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**Evolution of carnivory in the *Lentibulariaceae*: considerations based on  
molecular, morphological, and physiological evidence**

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## ABSTRACT

Data from recent molecular phylogenetic studies on the *Lentibulariaceae* and on related families in the Lamiales are discussed in the light of a potential scheme for the evolution of carnivory in this plant family. It is suggested that all 3 extant genera of the *Lentibulariaceae* (*Pinguicula*, *Utricularia* and *Genlisea*) evolved from a common, more or less aquatic (submerged under a very thin film of water or wetted regularly by rain or fog), and rootless (no central root system) rosetted ancestor. This ancestor would not be related to the Lamiales procarnivorous families, *Martyniaceae* and *Byblidaceae*. It might have been making use of sessile glands on its leaves (a potential speciation from an ancestral gland type shared with the rest of the Lamiales) to absorb nearby nutrients and secrete enzymes to digest surrounding organic matter and increase nutrient availability next to its leaves. With the end result of better securing catches (and thus becoming carnivorous) and limiting water losses on traps, 2 strategies have been developed. One has consisted in producing a novel gland type, a stalked gland, that secretes mucilage to glue preys and prevent dehydration, and that generates a bowl (curling effect) under the preys to retain digestive fluids. *Pinguicula* has probably evolved according to this pattern. The nearest ancestor of our *Pinguicula* sampling was living in the northern hemisphere and was producing an hibernaculum to resist frost. This hibernaculum has subsequently evolved into a drought resistant form (and in some cases later disappear altogether) in the Mexican taxa. A larger sampling is likely to shed light on a more ancient past of this genus. *Utricularia* and *Genlisea* are suggested to have worked out a separate strategy towards carnivory whereby they have closed (more or less tightly) their trap-leaves and inverted their tropism to generate upside-down bladders that suck and filter food permanently from

their aqueous environment (anything from already dissolved matter to microscopic organisms). Both bladder-bearing genera developed different ways of securing their catches. *Utricularia* traps have a proper door and doorstep system of closure while *Genlisea* traps have a long neck and 2 arms next to a permanently opened trap mouth. *Genlisea* neck and arms inside surfaces are lined with downwards-pointing hairs that force the flow of prey to only migrate towards the digestive cavity and never escape. Early diversification of *Utricularia* and *Genlisea* is thought to have taken place in the southern hemisphere.

## INTRODUCTION

The evolution of carnivorous structures in plants represents one of the most complex and most fascinating enigmas in plant sciences. Recent attempts to find their origins with genetic tools have been made on several groups of carnivorous plants such as the *Droseraceae* and related families (Albert *et al.* 1992), *Nepenthaceae* (Meimberg *et al.*, 2000), and the *Sarraceniaceae* and related families (Bayer *et al.*, 1996). However, the *Lentibulariaceae* family that makes up nearly half of the total number of carnivorous plant species had so far never been thoroughly investigated.

*Lentibulariaceae* comprise 3 carnivorous genera, *Pinguicula*, *Genlisea* and *Utricularia*. All 3 genera share characteristics in flower structure (zygomorphic flowers with a spur) but exhibit widely different carnivorous habits. *Pinguicula* species have the simplest trapping device. Their leaves possess sticky glands and they catch prey by acting like flypapers. Because of this apparent simplicity of action, it has been widely suggested that they could represent an ancestral form that led to the development of the more complex-looking *Utricularia* and *Genlisea* suction type traps (Taylor, 1989). In this respect, *Genlisea* species have been considered to be evolutionary intermediates between the 2 other genera since they generate bladder type traps but do not possess a sophisticated door system to secure any catch like *Utricularia*. Along the same line of thinking, *Byblidaceae* and some *Martyniaceae* (called procarnivores in this study) have been suggested to be descendants of an even more ancestral form since their leaves act as flypaper traps that cannot digest their prey (Lloyd, 1942, Hartmeyer, 1997, 1998; Meyer-Rice, 1999; Wallace and McGhee, 1999). Affinities of *Lentibulariaceae* and procarnivores within Lamiales are

suggested by morphology and were supported by broad scale molecular phylogenetic analyses (Albert *et al.*, 1992; Chase *et al.*, 1993; Soltis *et al.*, 2000; Albach *et al.*, 2001). However, exact relationships remained uncertain.

