Fluorescence Labelling of Phosphatase Activity in Digestive Glands of Carnivorous Plants

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Abstract: **A new ELF (enzyme labelled fluorescence) assay was applied to detect phosphatase activity in glandular structures of 47 carnivorous plant species, especially Lentibulariaceae, in order to understand their digestive activities. We address the following questions: (1) Are phosphatases produced by the plants and/or by inhabitants of the traps? (2) Which type of hairs/glands is involved in the production of phosphatases? (3) Is this phosphatase production a common feature among carnivorous plants or is it restricted to evolutionarily advanced species? Our results showed activity of the phosphatases in glandular structures of the majority of the plants tested, both from the greenhouse and from sterile culture. In addition, extracellular phosphatases can also be produced by trap inhabitants. In *Utricularia,* activity of phosphatase was detected in internal glands of 27 species from both primitive and advanced sections and different ecological groups. Further positive reactions were found in *Genlisea, Pinguicula, Aldrovanda, Dionaea, Drosera, Drosophyllum, Nepenthes,* and *Cephalotus*. In *Utricularia* and *Genlisea,* enzymatic secretion was independent of stimulation by prey. *Byblis* and *Roridula* are usually considered as "proto-carnivores", lacking digestive enzymes. However, we found high activity of phosphatases in both species. Thus, they should be classified as true carnivores. We suggest that the inflorescence of *Byblis* and some *Pinguicula* species might also be an additional "carnivorous organ", which can trap a prey, digest it, and finally absorb available nutrients.**

Key words: **Carnivorous plants, digestion, enzyme-labelled fluorescence, glandular cells, mutualism, enzymatic activity, trap functioning, Lentibulariaceae.**

Introduction

Carnivorous plants have the ability to trap animals and utilize nutrients from their carcasses. Carnivory is known to occur within different phylogenetic groups of angiosperms and has arisen as an adaptation to habitats with nutrient-deficient substrates (Givnish et al., 1984; Juniper et al., 1989; Albert et al., 1992; Adamec, 1997). Animals are an additional source of N, P, S, K, and Mg for carnivorous plants; some can even take up more than 50% of their N and P from prey (Lollar et al., 1971; Friday and Quarmby, 1994; Adamec, 1997). Both growth and flowering of carnivorous plants are promoted by feeding on animals, as was demonstrated under both greenhouse and field conditions (Darwin, 1978; Karlsson and Carlsson, 1984; Karlsson and Pate, 1992; Krafft and Handel, 1991; Adamec, 1997; Zamora et al., 1997; Otto, 1999; Jobson et al., 2000). During evolution, carnivorous plants have formed several types of specialized traps (for detailed descriptions see Darwin, 1875; Lloyd, 1942; Juniper et al., 1989).

So far, little is known about the production of hydrolytic enzymes and the mechanisms of digestion in carnivorous plants. The subcellular localization of hydrolytic enzymes has been studied in several genera, e.g., by Heslop-Harrison (1975, 1976 a, b). Detailed information is available only for *Dionaea, Nepenthes,* and *Pinguicula* (reviewed by Juniper et al., 1989). Enzymes in the traps might also be produced by associated organisms like bacteria, fungi, algae, and invertebrates which are often detected in the trap environment of both aquatic and terrestrial species (Fish, 1983; Beaver, 1983; Bradshaw, 1983; Istock et al., 1983; Juniper et al., 1989; Ellis and Midgley, 1996; Lowrie, 1998; Hartmeyer, 1998; Anderson and Midgley, 2002; Ellison et al., 2003; Sirová et al., 2003; Płachno et al., 2005 b). The role of these organisms in the digestion process is under debate (e.g., Richards, 2001). It is an open discussion how important a role these organisms play for these plants. For instance, Richards (2001) proposed that *Utricularia purpurea* might have more benefits from mutualism than carnivory. It is also well known that pitchers of carnivorous plants are phytotelms with a specific rich fauna and flora (Juniper et al., 1989; Ellison et al., 2003). Nevertheless, plants with adhesive types of traps also possess specific inhabitants: e.g., *Setocoris*/*Cyrtopeltis* species on the leaves of *Drosera* and *Byblis,* or *Pameridea* species on the leaves of *Roridula*. The latter insects first rob the prey but later defecate on the plant surface, and the host plant can then use these nutrients (Ellis and Midgley, 1996; Lowrie, 1998; Hartmeyer, 1998; Anderson and Midgley, 2002). Finally, certain enzymes from external aquatic environments might be accumulated in some types of traps (Sirová et al., 2003).

The Lentibulariaceae is the biggest carnivorous family, with three genera *(Pinguicula, Utricularia,* and *Genlisea)* and about 300 species (Jobson et al., 2003; Müller et al., 2004; Mabber-

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ley, 2000). In both *Utricularia* and *Genlisea,* little is known about the origin of digestive enzymes in the traps. There are few *in-situ* studies on the secretory glands of a few species (Vintéjoux, 1974; Heslop-Harrison, 1975, 1976b; Parkes, 1980 after Juniper et al., 1989; Sirová et al., 2003). In the latter genus, the ability to attract and catch diverse prey (both protozoa and metazoa) has recently been proved experimentally (Płachno et al., 2005b). The process of prey digestion is even less understood in the other carnivorous families, such as Droseraceae, Nepenthaceae, Cephalotaceae, or Byblidaceae (reviewed by Juniper et al., 1989).

