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A.G. Ellis · J.J. Midgley

A new plant-animal mutualism involving a plant with sticky leaves and a resident hemipteran insect

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Abstract We report on a new plant-animal mutualism in which the plant *Roridula gorgonias*, first suspected by Darwin (1875) to be carnivorous, is, at least in part, indirectly carnivorous. This plant has sticky leaves which trap many insects but it has no digestive enzymes. Instead, trapped invertebrates are rapidly consumed by a hemipteran *Pameridea roridulae*, only found on this plant. However, evidence from $\delta^{15}N$ experiments suggests that *R. gorgonias* does derive significant amounts of nitrogen from trapped prey, apparently via exudations of *P. roridulae*.

Key words *Roridula gorgonias* · Carnivorous plant · Hemipterans

Introduction

Since Darwin (1875) first suggested *Roridula gorgonias* (Linnaeus) (one of two species, both rare, of Roridulaceae, an endemic Cape family) was carnivorous, controversy has existed as to whether this is indeed so. Its leaves bear numerous stalked glands which secrete a very viscid substance which traps insects. This led Darwin (1875) and Marloth (1903) to consider it to be carnivorous. However, after investigating the viscid glandular leaf secretion, Marloth (1910) reversed his opinion, on the basis that the secretion was balsam-like. Lloyd (1934) later described the viscid secretion as a resinous substance which therefore lacked digestive properties. Furthermore, unlike *Drosera*, *Roridula* possesses glands which lack vasculature and, presumably, absorptive ability. It lacks the sessile glands which are involved in enzyme production and nutrient uptake (Lloyd 1934) in other carnivorous plants.

Also conspicuous on *Roridula* are large numbers of a carnivorous hemipteran, *Pameridea roridulae* (Reuter) (Hemiptera: Miridae), apparently only found only *R. gorgonias* (Dolling and Palmer 1991), which feeds on invertebrates trapped by the plant (Marloth 1903). Marloth (1910) suggested the benefit of the insect to the plant was as a pollinator. This hemipteran is carnivorous and is therefore potentially a kleptoparasite (i.e. steals the prey of *R. gorgonias*). This may further reduce any likelihood of the plant being functionally carnivorous. At present, *Roridula* is considered only as a near-carnivore (Juniper et al. 1989), possibly benefiting from nutrients of trapped insects after leaf fall and subsequent leaching.

However, similarities with other carnivorous plants suggest that it may be carnivorous. For example, *Roridula* has both a very poor root system (Marloth 1903) and a distribution limited to acidic leached soils in humid sites, an environment typical of most carnivorous plants (Givnish et al. 1984), co-occurring as it does with carnivorous *Drosera* species. Here we report on experiments to determine whether it is actually carnivorous and what impacts, if any, the hemipterans may have on the plant.

A.G. Ellis · J.J. Midgley (✉)
Botany Department, University of Cape Town,
P. Bag Rondebosch, 7700, South Africa

Methods

Study site

Field studies were undertaken at Fernkloof nature reserve in Hermanus, South Africa (34°23′30″S, 19°17′30″E) on 5–9 July, 1994. The study population was situated on a mountain seep and consisted of approximately 50 plants (c. 1 m in height) confined to an area of about 100 m^2. Plants are woody, sparsely branched shrubs with leaves in terminal clusters.

Enzyme production

We used the method of Heslop-Harrison and Knox (1971) to determine whether proteolytic enzymes are produced by *R. gorgonias*. We applied yeast etract to leaves on a laboratory-grown *R. gorgonias* in order to chemically stimulate production of enzymes. Eight hours after protein application pieces of exposed and developed (as yet unfixed) photographic film (Kodalith orthofilm) were attached to stimulated leaves. Film was removed after 24 h and inspected for sign of digestion. *Drosera capensis*, a species known to secrete digestive enzymes, was also investigated in an identical manner to provide comparative data.

