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ORIGINAL ARTICLE

Prey-induced changes in the accumulation of amino acids and phenolic metabolites in the leaves of *Drosera capensis* L

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Abstract Effect of prey feeding (ants *Formica fusca*) on the quantitative changes in the accumulation of free amino acids, soluble proteins, phenolic metabolites and mineral nutrients in the leaves of carnivorous plant *Drosera capensis* was studied. Arginine was the most abundant compound in *Drosera* leaves, while proline was abundant in ants. The amount of the majority of amino acids and their sum were elevated in the fed leaves after 3 and 21 days, and the same, but with further enhancement after 21 days, was observed in ants. Accumulation of amino acids also increased in young non-fed leaves of fed plants. Soluble proteins decreased in ants, but were not enhanced in fed leaves. This confirms the effectiveness of sundew's enzymatic machinery in digestion of prey and suggests that amino acids are not in situ deposited, but rather are allocated within the plant. The content of total soluble phenols, flavonoids and two selected flavonols (quercetin and kaempferol) was not affected by feeding in *Drosera* leaves, indicating that their high basal level was sufficient for the plant's metabolism and prey-induced changes were mainly N based. The prey also showed to be an important source of other nutrients besides N, and a stimulation of root uptake of some mineral nutrients is assumed (Mg, Cu, Zn). Accumulation of Ca and Na was not affected by feeding.

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Introduction

Carnivorous plants comprise up to 600 species and carnivory has been suggested to evolve to overcome deficit of mineral nutrients (Juniper et al. 1989; Adamec 1997). Owing to nutrient deficiency in soil, these plants produce a range of enzymes to gain nutrients from the prey they capture (Juniper et al. 1989; An et al. 2002) and/or enzymes that protect against pathogens carried by the prey (Okabe et al. 2005). On account of the low tissue content of nitrogen (N) in these plants in general, N is the main mineral element absorbed from insects. In *Drosera* species, efficient absorption of N and also of P, K and Mg from insect (>43%) was observed in *Drosera capillaris* and *D. capensis* and effective re-utilisation of selected nutrients from senescing leaves was also detected (Adamec 2002). In *Drosera rotundifolia* and *Drosera intermedia*, N derived from prey represented 50% of total N (Millett et al. 2003) and even higher values were found in pitcher carnivorous plants (Schulze et al. 1997). It can also be expected that allocation of N absorbed from the prey would follow a "logical" pattern, and it was found that the proportion of plant N derived from preys was higher in flowers than in roots of *D. rotundifolia* (Millett et al. 2003). Distribution of organic nitrogenous compounds may therefore follow a similar pattern, but exact data are not available. In a few of available reports, ^{14}C-label was absorbed by *D. capensis* from ^{14}C-labelled sugars and amino acids supplied to *Daphnia*, and similar experiment with ^{35}S-sulphate showed absorption from *Drosophila* by *D. binata* leaves (Chandler and Anderson 1976 and the references therein).

Phenolic metabolites are widespread secondary plant products with numerous functions in the life and protection of the plant (Dixon and Paiva 1995). Among others, they are important for colouration and UV light screening (Dixon and Paiva 1995) and reactive oxygen species (ROS) scavenging (Rice-Evans et al. 1996). Different carnivorous plants are full of phenolic metabolites (Juniper et al. 1989), and in *Drosera* species, mainly naphthoquinones (Kováčik and Repčák 2006) and flavonoids (Repčák et al. 2000) are abundant. This could be caused by lower N availability in the environment of carnivorous plants, since the accumulation of phenols is enhanced in N-deficient conditions (Kováčik and Bačkor 2007). Through phenylalanine ammonia-lyase activity, which produces cinnamic acid from phenylalanine for overall biosynthesis of phenols, there exists a direct connection between N and C metabolism. To our knowledge, responses of phenolic metabolites to prey presence on the leaves have not yet been studied. More recently, Galambosi et al. (2000) reported that feeding with powdered milk had a different effect on the accumulation of 7-methyljuglone and flavonol aglycones in two *Drosera* species.

Carnivorous plants have been shown to benefit from prey through increase in growth, flowering and seed production (Darwin 1878). Despite a considerable knowledge about the contribution of prey or nitrogenous compounds (protein) to N status (Ashley and Gennaro 1971; Shibata and Komiya 1972; Schulze et al. 1997; Millett et al. 2003), quantitative changes in organic nitrogenous compounds are not known. We therefore studied the accumulation of free amino acids and soluble proteins in the leaves of *D. capensis* fed with ants (*Formica fusca*) after 3 or 21 days. Quantitative changes in the accumulation of phenolic compounds and mineral nutrients were also recorded. We note that it was not the aim of this study to identify the effect of prey on growth, but rather the above-mentioned nutritional parameters that are prerequisites for any growth benefit of carnivorous plants. With the exception of phenolic metabolites that are absent in ants, all parameters were also measured in ants.