Ever since scientists have unveiled the heredity of the DNA molecules that are contained in each living cell, they have tried to access the information it contains to better understand the past history of modern life on earth. The recent complete sequencing of the human genome has been a giant step in this direction (Lander *et al.*, 2001; Venter *et al.*, 2001). It revealed that all of the genes that act to maintain our body living barely make up 1.5 % of our total DNA and that the rest is an immense junk yard of non-functional genetic information that has accumulated over billions of years of evolution like geological layers in an archeological site. But what can they tell us? How were they deposited? How has new DNA information been created? These are questions scientists still cannot answer fully. It is still out of the question to conduct a complete genome sequencing of every living organism. Current technologies (including computer technologies) do not allow it. What is possible at the moment is to look at small, carefully chosen, portions of the full genome whose variations among sampled individuals have enough statistical significance to draw conclusions.

Over the past three years, we have attempted to generate DNA sequence data of a large group of species of the *Lentibulariaceae* and related families within the Lamiales (Müller *et al.*, 2002a and b). Our DNA target has been the widely used chloroplast gene *matK* (Neuhaus and Link, 1987; Hilu & Liang, 1997) and the rarely used adjacent intron sequences (*trnK* intron). New computer software has been

designed to facilitate alignment, phylogenetic analysis and analysis of rate heterogeneity (Müller, 2000; Müller *et al.*, 2002b; Müller, 2002). Besides molecular work, a lot of progress has been made to understand the biology of *Lentibulariaceae*. For example, Barthlott *et al.*, 1998 showed *Genlisea* to be trapping protozoans. To understand the evolutionary diversification of the *Lentibulariaceae*, we attempted to link our molecular phylogenetic data to a careful analysis of morphological features, in particular the differences/similarities in gland anatomy (Müller *et al.*, 2002a and b). The present article will review some of the conclusions of this work and will outline one possible scenario for the evolution of members of the *Lentibulariaceae*.

## MATERIALS AND METHODS

Information concerning the methods used for DNA extraction, amplification and sequencing can be found in Müller *et al.* (2002a and b). Computer software used and developed for analysing the data are also described in the above manuscripts.

A set of 32 *Utricularia* species, 6 *Genlisea* species and 27 *Pinguicula* species with representatives from most known major sections of these 3 genera has been selected. Sections were derived from Fischer *et al.* (2000), Casper (1966), Legendre (2000) and Taylor (1989). In addition, species belonging to 22 related Lamiales genera were included in the data set. Voucher information and GenBank accession numbers can be found in Müller *et al.* (2002a and b).

## RESULTS

### *Phylogenetic relationships between procarnivorous taxa and Lentibulariaceae in Lamiales*

Parsimony and Likelihood analyses of *matK* and *trnK*-intron sequence data clearly support the current grouping of *Utricularia*, *Genlisea* and *Pinguicula* in one family (Müller *et al.*, 2002a), so that all 3 genera share common ancestry (i.e., *Lentibulariaceae* are monophyletic). There is strong evidence that the evolution of true carnivory in *Lentibulariaceae* is independent from that of procarnivory in *Byblidaceae* and *Martyniaceae*, i.e., preadaptations to carnivory were acquired at least twice independently in this angiosperm order. However, only limited statistical support was obtained for the respective phylogenetic relationships as inferred from *matK* and *trnK* sequences. Additional Molecular markers are now under scrutiny to better resolve this important issue. Earlier results obtained by Albert *et al.* (1992) with the chloroplast gene *rbcL* suggested that the procarnivorous *Byblis* might be the closest relative of the carnivorous *Lentibulariaceae*. However, this conclusion was based upon a very small Lamiales sampling and not supported statistically.

The distribution of morphological characters supports the phylogenetic relationships reconstructed with molecular data. This becomes especially evident when looking at the structure of the glands of the traps of carnivorous and procarnivorous plants. As shown in figures 1 and 2, the carnivorous/procarnivorous species of the Lamiales are the only insect trapping plants in which gland stalks are made of stacks of layers of single cells. While this may suggest common ancestry, significant differences among the various members do not support such a conclusion and it is just as likely that the



formation of these simple glands is easy enough to have occurred, or have been modified, several times in the evolution of the Lamiales. The glands of the traps of the members of the *Lentibulariaceae* are all positioned on a single epidermal cell, while those of *Byblidaceae* and *Martyniaceae* develop on a minimum of 2 epidermal cells (Juniper et al. 1989). The digestive glands of the members of the *Lentibulariaceae* are all attached to sap vessels unlike any other gland of the *Byblidaceae* and *Martyniaceae*. Finally, the central cell of the digestive glands of the *Lentibulariaceae* (endodermoid cell) possesses physiological characteristics (thickened cell wall/cuticle, endoplasmic reticulum organisation, accumulation of fluorescent substances) that have not been described in any other member of the Lamiales (Juniper et al., 1989; Legendre, 2000). All of this supports the hypothesis of an independent evolution of the incomplete carnivorous syndrome in the procarnivores.