Insect trapping rates

We cleared a leaf rosette, from each of 15 individual plants, of all captured insects on 6 July 1994. Eight weeks later we removed each rosette so as to sample the prey and trapping area of leaves. The rate of arrival of hemipterans at trapped prey was investigated by applying a single anaesthetized *Drosophila* individual to a leaf on 20 *R. gorgonias* plants. The initial time of arrival of hemipterans at these flies was recorded as well as at 15, 30 and 60 min after application.

^{15}N analyses

To determine whether prey nitrogen is absorbed by *R. gorgonias* we used the methods used by Dixon et al. (1980). Commercial bakers yeast was raised on a standard Difco-Bacto carbon base medium with $(^{15}NH4)_2SO_4$ (99 atom percent excess, APE ^{15}N) (from Sigma Chemical Co.) as the labelled nitrogen source. Yeast cultures were grown in sterile test tubes at 30° C for 48 h. Resulting yeast was collected by centrifugation and then incorporated into a *Drosophila* feeding medium.

D. melanogaster was cultured from the egg stage on a standard rearing medium including the labelled yeast. Egg-laying adult flies were introduced to the media for 5 days and then removed. An unenriched batch of flies was raised on brown sugar, maize meal, yeast and water.

Three field treatments were used. A non-exclusion treatment in which plants were fed ^{15}N-enriched *Drosophila* and the hemipteran population remained on the plants. An exclusion treatment in which plants were fed ^{15}N-enriched *Drosophila* but the hemipterans were removed and excluded. Finally, plants were fed unenriched *Drosophila* and the hemipterans remained on the plants. In the hemipteran exclusion treatment, initially hemipterans were cleared by hand and thereafter a sticky barrier was established around the stem of each plant in order to prevent the return of hemipteran individuals. Adult hemipterans, which make up less than 10% of the hemipteran population (A.G. Ellis, personal observations), are able to fly. For this reason, exclusion plants were examined regularly over the 3-day study period for the presence of hemipterans. In the event of their return (rarely observed), hemipterans were removed.

Prior to application of flies all natural prey carcasses were eliminated from the leaf clusters. Slightly anaesthetized *Drosophila* were then applied to recently mature, fully expanded leaves. Six flies were fed to each of the treatment plants, with two flies placed centrally on three leaves of a plant. Flies were only fed to one leaf rosette per treatment plant. Plants were then left for 72 h before sampling, a period considerably longer than that required by other carnivorous plants to effect nutrient uptake from prey (Heslop-Harison and Knox 1971; Clancy and Coffey 1977), but probably less than required by indirect feeders (e.g. those that rely on fungi for prey break down; Juniper et al. 1989). Fed leaves ("old") and the apical bud (with associated "new" leaves) of each treatment leaf rosette were then collected. Three untreated plants, used to obtain natural ^{15}N abundance, were also sampled, as well as hemipterans from both untreated and enriched treatment plants.

The possibly mutualistic role of the hemipterans was further analysed by feeding ^{15}N-labelled flies to *P. roridulae* individuals in such a way as to prevent any contact between labelled flies and *R. gorgonias*. Since this is a rare plant, only a single plant with soil, including a number of resident hemipterans, was dug up and then grown under greenhouse conditions for 2 months at U.C.T. [During this period the plant continued to trap and absorb prey, although urban prey apparently have a different $\delta^{15}N$ content from wild insects. This is reflected in a decreased leaf $\delta^{15}N$ (−3.87‰) relative to wild individuals ($\delta^{15}N$=14.96‰) (see Tables 1, 2)]. Hemipterans were then removed from the plant and fed six ^{15}N-enriched flies in a petri dish. After 24 h hemipterans were returned to the plant. Over the next 5 days they were fed in a similar manner on another ten labelled flies. Throughout the duration of this experiment no labelled flies came into contact with the plant surface. Furthermore, the terminal leaves of the plant were bagged in order to exclude hemipterans. After 5 days leaf and apical bud material were harvested for isotope analysis.