Materials and methods

Cultivation of plants and experimental design

Three-year-old plants of *D. capensis* L. (native to South Africa) were used in the present study and were 9–13 cm high with 14–20 adult leaves (Fig. 1a). Plants were cultured from root cuttings in a mixture of brown peat and silicate sand (10:1) and placed in 60-L glasshouses on the window in the Laboratory of Plant Stress Physiology (Department of Botany, P. J. Šafárik University in Košice, Slovak Republic) where they received full sunlight, ca. 7–11 a.m., with a maximum daily irradiance of ~650 μmol m^{-2} s^{-1} PAR; day/night temperature was ~25/20°C and relative humidity ~80%. Feeding experiments were conducted during May–July 2009 and plants were in the non-reproductive phase. Ants (*Formica fusca* L.) were collected from the Botanical Garden of P. J. Šafárik University in Košice, narcotised with ether and immediately used for feeding or quantification of soluble proteins. Part of the ants were dried in an oven for control purposes and stored in a desiccator (estimation of amino acids and mineral nutrients) or in liquid N_2 (estimation of proteins after 21 days). To overcome ontogenetic differences and to achieve enough biomass for measurements, four individual adult ants of similar size (ca. 1 cm) were placed on four individual fully developed leaves (one ant per leaf = 4 ants/plant). Leaves were coiled around the prey within 48–96 h (Fig. 1b) and were re-opened within 10–16 days. Leaves and ants were collected 3 or 21 days after initiation of feeding; four ants and four leaves from one plant were pooled and analysed as one sample after drying at 65°C (estimation of amino acids and mineral nutrients) or analysed immediately after

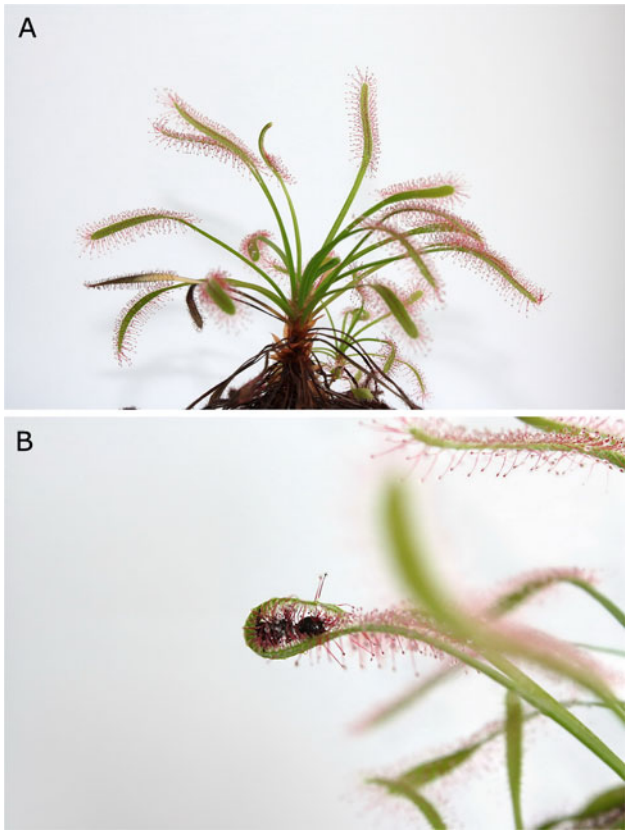

Fig. 1 Representative photo of *Drosera* plants used in this study (**a**) and leaves with ants 2–4 days after initiation of feeding (**b**)

collection (quantification of soluble proteins and phenolic metabolites) and re-calculated to DW. In ants, data were also calculated per one ant (Table 5) at the end of the experiment (21 days of feeding) because the prey is usually almost completely digested within 2 weeks (Płachno et al. 2009). Average DW of four ants used per plant (35.1 and 24.8 mg for unspent/control and spent/fed, respectively) was used for these calculations. Four individual plants were fed with ants (then $n = 4$ in tables/figures) for each parameter. All samples were powdered using liquid N_2 before being analysed. Data were evaluated using two-way ANOVA followed by a Tukey's test (MINITAB Release 11, Minitab Inc., State College, PA, USA) at $p < 0.05$ or by Student's t test as explained in the heads of figures/tables. The complete experiment included 52 plants.

Assay of free amino acids and soluble proteins

Free amino acids were quantified after stirred extraction from dried material using a computer-controlled commercially available IKA Werke 50 device (IKA-Werke, Germany) related to the Soxhlet apparatus. A two-step temperature program (first step, temperature of cooling/heating block 130°C for 30 min, cooling/heating block to 30°C for 5 min; second step, temperature of 120°C for 30 min, cooling to 30°C for 5 min) was applied for isolation into an 80% (v/v) aqueous ethanol. Aliquots of the extracts were evaporated to dryness under vacuum and the residue was dissolved in 500 µl 0.1 M HCl. The measurement of amino acid concentrations was performed using an HP 1100 liquid chromatograph (Hewlett-Packard, Waldbronn, Germany) with fluorimetric detector FLD HP 1100 and precolumn derivatisation with *o*-phthalaldehyde and 9-fluorenylmethyl chloroformate. Separation was carried out with a Zorbax Eclipse AAA column (4.6 × 150 mm, 3.5-µm particle size; Agilent Technologies, USA). A linear gradient profile of the mobile phase, consisting of 40 mM Na_2HPO_4, pH 7.8 (solvent A), and acetonitrile/MeOH/water 45/45/10 (v/v) (solvent B): 0% B (0–1.9 min), 0–57% B (1.9–18.1 min), 57–100% B (18.1–18.8 min), 100% B (18.8–22.3 min), 100–0% B (22.3–23.2 min), and 0% B (23.2–26 min) was applied at a flow rate of 2.0 ml min^{-1}. The column was equilibrated for 5 min under initial conditions before injection of the next sample. The column temperature was maintained at 40°C (Kováčik et al. 2009a).