It is interesting to note that carnivorous plants are the only angiosperm species that have glands (digestive ones) that are linked to sap vessels (Juniper et al., 1989). Lloyd (1942) argued that the spongy tissue on which *Byblis* glands sit may act as a conductive medium for the rapid absorption/release of substances. No experimental evidence has been provided. It is, therefore, possible that the linking of glands to sap vessels is a necessary condition for effective digestion and absorption of organic matter by speeding up the transport of enzymes and nutrients.

#### *Phylogeny of Lentibulariaceae*

The analysis of *trnK* sequences from 32 *Utricularia* species, 6 *Genlisea* species and of 27 *Pinguicula* species, revealed that *Lentibulariaceae* species cluster into 2 separate groups (Müller *et al.*, 2002a). One comprises the 2 bladder trap plants (*Utricularia* and *Genlisea*) and the other one is constituted by the flypaper trap genus, *Pinguicula*. *Pinguicula* is, therefore, a sister group of the 2 former genera. Our data show that all three genera are monophyletic. Each of these infrafamilial relationships receives maximum statistic support.

To our great surprise, the rate of mutation of *matK* and the noncoding *trnK* intron sequences was found to be much higher in *Utricularia* and *Genlisea* than in *Pinguicula* (Müller *et al.*, 2002b). Even when compared to other genera in flowering plants, *Utricularia* and *Genlisea* still exhibited the fastest evolutionary rates in *matK*. In *Pinguicula*, this gene was found to evolve just as fast as the fastest previously known rate observed in some parasitic plants. This increased DNA evolutionary rate of *Utricularia* and *Genlisea* was confirmed when other genes from other plant genome pools were studied (Müller *et al.*, 2002b).

An analysis of gland structures supports the relationships found with molecular data. All 3 genera share one type of gland. This gland is the sessile gland of *Pinguicula*, the bifid/quadrifid gland of *Utricularia* and the digestive gland of *Genlisea* (Fig. 1). It is made of an epidermal cell on which sits an endodermoid cell that supports a varying (always a multiple of 2) number of head cells. The number and shape of the head cells varies among genera and species but also on one given trap (Lloyd, 1942, Casper, 1966, Juniper *et al.*, 1989). This conserved gland structure also serves a common function since, in all 3 genera, it is involved into the secretion of digestive enzymes

and the absorption of nutrients. It does not secrete mucilage and is invariably attached to some tracheid element (xylem and/or phloem).

*Pinguicula* leaves harbour another type of gland, a pedunculate gland, which is specific to this genus. Compared to the digestive glands, this gland exhibits an additional elongated stem cell that elevates the endodermoid and radiating head cells from the surface of the epidermis. It also sits on an enlarged epidermal cell (reservoir cell) that further elevates the whole structure. This epidermal cell is not linked to a tracheid element but is in contact with numerous other cells via plasmadesmata (cell to cell communication structure). The pedunculate gland of *Pinguicula* is involved in mucilage production, enzyme and water secretion, and prey capture but does not seem to be involved in nutrient absorption (reviewed in Legendre, 2000). Its enzyme and water secretory mechanism is unique to the Lamiales in the sense that it is a one-off process whereby the head cells secrete their entire content once stimulated by a prey to then engage into a self-destructive mechanism. Thus, this gland can only secrete once in its lifetime. The stalked gland of *Pinguicula* secretes a smaller set of hydrolytic enzymes than the sessile glands (lack of leucine aminopeptidase for example). When similar enzymatic activities are present in both gland types, their intracellular distribution may vary (RNase activity for example – reviewed in Juniper, 1989). Even though *Utricularia* and *Genlisea* do possess mucilage-producing glands, these have a totally different anatomy (Fig. 1). The *Utricularia* mucilage glands are situated close to the entrance of the door of the trap (outside of the trap, i.e., back side of the leaf, see below) where they allow a good sealing of the trap door. Their stem is an elongated epidermal cell (not an additional stem cell). The mucilage producing glands of *Genlisea* are also situated on the outside of the trap contrary to *Pinguicula*

species (if *Pinguicula* species have mucilage glands on the under side of their leaves, they also contain digestive glands on that same leaf surface; see *P. gigantea* for example). They comprise an epidermal cell, a small intermediate cell and a small, round head cell.