After sampling, each leaf was examined under the dissecting microscope. All invertebrate remains, debris, hemipteran faeces and that portion of leaf in contact with applied flies was removed in order to eliminate the possibility of contamination, by both natural prey and labelled fly residue. Samples were then rinsed and freeze-dried and 1–1.5 mg of dried sample was then prepared for automated Dumas combustion in a Carlo-Erba NA 1500 Elemental Analyzer connected to a Finnigan MAT 252 mass spectrometer (Archeometry, University of Cape Town). Samples were analysed by continuous flow batch run techniques as presented by ISODAT controller software. A Merck gelatin standard ($\delta^{15}N$=7.0‰) was used. Precision throughout was found to be 1.46‰ in the natural abundance range. In order to eliminate the possibility of sample contamination during analyses, blanks and standards were inserted regularly throughout. Also samples were run in order of expected enrichment obtained from earlier trial runs. It was impossible to analyze labelled flies as the mass spectrometer was only appropriate for enrichments less than 5 APE ^{15}N. We assume that flies were at least 90% enriched in ^{15}N because parent flies and their off-spring were raised on a medium containing 99 APE ammonium sulphate as their only nitrogen source.

Results

The gelatin substrate film method showed that the leaves of *R. gorgonias* do not secrete digestive enzymes. Only the film applied to *Drosera capensis* showed the "spottiness" associated with the presence of digestive enzymes.

R. gorgonias is an efficient trapper of insects. Over the 8-week period a total of 32 macro-invertebrate (i.e. >2 mm in length) species and 109 individuals were trapped. They consisted mainly of dipterans (80.6% by length) and coleopterans (10.1% by length). We used prey length as the measure of importance because the rapid removal of body juices by the hemipterans makes mass a meaningless quantity. Mean prey length was 3.55 mm (SD 0.57 mm). Mean prey length/cm^2 of leaf was 0.47 cm^{-1}. Micro-invertebrates (i.e. <2 mm in length) numbered 112 (a mean of 2.3 per leaf) and consisted mainly of Thysanoptera (52% of individuals) and Diptera (35% of individuals).

P. roridulae probably finds and attacks any newly trapped insects within minutes. The mean period for initial location of experimentally placed flies was 11.09 min (SD=12.06 min). Of the experimentally applied flies 85% (17/20) were located within 15 min and 95% within the hour.

Leaves from untreated *R. gorgonias* have a high $\delta^{15}N$ value (mean 14.96‰, Table 1). The $\delta^{15}N$ from leaves to which experimental flies (both enriched and depleted in $\delta^{15}N$) were added, as well as that of control leaves in new buds, differed from that of control plants (Table 1). The degree of incorporation was greatest when *P. roridulae* was not excluded (Table 1). When the hemipterans were allowed access to a laboratory grown individual of *R. gorgonias*, the label from the enriched flies they had fed on was later found to be incorporated in the leaves, including previously bagged new leaves (Table 2).

Table 1 Results of $\delta^{15}N$ analyses of leaves from experimental and control field-grown plants of *Roridula gorgonias* to which enriched (>90% atom percent excess, APE) and unenriched *Drosophila melanogaster* flies were applied. Old leaves are fully expanded green leaves to which flies were applied and new leaves are distal immature leaves still in young buds. Leaves were removed 5 days after flies (two per leaf on each of three leaves per rosette, one rosette per plant) were applied

	$\delta^{15}N$ ‰ (SD)	Atom % excess (SD)
Natural abundance	(*n*=2 plants)	
Old	14.96 (2.23)	0.371 (0.0008)
New	11.61 (4.26)	0.370 (0.0015)
Unenriched treatment (*n*=2 plants)		
Old	2.54 (0.08)	0.367 (0.00003)
New	1.71 (2.10)	0.366 (0.00077)
Enriched treatment, hemipterans excluded (*n*=4 plants)		
Old	221.68 (116.67)	0.447 (0.04252)
New	73.72 (59.93)	0.393 (0.02186)
Enriched treatment, hemipterans present (*n*=4 plants)		
Old	1208.87 (483.86)	0.805 (0.17502)
New	560.67 (438.73)	0.570 (0.15925)
D. melanogaster (*N*=1)		
	−3.24	0.365