Hydrolysis of phosphate esters is a critical process of phosphorus metabolism at cellular, organism, and ecosystem levels. Phosphatases (phosphomonoesterases) represent a broad group of enzymes that catalyze the hydrolysis of phosphate esters (Feder, 1973). Acid phosphatases are common plant enzymes of low substrate specificity that appear to be important in the production, transport, and recycling of phosphorus (Duff et al., 1994). So far, a few authors (Clancy and Coffey, 1976; Robins and Juniper, 1980; Sirová et al., 2003) have studied production of acid phosphatases by carnivorous plants. While proteases may not occur in some typical carnivorous plants (e.g., in *Utricularia,* Sirová et al., 2003, or *Byblis,* Hartmeyer, 1997), we suggest the phosphatases as model digestive enzymes in this group. In our opinion, the phosphatases are indispensable for phosphate mobilization from prey carcasses and phosphate uptake may be essential for many carnivorous plants (e.g., Adamec, 1997). Last but not least, a commercial phosphatase substrate is available for the ELF (enzyme labelled fluorescence) assay, which exhibits both better resolution and sensitivity than classical histochemical techniques (Larison et al., 1995; Cox and Singer, 1999; van Aarle et al., 2001; Nedoma et al., 2003; Štrojsová et al., 2003; Štrojsová and Vrba, 2005).

In this study we test the following hypotheses: (1) phosphatases are produced by the plants or by inhabitants of the traps; (2) phosphatases are produced by glandular hairs inside the traps; and (3) phosphatase production is a common feature among carnivorous plants, even more frequent than protease production. Hence, we screened the following carnivorous genera for phosphatases: *Utricularia, Genlisea, Byblis, Nepenthes, Drosera, Aldrovanda, Dionaea, Cephalotus, Brocchinia,* and the putative carnivorous genera *Stylidium* (Darnowski, 2002; Darnowski et al., 2002) and *Roridula* (Ellis and Midgley, 1996).

Materials and Methods

Plants were obtained from greenhouse collections of the Department of Plant Cytology and Embryology and the Botanical Garden of the Jagiellonian University in Cracow, Poland, the Institute of Botany in Třeboň, Czech Republic, and the commercial grower Bestcarnivorousplants in Dobroslavice, Czech Republic (http:/www.bestcarnivorousplants.com), who also provided axenic *in-vitro* cultures. The species investigated are given in Table **1**. Unstained samples were used to check possible autofluorescence.

The traps were hand-sectioned with a razor blade and assayed with the ELF®97 phosphatase substrate (ELFP, Molecular Probes) following the protocol of Nedoma et al. (2003), with minor modifications. The traps were incubated in the substrate solution (250 µM of ELFP in distilled water) at room temperature in dark conditions for 5, 10, and 15 min. Then the traps were screened for green fluorescence in an epifluorescence microscope (Nikon Optiphot-2 or Nikon Eclipse E 800 with the UV-2A filter: Ex 330–380, DM 400, BA 420). Documentation was made on Fujichrome Provia 400 and Sensia 200 slide films as well as by a Nikon FDX-35 digital camera.

We screened traps of 46 species from 11 genera of carnivorous plants for phosphatase activity. We focused on the family Lentibulariaceae because of the outstanding taxonomic and functional diversity of this group of carnivorous plants and, for comparison, also included plants from other families with different types of traps. In addition, we assayed four axenic *in vitro* cultures.

Results

An overview of the results is given in Table **2**.

Eel and suction traps

In *Genlisea,* activity of phosphatases was detected in terminal cells of glands in the bulb (Fig. **1**), neck, arms, and in the trap openings in all species tested. In bulb hairs of the species of the subgenus *Tayloria,* enzymes are most active in the upper part of the radial walls of the terminal cells. Furthermore, we detected phosphatase activity in some stalked and sessilie glands of the inflorescence where small Dipterans were trapped and killed.

In all examined *Utricularia* species, quadrifid glands inside the traps produced phosphatases. The signal of activity was generally very intense, except for quadrifid glands of *U. warburgii* where the observed signal was weak. The enzymatic activity was also detected in arms of bifid glands, terminal cells of both pavement epithelium hairs, and external glands in some species. Especially high activity was found in large pyramidal pavement hairs of *Utricularia multifida*. Cyanobacteria and algae in the utricles (Fig. **2**) also showed phosphatase activity. However, they could not be the only source of phosphatases because quadrifids from *in vitro* cultured plants also showed positive reactions.

Adhesive traps

In *Pinguicula,* intensive phosphatase activity was found in the terminal cells of the sessile glands in both leaves and inflorescences. The enzymatic activity was connected with the upper part of the terminal cells. In stalked glands, activity of phosphatases was less pronounced than in sessile glands and was limited to their outer and radial walls. A positive response was also found in the terminal cells of hydathodes (Fig. **3**).

In *Drosophyllum,* we observed high phosphatase activity in both walls and cytoplasm of stalked and sessile glands. In the tentacles of *Drosera,* a positive reaction for phosphatases occurred only in the secretory head. In *D. binata,* we detected fluorescence directly in the outer walls, including the wall ingrowths.

