THE EFFECTS OF SIZE AND HABITAT ON δ N-15 OF CARNIVOROUS PLANTS (Drosera spp.)

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ABSTRACT:

The δ N-15 natural abundance method was used to investigate the role of nutrient-poor habitats in carnivorous *Drosera capensis* and *Drosera aliciae*, and how that role changes under sunny and shady environmental conditions. The main purpose of the study was to evaluate Givnish's (1984) cost/benefit model used to explain the evolution of carnivory in nutrient-poor, sunny and moist habitats. δ N-15and total nitrogen values of the *Drosera* species were compared against each other, as well as against the non-carnivorous reference plants collected from each of the two habitats. Generally, data indicated significant differences between the carnivorous plants and their reference plants in terms of δ N-15 values. However, there was no significant difference between plants collected from the shade and those collected from the sun for both *Drosera* species. Total nitrogen results revealed higher values for *Drosera* plants from Camp's Bay than those from Table Mountain did. This indicated that there was a bigger source of insect nitrogen at that site, meaning more insects were available and being caught by the plants at Camp's Bay. Further investigations were performed on the two *Drosera* species in order to find the influence of altitude, leaf-size and plant form, on the degree of carnivory. There was an overall, higher degree of carnivory at Camp's Bay where it is, seasonally wet and the plants have longer leaves and a stem-like rosette. On Table Mountain it is cooler, waterlogged, and the plants have short leaves and ground-level rosettes therefore, the degree of carnivory there was lower. Lastly, chlorophyll and anthocyanin contents were measured and compared between the sun and shade collected *D. capensis* plants, with tentacles intact and with them removed. Chlorophyll investigations showed significant differences between sun and shade collected *D. capensis* plants but these were not affected by the removal of tentacles. Alternatively, anthocyanin measurements indicated that sun and shade collected *D. capensis* plants have similar amounts of anthocyanins, but the removal of tentacles results in a decrease (about four times lower) in the anthocyanin content.

1] INTRODUCTION

1.1) Carnivory defined:

According to Givnish (1984) the definition of plant carnivory is composed of two distinguishing characteristics: an ability to attract, capture and digest prey; and an adaptation to absorb nutrients from prey using the plant's surface, thereby increasing the plant's fitness (i.e. increased growth, survival and reproduction).

1.2.1) Evolution of carnivory:

Carnivorous plants are usually found in nutrient-poor environments (Darwin, 1875). However, when Givnish (1984) studied an epiphytic Bromiliad, *Brocchinia reducta*, which also occurs in nutrient limited environments, he noticed that the Bromeliad was not carnivorous and that few epiphytes were carnivorous. The epiphytes were found in nutrient-poor environments that were either shady or exposed to sunlight and regular desiccation. In contrast, carnivorous plants were found in sunny, moist (at least seasonally) nutrient-poor habitats. Similar observations (Darwin, 1875)on the conditions in which carnivorous plants were found had been published prior to Givnish (1984) but no explanations for the preferences were given.

Givnish (1984) explained the carnivory in plants using a cost/benefit model. The model considers the energetic benefits and costs of carnivory in various habitats. It is based on the theory that carnivory should evolve if the benefits are greater than the cost of investments of adaptations to carnivory, so that the plants with the mutations for such investments should have an advantage when competing with other plants (Givnish 1979, 1982). The cost/benefit model of plant carnivory is made up of three potential benefits and these all support the preference of sunny, moist nutrient-poor habitats by such plants.

The primary benefit of this model is the enhancement of photosynthesis due to an increase in nutrient absorption and consequently increased growth and reproduction rates. The enhancement of photosynthesis by the increased nutrient absorption rate depends on environmental conditions (Givnish, 1984). As the amount of energy for carnivory (e.g. traps and/or digestion enzymes) increases, the amount of nutrients absorbed should also increase. Furthermore, as the amount of energy for carnivory

continues to increase, the photosynthetic benefits should rapidly increase and then slowly level-off as an equilibrium is reached. This indicates that factors other than nutrients limit photosynthesis and hence, carnivory in plants.

The effective rate of photosynthesis is unlikely to increase if nutrient availability increases, unless nutrients are in short supply and limit photosynthesis (Givnish, 1984). For example, studies by Soreson and Jackson (1968) on a *Utricularia* species and Chandler and Anderson (1976) on a *Drosera* species showed that usual increase in growth of carnivorous plants supplied with prey on nutrient-poor habitats largely disappears as nutrient availability in the habitat increases. Therefore, the greatest benefit is expected in nutrient-poor sites (Givnish, 1984). However, if factors like light or water are limiting then the extent to which nutrients added by carnivory can increase photosynthesis decreases (Bannister, 1976). A study by Gulmon and Chu (1981) showed that photosynthesis increases more slowly with added leaf nitrogen at low light intensities than at high ones. Thus, sunny environments are more favourable for maximum benefits from carnivory (i.e. photosynthesis) than shady habitats.

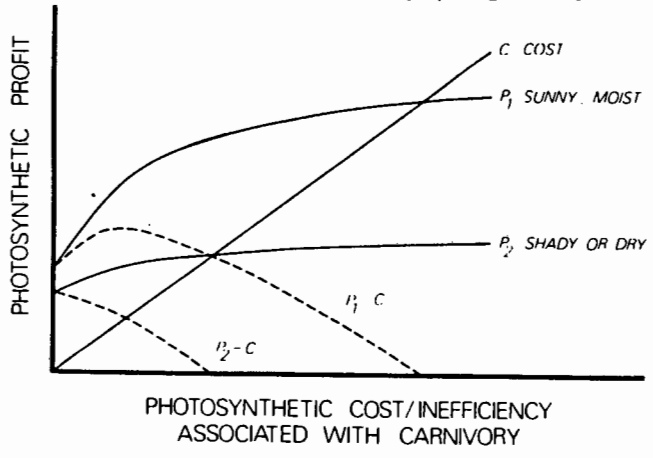


Figure1: Photosynthetic benefits and costs associated with differential levels of investment in carnivory adaptations in nutrient-poor sites, as a function of environmental conditions [Taken from Givnish, 1984]

Figure 1 shows that the benefits of carnivory and consequently, photosynthesis, are limited by the availability of water and light. The relationship is such that in habitats that are sunny and moist carnivory yields greater benefits than in shady and/or dry habitats. Overall, Givnish's cost/benefit model suggests that the differences in photosynthetic benefits and costs are positive (i.e. benefits > costs) when there is not so much investment in carnivory. This happens in nutrient-poor environments that are also sunny and moist.