Soluble proteins were quantified according to Bradford (1976) using 20 µl of supernatants (prepared with 50 mM K-phosphate buffer, pH 7.0) and bovine serum albumin as standard (Kováčik and Bačkor 2007). Spectrophotometry was carried out with an Uvi Light XTD 2 (Secomam, ALES Cedex, France).

Quantification of phenolic metabolites

Total soluble phenols and flavonoids were extracted from fresh tissue with 80% methanol (dilution ratio 10 mg FW/1 ml) and assayed using Folin–Ciocalteu method (gallic acid as standard, 750 nm) and $AlCl_3$ procedure (quercetin as standard, 420 nm), respectively (Kováčik and Bačkor 2007; Ordoñez et al. 2006). For quantification of selected flavonols (quercetin and kaempferol), 0.2 ml 1 M HCl was added to 0.3 ml of methanol supernatants and samples were heated for 1 h at 80°C. Measurement was done using an HPLC system at 370 nm and calculation using a peak of quercetin and kaempferol standard compounds (Repčák et al. 2000).

Analyses of mineral nutrients

The nitrogen content was estimated by the Kjeldahl method (Kováčik et al. 2006). Briefly, dry material was mineralised using concentrated H_2SO_4 (dilution ratio 10 mg DW/1 ml) with selenium catalyst in Kjeldahl flasks heated with a sand bath. Clear solutions were then neutralised in Parnass–Wagner distilling apparatus with 40% NaOH and released ammonium was absorbed in 3% H_3BO_3. Nitrogen content was calculated after titration with 0.01 M H_2SO_4. The reproducibility of apparatus was verified using standard solution with known amount of N (1 M $(NH_4)_2SO_4$) and found to be ca. 97%. We note that this type of Kjeldahl method detects only N in ammonium form (proteins, amino acids and chitin in ants) and it was used to prevent interferences caused by inorganic N.

Samples for quantification of metals were prepared as described previously (Kováčik and Klejdus 2008; Kováčik et al. 2009b): dry material was kept overnight in HNO_3 and H_2O_2 mixture (10 ml + 10 ml, Suprapur, Merck) at laboratory temperature and on the next day evaporated to dryness at 90°C in a water bath (5–6 h). Dry residue was dissolved in 5% HNO_3 and diluted to a final volume of 10 ml. All measurements were carried out using an atomic absorption spectrometer AA30 (Varian Ltd., Mulgrave, Australia) and an air–acetylene flame.

Results

Amino acids and soluble proteins in *Drosera* leaves

Among 17 free amino acids, arginine was the most abundant compound in control plants (Table 1). Controls were roughly similar and both increase and decrease in individual compounds were visible on comparing days 3 and 21. Mainly serine increased in fed plants on day 3 (2.7-fold over control), thus contributing to increase in the

Table 1 Quantitative changes in the accumulation of free amino acids (μmol g^{-1} DW) and soluble proteins (mg g^{-1} DW) in the leaves of *Drosera capensis* fed with ants (*Formica fusca*) for 3 or 21 days

	3 days		21 days	
	Control	Fed	Control	Fed
Aspartic acid	0.61 ± 0.10^{c}	2.76 ± 0.55^{a}	0.62 ± 0.03^{c}	0.88 ± 0.04^{b}
Glutamic acid	1.12 ± 0.10^{b}	2.52 ± 0.22^{a}	1.41 ± 0.28^{b}	2.34 ± 0.25^{a}
Serine	3.94 ± 0.84^{c}	10.7 ± 0.75^{a}	5.41 ± 0.35^{c}	8.24 ± 0.65^{b}
Histidine	0.36 ± 0.04^{b}	0.37 ± 0.05^{b}	0.78 ± 0.11^{a}	0.90 ± 0.06^{a}
Glycine	1.07 ± 0.15^{c}	2.73 ± 0.29^{b}	2.95 ± 0.20^{ab}	3.45 ± 0.24^{a}
Threonine	2.35 ± 0.13^{c}	3.32 ± 0.32^{b}	3.09 ± 0.32^{bc}	4.22 ± 0.46^{a}
Arginine	26.9 ± 3.70^{a}	27.8 ± 2.79^{a}	28.1 ± 3.94^{a}	26.7 ± 4.51^{a}
Alanine	15.6 ± 1.41^{a}	14.4 ± 2.98^{a}	16.9 ± 2.85^{a}	19.9 ± 2.76^{a}
Tyrosine	0.38 ± 0.05^{b}	0.34 ± 0.03^{b}	0.61 ± 0.04^{a}	0.69 ± 0.05^{a}
Cysteine	0.63 ± 0.10^{c}	0.98 ± 0.05^{b}	1.07 ± 0.13^{b}	1.74 ± 0.06^{a}
Valine	2.37 ± 0.18^{c}	4.71 ± 0.27^{a}	2.42 ± 0.24^{c}	3.44 ± 0.47^{b}
Methionine	0.074 ± 0.007^{a}	0.071 ± 0.003^{ab}	0.047 ± 0.004^{c}	0.061 ± 0.002^{b}
Phenylalanine	0.23 ± 0.04^{c}	0.51 ± 0.02^{a}	0.40 ± 0.02^{b}	0.44 ± 0.05^{ab}
Isoleucine	0.69 ± 0.02^{b}	1.47 ± 0.19^{a}	1.01 ± 0.09^{b}	1.04 ± 0.15^{b}
Leucine	0.71 ± 0.06^{a}	0.75 ± 0.09^{a}	0.79 ± 0.12^{a}	0.77 ± 0.09^{a}
Lysine	0.35 ± 0.03^{b}	0.62 ± 0.03^{a}	0.34 ± 0.04^{b}	0.67 ± 0.05^{a}
Proline	0.75 ± 0.09^{c}	0.77 ± 0.13^{c}	1.08 ± 0.07^{b}	1.57 ± 0.08^{a}
Sum	58.3 ± 4.67^{b}	74.8 ± 2.93^{a}	67.2 ± 6.23^{ab}	77.0 ± 2.10^{a}
Proteins	na	na	2.61 ± 0.19	2.38 ± 0.26ns

