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The carnivorous syndrome in Nepenthes pitcher plants

Current state of knowledge and potential future directions

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Nepenthes is the largest genus of pitcher plants, with its center of diversity in SE Asia. The plants grow in substrates that are deficient in N and offset this deficiency by trapping animal prey, primarily arthropods. Recent research has provided new insights into the function of the pitchers, particularly with regard to prey tapping and retention. Species examined to date use combinations of wettable peristomes, wax layers and viscoelastic fluid to trap and retain prey. In many respects, this has redefined our understanding of the functioning of Nepenthes pitchers. In addition, recent research has shown that several Nepenthes species target specific groups of prey animals, or are even evolving away from a strictly carnivorous mode of operation. Future research into nutrient sequestration strategies and mechanisms of prey attraction would no doubt further enhance our knowledge of the ecology of this remarkable genus.

Introduction

Carnivory in plants is a relatively rare phenomenon: there are believed to be a little more than 600 species, the majority of which belong to the Orders Caryophyllales and Lamiales.[1] The monotypic Nepenthaceae (Caryophyllales) contains >100 species, making it the largest Family of pitcher plants. Nepenthes pitcher plants are restricted to the Palaeotropics, ranging from Madagascar eastwards to New Caledonia and a small number of outlying western Pacific islands.[2] The center of diversity lies in the Indonesian archipelago, with the Philippines, Sulawesi and the Greater Sunda islands of Borneo and Sumatra being particularly species-rich.[3,4]

Most Nepenthes are vines or subscandent shrubs in habit, attaching themselves to adjacent vegetation by the use of looped tendrils which develop from the tips of the leaf blades. The pitchers are in turn produced at the tips of the tendrils. The majority of species are terrestrial, but a small number of species grow epiphytically, primarily in montane habitats.[4,5] In general, the fluid-filled pitchers are produced to trap and digest invertebrate prey,[6,7] and Nepenthes are notable for their dimorphism, i.e., the production of two distinct pitcher types.[5] Young plants produce rosettes of "terrestrial" or "lower" pitchers. These are usually ovoid or globose in form, rest on the substrate, and are characterized by the presence of a pair of vertically-oriented, wing-like processes. It is popularly held that these structures serve to channel prey from the pitcher base into the mouth, facilitating capture. However, this has been found not to be the case with *Nepenthes rafflesiana* Jack, the only species in which this idea appears to have been tested to date.[8] As the plant grows, it may then produce a second pitcher type, the "aerial" or "upper" form. These pitchers are normally funnel-shaped or cylindrical, and lack the wing-like structures. Occasionally, pitchers of intermediate form are produced. In some species, the two pitcher types have been shown to target different prey taxa.[9-12]

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Scientific interest in Nepenthes biology has grown steadily over the last two decades: from one or two peer-reviewed papers published annually in the early 1990s, the number has increased more than five-fold in the last two years.[13] New insights into the genetics, physiology, structure and ecology of the Nepenthaceae are now being gained at an increasingly rapid rate. This increase in our understanding of the biology of these fascinating plants suggests that a review of the current state of knowledge may now be in order. It is our intention to provide a brief overview of the carnivorous syndrome in the Nepenthaceae, and to suggest some potentially fruitful directions for future investigation.

Prey Attraction

Nepenthes pitchers are passive, gravity-driven traps that show distinct functional zonation on their inner surfaces.[14-17] The pitcher lid and peristome (a collar-shaped structure surrounding and overhanging the mouth) are the sites of the highest density of extrafloral nectaries (**Fig. 1A**). In some species, the aerial pitchers produce fragrance; this has been demonstrated to attract anthophilous (flower-visiting) insects.[9,11] In addition, color patterns may serve to attract prey in some species. For example, pitchers of *N. rafflesiana* possess peristomes that stand out in high contrast against the pitcher body in the ultraviolet, blue and green wavebands (350–370, 430–470 and 490–540 nm, respectively). These regions correspond to insect visual sensitivity maxima, making