1.2.2) Alternatives for the evolution of carnivory:

Zamora (1999) has suggested other reasons for carnivory being abundant in sunny and moist, nutrient-poor habitats, besides the cost/benefit reasons put forward by Givnish (1984). Zamora (1999) does not dispute Givnish's (1984) theory; he adds dimension to the subject by giving alternative reasons of carnivory dominating in sunny environments. He looked at the bladders in an *Utricularia* species, which are used by the plant to trap insects, and noted that the number of bladders increased in nutrient-rich soils. This result is opposite to expectations suggested by Givnish (1984). Therefore, according to Zamora (1999) the degree of carnivory is higher in nutrient-rich environments than nutrient limited ones. Bronstein (1994) showed that results of interactions between organisms are affected by current ecological conditions.

Factors such as size and age of carnivorous plants as well as density and population structures of insects can determine the nature of plant-insect interactions. This is such that older and larger leaves have the capacity and opportunity to catch more insects and areas abundant in insects increase the likelihood of insects being trapped by carnivorous plants. Also, abiotic conditions can play a major role in determining the outcome of such plant-insect interactions (Dunson and Travis, 1991) because both plants and insects are affected by environmental conditions, these conditions either promoting the survival of plants and insects or leading to a decline in their numbers. This latter concept supports Givnish's reasons for carnivory being most successful in sunny, moist environments. The site-specific effects (i.e. shady, sunny) in plant-insect relationships are particularly important because the environment governs the ecophysiology of both plants due to their sessile lifestyle, and insects due to their small size (Zamora, 1999).

With all these points in mind, reasons for sunny nutrient-poor habitats being favoured by carnivorous plants, may also be due to the greater abundance of insects of all sizes in open sunny habitats than in shady and dry ones. In shady habitats the presence of many ectothermal insect species is strongly limited, because such insects cannot remain active below specific threshold temperatures (Corbet 1990, Herrera 1995a). Furthermore, the knowledge of differences in the presence of insects in shady and sunny habitats suggests that altitude may influence carnivory. Areas that are higher up

in terms of altitude (i.e. mountaintops) which are cooler, will have fewer insects because most insects won't be able to remain active there, and incidents of prey capture will be low. On the other hand, habitats that are lower down in altitude (i.e. below mountains or valleys) are warmer and hence, have an abundance of insects that are potential prey for carnivorous plants growing around those premises. The above mentioned points show that there are alternative reasons for carnivory occurring largely in sunny areas (also in other specific habitats) besides Givnish's (1984) suggestions.

1.3) Aims:

The primary aim of the paper is to investigate whether or not Givnish's proposed cost/benefit explanation of sunny environments being favoured by carnivorous plants is correct. This was performed on two *Drosera* species; *Drosera aliciae*, found on Table Mountain and *Drosera capensis*, found in Camp's Bay. The environments in which the two species of *Drosera* occur are similar in soil nutrient content, but differ in moisture and altitude. However, both locations of the two species are similar according to Givnish's requirements for successful carnivory (i.e. both nutrient poor and moist) therefore, the only variable tested was the amount of sunlight (sunny vs shady). Previous studies have shown that the uptake of metabolites from prey can be demonstrated using labelled compounds including nitrogen (N), phosphorus (P) and carbon (C).

From such studies it has been illustrated that carnivorous plants, like *D. aliciae* and *D. capensis* use mineral elements (nutrients) from prey. It is appropriate therefore to investigate the degree of carnivory in these plants in both sunny and shady conditions by measuring the amount of one of the major nutrients present in the carnivorous plants compared to the surrounding vegetation. Past studies(including Givnish, 1984) isolated nitrogen as the major insect-derived nutrient. For this reason, the strength of carnivory in sunny and shady conditions of the habitats of the two *Drosera* species was measured using the δ N-15 natural abundance method. This method measures the N-15/N-14 ratio present in each of the carnivorous plants collected from sunny and shady conditions in their habitats. The values obtained for the carnivorous plants can

then be compared to those of the surrounding reference plants to indicate whether a different source of nitrogen is being used by carnivorous plants than that used by non-carnivorous reference plants. Previous studies have shown that insects have higher N-15/N-14 ratios because they are higher up the food chain relative to the soil where the non-carnivorous plants are getting their nitrogen. Thus the use of insect nitrogen leads to the carnivorous plants having a higher δ N-15 value. Moreover, the resulting values can be compared between the carnivorous plants themselves to see in which conditions (sunny or shady) the most insects (degree of carnivory) are being captured and digested.

Further aims for this paper include looking at the influences of plant leaf size and form on the δ N-15 natural abundance values, in order to give insight to whether size and form of leaves play a role in the strength of carnivory. This is particularly interesting because of the obvious and distinct difference in leaf size and rosette form of the two species of *Drosera* being investigated. The paper also aims to investigate the influence of altitude on carnivory because the plants being studied in this paper are collected from locations that are in different points of altitude. In Camp's Bay it is warmer and seasonally wet due to the point of altitude there, and according to Zamora (1999) and other authors (Corbet, 1990;Herrera, 1995a), more insects are expected to be found and captured in those conditions. Table Mountain on the other hand, is on a higher point of altitude, resulting in cooler and waterlogged conditions, and that suggests fewer insect occurrences and thus fewer capture incidences by the plants growing there. The last two aims are important because if they play a role in the degree of carnivory in plants, then their influence on the δ N-15 natural abundance results might be more important in explaining the preferences of certain habitats by carnivorous plants than the cost/benefit theory.