Finally, *Utricularia* possesses some glands that are unique to their genus. *Utricularia* traps harbour a type of small gland on their outside surface. It is made of an epidermal cell, an intermediate cell and one head cell. Interestingly, this gland resembles the mucilage gland of *Genlisea* in both shape and position even though its function has been hypothesised to be different (absorption of nutrients in immature traps, Lloyd, 1942). *Genlisea* harbour a large variety of trichome hairs inside their traps and at the edge of their trap arms to clip them. *Pinguicula* and *Utricularia* also express a large variety of hairs but none are located among the digestive glands.

#### *Phylogeny of Pinguicula*

An analysis of the genetic relationships among *Pinguicula* species generated a very surprising result (Müller *et al.*, 2002a). Based on our current sampling, *Pinguicula* species can be divided into 2 separate subgroups. One (called group A) constitutes all of the temperate *Pinguicula* spp. minus *P. alpina* which belongs to the other group (called group B) that includes all Mexican/central American/Cuban species. Even though hybrids between *P. alpina* and *P. vulgaris* (temperate species) have been reported (Casper, 1966), the authors have not succeeded in hybridising these 2 species, as expected if the above genetic grouping is correct. In contrast, many

temperate and Mexican species have been reported to hybridise well with members of their own clade (group of species sharing common ancestry). The temperate species (group A) are subdivided into 2 subgroups. One comprises *P. leptoceras* and *P. poldinii* while the other subset contains the remaining species. These 2 groups are differentiated by a large number of genetic differences. However, species of the large group (comprising *P. vulgaris*, *P. vallisneriifolia*, *P. grandiflora*, *P. macroceras*, *P. corsica*, *P. longifolia*, and others) exhibit no significant differences in DNA sequences of *trnK*. In contrast, the Mexican species are separated by longer branches. *Pinguicula alpina* is sister to the Mexican clade. Within this clade the basal branching taxa are *P. filifolia*, *P. gracilis* and *P. rotundiflora*. Then follows as separate clusters *P. ehlersiae* and a clade consisting of *P. sharpii*, *P. gigantea* and *P. aganta*. Finally, the most terminal clades consist of *P. moctezumae* and *P. moranensis* on one hand, and *P. emarginata* and *P. rectifolia* on the other. This phylogeny of *Pinguicula* based on molecular data contradicts the current subclassification of the genus into 3 subgenera, *Isoloba*, *Temnoceras* and *Pinguicula*. Even though our sampling of species allowed wide representation of most previously defined sections of *Pinguicula* (Casper, 1966; Legendre, 2000), we are currently adding crucial species that will be needed to better understand the evolutionary history of the genus.

All *Pinguicula* species harbour similar sets of glands. They, however, exhibit different growth characteristics. Casper (1966) proposed to distinguish the temperate growth type (forming an hibernaculum) from the tropical growth type, both being subdivided into homophyllous and heterophyllous subgroups depending on whether the plants generate only one or 2 types of leaves during the year. This classification does not match the results of our cladistic analysis based on DNA sequences (Müller *et al.*,

2002a). Both group A and B contain temperate and homophyllous species. Rather, species of groups A and B differ in their time of flowering. All species of group A flower (create flower buds) in the spring after producing a first set of carnivorous leaves while most species of group B, and at least all of the basal ones (*P. alpina*, *P. rotundiflora* and *P. gracilis*), generate flower buds from their winter resting bud before the onset of production of carnivorous leaves. However, this floral characteristic has been poorly recorded on wild Mexican specimens and has sometimes been faulted by observations made on greenhouse grown plants. Further research is needed to validate this observation. We have seen no morphological trait that can associate *P. leptoceras* and *P. poldinii* while distinguishing them from all other European temperate species. The grouping of Mexican species based on their genetic characteristics is as difficult to link to any physiological structure previously used by taxonomists. Further research and a larger sampling will be needed to find morphological characters supporting the above grouping of species.

### *Phylogeny of Utricularia*

*Utricularia* phylogeny was found (Müller *et al.*, 2002a and b) to support the latest intrageneric classification of the genus by Taylor (1989) that was based on morphological data, predominantly from the traps. Pollen morphology was studied by Lobreaan-Callen *et al.* (1999) and seems to be in line with the other phenotypic characters. However, in contradiction to Taylor's concept, our results indicate that section *Polypompholyx* is an integral part of the genus *Utricularia*. As already suggested by Lloyd (1942) and Taylor (1989), this section was found to represent an

ancestral lineage in the genus, along with section *Pleiochasia* (called group A). Following Taylor's division of *Utricularia* into terrestrial, epiphytic or aquatic species, all so-called epiphytes (called group B' here) and aquatic species (called group B) were found to form separate clades that evolved from within a group of terrestrial species (group A, for sections *polypompholyx* and *Pleiochasia*, and group A' for the remaining sections).