Na not analysed

ns non-significant difference according to Student's *t* test

Control non-fed ones, *Fed* leaves from plants where ants were placed (only leaves directly in contact with ants were collected and analysed)

Data are means ± SDs ($n = 4$)

Values within *horizontal lines* followed by the *same letter*(*s*) are not significantly different according to Tukey's test ($p < 0.05$)

sum of amino acids (+28%). However, no further enhancement of amino acids accumulation after prolonged feeding was found, as also visible by non-significant difference in their sum in fed plants (day 3 vs. day 21). Surprisingly, aromatic amino acids (phenylalanine and tyrosine) and proline showed only negligible changes in response to feeding (Table 1). Accumulation of soluble proteins in *Drosera* leaves was not affected by feeding (Table 1).

Amino acids and soluble proteins in ants

Proline was the most accumulated amino acid in control ants (Table 2). Especially glutamic acid, valine, isoleucine and leucine increased in fed plants on day 3 (+49, +115, +132 and +32%, respectively). All these compounds were further accumulated after prolonged feeding (21 days, Table 2). Accumulation of aromatic amino acids was also elevated in ants used for feeding at both time points. Only methionine and proline showed no quantitative changes in relation to feeding. For these reasons, the sum of free amino acids was significantly higher in ants used for feeding (+18 and 93% in comparison with control on day 3 and 21, respectively) and also in ones used for feeding on comparing days 21 and 3 (Table 2). Soluble proteins decreased in ants used for feeding by 75% after 21 days (Table 2). On calculation per ant after 21 days of feeding, the sum of amino acids increased (+37%) and soluble proteins decreased (−83%) in comparison with respective controls (Table 5).

Ontogenetic differences in amino acids content in *Drosera* leaves

Within 17 detected amino acids, only aspartic acid, cysteine, methionine and proline did not show the highest content in mature leaves (Table 3). Accumulation of arginine was ca. twice in mature and old leaves in comparison with young leaves. Aromatic amino acids were present in the lowest quantities in old leaves. Overall, the sum of free amino acids decreased in the order: mature > old > young leaves (Table 3).

Table 2 Quantitative changes in the accumulation of free amino acids (μmol g^{-1} DW) and soluble proteins (mg g^{-1} DW) in the ants (*Formica fusca*) used for feeding of *Drosera capensis* after 3 or 21 days of presence at the leaves

	3 days		21 days	
	Control	Fed	Control	Fed
Aspartic acid	1.42 ± 0.17^{c}	2.27 ± 0.12^{b}	0.86 ± 0.13^{c}	5.36 ± 0.37^{a}
Glutamic acid	4.30 ± 0.21^{c}	6.44 ± 0.14^{b}	3.98 ± 0.39^{c}	9.90 ± 1.02^{a}
Serine	9.52 ± 0.53^{b}	10.9 ± 0.48^{ab}	8.93 ± 0.85^{b}	13.1 ± 1.79^{a}
Histidine	1.04 ± 0.12^{c}	1.22 ± 0.07^{bc}	1.61 ± 0.13^{b}	2.43 ± 0.42^{a}
Glycine	3.94 ± 0.24^{c}	4.83 ± 0.27^{bc}	5.69 ± 0.20^{b}	8.77 ± 0.92^{a}
Threonine	1.17 ± 0.15^{c}	1.93 ± 0.05^{b}	1.36 ± 0.16^{c}	5.77 ± 0.34^{a}
Arginine	5.01 ± 0.61^{c}	5.50 ± 0.29^{bc}	6.56 ± 0.35^{b}	8.51 ± 0.55^{a}
Alanine	13.4 ± 1.46^{b}	14.3 ± 2.86^{b}	15.3 ± 1.89^{b}	24.0 ± 2.79^{a}
Tyrosine	1.16 ± 0.09^{c}	2.05 ± 0.07^{b}	1.25 ± 0.09^{c}	6.20 ± 0.37^{a}
Cysteine	1.45 ± 0.18^{c}	1.89 ± 0.03^{ab}	1.82 ± 0.19^{bc}	2.26 ± 0.12^{a}
Valine	1.20 ± 0.16^{c}	2.58 ± 0.29^{b}	2.29 ± 0.14^{b}	9.32 ± 0.84^{a}
Methionine	0.49 ± 0.04^{a}	0.51 ± 0.07^{a}	0.42 ± 0.03^{a}	0.46 ± 0.05^{a}
Phenylalanine	1.35 ± 0.09^{c}	2.03 ± 0.17^{b}	0.71 ± 0.04^{d}	4.40 ± 0.18^{a}
Isoleucine	0.89 ± 0.09^{c}	2.07 ± 0.15^{b}	2.03 ± 0.19^{b}	9.11 ± 1.09^{a}
Leucine	4.35 ± 0.32^{c}	5.78 ± 0.23^{b}	2.83 ± 0.36^{d}	18.2 ± 2.80^{a}
Lysine	0.54 ± 0.04^{c}	1.32 ± 0.26^{b}	0.86 ± 0.09^{b}	4.27 ± 0.56^{a}
Proline	25.8 ± 2.68^{a}	25.7 ± 1.84^{a}	24.4 ± 3.59^{a}	24.5 ± 4.06^{a}
Sum	77.1 ± 5.27^{c}	91.5 ± 2.46^{b}	81.0 ± 5.35^{bc}	156.5 ± 4.50^{a}
Proteins	na	na	41.6 ± 2.75	$10.2 \pm 1.71^{***}$