In *Byblis,* phosphatase activity was found in the sessile glands (Fig. **4**) all over the shoot. In *Roridula,* phosphatases were missing in the tentacles, whereas the epidermis showed strong

Table 1 Carnivorous plant species investigated in this study. Species marked with * were from sterile culture

Family	Genus	Species
Lentibulariaceae	*Pinguicula*	*P. vulgaris ssp. bicolor, P. moranensis, P. gypsicola*
	Utricularia sect. *Polypompholyx*	*U. multifida*
	Utricularia sect. *Pleiochasia*	*U. dichotoma, U. monanthos, U. volubilis*
	Utricularia sect. *Nigrescentes*	*U. warburgii*
	Utricularia sect. *Calpidisca*	*U. livida, U. sandersonii*
	Utricularia sect. *Lloydia*	*U. pubescens*
	Utricularia sect. *Aranella*	*U. fimbriata*
	Utricularia sect. *Setiscapella*	*U. subulata*
	Utricularia sect. *Foliosa*	*U. tricolor, U. tridentata*
	Utricularia sect. *Psyllosperma*	*U. calycifida, U. longifolia, U. praelonga*
	Utricularia sect. *Orchidioides*	*U. alpina*
	Utricularia sect. *Iperua*	*U. humboldtii**
	Utricularia sect. *Lecticulata*	*U. resupinata*
	Utricularia sect. *Utricularia*	*U. gibba, U. floridana, U. intermedia, U. stygia, U. minor**, *U. dimorphantha, U. radiata, U. foliosa, U. foliosa**
	Utricularia sect. Vesiculina	*U. purpurea*
	Genlisea sub. *Tayloria*	*G. lobata, G. violacea* f. Giant, *G. lobata* × *G. violacea* f. Giant, *G. lobata* × *G. violacea* f. Giant*, *G.* sp. "Itacambira Beauty"
	Genlisea sub. *Genlisea*	*Genlisea pygmaea, Genlisea hispidula*
Byblidaceae	*Byblis*	*B. liniflora*
Drosophyllaceae	*Drosophyllum*	*D. lusitanicum*
Droseraceae	*Drosera*	*D. pygmaea, D. binata var. multifida*
	Aldrovanda	*A. vesiculosa*
	Dionaea	*D. muscipula*
Roridulaceae	*Roridula*	*R. gorgonias*
Cephalotaceae	*Cephalotus*	*C. follicularis*
Nepenthaceae	*Nepenthes*	*N. tobaica*
Stylidiaceae	*Stylidium*	*S. fimbriatum*
Bromeliaceae	*Brocchinia*	*B. reducta*

fluorescence (Fig. **5**). In *Stylidium fimbriatum,* no positive reaction was observed (Figs. **6, 7**).

Snapping traps

In *Aldrovanda,* activity of phosphatases was observed in all types of glands (Fig. **8**), especially in quadrifid hairs. In *Dionaea muscipula,* phosphatase activity occurred in the glandular heads in the digestive zone.

Pitcher traps

In *Cephalotus,* activity of phosphatases was recorded in both large and small glands (Figs. **9,10**). In the large glands, the enzymatic activity was connected with the cell walls. Further phosphatases were produced by bacteria on the inner pitcher surface.

In *Nepenthes tobaica,* activity of phosphatases was found in the radial cell walls of the digestive glands (Fig. **11**). As in *Cephalotus,* organisms in the pitcher fluid also produced phosphatase.

In *Brocchinia reducta,* the glandular heads next to the leaf base showed phosphatase activity. However, it was much weaker than in all other species mentioned previously.

Discussion

Plants cope with a deficiency of phosphorus in the soil by modifications of root morphology and by changes in phosphorus uptake and metabolism; in addition, the production of extracellular phosphatases can help to release inorganic phosphate from the environment (Chróst, 1991; Olczak, 1996; van Aarle et al., 2001; Nedoma et al., 2003; Štrojsová et al., 2003; Hammond et al., 2004). Carnivorous plants are an example for the latter strategy since they use enzymatic exudates to digest phosphate compounds of their prey.

The ELFP assay developed by Nedoma et al. (2003) specifically labels extracellular enzymes on the cell surface, since the ELFP hardly penetrates biomembranes (Štrojsová et al., 2003). However, intracellular structures may also be labelled in glandular cells after intensive endocytosis of the dye, as was observed in the intestine of rotifers (Štrojsová and Vrba, 2005).

Table 2 Phosphatase activities found in different types of glandular and epidermal cells. +++ means strong positive, ++ less, + only small reaction, – negative reaction

Species	Type of glands	Reaction
Pinguicula, all species	sessile glands	+++
	stalked glands	+/++
	hydathodes	++
Utricularia, all species	quadrifids	+++/++
	bifids	+++/++
Genlisea, all species	arm hairs	+++
	neck hairs	+++
	bulb hairs	+++
Byblis liniflora	sessile glands	+++
Drosophyllum lusitanicum	sessile glands	+++
	emergences	+++
Drosera, all species	emergences	+++
Aldrovanda vesiculosa	glandular heads	++
	quadrifids and bifids	+++
	external bifids	++
Dionaea muscipula	glandular heads	+++
Roridula gorgonias	epidermis	+++
	emergences	–
Cephalotus follicularis	small glands	+++
	large glands	+++/–
Nepenthes tobaica	digestive glands	+++
Stylidium fimbriatum	petiole glands	–
Brocchinia reducta	glands	(+)

Phosphatases are common in all Lentibulariaceae

In *Pinguicula,* our results verify the observations of Heslop-Harrison and Knox (1971). The sessile hairs digest prey and absorb nutrients; accordingly, high activity of phosphatases could be found in them. Interestingly, the stalked glands – that are specialized in the production of trapping mucilage – also produced phosphatases, although to a lesser degree. This could, however, be due to their own high metabolic activity.