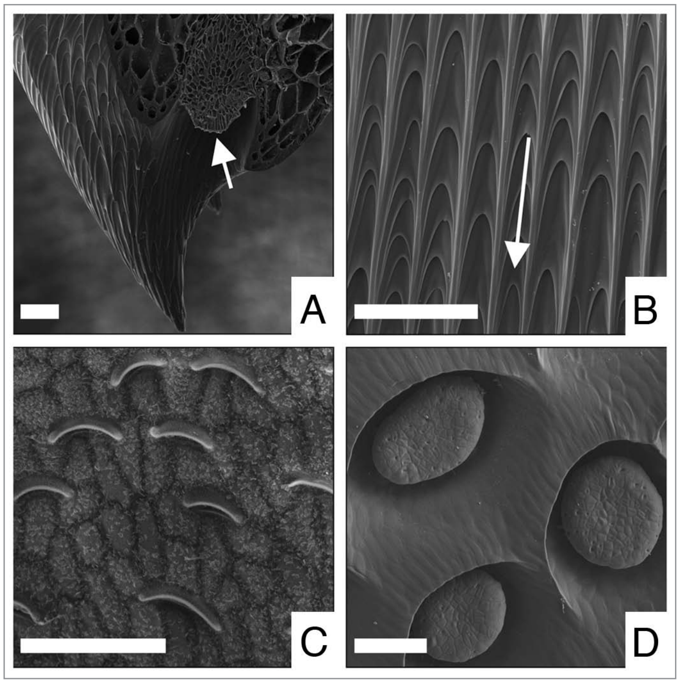

Figure 1. Scanning electron micrographs of some features of Nepenthes pitchers. (A) Vertical section through peristome of *N. villosa*. Arrow points to position of extrafloral nectary at top of nectar duct. (B) Peristome of *N. rafflesiana*, showing anisotropic arrangement of epidermal cells. Arrow points in direction of pitcher mouth. (C) Lunate cells and wax plates inside upper wall of *N. diatas* pitcher. (D) Digestive glands towards base of *N. fusca* pitcher. In all cases, white bar = 100 μm.

the pitchers highly conspicuous to many anthophilous taxa.[9,10] The possession of apparently floral traits (nectar, fragrance and visual orientation cues to a nectar source) by Nepenthes pitchers represents a powerful example of convergent evolution. Both insect-pollinated flowers and Nepenthes pitchers have evolved to attract and retain insects at the site of optimum benefit to the plant, the first through transfer of gametes, the second, via the acquisition of scarce nutrients.

Not all Nepenthes species rely exclusively on nectar as a reward to visitors. The pitchers of *Nepenthes albomarginata* T. Lobb ex Lindl. produce only small quantities of nectar in comparison to other species.[18] However, immediately below the peristome, on the outside surface of the pitcher, lies a conspicuous cream-colored band of tomentose tissue from which the species derives its name. This tissue attracts lichen-eating termites of the genus Hospitalitermes (Isoptera), which forage in columns containing thousands of workers.[19] These termites are often trapped in large quantities by *N. albomarginata* pitchers; using stable isotope analysis, it has been conservatively estimated that termites account for >50% of foliar nitrogen (N) in this species.[20]

Prey Capture and Retention

As noted above, the peristome is a region of high nectary density, making it a key component of the prey attraction system. A series of elegant studies has identified the role of the peristome in the mechanics of prey capture itself.[21-23] The surface of the peristome comprises rows of overlapping epidermal cells aligned in radial ridges (**Fig. 1B**). This arrangement confers two characteristics that contribute to prey capture. The first is anisotropy: the individual epidermal cells all overlap in the same direction, i.e., from the outer edge of the peristome inwards. Thus, although it is relatively easy for an insect to gain purchase with its claws while traveling towards the pitcher mouth (and capture), it finds difficulty in maintaining a grip while traveling in the opposite direction. The second characteristic is wettability via capillary action. The peristome may be wetted by moisture in the environment (e.g., condensation or rainfall), or by the nectar produced at the inner rim of the peristome itself (see **Fig. 1A**); under these conditions it becomes highly slippery, causing invertebrates to lose traction and fall towards the pitcher mouth. The research was carried out on *N. rafflesiana* and *Nepenthes bicalcarata* Hook. f; however, given the near universality of the typical peristome structure among Nepenthes, it is likely that the mechanisms outlined above are prevalent throughout the genus.