The last investigation in the paper will involve measuring the two pigments found in the two *Drosera* species. The pigments of interest are Chlorophyll (chl a & chl b) and Anthocyanins. However, the pigments will be tested in only one of the *Drosera* species, *D. capensis*, which was more abundant in its habitat. This last investigation was inspired by the distinct differences in colour in the plants of both species collected from sunny and shady environments. Plants collected from sunny conditions

had very bright red tentacles and plants from shady conditions were much greener and their tentacles were only slightly red in colour. Such an observation suggested there was a difference in pigment content in the leaves and tentacles of the plants growing under the different conditions. The questions invoked by these colour differences are: are the colours adaptations to succeeding in each of the conditions in the habitats? If yes, are these adaptations costs to being carnivorous or do the benefits from such investments exceed the costs? Overall, the investigation is to test the cost/benefit theory in *D. aliciae* and *D. capensis*.

1.3) Predictions or Hypotheses:

In light of all the aims and the evidence of past studies on similar investigations as what was done in this paper, a few starting hypotheses or predictions are proposed. These will either be accepted or rejected at the end of this paper depending on the results obtained. These hypotheses are:

1. δ N-15 values will be greater in *Drosera* plants growing in sunny conditions because carnivory is favoured in sunny and moist nutrient-poor conditions according to Givnish (1984).

    - δ N-15 >> sunny conditions

2. δ N-15 values will be greater in *Drosera* plants with taller rosettes and longer leaves because long-leafed plants have more surface area and thus, can catch more insects and taller plants greater opportunity to catch flying insects.

    - δ N-15 >> taller plants with longer leaves

3. δ N-15 values will be greater at lower altitudes (warmer, seasonally wet conditions) because more insects are found in conditions where the temperature is above the minimum temperature required for them to survive (i.e. Camp's Bay).

    - δ N-15 >> warm, seasonally wet conditions

4. High chlorophyll content in plants from shady conditions than in sunny conditions because the plants collected from shady areas had greener leaves than the ones from the sun.

   - Chlorophyll >> shady conditions

5. High anthocyanin content in plants from sunny conditions compared to those from shady conditions because plants collected from sunny conditions were more red than the plants collected from the shade.

   - Anthocyanin >> sunny conditions

2] MATERIALS AND METHODS

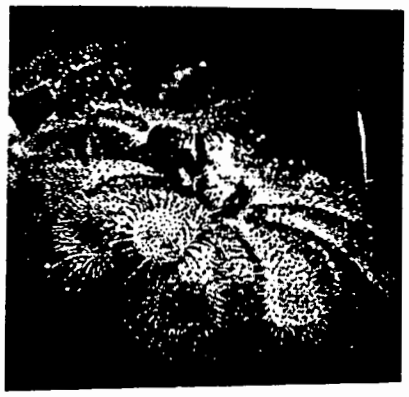
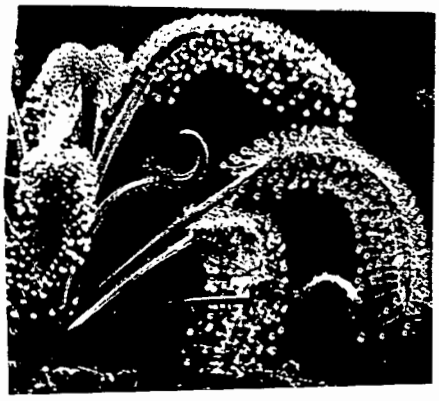
2.1) Materials

Drosera is a genus of the sundew group of plants, diverse in shape and size. There are more than 100 species of *Drosera* in the world, with the greatest concentration of species in Australia. This genus, of the family Droseraceae, has a characteristic leaf surface thickly covered with tentacles. The tentacles are usually reddish in colour (especially in plants exposed to direct sunlight), and each has a drop of clear colourless mucilage at the tip, which glistens. Species of *Drosera* occur in both hemispheres and in most countries and climates. Almost all are found in poor, generally acid soils, usually in marshy habitats, but in Australia many species have evolved which are adapted to growing in much drier habitats.

The species of *Drosera* differ largely in size and form/habit. The leaf-blades varying in length from as small as 1.5 cm to over 60cm. The leaf-blade may be simple or divided, and can be in a rosette at ground level or singly on a tall or even climbing stem. The flowers, roots and tentacle sizes and lengths may also vary. In this paper, two members of the South African sundews were studied. The first is a rosetted sundew, *Drosera aliciae*. This species has a ground level rosette, up to 5cm in diameter. The leaves are dark green (more dark in shade than in sunny conditions), wedge-shaped and have blunt, rounded ends with red tentacles. The leaves are up to three times longer than the widest point and the tentacles continue almost to the base. The flowers are light purplish pink and about 1.3cm in diameter. The flower-stalk grows up to 46cm high.

The other South African *Drosera* species investigated in this paper *is Drosera capensis*. This sundew is a member of the stem forming South African species. It is a large and very showy sundew, made up of attractive leaves loosely arranged in a rosette up to 15cm in diameter. The leaf-blade is ribbon-like, commonly about 5mm wide and 3.5cm long, and ends in a rounded point. The tentacles are bright red and contrast effectively with the green of the leaf itself. The petiole is usually about the same length as the leaf, flat and usually wide (about 2.5mm). The plants of this

species are also peculiar in that they gradually form woody, occasionally branched stems that become trailing and keep the dead leaves of previous seasons. The flower-stalks are about 30cm high, and bear up to 20 rosy pink flowers, some 2cm in diameter.

(I)  (II) 

Figures 2 & 3: Photographs of the two *Drosera* species, showing the differences in size and form/habit. (I) *D. aliciae* and (II) *D. capensis*.

2.2) Study Sites:

The first species, *D. aliciae*, was collected along the inner banks of a perennial Disa stream on Table Mountain. Twenty plants, ten from shady conditions and ten from sunny conditions were collected and put into paper packets suitably labelled. Five species of reference plants, each with three representative plant samples, were collected from the same vicinity as the carnivorous plants. The second species, *D. capensis*, was collected from Camp's Bay. This site is warmer and is only seasonally wet. Again twenty carnivorous plants were collected, with ten samples each from sunny and shady conditions. Also five reference plants, each with three representatives, were collected and placed in suitably labelled packets. These were *Berzelia lanuginosa*, *Osmitopsis asteriscoides*, *Psorolea pinnata*, *Pteridium aquilinum* and *Cliffotia ordorata* from Table Mountain; and from Camp's Bay, *B. languginosa*, *O. asteriscoides*, *Laurembergia repens*, *Rhodocoma sp.* and *Hymenophyllum capense*.