On the lower leaf surface, hydathodes also produced phosphatases. The enzymes are probably secreted into a thin water layer between the leaf and the soil, where they might release inorganic phosphate, as known from phytoplankton (Nedoma et al., 2003). An additional source of phosphatases were the secretory cells within the inflorescence. This gives weight to the suggestion that the *Pinguicula* inflorescence serves as an additional carnivorous organ (Hanslin and Karlsson, 1996).

In *Genlisea,* digestive enzymes have so far been localized cytochemically only in the trap of *G. africana* (Heslop-Harrison, 1975, 1976 b), but it is obviously a common feature within the whole genus. As in *Pinguicula,* also in *Genlisea,* all types of glands all over the plant, including the inflorescence, produce phosphatase.

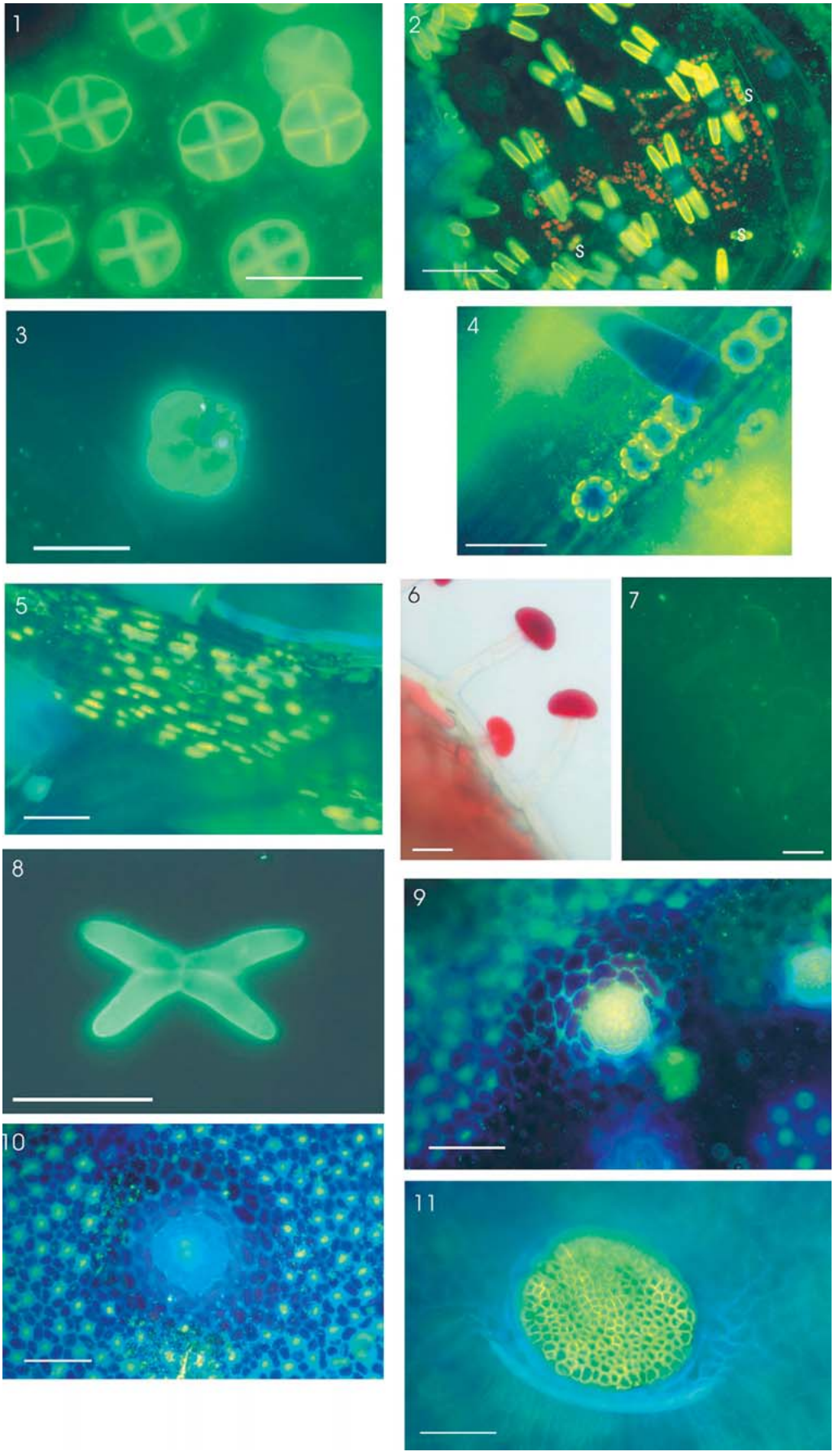
Prior to the present study, within the large genus *Utricularia,* cytochemical studies on phosphatases had only been done in *Utricularia* sp. (Heslop-Harrison, 1975), *U. australis* (Vintéjoux, 1973, 1974; Sirová et al., 2003), *U. aurea* (Parkes, 1980 after Juniper et al., 1989), and *U. ochroleuca* s. lat. (Sirová et al., 2003). All these species are aquatic and belong to the advanced section of *Utricularia*. In some other species, e.g., *U. aurea, U. foliosa, U. vulgaris, U. bremii, U. floridana,* and *U. purpurea,* Sirová et al. (2003) found no phosphatases when using a less advanced ELFP protocol. We observed a positive reaction in *Utricularia* species from all evolutionary levels and all ecological groups (for a detailed description, see Taylor, 1989), i.e., hydrophytes, lithophytes, epiphytes, and terrestrials. Thus, it seems to be a common feature within this genus.