Table 2 Results of $\delta^{15}N$ analyses of a single laboratory grown *R. gorgonias* individual and its associated hemipteran *Pameridea roridulae*. The treatment consisted of feeding labelled flies off the plant to *P. roridulae* but allowing it access to the plant, except bagged new leaves

	$\delta^{15}N$ (SD) ‰	Atom % excess (SD)
R. gorgonias		
Before treatment (*n*=1 plant)	−3.87	0.365
After treatment		
Old leaves (*n*=1)	4497.6	1.980
New leaves (*n*=2)	4337.4 (192.4)	1.924 (0.068)
P. roridulae		
Natural abundance (*n*=1)	6.56	0.364
Enriched	10317.1	3.994

Discussion

The fact that *P. roridulae* rapidly finds and attacks trapped insects confirms that direct carnivory is unlikely, given that digestion of prey by plants takes many hours (Heslop-Harison and Knox 1971; Clancy and Coffey 1977). It also suggests that indirect carnivory either after leaf fall (Juniper et al. 1989), or by fungi or bacteria, is unlikely to be as important. This is because all that is left after the hemipterans have attacked captured insects, is the empty exoskeleton.

The $\delta^{15}N$ studies suggested that *R. gorgonias* is carnivorous. Natural abundance value of ^{15}N is high. Plants obtaining nitrogen only from the soil have values usually between −8 and 10‰. Stock et al. (1995) presented values for non-N_2 fixing Cape fynbos plants of between −3 and 5‰ and soil values between 3 and 6‰. The $\delta^{15}N$ value for *R. gorgonias* is considerably higher than those reported for other carnivorous plant species, i.e. 0.819–3.302‰ for Australian *Drosera* sp. (Schulze et al. 1991), and 0.37‰ for *Utricularia vulgaris* (Friday and Quarmby 1994).

Isotope evidence also suggests that nitrogen from labelled flies is incorporated and translocated to young leaves, despite the lack of enzymes and the kleptoparasitism. The route of absorption is probably via exudations of the hemipteran.

Observations of *P. roridulae* indicate that it produces a liquid secretion, commonly produced by hemipterans, as well as more solid excreta. The liquid is excreted within a few minutes of feeding on a trapped insect. Both of these exudations are deposited on the leaves, especially the undersides. We suggest that these waste products are then absorbed by the leaf.

This is the first report of this kind of mutualism between a carnivorous plant and an invertebrate. However, it may not be unique. The closely related *R. dentata* (Planchon) – *P. marlothi* (Poppius) pair (Marloth 1910) are likely to display similar nutritional dynamics. Also, the nutrition of the Australian Byblidaceae may be similar. The plants in this genus resemble *Roridula*; they also do not manufacture digestive enzymes (Juniper et al. 1989) and also support obligate hemipteran colonies (Lloyd 1942). *Byblis* is currently regarded as truly carnivorous, although it is thought to derive prey nutrients indirectly through fungal activity (Juniper et al. 1989), as do many plant-carnivores such as pitcher-plants (Lloyd 1942). We suspect that the nutritional dynamics of *Byblis*, and some other plant carnivores, therefore may actually resemble those of *R. gorgonias*.

Although we have demonstrated carnivory, we have not been able to quantify it. For the following reasons we suspect that levels of carnivory are significant. *R. gorgonias* trapping rates and prey sizes, after only an 8-week trapping period, compare favourably with leaf-life time trapping rates of *Drosera* (Verbeek and Boasson 1993) and *Pinguicula* (Zamora 1990). It has a high $\delta^{15}N$ and new leaves on plants to which enriched flies had been applied were also enriched.

Our quantification problems involved dealing with dilution and equilibration effects (Deleens et al. 1994) associated with using small amounts of labelled ^{15}N which are translocated around a rare, large, woody perennial plant, and also measuring the quantity and quality of the exudates of a small mobile carnivorous mediator.

Our results elevate Roridula to being the tallest (up to 2 m tall) and woodiest carnivorous plant. Darwin (1875) was right about *Roridula*, but for the wrong reason.

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