Once the prey has slipped from the peristome and entered the pitcher proper, it encounters additional features that facilitate its downward trajectory. Depending upon species, the upper region of the inner pitcher surface may or may not possess large numbers of lunate cells. These are modified stomatal guard cells that are oriented to overhang the pitcher wall, denying traction to the claws of invertebrates travelling in an upward direction[16,17,24-27] (**Fig. 1C**). The second feature of this zone (again, depending on species) is the presence of layered epicuticular waxes (**Fig. 1C**). Although the chemical composition may vary between species, the waxes all comprise very-long-chain aldehydes (C_{30} to C_{32}) and primary alcohols.[28,29] The waxy zone inhibits traction in two ways: firstly, the wax crystals of the outermost layer clog the claws, reducing their effectiveness, and secondly, the wax surface presents a surface of low free surface energy. This renders the inner pitcher wall unwettable, and prevents insects that possess hairy pulvillae from gaining traction via capillary action.[17,25,27,30-33] Eventually, the prey will fall into the fluid that collects in the lower part of the pitcher. In *N. rafflesiana*, this fluid is highly viscoelastic, preventing the prey from extricating itself, and ensuring that it is held securely until it drowns.[11,34] A recent study suggests that in this species, viscoelasticity of the pitcher fluid may play a greater role in prey retention than do the lunate cells and/or epicuticular waxes.[35] *N. rafflesiana* is not unique in this regard: fluid with similar properties is produced by pitchers of *Nepenthes inermis* Danser, *Nepenthes aristolochioides* Jebb & Cheek, *Nepenthes jacquelinae* Clarke, Davis & Tamin, *Nepenthes dubia* Danser and *Nepenthes talangensis* Nerz & Wistuba.[4]

Biomimicry (the use of biological principles in, for example, engineering) is a rapidly developing field, as scientists attempt to emulate natural systems in a sustainable fashion.[36] It is interesting to note that the effectiveness of the mechanisms, outlined above in trapping insects, has prompted some authors to suggest that analogues might be developed as pest control measures, reducing the need for application of chemical pesticides.[27,34]

Digestion of Prey and Uptake of Nutrients

As well as drowning the prey, the pitcher fluid allows enzymatic degradation of the remains, and uptake of the products of digestion. The lower part of the inner pitcher wall possesses large numbers of digestive glands[26,33,37,38] (**Fig. 1D**), which carry out two ontogenically-determined functions. In young pitchers, the glands secrete the aqueous digestive fluid.[17,37] This has been shown to contain a variety of enzymes that include proteases, peptidases, phosphatases, esterases, ribonucleases and chitinases.[26,38-47] In addition, the glands may also produce free radicals to aid in the degradation of prey tissue,[48] as well as a thaumatin-like protein (TLP) that possesses antibacterial and antifungal properties.[47,49] Once the pitcher has matured, the glands switch from secretion of enzymes to absorption of the products of enzymatic breakdown,[17,37] in the form of amino acids, peptides and ammonium ions.[50,51] The optimum pH for enzymatic function is maintained through the action of proton pumps.[52-54]

Costs and Benefits of Carnivory

In common with other carnivorous plants,[55,56] Nepenthes are N-limited in their natural habitats.[57] The degree to which other nutrients may be supplemented via carnivory has been reported for other taxa.[58] However, it remains to be investigated in the Nepenthaceae. Pitcher production imposes significant costs on the plant, in terms of the resources required for their construction.[57,59] The production of a leaf with the dual roles of carnivory and light harvesting also represents a functional compromise that ultimately leads to a reduction in photosynthetic efficiency.[60] The deployment of sophisticated and costly trapping structures by Nepenthes implies strong evolutionary pressure to augment or even supplant root-mediated nutrition with animal-derived inputs, and research to date supports this view. For example, stable isotope studies have estimated that the capture of ants (Formicidae) contributes up to 68% of foliar N in *N. rafflesiana*[20] and that in *Nepenthes mirabilis* Druce, there is an ontogenic switch from primarily root-derived N uptake to chiefly pitcher-derived N uptake from prey inputs, as the plant matures.[61] By ameliorating N deficits, prey capture confers physiological benefits: a starvation study, in which *N. rafflesiana* plants were denied prey inputs, showed increased foliar reflectance in the photosynthetically-active waveband (608–738 nm) compared to control plants, signifying degradation of photosynthetic capacity. The effects of this loss were manifested in a significant reduction in the rate of pitcher production, as well as a decrease in pitcher size, compared to controls.[62] Similarly, feeding of insect prey was found to increase photosynthetic efficiency significantly in *N. talangensis*.[63]

Non-Carnivorous Nutrient Inputs

Nepenthes typically inhabit open, sunny environments, a habit typical of botanical carnivores.[55,64-66] *Nepenthes ampullaria* Jack is an exception in that it can often be found growing under the canopy of tropical heath forest.[67] Litterfall is a key component of forest nutrient cycling that represents a significant nutrient resource for organisms able to intercept and utilize it.[68,69] *N. ampullaria* is one such species: by producing "carpets" of pitchers at ground level, it intercepts falling leaves and derives up to 35% of its N from this source.[70] The balance probably comes from a combination of invertebrate prey capture and root uptake.