In addition to the plant glandular cells, some trap inhabitants also produce digestive enzymes. This confirms the suggestions of various authors that inhabitants of the *Utricularia* trap bacteria, algae, and rotifers that might contribute to digestion (Schumacher, 1960; Jobson et al., 2000; Richards, 2001; Sirová et al., 2003) and benefit from the nutrient-rich environment of the trap (Cohn, 1874). They are, however, not the only source of digestive enzymes, as was determined by our data and also by our analysis of sterile plants. The experiments of Jobson et al. (2000) provided evidence that some inhabitants may affect the fitness of *Utricularia,* probably because of competition for nutrients. Our results with sterile plants show that stimulation by prey is not necessary for enzyme production.

The staining of quadrifid glands starts in the vacuolated tip of the terminal cell and proceeds to the basal part containing the bulk cytoplasm and the nucleus. This suggests that, on top of the arm, the cuticle is more permeable and, therefore, it is the first point of contact of ELFP with the enzymes. Phosphatase activity was also found in the arms of the bifid hairs, which are very similar to the quadrifid glands, as was shown by ultrastructural research (Fineran and Lee, 1975; Płachno and Jankun, 2004).

On the basis of this finding, we suggest that both bifid and quadrifid hairs play a role in prey digestion. Phosphatases in *Utricularia* traps might have many functions and their activity might also be coupled with the cellular transport. Vintéjoux (1974) suggested that the activity of acid phosphatase in the digestive hairs of *Utricularia* was coupled with the transport and excretion of protease. However, Sirová et al. (2003)

Figs. 1 – 11 (**1**) Phosphatase activity in bulb hairs of *Genlisea lobata* × *G. violacea* f. Giant, bar = 50 µm. (**2**) Phosphatase activity in *Utricularia pubescens* quadrifids and also in trap inhabitants (s). Bar = 100 µm. (**3**) Phosphatase activity in *Pinguicula gypsicola* hydathode. Bar = 20 µm. (**4**) Phosphatase activity in sessile hairs of *Byblis liniflora.* Bar = 100 µm. (**5**) Phosphatase activity in leaf epidermis of *Roridula gorgonias.* Bar = 100 µm. (**6**) Secretory hairs of *Stylidium fimbriatum.* Bar = 50 µm. (**7**) Negative reaction of phosphatase activity in hairs of *Stylidium fimbriatum* (the same hairs as in Fig. **6**). Bar = 50 µm. (**8**) Phosphatase activity in an *Aldrovanda vesiculosa* quadrifid hair. Bar = 50 µm. (**9**) Phosphatase activity in a large gland of *Cephalotus follicularis.* Bar = 100 µm. (**10**) Phosphatase activity in small glands of *Cephalotus follicularis.* Bar = 100 µm. (**11**) Phosphatase activity in a digestive gland of *Nepenthes tobaica*. The activity is connected with walls of secretory cells. Bar = 100 µm. ►



Figs. 1 – 11

showed very low activity of aminopeptidases in the trap fluid in four *Utricularia* species. We have also detected phosphatase activity in some pavement hairs in *Utricularia* species. These hairs might possess transfer cells and participate in excreting water from the traps (Sydenham and Findlay, 1975; Broussaud and Vintéjoux, 1982; Fineran, 1985; Sasago and Sibaoka, 1985; Płachno and Jankun, 2004; Płachno et al., 2005 a). Finally, we suggest that the activity of phosphatases in internal hairs (bifids and quadrifids) is coupled both with prey digestion and transport of water and ions, but in the typical pavement hairs, it is mainly associated with water and ion transport.

Phosphatases are distributed in diverse families and types of carnivorous plants

In all tested species of Droseraceae, we found phosphatase activity, which is in accordance with the rich literature on this familiy (e.g., Heslop-Harrison, 1975; Clancy and Coffey, 1976; Robins, 1978; Henry and Steer, 1985). In *Aldrovanda,* phosphatases occur not only in the digestive glands within the traps, but also in external glands. These hairs obviously release phosphatases to the environment, as shown in phytoplankton (Nedoma et al., 2003; Štrojsová et al., 2003) and in *Pinguicula* (see above).