All these plants were carried back to the laboratory and kept in the 0 degrees freezer until measurements were done. Of the five reference plants from both locations, three were the same. Plants of approximately the same size and age were collected for each of the *Drosera* species (i.e. same size and age for *D. aliciae* and same size and age for *D. capensis*) and for each of the reference plants.

2.3) δ N-15 Natural Abundance Measurement:

The plant samples were dried in an oven at 70 degrees Celsius for 24 hours. Before drying, each of the *Drosera* samples were measure for their diameters or height to ensure that plants of the same sizes for each species were being analysed. Also each of the carnivorous plants were cleaned of any insects or debris on the leaves using alcohol. After drying, the entire rosette for each both species of *Drosera* were ground-up using a grinding machine for about 3 minutes each. The ground material of each of the species samples was scraped into a labelled eppendorf vial, ready for mass spectrometry. A couple of leaves were removed from each of the three reference plant representatives, and ground up. These were also placed into labelled eppendorf vials before they were to be analysed for the N-15 isotope using the mass spectrometer. The grinding container was cleaned with alcohol between each carnivorous plant sample and reference plant sample to prevent contamination.

For the final stage, approximately 3-mg of ground material of both *Drosera* species and reference plants, were measured for running in the Finnigan-MAT mass spectrometer. This procedure was for measuring the δ N-15 and total nitrogen values for each of the carnivorous and reference plants being tested. The detailed methods for these measurements (δ N-15 and total nitrogen) using the Finnigan-MAT2 mass spectrometer are present in Gebauer and Schulze (1991). Output total nitrogen and δ N-15 values were corrected before being statistically analysed. Since 'Nasturtium' was used as a standard at different intervals when running the samples in the mass spectrometer and the expected 'Nasturtium' δ N-15 and total nitrogen values are known, this was used to correct the rest of the δ N-15 and total nitrogen values. This is done by calculating the average of the Nasturtium δ N-15 and total nitrogen values obtained, and from that subtracting the expected Nasturtium values. The resulting

value from that subtraction is then subtracted from each of the δ N-15 and total nitrogen values of all the plant samples.

2.4) Measures of chlorophyll and Anthocyanins:

2.4.1) Chlorophyll

Plant samples were collected from Camp's Bay (i.e. *D. capensis*) only for this analysis. A pair of leaves of approximately the same length and size, were cut off each of 5 plants from shady conditions and 5 plants from sunny conditions. Both pairs of each of the 5 plants from the shade and 5 plants from the sun, were measured for wet weight, and only one of each pair for all the plants was then dried at 70 degrees Celsius for 24 hours. These were later measured for their dry weights that would be used to estimate the dry weights of the other pair that was used for the pigment extraction.

The extraction pair of leaves were each cut into small pieces and ground up with a mortar and pestle in 8ml pure acetone. Each mixture was then poured into a test tube with a stopper and left in a dark cupboard for 24 hours for the extraction to take place. The absorbance values were read in a CARY spectrometer after 24 hours, at 644.8 nm and 661.1 nm wavelengths. The total chlorophyll content, made up of chlorophyll a and chlorophyll b, were calculated using the following equation:

$$\textbf{Chl a+b} = 7.02\ A\ (644.8) + 18.09\ A\ (661.6)$$

The same procedure was followed for 10 plant samples (5 from shade and 5 from sun) with the tentacles removed. The tentacles were removed using a sharp blade and gently scraped off the leaves.

2.4.2) Anthocyanins

The wet weights of two approximately same sized leaves each of twenty plants were measured (10 with tentacles, made up of 5 plants from the shade and 5 plants from the sun; and 10 without made up as the ones with tentacles). The one pair was dried at 70 degrees Celsius for 24 hours, and then measured for its dry weight. This dry weight was used to calculate the dry weight of the other leaf pair that was used for the extraction. The extraction was done in acidified methanol made up of the following ratio of chemicals; 79 methanol: 20 distilled water: 1 HCL. Each of the other pair of 30 leaves to be extracted is cut up into small pieces into labelled eppendorf vials and briefly ground up with a glass rod in 1ml of acidified methanol. The eppendorf vials were then closed and covered tightly with tinfoil and placed in a dark freezer at 4 degrees Celsius for 48 hours. The mixture was then centrifuged to remove insoluble leaf material. The mixtures were each made up to 8ml with acidified methanol and the absorbance values read off the CARY spectrometer. The absorbance values were read off at 530 nm and 657 nm wavelengths. The anthocyanin concentration [A] was determined by the following formula:

$$\mathbf{[A]} = A(530) - \{1/3\ A(657)\}$$

3] RESULTS

3.1) δ N-15 Measurements

Location/ Drosera spp.	Conditions	Ave.Corr.delta N	Ave.Corr. % N	Ave. *D. capensis* vs ave. refs.
***D. capensis* (Camp's Bay)**	Sunny	3.244	1.105	sun vs refs = 1.970 *
	Shady	2.817	1.393	
Berzelia lanuginosa		-2.739	0.985	(p = 0.089)
Osmitopsis asteriscoides		1.254	1.097	
Laurembergia repens		4.777	1.495	shade vs refs = 1.543
Rhodocoma sp.		3.587	0.798	
Hymenophyllum capense		-0.509	0.695	(p = 0.140)
D. aliciae (Table Mt.)	Sunny	1.455	0.330	**Ave. D. aliciae vs ave. refs.**
	Shady	1.160	0.378	sun vs refs = 3.826 ***
B. lanuginosa		-4.919	-2.518	(p = 0.004)
O. asteriscoides		-3.796	-3.242	
Psorolea pinnata		-1.009	-1.693	shade vs refs = 3.531***
Pteridium aquilinum		-0.273	-1.046	
Cliffota ordorata		-1.856	-0.710	(p = 0.000)

Table 1: Average values for all the measurements done on *D. capensis*, *D. aliciae* and Reference plants.