Trap functioning and overall gland structures have previously been described as being conserved within the genus *Utricularia* (Lloyd, 1942). The shape of the head cells of the digestive glands has, however, been reported to vary among species. These variations have been used successfully as a taxonomic character (Taylor, 1989). The decorations on the outside of the trap (such as ventral and mouth appendages) also vary among species and have been used at a lower taxonomic level in agreement with our data. Heslop-Harrison (1975, 1976), has suggested that aquatic *Utricularia* species are the only ones to bear active traps and thus represent the most evolved/advanced members of the genus. Taylor (1989) did not support the idea that *Utricularia* traps can be divided into active and passive ones. He suggested that European aquatic *Utricularia* could be the most advanced form of the genus since they do not produce rosettes and make the largest use of stolons. These claims are, however, not supported by our data (Müller *et al.*, 2002a).

Even though trap functioning is conserved within the genus *Utricularia* (Taylor, 1989), the position of the traps on the plant varies and has been used to distinguish groups of sections of this genus (Taylor, 1989). Our data (Müller *et al.*, 2002a) support this observation. The central stem of *Genlisea* species and the species of the

basal sections of *Utricularia* (group A) forms a dense rosette of leaf-like (see later for a discussion of the organs in *Utricularia*) organs and traps. In contrast, species of group A' generate traps on either the leaf-like structures or on stolons but never from the central rosette (main stem). In group B', members of the so-called epiphytes bear traps on the main stem of their rosettes, while the so-called aquatic group members (group B) bear traps on the leaf-like structures only. The branching of the photosynthetic organs is a feature that distinguishes most species of groups A/A' from those of groups B/B' even though it has not been previously used taxonomically. With the exception of *U. sandersonii* and related species, all members of groups A/A' possess leaf-like structures that contain only one undivided vein. Conversely, with the exception of *U. subulata*, all members of group B/B' harbour leaf-like structures whose veins fork many times, the laminae either joining the veins as in the so-called epiphyte group (group B') or leaving them separate as in the so-called aquatic group (group B).



## DISCUSSION

With the above data, we will now attempt to discuss a scheme for the evolution of the carnivorous syndrome in the *Lentibulariaceae*. Such a scheme assumes that the simplest evolutionary path has been followed. It is highly dependent on the amount of information available at the moment and may be faulted by past relatives that may have disappeared. Unfortunately, the fossil record of our group of plants is extremely poor. Our evolutionary scheme is presented as an educated guess to be used for future studies and discussions on this topic.

Physiological characters that are shared by all *Pinguicula*, *Utricularia* and *Genlisea* species are most likely so because they were present in their common ancestor. Examples of independent and convergent evolution of specific morphological traits are common in the plant kingdom but they usually only concern 2 distant genera and not 3 closely related ones. Based on this assumption, the nearest original ancestor of the *Lentibulariaceae* would have been a rosetted plant with no central root system and only one type of gland on the surface of its leaves (the digestive gland common to all 3 modern genera). All 3 genera generate rosetted plants (admittedly less obvious in the more recent *Utricularia* species). Their central root system never develops out of the seed coat during germination so that one end of the central stem grows while the other one dies off (only adventitious roots, i.e., side roots developing from the main stem, are generated by *Pinguicula* species). With just digestive glands, it is hard to foresee how this original ancestor could have caught any prey since *Lentibulariaceae* digestive glands do not secrete mucilage and they lay flat on the leaf surface. Moreover, this type of gland secretes and absorbs via cracks in its cuticular surface

(free exchange of substances between the outside environment and the inter-cellular apoplastic fluids) so that these mechanisms can only take place in an aqueous environment and death of the plant by dehydration can quickly occur if the glands are exposed to open and dry air. This need for an aqueous, or at least very humid, environment next to the digestive glands is still valid for all 3 modern genera. Therefore, it is very probable that the original ancestor was acting like a partially saprophytic-type plant (still carnivorous) in or next to a rich liquid broth. Most likely, the amount of water surrounding the leaves was small, like a thin film above the leaves or leaves wetted very often by rain or fog, to prevent the secreted enzymes from being diluted away. In such an environment, the secretion of enzymes would have helped degrade the surrounding organic debris to further enrich the broth next to the leaves. The attachment of the glands to tracheid element would have facilitated the distribution of these nutrients through the plant. So, the leaves (via the digestive glands) would have done the roots' job in feeding the plant. This may explain why an elaborate root system may have disappeared.