Our localization of phosphatase in *Nepenthes tobaica* confirmed Heslop-Harrison's (1975) observations on *N. rafflesiana,* using another cytochemical approach (As-BI-pararosanilin reaction). Whereas Parkes (1980, after Juniper et al., 1989) found acid phosphatase in *Nepenthes khasiana, N. rufescens,* and *N. maxima* ssp. *superba* in the cytoplasm, but glucose 6-phosphatase in cell walls. Parkes (1980, after Juniper et al., 1989) also detected the acid phosphatase in *Cephalotus,* but only in the vacuoles of the multicellular large glands. We found activity of phosphatases both in large and small glands.

Phosphatases also occur in some protocarnivorous species

In *Byblis,* only one author (Bruce, 1905, after Juniper et al., 1989) found evidence for the production of digestive enzymes, whereas Hartmeyer (1997) failed to detect proteases and, therefore, questioned the presence of carnivory in *Byblis*. Our results show that *Byblis* is able at least to digest phosphorus compounds. The total degradation of proteins, however, may depend on symbiotic organisms.

So far, no digestive enzymes are known from *Roridula*. Prey degradation was thought to be performed only by symbiotic hemipterans (Ellis and Midgley, 1996; Anderson, 2005). In the leaf epidermis, however, we found phosphatase activity, but not in the glandular tentacles. Future research will show if the epidermis takes part in the utilization of prey, or if the phosphatases only reflect high metabolic activity in general.

In *Brocchinia reducta,* prey capture and nutrient uptake from the pitcher are well known but digestive enzymes have not yet been found (Givnish et al., 1984; Gonzalez et al., 1991). Our own experiments concerning phosphatase produced no clear result. Since living trap inhabitants are known from *Brocchinia* (Gonzalez et al., 1991), we suggest that symbionts play the key role in prey degradation.

No phospholytic activities could be found in *Stylidium fimbriatum*. Darnowski et al. (2002) showed that some species of *Stylidium* trap animals, digest proteins, and absorb amino acids. *Stylidium* probably either utilizes only nitrogenous compounds from its prey, or phosphatase is produced only upon stimulation.

Conclusion

We found phosphatases in diverse cell compartments, i.e., the cytoplasm, vacuoles, and cell walls of all investigated carnivorous and most protocarnivorous plants. The mechanism of translocation of the ELFP within the cell deserves detailed examination, as well as the regulation of enzyme production in general. Enzyme production by the plant and also by symbionts enables the release of inorganic phosphate from prey. In P-limited habitats, this ability significantly increases plant fitness.

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References

Adamec, L. (1997) Mineral nutrition of carnivorous plants: a review. The Botanical Review 63, 273 – 299.

Albert, V. A., Stephen, E. W., and Chase, M. W. (1992) Carnivorous plants: phylogeny and structural evolution. Science 257, 1491 – 1495.

Anderson, B. (2005) Adaptation to foliar absorption of faeces: a pathway in plant carnivory. Annals of Botany 95, 757 – 761.

Anderson, B. and Midgley, J. J. (2002) It takes two to tango but three is a tangle: mutualists and cheaters on the carnivorous plant *Roridula*. Oecologia 132, 369 – 373.

Beaver, R. A. (1983) The communities living in *Nepenthes* pitcher plants: fauna and food webs. In Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities (Frank, J. H. and Lounibos, L. P., eds.), Medford: Plexus, pp. 129 – 159.

Bradshaw, W. (1983) Interaction between the mosquito *Wyeomyia smithii,* the midge *Metriocnemus knabi,* and their carnivorous host *Sarracenia purpurea.* In Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities (Frank, J. H. and Lounibos, L. P., eds.), Medford: Plexus, pp. 161 – 189.

Broussaud, F. and Vintéjoux, C. (1982) Etudes ultrastructurales et cytochimiques des tissues superficiels placés à l'entrée des urnes d'*Utricularia* (Lentibulariacée). Bulletin de la Société Botanique de France 129, Lettres Botaniques 3, 191 – 201.

Chróst, R. J. (1991) Environmental control of the synthesis and activities of aquatic microbial ectoenzymes. In Microbial Enzymes in Aquatic Environments (Chróst, R. J., ed.), New York: Springer Verlag, pp. 29 – 59.

Clancy, F. G. A. and Coffey, M. D. (1976) Acid phosphatase and protease release by the insectivorous plant *Drosera rotundifolia*. Canadian Journal of Botany 56, 480 – 488.

Cohn, F. (1874) Über die Function der Blasen von *Aldrovanda* und *Utricularia*. Beiträge der Biologie der Pflanzen 3, 71 – 92.

Cox, G. W. and Singer, V. L. (1999) A high-resolution, fluorescence-based method for localization of endogenous alkaline phosphatase activity. Journal of Histochemistry and Cytochemistry 47, 1443 – 1455.

Darnowski, D. W. (2002) Triggerplants. Dural, New South Wales, Australia: Rosenberg Publishers.

Darnowski, D. W, Carroll, D., and Stielper, J. M. (2002) Are triggerplants (*Stylidium*; Stylidiaceae) carnivorous? Proceedings of the 4th International Carnivorous Plant Conference, Tokyo, Japan, 2002, pp. 209 – 213.

Darwin, C. R. (1875) Insectivorous Plants. London: J. Murray.

Darwin, F. (1978) Experiments on the nutrition of *Drosera rotundifolia*. Journal of the Linnaean Society of Botany 17, 17 – 32.