Where: P< 0.1 = *** (99 % significance level)

P < 0.05 = ** (95 % significance level)

P < 0.01 = * (90 % significance level)

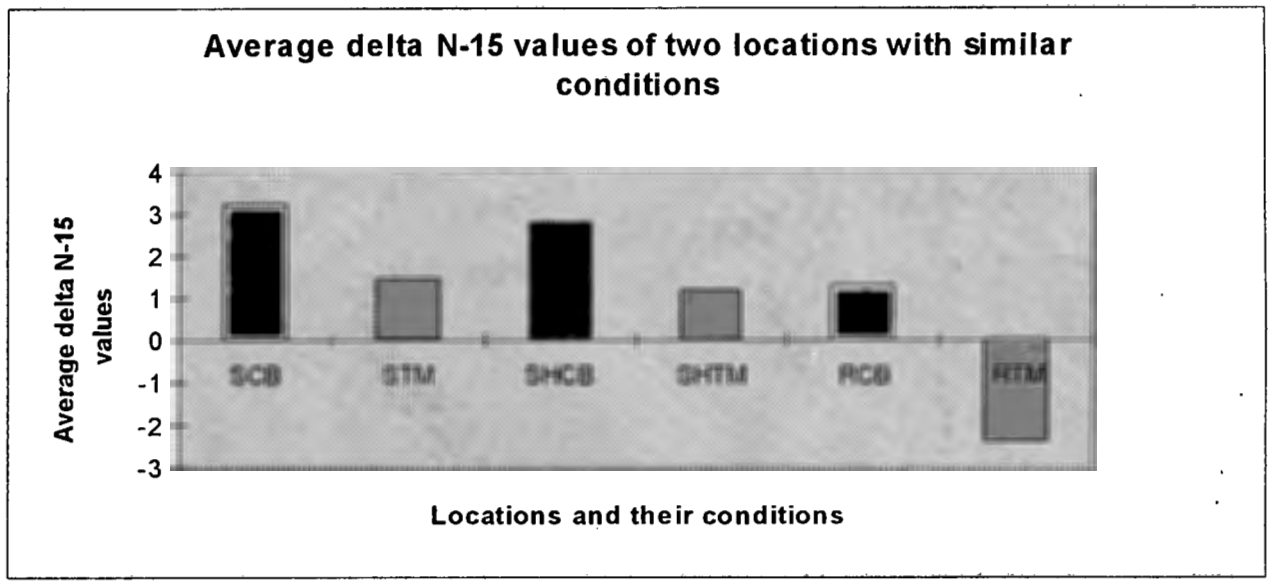


Figure 4: Graphical representation of average δ N-15 values of carnivorous and reference plants, from two different locations and under two environmental conditions. [Where: SCB= sunny, Camps Bay's *D. capensis*; SHCB= shady, Camps Bay's *D. capensis*; STM= sunny, Table Mountain's *D. aliciae*; SHTM= shady, Table Mountain's *D. aliciae*]

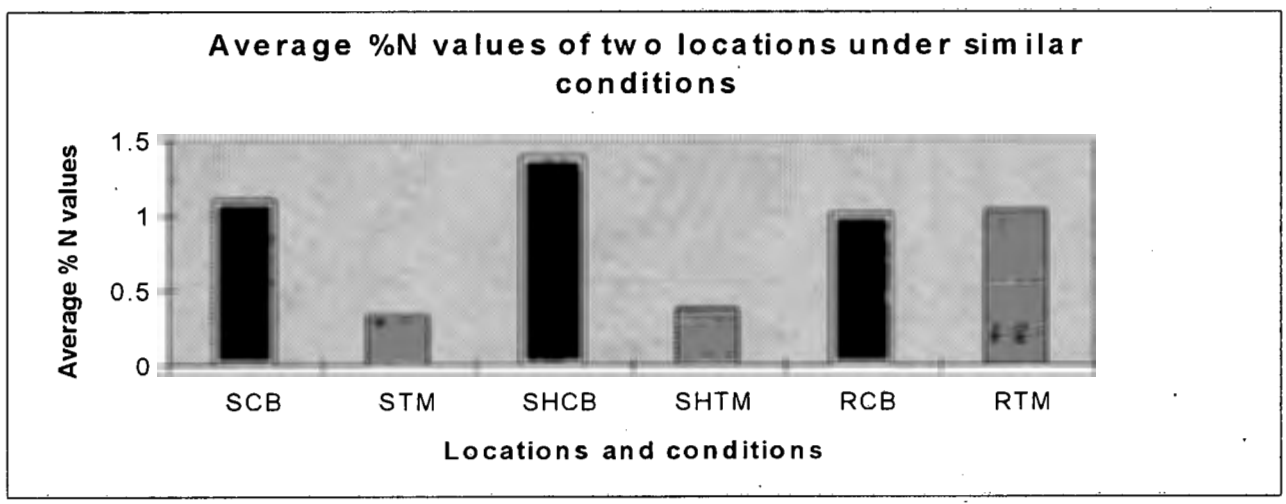


Figure 5: Graphical representation of the average % nitrogen values of the carnivorous plants and their reference plants from Camp's Bay and Table Mountain. [Where: SCB= sunny, Camps Bay's *D. capensis*; SHCB= shady, Camps Bay's *D. capensis*; STM= sunny, Table Mountain's *D. aliciae*; SHTM= shady, Table Mountain's *D. aliciae*]

3.2) Chlorophyll and Anthocyanins

***Drosera* spp.**	**Conditions**	**Tentacle treatment**	**Ave.Chlorophyll**
D. capensis	Sunny	present removed	3.046 2.238
D. capensis	Shady	present removed	1.881 1.111
***Drosera* spp.**	**Conditions**	**Tentacle treatment**	**Ave. Anthocyanins**
D. capensis	Sunny	present removed	0.0049 0.0026
D. capensis	Shady	present removed	0.0047 0.0022
Chl. comparisons		**Anth. comparisons**	
sun vs shade p = 0.011**		sun vs shade p = 0.205	
present vs removed p = 0.015**		present vs removed p = 0.027**	

Table 2: The Average chlorophyll and anthocyanin contents of *D. capensis* plants with different tentacle treatments.