Hypothesising that carnivory in the *Lentibulariaceae* could have evolved from the above mentioned nutritional specialisations is new and different from previous hypotheses elaborated for other carnivorous plant groups. It has been suggested that carnivorous structures in *Droseraceae* (Schlauer, 1997) evolved from salt-secretion glands and/or as a flower defence mechanism aimed at gluing crawling insects to prevent them from pollinating or eating flower parts. Possibly, this explains why *Drosera* glands both secrete mucilage and digestive enzymes in contrast to *Pinguicula* which have 2 separate gland types to conduct these 2 activities.

The geographical distribution of the common ancestor of the *Lentibulariaceae* is impossible to guess with the current data. Except for their most derived members, *Pinguicula* is a northern hemisphere genus while *Utricularia* and *Genlisea* are southern hemisphere genera (see below). Possible explanations include that the ancestor of *Lentibulariaceae* was present before the split of Gondwana and Laurasia, or that in some stage of the evolution of the lineage diaspores were dispersed into both hemispheres to give rise to all 3 genera. Rather rapid distribution must have also occurred in the aquatic members of *Utricularia* to and throughout the northern hemisphere. This, however, seems rather likely given that many aquatics possess cosmopolitan ranges and are easily distributed by waterfowl.

For some reason, the common ancestor of the *Lentibulariaceae* did not survive and its descendants developed 2 separate strategies to better retain their catches and limit dehydration of their leaves (Müller *et al.*, 2002). One involved the production of a novel type of gland, a pedunculate gland, by the current *Pinguicula*. This has led to a slowing-down of the rate of DNA evolution of its members. This genus-specific gland serves many new functions (reviewed in Legendre, 2000). It allows good retention of the prey via the production of mucilage and the creation of a bowl (leaf curling) under the prey. This movement is achieved thanks to the basal, epidermal cell that is swollen (water reservoir) in the resting state but secretes all of its content upon mechanical stimulation by a prey. In doing so, it loses turgor and sinks within the epidermis, creating a small depression under the prey where digestive fluids can be kept. This novel type of gland also prevents dehydration of the leaf thanks to the mucilage that surmounts it.

The different phenotypes of *Utricularia* and *Genlisea* apparently evolved via a separate option that consisted of closing the leaf on itself and forming inverted traps. Several species of *Pinguicula* have been observed to accidentally generate Champagne cup-shaped leaves under cultivation. This phenomenon rarely repeats itself on the same plant and seems to be a one-time error in the developmental program of the leaves. Close examination of a herbarium specimen of *P. utricularioides*, a *Pinguicula* species that naturally forms bladders, did not reveal any clue to the way these bladders evolved or whether they are a constant phenomenon for these plants. It is possible that some evolutionary intermediates of the bladder plants harboured Champagne cup-shaped leaves on a regular basis. *Pinguicula*-derived Champagne cup leaves are small and so are most of the bladders of *Utricularia* and *Genlisea*. Thus, *Utricularia* and *Genlisea* traps would technically be leaves in agreement with Lloyd (1942). Even though this leaf configuration offers an intermediate stage towards the development of a proper bladder, it does not allow the catching of many preys, nor their complete retention. Inverting tropism is one way to solve this issue and this is what *Utricularia*'s and *Genlisea*'s common ancestor may have done. Once the traps grow downwards, the flow of sap through their petiole will move backwards, pulled by dehydration of the top portion of the plant. Because of this inverted sap flow, the trap will now act as a permanent suction device that will filter its bathing medium. It will also be able to sit in a moist environment while allowing the rest of the plant to develop above the soil and/or water. The catches will be more numerous and can be anything from dissolved nutrients to microscopic organisms that will be caught and digested to be later assimilated. This scheme is further supported by a recent observation that *Genlisea* feeds on protozoans (Barthlott *et al.*, 1998) and the fact that some of the most ancient *Utricularia* species (members

of section *Pleiochasia* like *U. volubilis* and *U. helix*) produce traps on the top of green petioles and that these petioles grow in several directions from upwards, to horizontal, to downwards. At present, no plants with inverted Champagne cup-shaped leaves are known. Probably, they have not survived the competition with their more modern descendants. One possible reason for this is that such a trap is too open and can be visited by large creatures. During such visits, previously trapped microscopic organisms can be stolen and the trap or the head cells of the glands damaged. The head cells occupy a vulnerable position on top of a fragile stem. Because the traps are thought to exist in water, or at least in a very moist environment, infections would spread easily from such wounds. It is suggested that the closing of the leaf was the safest option and the best way to retain catches (formation of trap). *Genlisea* and *Utricularia* have developed 2 separate trap designs (Müller *et al.*, 2002a).