Na not analysed

Control non-fed ones, *fed* ants placed on the leaves

Data are means ± SDs ($n = 4$)

Other details are as in Tables 1 and 4

Accumulation of phenolic metabolites in *Drosera* leaves

Amounts of total soluble phenols and flavonoids were not affected by feeding in *Drosera* leaves at any harvest time (Fig. 2). Selected flavonols (quercetin and kaempferol) exhibited similar pattern, and no significant changes in response to feeding were observed (Fig. 3).

Quantitative changes of mineral nutrients

Tissue N content was ca. five times higher in ants in comparison with *Drosera* (Table 4). Feeding elevated N content in *Drosera* leaves after 21 days (+29%) was concomitant with depletion in ants used for this feeding (−12%). Accumulation of K and Mg also increased in fed leaves (+25 and +42%, respectively) and decreased in ants (−40 and −19%, respectively). Contents of Na, Ca and Fe were affected neither in leaves nor in ants (Table 4). Accumulations of Zn and Cu were elevated in fed *Drosera* leaves (2.7- and 1.6-fold over control), but no changes were found in ants (Table 4). If calculated per ant after 21 days of feeding, the majority of minerals showed similar trend and only Fe and Zn contents were reduced in fed (spent) ants by 35 and 31%, respectively (Table 5).

Discussion

Although ants are not a typical prey for *Drosera* species, we used them to achieve sufficient biomass for individual measurements. Because of their higher biomass, ants were not fully digested after 21 days (see DW of unspent and spent ants mentioned in "Materials and methods" section), although smaller preys (such as *Drosophila* flies) are usually almost completely digested within 2 weeks (Adamec 2002; Płachno et al. 2009). The amount of N we found in ants (ca. 9% in control, Table 4) was similar to values recorded in other *Formica* species (6–11%, Pekár et al. 2010), but we failed to find data about the accumulation of soluble proteins and amino acids in ants. Our quantitative data indicate that soluble proteins and free amino acids form only a part of detected N content and suggest that the remaining N is deposited in insoluble form such as chitin (poly *N*-acetyl-glucose-amine, Adamec 2002). The decrease in the ant's soluble proteins and increase in free

Table 3 Ontogenetic differences in the accumulation of free amino acids (μmol g^{-1} DW) in the leaves of *Drosera capensis* from non-fed plants

	Young leaves	Mature leaves	Old leaves
Aspartic acid	0.72 ± 0.07[b]	0.78 ± 0.04[b]	1.22 ± 0.23[a]
Glutamic acid	1.26 ± 0.12[a]	1.10 ± 0.17[a]	0.62 ± 0.03[b]
Serine	2.57 ± 0.42[b]	4.02 ± 0.53[a]	2.67 ± 0.51[b]
Histidine	0.26 ± 0.03[b]	0.37 ± 0.04[a]	0.18 ± 0.04[b]
Glycine	1.34 ± 0.46[a]	1.58 ± 0.44[a]	1.33 ± 0.36[a]
Threonine	1.52 ± 0.35[b]	2.82 ± 0.26[a]	1.81 ± 0.27[b]
Arginine	12.9 ± 2.61[b]	26.2 ± 3.31[a]	28.1 ± 3.64[a]
Alanine	8.93 ± 1.82[b]	15.6 ± 2.12[a]	8.28 ± 0.91[b]
Tyrosine	0.31 ± 0.02[a]	0.29 ± 0.03[a]	0.21 ± 0.02[b]
Cysteine	0.66 ± 0.03[a]	0.51 ± 0.02[b]	0.57 ± 0.03[b]
Valine	2.31 ± 0.11[a]	2.53 ± 0.18[a]	1.80 ± 0.21[b]
Methionine	0.101 ± 0.013[a]	0.064 ± 0.004[b]	0.028 ± 0.003[c]
Phenylalanine	0.48 ± 0.04[a]	0.43 ± 0.03[a]	0.29 ± 0.03[b]
Isoleucine	0.66 ± 0.04[b]	0.92 ± 0.09[a]	0.42 ± 0.02[c]
Leucine	0.64 ± 0.03[a]	0.60 ± 0.04[a]	0.43 ± 0.03[b]
Lysine	0.43 ± 0.03[a]	0.38 ± 0.04[a]	0.28 ± 0.02[b]
Proline	1.53 ± 0.22[a]	0.74 ± 0.04[b]	0.33 ± 0.02[c]
Sum	36.6 ± 3.14[c]	58.9 ± 3.85[a]	46.2 ± 3.47[b]