Duff, S. M. G., Sarath, G., and Plaxton, W. C. (1994) The role of acid phosphatases in plant phosphorus metabolism. Physiologia Plantarum 90, 791 – 800.

Ellis, A. G. and Midgley, J. J. (1996) A new plant-animal mutualism involving a plant with sticky leaves and resident hemipteran. Oecologia 106, 478 – 481.

Ellison, A. M., Gotelli, N. J., Brewer, J. S., Cochran-Stafira, D. L., Kneitel, J. M., Miller, T. E., Worley, A. C., and Zamora, R. (2003) The evolutionary ecology of carnivorous plants. Advances in Ecological Research 33, 1 – 74.

Feder, J. (1973) The phosphatases. In Environmental Phosphorus Handbook (Griffith, E. J., ed.), New York: J. Willey, pp. 475 – 505.

Fineran, B. A. (1985) Glandular trichomes in *Utricularia*: a review of their structure and function. Israel Journal of Botany 34, 295 – 330.

Fineran, B. A. and Lee, M. S. L. (1975) Organization of quadrifid and bifid hairs in the trap of *Utricularia monanthos*. Protoplasma 84, 43 – 70.

Fish, D. (1983) Phytotelmata: Flora and Fauna. In Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities (Frank, J. H. and Lounibos, L. P., eds.), Medford: Plexus, pp. 1 – 27.

Friday, L. E. and Quarmby, C. (1994) Uptake and translocation of prey-derived ^{15}N and ^{32}P in *Utricularia vulgaris* L. New Phytologist 126, 273 – 281.

Givnish, T., Burkhardt, E., Happel, R., and Weintraub, J. (1984) Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. The American Naturalist 124, 479 – 497.

Gonzalez, J. M., Jaffe, K., and Michelangeli, F. (1991) Competition for prey between the carnivorous Bromeliaceae *Brocchinia reducta* and Sarraceniaceae *Heliamphora nutans*. Biotropica 23, 602 – 604.

Hammond, J. P., Broadley, M. R., and White, P. J. (2004) Genetic responses to phosphorus deficiency. Annals of Botany 94, 323 – 332.

Hanslin, H. M. and Karlsson, P. S. (1996) Nitrogen uptake from prey and substrate as affected by prey capture level and plant reproductive status in four carnivorous plant species. Oecologia 106, 370 – 375.

Hartmeyer, S. (1997) Carnivory of *Byblis* revisited – a simple method for enzyme testing on carnivorous plants. Carnivorous Plant Newsletter 26, 39 – 45.

Hartmeyer, S. (1998) Carnivory in *Byblis* revisited II: the phenomenon of symbiosis on insect trapping plants. Carnivorous Plant Newsletter 27, 110 – 113.

Henry, Y. and Steer, M. W. (1985) Acid phosphatase localization in the digestive glands of *Dionaea muscipula* Ellis flytrap. Journal of Histochemistry and Cytochemistry 33, 339 – 344.

Heslop-Harrison, Y. (1975) Enzyme release in carnivorous plants. In Lysozymes in Biology and Pathology (Dingle, J. T. and Dean, R. T., eds.), Amsterdam: North Holland Publishing Company, pp. 525 – 578.

Heslop-Harrison, Y. (1976 a) Enzyme secretion and digest uptake in carnivorous plants. In Perspectives in Experimental Biology (Sunderland, N., ed.), S.E.B. Symposial Volume 2, Oxford: Pergamon Press, pp. 463 – 476.

Heslop-Harrison, Y. (1976 b) Carnivorous plants a century after Darwin. Endeavour 35, 114 – 122.

Heslop-Harrison, Y. and Knox, R. B. (1971) A cytochemical study of the leaf-gland enzymes of insectivorous plants of the genus *Pinguicula*. Planta 96, 183 – 211.

Istock, C. A., Tanner, K., and Zimmer, H. (1983) Habitat selection by the pitcher plant mosquito, *Wyeomyia smithii*: behavioral and genetic aspects. In Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities (Frank, J. H. and Lounibos, L. P., eds.), Medford: Plexus, pp. 191 – 204.

Jobson, R. W., Morris, E. C., and Burgin, S. (2000) Carnivory and nitrogen supply affect the growth of the bladderwort *Utricularia uliginosa*. Australian Journal of Botany 48, 549 – 560.

Jobson, R. W., Playford, J., Cameron, K. M., and Albert, V. A. (2003) Molecular phylogenetics of Lentibulariaceae inferred from plastid rps16 intron and trnL-F DNA sequences: implications for character evolution and biogeography. Systematic Botany 28, 157 – 171.

Juniper, B. E., Robins, R. J., and Joel, D. M. (1989) The Carnivorous Plants. London: Academic Press.

Karlsson, P. S. and Carlsson, B. (1984) Why does *Pinguicula vulgaris* L. trap insects? New Phytologist 97, 25 – 30.

Karlsson, P. S. and Pate, J. S. (1992) Contrasting effects of supplementary feeding of insects or mineral nutrients on the growth and nitrogen and phosphorus economy of pygmy species of *Drosera*. Oecologia 92, 8 – 13.