*Genlisea* traps produce 2 long arms to allow the pumping of water from a larger surface area. Trichome hairs are produced inside the trap. They are oriented towards the inside of the trap to prevent large preys from escaping. Attachment hairs are also generated to attach the 2 edges of the arms, while still leaving entry holes in between them. The role of the mucilage glands on the outside surface of the traps is unknown but Lloyd (1942) has been hypothesised that they generate a lubricant to facilitate the flow of prey towards the digestive cavity where the main digestive glands are found.

Instead of developing parallel series of hairs to retain prey, *Utricularia* generate a door and a doorstep to shut the trap. Interestingly, *Genlisea* species possess a non-functional door and doorstep on their dorsal and ventral sides respectively, close to the mouth of their traps. The proper functioning of a door system requires the

presence of side features such as mucilage glands to seal the door (morphologically different from those of *Genlisea*), nectar glands to attract prey towards a tiny trap, and trigger hairs to allow future meals to induce the opening of the door (morphologically different from those of *Genlisea*). If our original hypothesis that *Genlisea* and *Utricularia* traps are in fact leaves that replace the function of the missing roots, how can *Utricularia* plants survive with tightly closed traps? The answer probably lies with the last type of gland that we haven't discussed so far. It is a tiny gland situated on the outside surface of the *Utricularia* trap that resembles the mucilage gland of *Genlisea* or the bifid/quadrifid glands of the inside of the *Utricularia* trap, except that it harbours only one small head cell. These glands have been observed to absorb nutrients readily (Lloyd, 1942). Their smaller size may make them less subject to mechanical damage by passing macro-organisms.

Even though complex in structure, the *Utricularia* trap has obviously been successful judging by the diversity of habitats in which these plants grow, the number of species in this genus and their geographical distribution. Their success has been suggested to have more than one origin (Müller *et al.*, 2002a). First of all, the transformation of leaves into underground structures that may compensate for the lack of roots has led to some unique changes in the Biology of other organs. Lloyd (1942) proposed that the green leaf-like organs could in fact be flattened stems. This proposal was later rejected by Taylor (1989) without specific reasons. Lloyd's conclusion was based on the study of organ development in newly developing seedlings. For many *Utricularia* species, he observed that the tiny seeds contain small food stores, develop small cotyledons and then form traps before green shoots develop from their base like a side bud at the base of a leaf petiole. The function of this early trap is most likely to

provide food for the seedling in the absence of stores and photosynthesis. This may explain why the germination of *Utricularia* seeds in collections is so difficult since most of the germination media are made fresh and are, therefore, devoid of established microflora on which the young seedling (invisible above the ground at that time) could feed. The proposal by Lloyd (1942) is supported by the fact that many *Utricularia* species generate traps (i.e. leaves) along the veins of their green organs (i.e. lateral stems). Some of these lateral stems sometimes serve parallel functions such as underground storage organs (tubers, or tuber-like just like potatoes) or stolons along which traps (i.e. leaves) can develop. At the base of these traps new lateral green shoots can be formed that generate a new rosette. It is clear from our genetic analysis that these side shoots have become more diverse through evolution to prevent concentrating all of the traps near the base of the main rosette as in the early forms of *Utricularia* (section *Polypompholyx* and *Pleiochasia*) and in *Genlisea*. Not all of the green organs have to be stems. As stated earlier, Some *Utricularia* species of section *Pleiochasia* harbour traps at the end of green petioles. Thus, for some species there is a possibility that the green organs are petioles that do not bear any trap and express positive tropism. The unrelated carnivorous genus *Nepenthes* also produces leaves with well-formed traps and small green laminae upon germination. Later, when *Nepenthes* plants reach abundant food supplies, they may only form green leaf petioles without the expense of producing traps on their tips (Juniper *et al.*, 1989). A similar situation occurs in a third unrelated carnivorous genus *Triphyophyllum* (Bringmann *et al.*, 1999). Carnivory may, therefore, just be a more efficient way than photosynthesis to obtain food for a fast developing seedling. After all, meat is more calorific than vegetables (Juniper *et al.*, 1989).