Young leaves undeveloped leaves without mature stalked glands, *mature leaves* fully developed leaves with functional glands (used for feeding in Table 1), *old leaves* drops of stalked glands are absent but leaves are still green. Five to seven leaves per "fraction" were pooled to achieve enough biomass and analysed as one sample

Samples were harvested from five individual plants. Data are means ± SDs. Results of statistics are as in Table 1

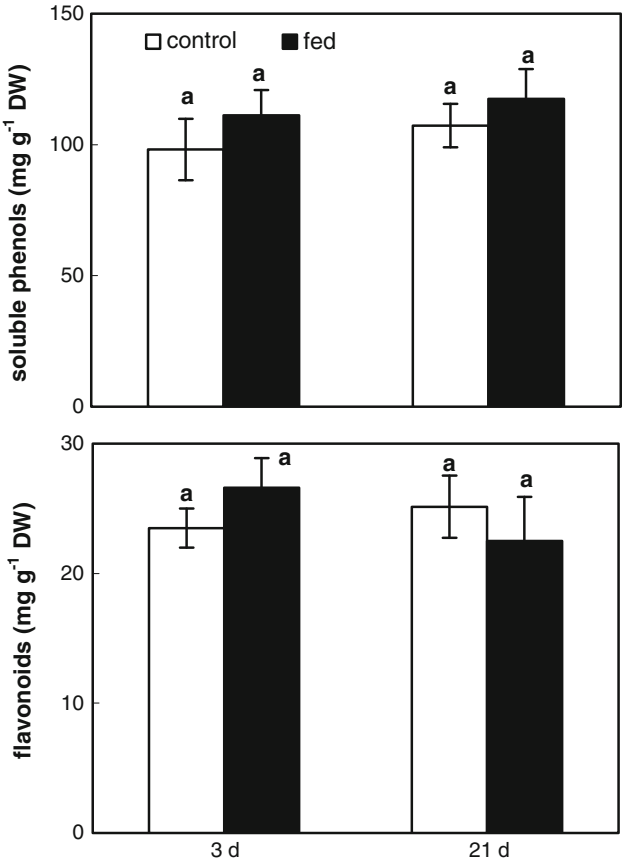

Fig. 2 Quantitative changes in the accumulation of total soluble phenols and flavonoids (flavonols) in *Drosera* leaves 3 or 21 days after initiation of feeding. Data are means ± SDs ($n = 4$). Values within each graph followed by the *same letter* are not significantly different according to Tukey's test ($P < 0.05$)

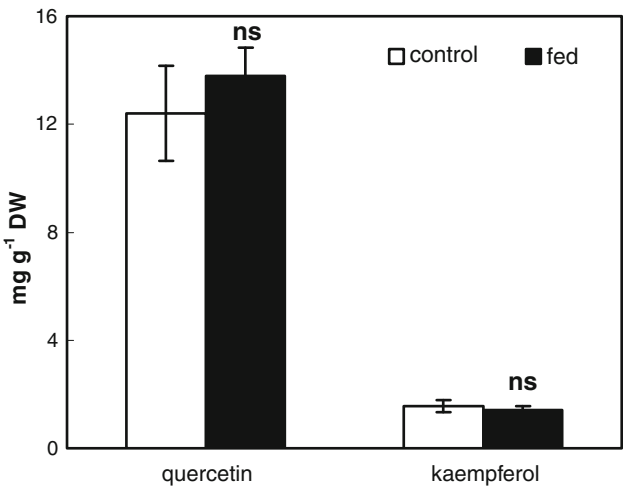

Fig. 3 Accumulation of two flavonols (quercetin and kaempferol) in *Drosera* leaves 21 days after initiation of feeding. Data are means ± SDs ($n = 4$). *Ns* non-significant difference according to Student's *t* test

amino acids observed (Table 2) suggest the effectiveness of sundew's enzymatic machinery. This trend was also visible after re-calculation per ant to consider the decrease in DW of ants owing to digestion by plants (Table 5). Amino acids may be released from a prey by different proteases (Juniper et al. 1989) and the resulting compounds may serve as a pool for the synthesis of important sundew proteins. These proteins may include, e.g., constitutively expressed RNAase recorded in *D. adelae* (Okabe et al. 2005) or phenylalanine ammonia-lyase (PAL), since we found its higher activity in different carnivorous plants (mainly in *D. capensis*) in comparison with a non-carnivorous plant (Kováčik, submitted results). Another interesting aspect of our study was visible in the level of changes to nitrogenous metabolites in *Drosera* leaves. Increase in N content in fed leaves was correlated with increase in free amino acids, but not with changes of soluble proteins (cf. Tables 1, 4). This partial discrepancy may be explained by increase in free amino acids in young non-fed leaves from fed plants (10 from 17 compounds, data not shown), indicating that amino acids are transported throughout the plants to serve as a pool for growth and