Krafft, C. C. and Handel, S. N. (1991) The role of carnivory in the growth and reproduction of *Drosera filiformis* and *D. rotundifolia*. Bulletin of the Torrey Botanical Club 118, 12 – 19.

Larison, K. D., Bremiller, R., Wells, K. S., Clements, I., and Haugland, R. P. (1995) Use of a new fluorogenic phosphatase substrate in immunohistochemical applications. Journal of Histochemistry and Cytochemistry 43, 77 – 83.

Lollar, A. Q., Coleman, D. C., and Boyd C. E. (1971) Carnivorous pathway of phosphorus uptake by *Utricularia inflata*. Archiv für Hydrobiologie 69, 400 – 404.

Lloyd, F. E. (1942) The Carnivorous Plants. Waltham, MS, USA: Chronica Botanica Company.

Lowrie, A. (1998) Carnivorous Plants of Australia, Vol. 3. Nedlands: University of Western Australia Press.

Mabberley, W. J. (2000) The Plant Book. Cambridge: Cambridge University Press.

Müller, K., Borsch, T., Legendre, L., Porembski, S., Theisen, I., and Barthlott, W. (2004) Evolution of carnivory in Lentibulariaceae and the Lamiales. Plant Biology 6, 477 – 490.

Nedoma, J., Štrojsová, A., Vrba, J., Komárková, J., and Šimek, K. (2003) Extracellular phosphatase activity of natural plankton studied with ELF97 phosphate: fluorescence quantification and labelling kinetics. Environmental Microbiology 5, 462 – 472.

Olczak, M. (1996) Acid phosphatases from higher plants. Wiadomości Botaniczne 40, 39 – 51.

Otto, C. (1999) Effects of prey and turion size on the growth and turion production of the carnivorous bladderwort, *Utricularia vulgaris* L. Archiv für Hydrobiologie 145, 469 – 478.

Płachno, B. J. and Jankun, A. (2004) Transfer cell wall architecture in secretory hairs of *Utricularia intermedia* Traps. Acta Biologica Cracoviensia, Series Botanica 46, 193 – 200.

Płachno, B. J., Jankun, A., and Faber, J. (2005 a) Development of the wall labyrinth in pavement epithelium hairs of some *Utricularia* species. Acta Biologica Cracoviensia, Series Botanica 47, 109 – 113.

Płachno, B. J., Adamus, K., Faber, J., and Kozłowski, J. (2005 b) Feeding behaviour of carnivorous *Genlisea* plants in the laboratory. Acta Botanica Gallica, 152, 159 – 164.

Richards, J. H. (2001) Bladder function in Utricularia purpurea (Lentibulariaceae): is carnivory important? American Journal of Botany 88, 170 – 176.
Robins, R. J. (1978) Studies in secretion and absorption in *Dionaea muscipula* Ellis. PhD Thesis, University of Oxford.
Robins, R. J. and Juniper, B. E. (1980) The secretory cycle of *Dionaea muscipula* Ellis. IV. The enzymology of the secretion. New Phytologist 86, 401 – 412.
Sasago, A. and Sibaoka, T. (1985) Water extrusion in the trap bladders of *Utricularia vulgaris*. I. A possible pathway of water across the bladder wall. Botanical Magazine (Tokyo) 98, 55 – 66.
Schumacher, G. J. (1960) Further notes on the occurrence of desmids in *Utricularia* bladders. Castanea 25, 62 – 65.
Sirová, D., Adamec, L., and Vrba, J. (2003) Enzymatic activities in traps of four aquatic species of the carnivorous genus *Utricularia*. New Phytologist 159, 669 – 675.
Sydenham, P. H. and Findlay, G. P. (1975) Transport of solutes and water by resetting bladders of *Utricularia*. Australian Journal of Plant Physiology 2, 335 – 351.
Štrojsová, A., Vrba, J., Nedoma, J., Komárková, J., and Znachor, P. (2003) Seasonal study of extracellular phosphate expression in the phytoplankton of a eutrophic reservoir. European Journal of Phycology 38, 295 – 306.
Štrojsová, M. and Vrba, J. (2005) Direct detection of digestive enzymes in planktonic rotifers using enzyme labelled fluorescence (ELF). Marine and Freshwater Research 56, 189 – 195.
Taylor, P. (1989) The Genus *Utricularia* – A Taxonomic Monograph. Kew Bulletin Additional Series 14. London: HMSO.
van Aarle, I. M., Olsson, P. A., and Söderström, B. (2001) Microscopic detection of phosphatase activity of saprophytic and arbuscular mycorrhiza using a fluorogenic substrate. Mycologia 93, 17 – 24.
Vintéjoux, C. (1973) Études des aspects ultrastructuraux de certaines cellules glandulaires en rapport avec leur activité secretive chez l'*Utricularia neglecta* L. (Lentibulariaceae). Comptes Rendues 277D, 2345 – 2348.
Vintéjoux, C. (1974) Ultrastructural and cytochemical observations on the digestive glands of *Utricularia neglecta* L. (Lentibulariaceae). Distribution of protease and acid phosphatase activities. Portugaliae Acta Biologica, Series A 14, 463 – 474.
Zamora, R., Gómez, J. M., and Hódar, J. A. (1997) Responses of a carnivorous plant to prey and inorganic nutrients in a Mediterranean environment. Oecologia 111, 443 – 451.

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