Figure 6: Average chlorophyll content of *D. capensis* samples from sunny (lined blocks) and shady (dotted blocks) conditions,with (1 & 4) and without (2 & 5) tentacles

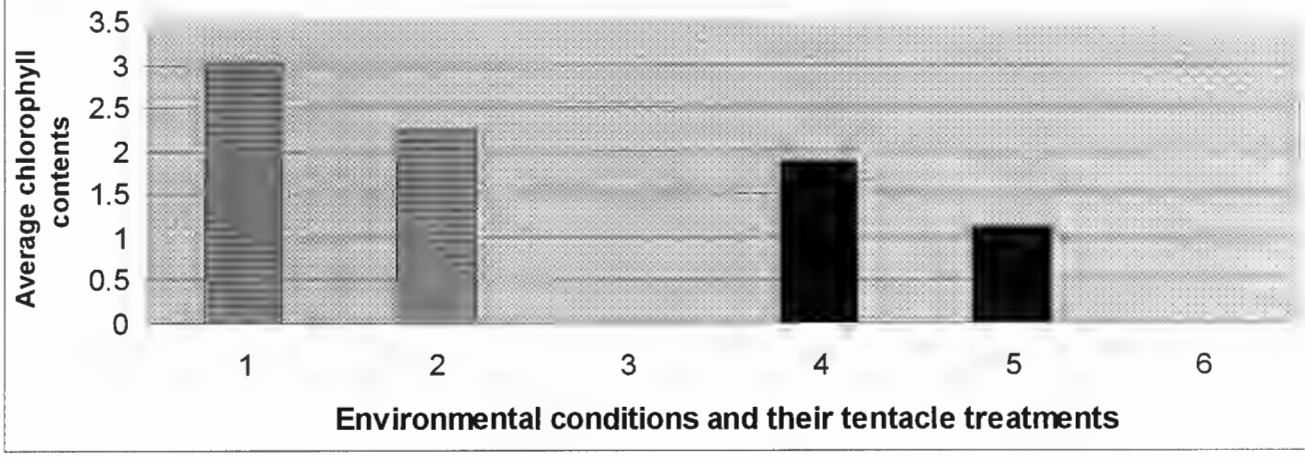
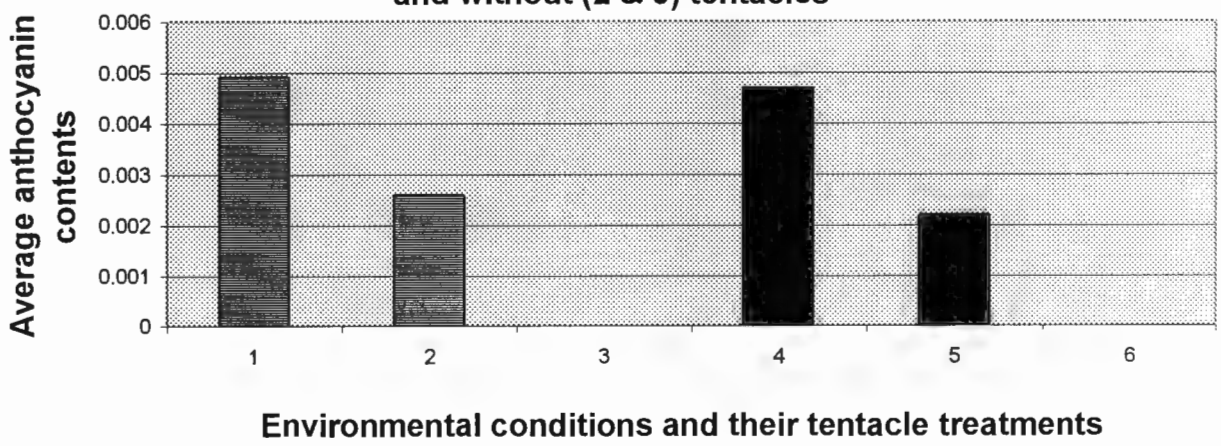


Figure 7: Average anthocyanin contents of D. capensis sampled from sunny (lined blocks) and shady (dotted blocks) conditions, with (1 & 4) and without (2 & 5) tentacles



Figures 6 & 7: The graphical illustration of chlorophyll and anthocyanins in *D. capensis* plant samples, with tentacles and without tentacles.

4] DISCUSSION

4.1) Sun vs Shade:

Results obtained for the two *Drosera* species being investigated in this paper compared to the reference plants (see Figure 4) showed that the carnivorous plants are more N-15 enriched, this was indicated by the more positive δ N-15 values obtained for these plants. The differences in δ N-15 values between the carnivorous plants and their reference plants are represented clearly in the graph (Figure 4) but statistically the difference is not as expected (see Table 1). Results of plants from Camp's Bay showed that there is a significant difference between the sun collected *D. capensis* plants and their reference plants ($p = 0.089, p < 0.1$; 90% significance level). However, similar statistical analyses (one-way ANOVA) performed between the shade *D. capensis* plants and reference plants of Camp's Bay, showed no significant difference between the shade collected *D. capensis* plants and the reference plants ($p = 0.140$, $p > 0.1$; at the lowest significance level).

Such lack of statistical significant difference between carnivorous plants and their reference plants was previously noted by Schulze et al. (1991) where they were comparing Southwest Australian Drosera species. They found that the rosette species did not generally differ from reference plants. They proposed two possible reasons for such results: either that the *Drosera* plants were acquiring relatively little insect nitrogen and/or the technique was not sufficiently sensitive to determine how much nitrogen originated from prey.

