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Adaptive radiation with regard to nutrient sequestration strategies in the carnivorous plants of the genus Nepenthes

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Carnivorous pitcher plants of the genus Nepenthes have evolved a great diversity of pitcher morphologies. Selective pressures for maximizing nutrient uptake have driven speciation and diversification of the genus in a process known as adaptive radiation. This leads to the evolution of pitchers adapted to specific and often bizarre source of nutrients, which are not strictly animal-derived. One example is *Nepenthes ampullaria* with unusual growth pattern and pitcher morphology what enables the plant to capture a leaf litter from the canopy above. We showed that the plant benefits from nitrogen uptake by increased rate of photosynthesis and growth what may provide competitive advantage over others co-habiting plants. A possible impact of such specialization toward hybridization, an important mechanism in speciation, is discussed.

Carnivorous plants of the genus Nepenthes have gained considerable attention during last few years. And it is not only due to the fact that new and often bizarre species of the genus are being discovered every year in remote highlands (*N. attenboroughii* described by Robinson et al.[1] was even included into the Top 10 new species for 2010, species.asu.edu./Top10), but also because of the new nutrient sequestration strategies described. Our perception of pitcher plants as merciless killers catching anything what is moving and careless is being changed. Instead of, the pitcher plants are under strong selective pressures of their prey and environment and some species gave up carnivory almost completely and rely on other source of nutrients. It is tempting to assume that such specialization helps to avoid interspecific competition and the different species can thus co-exist together. This is a good example of adaptive radiation with regard to nutrient sequestration strategies in plants—a powerful tool for speciation and diversification of the genus.[2,3]

The genus Nepenthes demonstrates remarkable variety in pitcher morphologies mirrored by the variety of strategies employed to obtain nutrients. It is believed that simple modification of trap geometry and its physiology makes possible to utilize different source of nutrients (like beaks of famous Darwin's finches).[2,4,5] The pitchers have evolved either waxy zone or wide peristom for prey capture, and waxy zone or viscoelastic digestive fluid for prey retention at each other's expanse.[3,5,6] The pitchers with viscoelastic digestive fluid are ussualy funnel-shaped and are more efficient at trapping flies than cylindrical pitchers with wax, which are more efficient on ants. The scarcity of ants in tropical mountains and the relative abundance of flying insect may favor the development of viscoelastic digestive fluid and may explain why such fluid is more common in mountain species.[5] The tropical mountains have also wetter habitat what may favor the development of large peristome, which is much more effective in insect trapping when is wet.[3,7] Pitchers of *N. albomarginata* produce a white rim of lichen-mimicking tissue to target termites of the genus Hospitalitermes.[8,9] *N. bicalcarata* hosts ants *Camponotus schmitzii* within its wide tendrils. They help its carnivorous host-plant to catch prey, may help with digestion, or can

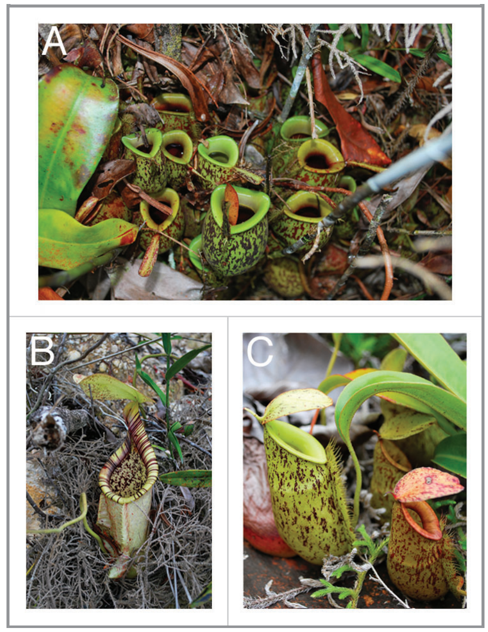

Figure 1. *N. ampullaria* (A), *N. rafflesiana* (B) and their natural hybrids *N.* × *hookeriana* (C).

effectively protect the plant against damage caused by weevil.[10-12] The upper pitchers of *N. lowii* lack the features associated with carnivory (waxy zone and reduced peristome) and are instead visited by a tree shrew *Tupaia montana*, which defecate into the pitchers after feeding on exudates produced by the pitcher lid.[13] The pitchers of *N. rajah* and *N. macrophylla* have retained the ability to trap live prey, but have also evolved mutualistic association with *T. montana* and even with summit rat (*Rattus baluensis*).[2,14-16] The pitcher size and geometry of *N. lowii*, *N. macrophylla* and *N. rajah* with large orifices and lids that are concave, elongated, and oriented approximately at right angles to the orifice, is fundamental to the positioning of the tree shrew hindquarters over the pitcher orifice while it feeds.[2] The elongated pitchers of *N. rafflesiana var elongata* provide roost for the bat *Kerivoula hardwickii*, which also defecate into the pitchers.[17] *N. ampullaria* has pitchers with lid reflexed away from pitcher orifice and allows debris to fall directly into the pitcher. The pitchers sit above the soil surface in a tighly-packed "carpet," waxy zone and lunate cells, the important mechanisms involved in prey capture and retention, are absent.[18-20] We tested the hypothesis, whether *N. ampullaria* can benefit from leaf litter, because only then the new nutrient sequestration strategy may be considered as adaptive.[21] We found that *N. ampullaria* can obtain ~40% of its leaf (lamina) nitrogen from leaf litter, what is in accordance with data obtained by Moran et al.[19,22] This resulted in slightly higher lamina N concentration and increased rate of photosynthesis. Because the leaves of co-habiting plant species are only a poor source of nitrogen and the nutrient stress was not alleviate completely, *N. ampullaria* has retained some structures responsible for prey capture (e.g., wide peristome, endochitinase activity).[22,23] These examples demonstrate that the genus Nepenthes is under strong selective pressure of the prey and environment.[3,5]

As we have shown, a lot of species is highly specialized to avoid interspecific competition. The unique pitcher morphological attributes important for different nutrient acquisition strategies may provide explanation why the Nepenthes hybrids fail to become established in large numbers in natural habitats, despite the fact that horticultural hybrids are more vigorous through the effect known as heterosis. In the case of natural hybrids, the unique features of the parent species may be significantly reduced in the hybrids (Nepenthes hybrids are intermediate in appearance between parent species), which attract, capture and digest fewer prey items (faeces or leaf litter) than either parent.[20] A natural hybrid *N.* × *hookeriana* between *N. rafflesiana* and studied *N. ampullaria* is a nice example (**Fig. 1**). As we mentioned above, *N. ampullaria* exhibits leaf litter trapping syndrome, whereas *N. rafflesiana* is an insect hunter which attracts prey using combination of visual and olfactory cues.[24] At the first glance, you can note that the lower pitchers of *N.* × *hookeriana* have not the lid reflexed away from pitcher orifice, typical character for *N. ampullaria*—a key structure for leaf litter capture syndrome. N × *hookeriana* also does not produce tighly-packed "carpet" of pitchers. One may also suggest that *N.* × *hookeriana* is also less atractive and less efficient in trapping insect prey, because *N. ampullaria* has lost many characters related to insectivorous lifestyle (e.g., nectar glands, waxy zone, viscoelastic digestive fluid, lunate cells, low pH in digestive fluid).[4,20] We can suggest that this may hold also for other Nepenthes hybrids, what makes them a suitable model for studying the relationship between structure and function of the pitcher. The scarcity of Nepenthes hybrids in natural habitat makes this type of research difficult, but not impossible task.

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