowers, i.e. these genomic disparities must not interfere with the majority of normal plant processes. Clearly then, minimal bladderwort and corkscrew-plant genomes will provide insight into the minimal set of angiosperm genes.

Bladderworts grow in such a diversity of habitats, ranging from wet terrestrial to affixed on rocks in streams, attached at lake bottoms, floating aquatic, epiphytic in cloud forests, and even within the tanks of tree-dwelling bromeliads, and they bear their bladders species-specifically, but so heterogeneously, on stolon-like rhizoids, at leaf tips, on leaf edges, from inflorescence floats or leaf lamina surfaces, or even in entire leaf positions during embryonic shoot development (Taylor, 1989). As such, Sirová *et al.*'s article (Sirová *et al.*, 2009*a*) reminds us that *Utricularia* and *Genlisea* are prime candidates for further research on the complexities of plant physiology associated with carnivory, metagenomic surveys of periphyton communities (see a first experiment in Fig. 2), novel plant nitrogen/nutrient utilization pathways, the ecology of prey attraction, whole-plant and bladder comparative ontogeny, and, finally, evolution of the minimal plant genome.

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