Their results are similar to the ones obtained in this study because even though there was an indication of a significant difference between the sun collected *D. capensis* plants and their reference plants, the significance level was very low ($p = 0.089$). Moreover, as already mentioned there was no significance difference between the shade collected *D. capensis* plants and their reference plants. Another possible reason for such results, could be the difference in soil depths of the reference and carnivorous plants. This is such that reference plants could be absorbing nitrogen from soil depths different from those supplying the *Drosera* plants and thus, have their δ N-15 values increased because according to Gebauer and Schulze (1991) δ N-15 values increase

with soil depth. However, to confidently imply the above reasons for the results obtained, further investigations would need to be done.

The plants collected on Table Mountain gave results that definitely agreed with the prediction that carnivorous plants are more enriched in N-15 than the reference plants. Sun and shade collected *D. aliciae* plants were significantly different from the reference plants growing with them (sun vs reference plants $p = 0.004$, $p<0.01$; shade vs reference plants $p = 0.0002$, $p< 0.01$). Even though the shade *D. capensis* plants gave no significant difference when compared to their reference plants, the rest of the results show that carnivorous plants (both *Drosera* species) are more enriched in N-15 than their surrounding plants. Since all the plants are growing under similar environmental conditions, the only reason for the more positive natural abundance values in these plants must be that they are a getting different nitrogen source than the other plants. Since the difference is in the utilisation of insects by the carnivorous plants, the 'new' sources must be from the insects that are expected to have higher δ N-15 values because they are higher up in the food chain, but actual insect N-15 values are needed to confirm this.

The differences that were expected between sun and shade collected *Drosera* plants, according to Givnish's (1984) cost/benefit model theory, were not observed in this study. The δ N-15 values for the sun collected carnivorous plants of both species look different, the sun collected plants have higher values than those collected from the shade (sun *D. capensis* ave. δ N-15 = 3.24, shade *D. capensis* ave. δ N-15 = 2.82; sun *D. aliciae* ave. δ N-15 = 1.46, shade *D. aliciae* ave. δ N-15 = 1.16). However, statistical analyses (one-way ANOVA) show no significant differences between the sun and shade collected *Drosera* plants ($p = 0.348$ for sun vs shade in *D. capensis*; and $p = 0.669$ for sun vs shade in *D. aliciae*).

Overall, Givnish's (1984) theory to explain that carnivorous plants prefer sunny conditions rather than shady ones was not supported by the results of this study. Therefore, the first starting hypothesis of this paper that stated that *Drosera* plants collected from sunny conditions would have higher δ N-15 values than the shade collected plants is rejected. Such results suggest that there are other reasons for

carnivorous plants occurring in both shady and sunny habitats. Moreover, there must be alternative reasons to why carnivorous plants might prefer to grow in sunny conditions than in the shade. A possible alternative reasons for the carnivorous species occurring in both sunny and shady conditions is to increase the niche these plants can exploit, which in turn leads to an increase in the overall fitness of the species. An alternative explanation to the abundance of carnivorous plants occurring in sunny rather than shady conditions is that more insects can be caught and then digested from sunny habitats.

The δ N-15 of the Camp's Bay *Drosera* species are higher (more positive) than those from Table Mountain in both sunny and shady environments (see Table 1 & Figure 4). This is also indicated by the high average % (total) nitrogen present in *Drosera* species from Camp's Bay that those of Table Mountain (Figure 5). Total nitrogen results of the reference plants from the two locations are almost exactly equal (see Table 1 and Figure 5), meaning that there are equal soil nitrogen resources in the two habitats. These results show not only that both carnivorous are receiving a different source of nitrogen (e.g. insects) than the surrounding vegetation, but also that the carnivorous plants from Camp's Bay are receiving a considerably higher amount of this other source than the Table Mountain carnivorous plants.

It is important to note that even though there seems to be a greater difference between carnivorous and reference plants of Table Mountain (see Table 1), the difference is due to the negative δ N-15 values obtained for the reference plants on Table Mountain. Such a difference is due to the moisture conditions in the habitat (Schmidt and Stewart, 1997; Midgley and Stock, 1998), and not the degree of carnivory between the two *Drosera* species. Therefore, the differences between the total nitrogen values at the two locations (high at Camps Bay and Low on Table Mountain) can only be explained by suggesting that the carnivorous plants of Camps Bay are catching more insects than those on Table Mountain. Reasons to explain this will be looked into later on in the paper, and these are related to the difference in altitude that results in temperature differences in the habitats (see 4.3), and plant-form and leaf-size (see 4.2).

4.2) Tall vs Short *Drosera* plants:

The second hypothesis proposed at the beginning of this paper was that carnivorous plants with taller (and longer leaves) rosettes, would be more carnivorous (i.e. would have higher δ N-15 values. Since it is know that *D. capensis* has considerably taller rosettes and longer leaves (stem-forming rosette, leaves about 15cm in diameter) than *D. aliciae (*ground-level rosette, leaves about 5cm in diameter), *D. capensis* was expected to yield higher δ N-15 values according to the hypothesis. The results of *D. capensis* δ N-15 values support the hypothesis by being generally higher than those of *D. aliciae* are ($p = 0.027$ for the sun *D. capensis* vs sun *D. aliciae*; $p = 0.000$ for the shade comparisons). The reason taller rosettes and longer leaves yield higher δ N-15 values is that such plants have bigger surface areas to catch, kill and digest prey. Moreover, longer leaves allow the plant to be able to catch bigger prey as well as small ones, and that will provide the plant with more nutrients than only small prey or very few large insects by small leaves. Previously Zamora (1995), had also encountered the role of leaf size, form and spatial distribution on plant-prey relationships. The results of this study support his earlier findings.

4.3) Cool/ damp vs warm/ seasonally wet:

The two *Drosera* species investigated in this study were collected from two locations differing in their points in altitude and moisture. Previous studies have indicated that different points of altitude result in differences in temperature. The site, Camp's Bay at a low point of latitude, has a generally warm temperature. Table Mountain however, is at a much higher point of altitude and as a result is cooler. The amount of moisture in the two locations is also different. In Camp's Bay, the habitat from which the plant samples were collected is seasonally wet; on Table Mountain the plant samples were collected from a habitat that is constantly moist and thus waterlogged.