Part of the success of *Utricularia* and *Genlisea* stems from their high DNA mutation rates. Such increased mutation rates (highest of the plant kingdom) may be due to the greater reliance of these species on carnivory (Müller *et al.*, 2002b). These plants may be considered to be the only obligate carnivores of the plant kingdom because they permanently suck nutrients and preys (at least microscopic ones) from their liquid environment unlike the other carnivorous plants which may be considered as occasional carnivores because they rely on an unpredictable source of prey. Additionally, and as stated above, the unique biology of these plants forces them to rely purely on their carnivorous nature at the seedling stage. Consequently, these plants have a lower dependence on basic metabolic processes such as photosynthesis, energy production, or amino acid and nucleotide synthesis. This, in turn, relaxes the selection pressure for maintaining the same composition of the DNA molecule that codes for these metabolic processes. Even though only metabolic genes should be affected by this increased evolutionary rate, recent findings (Müller *et al.*, 2002b) suggest that increased mutation rates affect a larger part of the genomes of these plants. This gives them a unique opportunity to generate new species faster for varying needs or to occupy new ecological niches. Because of the large genetic differences that quickly arise among species, it is not surprising that *Utricularia* and *Genlisea* are genera where no interspecific hybrids have been reported (even between closely related species that live together). This lack of back crossing to wild-type populations probably adds a snowball effect for increased genetic differences among species.

The shorter-term history of *Pinguicula*, *Utricularia* and *Genlisea* is much more difficult to reconstruct from the available data. For *Pinguicula*, our current sampling



is admittedly too weak to provide a full picture of this genus. Nevertheless, it seems likely that *Utricularia* and *Genlisea* originate from the southern hemisphere since only their most recent members are in the northern hemisphere. Only some of these recent members can withstand a long period of frost. A contrary picture can be drawn for the set of *Pinguicula* sampled for this study. This set of *Pinguicula* species would have originated from Eurasia (between the Alps and the Korean peninsula) because *P. alpina* is the modern descendant of the ancestor of the *Pinguicula* species of the Americas and West Indies. The genus entered North America via the Bering sea or via Greenland. Upon arrival in Mexico and/or southern USA, the winter hibernaculum would have been transformed into a drought-resistant *Sempervivum*-type rosette (only a recent group of species never forms any winter-resting structure; Ex: *P. filifolia*). This change may seem big but is not because frost-resistance and drought-resistance are achieved by the fixation of water molecules to prevent them from forming ice crystals or evaporating away. During the alternating periods of cold and warm times, it is probable that *Pinguicula* plants have repeatedly moved down into valleys where they could merge and hybridise to then isolate themselves (and differentiate from each other via independent evolution) by following the glaciers north or towards the top of mountains. Most of the European *Pinguicula* species (plus *P. vulgaris* and *P. macroceras* in North America and Asia) would thus have been generated because of the geographic isolation of their populations after the last glacial ages. Because this event is very recent, it may explain why we could not find any genetic differences among most members (*P. leptoceras* and *P. poldinii* excluded). This theory supports the hypothesis of Steiger (1998) that these species evolved from southern to northern Europe by increasing their ploidy. Casper (1966) has, however, suggested a very different scheme of evolution for *Pinguicula* species. He proposed that the genus

originated during the Miocene on the Pacific ocean side of the Americas somewhere between Columbia/Venezuela and Mexico/California. Then, some plants would have moved eastwards to reach the Atlantic coast and the West Indies to later spread north and reach Europe. Finally, the genus would have developed eastwards to reach Japan. This scheme was based on a subdivision of the genus into 3 subgenera and the crossing of the north Atlantic by the genus during the Miocene. However, this subdivision of the genus is not supported by our genetic data. Rather, our data suggest that more research efforts should be placed on the north-east Asian *Pinguicula* species (*P. ramosa*, *P. variegata*, *P. albida*) and the Bering sea area to explain the early history of this genus.

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## FIGURE LEGENDS

*Figure 1.* Anatomy of the glands of the *Lentibulariaceae*. (a) Digestive gland (quadrifid) of *Utricularia*. Some digestive glands possess only 2 head cells (bifid glands); (b) Mucilage glands of *Utricularia*; (c) nectar gland of *Utricularia*; (d) external gland of *Utricularia* trap; (e) digestive gland of *Genlisea*; (f) mucilage gland of *Genlisea*; (g) digestive gland of *Pinguicula*. They may possess 4 to 8 head cells; (h) mucilage gland of *Pinguicula*. They usually possess 8-32 head cells. Reproduced from Lloyd (1942) and Juniper *et al.* (1989).

*Figure 2.* Anatomy of the glands of carnivorous genera not belonging to the *Lentibulariaceae*. (a) digestive gland of *Byblis*; (b) Mucilage gland of *Byblis*; (c) digestive gland of *Ibicella*; (d) digestive gland of *Dionaea*; (e) digestive gland of *Drosera*; (f) digestive gland of *Triphyophyllum*; (g) digestive gland of *Cephalotus*; (h) digestive gland of *Sarracenia*; (i) digestive gland of *Nepenthes*. Reproduced from Lloyd (1942) and Juniper *et al.* (1989).