Although the density of potential invertebrate prey is high in tropical lowlands, the number of available prey animals declines rapidly with increasing altitude.[71] Many Nepenthes species are restricted to montane habitats,[3,4] and are therefore under pressure to exploit alternative sources of nutrients. One Bornean montane species, *Nepenthes lowii* Hook. f., deploys large, funnel-shaped aerial pitchers that attract the mountain tree-shrew, *Tupaia montana* (Tupaiidae), via the production of copious amounts of nectar. The large size of the pitcher, combined with the orientation of the nectary-rich lid, ensures capture and retention of any excreta produced by the animal while feeding. Isotopic modeling suggests that *N. lowii* obtains between 57 and 100% of its N through the operation of these specialised pitchers.[12] It has recently been demonstrated that *N. lowii* is not unique in this respect: *Nepenthes rajah* Hook. f., and *Nepenthes macrophylla* (Marabini) Jebb & Cheek, two very large Bornean montane species, also provide nectar for, and harvest excreta from, *T. montana*.[72] It is interesting to note that this syndrome is not unique to the Nepenthaceae: leaves of Roridula spp. have recently been shown to utilize insect faeces as a nutrient source.[73]

Mutualism

Clearly, mutualism is an integral component of the nutrient sequestration strategies of the above-mentioned montane species. There is also evidence for mutualism in lowland species, perhaps the most well-known example being *N. bicalcarata*, a Bornean endemic. It provides swollen pitcher tendrils as domatia for a species of ant, *Camponotus schmitzi* Stärke (Formicidae), which is found only in association with this plant.[74] In addition to domatia, the plant supplies copious amounts of nectar from an array of extrafloral nectaries, the most conspicuous being the two giant "thorns" pointing downward from the upper portion of the peristome, beneath the pitcher lid.[18,75] In return for the services provided by the plant, *C. schmitzi* aids its host in two ways. Weevils of the genus Alcidodes (Curculionidae) can cause considerable damage to *N. bicalcarata* by feeding on developing leaf tips. *C. schmitzi* selectively attacks Alcidodes sp., thus protecting the host plant.[76] The second service provided by the ant is the removal of oversized prey items from the pitcher. Such items can overwhelm the plant's digestive capacity, resulting in putrefaction, which can eventually kill the pitcher. Remarkably, *C. schmitzi* can swim, which enables groups of them to enter the pitcher fluid, haul the item to the underside of the peristome, disarticulate and consume it. It is important to note that this activity targets only large items; smaller prey are left to the host plant.[3,74]

Future Directions

It is evident from this short review that over recent decades, researchers from many disciplines have elucidated a number of fundamental aspects of the carnivorous syndrome in Nepenthes. Perhaps equally importantly, many of the findings presented here have posed further questions. Below are a few avenues that we feel might reward future research efforts; it is by no means intended to be an exclusive list.

Nutrient sequestration strategies. The Nepenthaceae is the largest Family of pitcher plants, and new species are being described on a regular basis.[77-85] Nonetheless, only a relatively small number of Nepenthes have been studied in their natural habitats and we still know very little about the ecology or physiology of the majority of species. Given the range of unusual pitcher morphologies, particularly in regards to the elaborate peristomes of many montane species,[3,4] it is likely that novel nutrient sequestration strategies await discovery. As has been pointed out recently, field-based comparisons between prey spectra of pitchers and neutrally-selective analogues (e.g., pitfall traps), will help elucidate the degree to which individual species are selectively targeting particular taxa from the range potentially available in the habitat.[1,86]

Mechanisms of attraction. Despite accounts of the role of fragrance in prey attraction in *N. rafflesiana*,[9,11] the volatile compounds involved remain unidentified. The extent to which fragrance may be employed by the pitchers of other Nepenthes species, as has recently been carried out for other carnivorous plant genera,[87] awaits investigation. The role of color patterns to orient prey to the nectar source has also been investigated in only a very small number of species.[9,10] The degree to which nectar composition (e.g., sugar composition/concentration, amino acids, etc.) may reflect the requirements of specific prey, in the way that many angiosperms tailor their nectar to the needs of their pollinators,[88,89] might also repay investigation.

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