Table 4 Quantitative changes in the accumulation of selected mineral nutrients in the leaves of fed or non-fed (control) *Drosera capensis* with ants (*Formica fusca*) and in these ants after 21 days

	Leaves		Ants	
	Control	Fed	Control	Fed
N (mg g^{-1} DW)	17.3 ± 1.29	22.4 ± 1.68**	96.2 ± 4.43	85.1 ± 2.77*
K (mg g^{-1} DW)	15.8 ± 1.81	19.9 ± 1.13**	14.7 ± 1.66	8.96 ± 0.57***
Na (mg g^{-1} DW)	11.7 ± 1.93	14.5 ± 2.58ns	18.6 ± 2.43	18.5 ± 2.10ns
Mg (mg g^{-1} DW)	3.91 ± 0.58	5.57 ± 0.61**	1.87 ± 0.13	1.49 ± 0.20*
Ca (mg g^{-1} DW)	1.71 ± 0.29	2.09 ± 0.40ns	1.43 ± 0.15	1.64 ± 0.18ns
Fe (mg g^{-1} DW)	0.41 ± 0.04	0.34 ± 0.05ns	0.53 ± 0.06	0.49 ± 0.04ns
Zn (μg g^{-1} DW)	67.9 ± 3.71	185.3 ± 24.3***	711.3 ± 48.7	695.9 ± 47.2ns
Cu (μg g^{-1} DW)	3.97 ± 0.28	6.32 ± 1.14**	17.5 ± 3.43	18.3 ± 3.11ns

ns non-significant

Data are means ± SDs ($n = 4$)

*, **, *** Significant differences at 0.05, 0.01 and 0.001 level of Student's *t* test in comparison with respective controls

Table 5 Accumulation of nitrogenous compounds and mineral nutrients re-calculated per ant (from Tables 2 and 4) after 21 days of feeding

	Control (unspent)	Fed (spent)
Sum of amino acids (μmol)	0.71 ± 0.05	0.97 ± 0.03**
Soluble proteins (μg)	365.2 ± 24.1	63.1 ± 10.6***
N (mg)	0.844 ± 0.04	0.527 ± 0.02**
K (μg)	128.9 ± 14.5	56.1 ± 3.56***
Na (μg)	162.2 ± 31.3	114.8 ± 13.0ns
Mg (μg)	16.4 ± 2.15	9.25 ± 1.24*
Ca (μg)	12.5 ± 1.32	10.2 ± 1.13ns
Fe (μg)	4.65 ± 0.53	3.04 ± 0.25*
Zn (μg)	6.24 ± 0.42	4.32 ± 0.29*
Cu (μg)	0.153 ± 0.03	0.114 ± 0.02ns

ns non-significant

Data are means ± SDs

*, **, *** Significant differences at 0.05, 0.01 and 0.001 level of Student's *t* test in comparison with respective control

other processes. This increase in non-fed leaves was lower in comparison with that found in leaves in direct contact with ants, providing further evidence that amino acids from prey were transported within plant. Additionally, non-significant difference in amino acids accumulation was found in fed plants on comparing days 3 and 21 (Table 1), but further enhancement in ants (Table 2) support the view that amino acids were not in situ deposited but were rather redistributed. Thus, the prey may serve as an N source for a longer time. We found no data on organic N distribution that we could discuss in detail, but irregular distribution of inorganic N was observed in *D. rotundifolia* mainly accumulated in flowers (Millett et al. 2003). This is a logical consequence of the carnivorous nature of these plants because, as observed already by Darwin (1878), the prey stimulates growth, flowering, seed production and thus investment into the new generation. In terms of ontogenetic differences in amino acid content, the highest accumulation in mature leaves could indicate accumulation of sufficient pool for digestive enzymes, since mainly these leaves capture and digest the prey. Higher content of amino acids in old leaves in comparison with young leaves (Table 3) suggests the degradation of proteins aimed to re-utilise this source of nitrogen. In fact, Adamec (2002) has found efficient re-utilisation of N (72–80%) from senescing leaves in different *Drosera* species including *D. capensis*. However, differences in free amino acids in relation to ontogenetic stage were not as strong as we would expect. It should also be noted that the sum of free amino acids we found was similar to that found in plants with sufficiently cultured N (such as chamomile, Kováčik et al. 2009b), indicating that despite strongly different N content (ca. 17 and 50 mg g^{-1} DW in *Drosera* and chamomile, respectively) and soluble proteins (ca. 3 and 100 mg g^{-1} DW in *Drosera* and chamomile, respectively), free amino acids were maintained in the free form. In this free form, they can serve as a mobile pool for growth and to save metabolic energy, which would increase if both digestion of prey and proteolysis for re-utilisation of N functioned simultaneously (Vierstra 1996). The same conclusion could be seen if the sum of amino acids in control *Drosera* leaves and ants are compared, because these values are also similar (cf. Tables 1, 2). Besides, uptake of two amino acids (phenylalanine and glycine) by *Sarracenia purpurea* pitchers was higher or similar in comparison with uptake of inorganic N (ammonium nitrate), depending on soil N content, suggesting that organic N saves energy needed for mineralisation and re-assimilation of inorganic N by carnivorous plants (Karagatzides et al. 2009). On the other

hand, a study by Shibata and Komiya (1972) has found that *D. rotundifolia* leaves did not absorb more than 10% of N from a protein.