Previous studies have looked at the influence of altitude (Corbet, 1990 and Herrera, 1995a) and moisture (Schmidt and Stewart, 1997; Midgley and Stock, 1998) on plant-insect relationships. Corbet (1990) and Herrera (1995a) showed that cool habitats are

limited in insects, and Midgley and Stock (1998) in their study found that waterlogged habitats have more negative δ N-15 values. The results of this study are similar to those by the previous studies. The δ N-15 values were higher in *D. capensis* that was collected from camp's Bay, a warm and seasonally moist habitat. This is due to there being a greater number of insects in warmer habitats than in cool ones (e.g. Table Mountain). A study by Zamora (1999) and other previous studies by Corbet (1990) and Herrera (1995a) support this notion because they show that in cool and shady habitats, most ecto-thermal insects cannot remain active below specific temperatures. The results of this paper then, support the initial prediction made at the beginning of the paper that higher δ N-15 values are expected from *D. capensis* than *D. aliciae*. This means only insects that can survive cool temperatures would be available as prey to the plants from Table Mountain. Since such insects are few, there are few captures on Table Mountain hence, the low δ N-15 values.

The differences in δ N-15 values between the two localities are further enhanced by the differences in the nature of the moisture in the habitats. The values are low at Table Mountain where there are possibilities of waterlogging. Water-logging according to studies by Schmidt and Stewart (1997) and Midgley and Stock (1998), has effects of lowering the δ N-15 natural abundance levels because of the depletion in the heavier N-15 isotope. Seasonal moisture is ideal for carnivory as proposed in Givnish's (1984) cost/benefit model. This is because the seasonal availability of water prevents the incidence of water being a limiting factor to photosynthesis and thus, carnivory (measured as the δ N-15 natural abundance levels).

4.4) Chlorophyll and Anthocyanins:

The enhancement of photosynthesis is the primary benefit from carnivory according to Givnish (1984). For the theory proposed by Givnish to be accurate, the benefits of photosynthesis should be greater than the costs of carnivory. There, the observed differences in colour in both the *Drosera* species should not result in higher costs of carnivory than the benefit of photosynthesis. The chlorophyll and anthocyanins contents of both species, in sunny and shady conditions, should not be a high cost for the plants to pay for their being carnivorous. This means the production of additional

chlorophyll and anthocyanins, which give the plants' leaves their 'darker' colouring, should not cost the plants more energy than what they will be benefiting from such additional pigment contents. Results of chlorophyll measurements between sun and shade collected *D. capensis* plants, showed that there is more chlorophyll in the plants growing in the sun (3.046 average chlorophyll content) than those growing in the shade (1.881 average chlorophyll content) {see Table 2 and Figures 6 & 7}. To test whether the tentacles played a role in the differences in chlorophyll content, they were removed and the chlorophyll content measured.

Results from that investigation showed similarities in the tests with the tentacles intact (sun: 2.238 average chlorophyll content; shade 1.111 average chlorophyll content) {see Table 2 and Figures 6 & 7}. These results indicate that the chlorophyll content is higher in plants growing in the sun and that the tentacles have no effect on chlorophyll. This suggests that there is a cost for carnivorous plants growing in the sun, but because these plants are the ones that catch more insects (high δ N-15 values) the benefits are greater than the costs of carnivory. Since there is no significant amount of chlorophyll on the tentacles themselves, this indicates that chlorophyll has nothing to do with carnivory (unlike the tentacles) and that the difference between sun and shade collected plants is biochemical and found in all green plants. The difference in chlorophyll content (due to biochemical characteristics) in sun and shade collected *D. capensis* plants is related to the chlorophyll *a/b* ratio. According to Packer and Dounce (1987) high-light and sun-exposed plants (high-light chloroplasts) exhibit a/b ratios that are high (e.g. 3.2 to 4), whereas shade plants (low-light chloroplasts) possess lower values for the *a/b* ratio (e.g. 2.5 to 2.9).

The anthocyanin measurements in leaves of *D. capensis* plants with the tentacles intact, gave results showing that there is generally no difference in anthocyanins content in the sun and shade collected *D. capensis* plants (sun [A] = 0.0049; shade [A] = 0.0047) {see table 2 and Figures 6 & 7}. When similar measurements were done with the tentacles removed from the leaves, the anthocyanins content decreased but was still relatively similar between sun and shade collected plants (sun [A] = 0.0026; shade [A] = 0.0022). These results show that the tentacles are the parts that contain the anthocyanins, but that there is no difference in content between sun and shade

plants. This means anthocyanins are not adaptations for the plants' protection against too much sun, for which shade collected plants would have much lower values as they would not need as much protection. The only other reason for the presence of this pigment is as an adaptation of carnivory, possibly to attract insects in both sunny and shady conditions equally.

When taking Givnish's (1984) cost/benefit theory into account, the presence of anthocyanins in *D. capensis* plants growing in the shade are more of a cost than a benefit because the plants there are not catching that many insects. Therefore, in the case of anthocyanins being an adaptation to carnivory in *Drosera* species by their colour that aids in the attraction of insects, Givnish's (1984) proposed cost/benefit model explanation of carnivory favouring sunny conditions is supported. At this stage the chlorophyll hypothesis that there would be more in shade collected plants than ones from the sun, is rejected; and the last hypothesis concerning anthocyanins is accepted.

5] CONCLUSION

Drosera species can grow in a number of different habitats, each with different environmental conditions. The differences occur between species, and within the species plants can utilise a wide range of habitat conditions as long as they are adapted to the particular environment. Overall results indicate that *D. capensis* growing in a warm environment that is seasonally wet, is superior in being carnivorous than *D. aliciae* which has smaller leaves, a ground-level rosette and grows in a cool and water-logged environment. Generally, both *Drosera* species have a capacity to vary their investment in carnivory in complex responses to differences in environmental conditions (e.g. sunny and shady).

This capacity is probably the reason the *Drosera* genus in general, has such a broad distribution range. Evidence was obtained in this study that supports Givnish's (1984) cost/benefit model explaining why sunny conditions are favoured by carnivory (e.g. anthocyanins results). However, there are other environmental conditions that affect carnivory and it is some of these that are the reason for carnivory being more abundant in sunny environment.

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