Considering the environment of carnivorous plants as nutrient deficient, this may partially explain the high accumulation of phenols, since the activity of phenylalanine ammonia-lyase (PAL, a pivotal step in their biosynthesis) is enhanced in N-deficient conditions (Kováčik and Bačkor 2007) and this could also be the main reason why we found its higher activity in three different carnivorous taxa as mentioned above. Besides, carnivorous plants may gain amino acids from their prey (An et al. 2002) and phenylalanine is a substrate for PAL leading to a common precursor of numerous phenolic metabolites. It is therefore surprising that accumulation of free phenylalanine was not elevated in fed leaves after prolonged exposure (21 days), but increased in ants used for feeding in this time point (Table 2). This is in accordance with an overall pattern of accumulation of amino acids within plants (redistribution) and highlights that the prey is a source of N also after prolonged exposure if leaves are not already coiled around the prey. Increase in the sum of amino acids in ants after 21 days (calculated per gram DW and one ant, Tables 2, 5) could also indicate the participation of bacteria in the digestion of ants, and this participation has previously been found in N cycling of *Sarracenia purpurea* pitchers (Karagatzides et al. 2009 and the references therein).

High abundance of total soluble phenols in *Drosera* leaves suggest their essentiality for plant's life and this value is even similar to that recorded in commercial green tea (data not shown). It can only be speculated if such a high content of phenol is caused, e.g. by photooxidative protection (since carnivorous plants prefer sunny localities), attraction of insect (red colour of leaves) or antioxidative protection during the digestion of prey (ROS aid in digesting prey in pitchers of *Nepenthes*, Chia et al. 2004), or by a combination of these factors. Further study will be focused on the manipulation of phenolic metabolism in vitro in *Drosera* plants using specific inhibitor of PAL (Kováčik et al. 2010) to highlight the significance of phenols for growth and stress protection. Our present data showed that the presence of prey had no significant effect on the accumulation of phenols, flavonoids and two flavonols (Figs. 2, 3), suggesting that the high basal level of these metabolites is sufficient for the plant's metabolism and prey-induced changes are mainly N based. Higher accumulation of quercetin in comparison with kaempferol is in accordance with the observation from other *Drosera* species such as *D. rotundifolia* and *D. anglica* (Repčák et al. 2000). Non-significant differences observed in the amount of these two flavonols in response to feeding are in accordance with data from *D. anglica*, but they decreased in *D. rotundifolia* in response to application of powdered milk. However, accumulation of biomass was strongly enhanced by this milk in both species during a long-term experiment (Galambosi et al. 2000).

Uptake of minerals from prey is one of the most important criteria for carnivory and calculation per prey (considering the reduction in biomass of prey during digestion) was found to be a useful method (Płachno et al. 2009). Therefore, we did the same (Table 5) for comparison with the standard unit (per g DW, Table 4). At the level of mineral nutrients, quantitative changes in N content were more correlated with the accumulation of amino acids than with soluble proteins as discussed above. No effect of prey on the accumulation of Ca has been observed in *D. capensis* (Adamec 2002), but a similar response of Na (Table 4) is not known. Only a small decrease in the ant's Fe content (Table 5) and unaltered leaf's Fe in *Drosera* (Table 4) suggest that this nutrient is not intensively taken up from the prey. Based on the comparison of Mg accumulation per gram DW of ants and per ant (Tables 4, 5) with that in *Drosera* (Table 4), it seems that the prey partially stimulates root uptake of Mg (Adamec 2002). Prey-stimulated root uptake is highly probable for Zn accumulation and this phenomenon was clearly visible for Cu accumulation (Tables 4, 5). Interestingly, the ratio of N:K and K:Mg in control ants was similar to that found in *Drosophila* files (Płachno et al. 2009), but absolute values were higher in our present study, reinforcing different efficiency of different carnivorous plants to use various preys.

In conclusion, our present study revealed higher amount of N and proteins in ants in comparison with *Drosera* plants; therefore, this prey is a good source of N as observed by its decrease in ants used for feeding. Surprising increase in free amino acid content in ants used for feeding confirms the effectiveness of sundew's enzymatic machinery. The absence of the extensive changes in fed leaves indicates, together with unaltered accumulation of soluble proteins, that amino acids are not in situ deposited, but rather are allocated within the plant. Absence of significant changes in phenolic metabolites indicates that their high basal level is sufficient for plant's metabolism, and prey-induced changes are mainly N based. The prey also seems to be an important source of several mineral nutrients and stimulation of root uptake of some minerals is assumed. Manipulation of phenolic metabolism through in vitro cultures will be a focus of our further studies to highlight the significance for growth, and nitrogenous and overall metabolism.

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