

REVIEW

Traps of carnivorous pitcher plants as a habitat: composition of the fluid, biodiversity and mutualistic activities

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- **Background** Carnivorous pitcher plants (CPPs) use cone-shaped leaves to trap animals for nutrient supply but are not able to kill all intruders of their traps. Numerous species, ranging from bacteria to vertebrates, survive and propagate in the otherwise deadly traps. This paper reviews the literature on phytotelmata of CPPs.
- **Pitcher Fluid as a Habitat** The volumes of pitchers range from 0.2 mL to 1.5 L. In *Nepenthes* and *Cephalotus*, the fluid is secreted by the trap; the other genera collect rain water. The fluid is usually acidic, rich in O₂ and contains digestive enzymes. In some taxa, toxins or detergents are found, or the fluid is extremely viscous. In *Heliamphora* or *Sarracenia*, the fluid differs little from pure water.
- **Inquiline Diversity** Pitcher inquilines comprise bacteria, protozoa, algae, fungi, rotifers, crustaceans, arachnids, insects and amphibia. The dominant groups are protists and Dipteran larvae. The various species of CPPs host different sets of inquilines. *Sarracenia purpurea* hosts up to 165 species of inquilines, followed by *Nepenthes ampullaria* with 59 species, compared with only three species from *Brocchinia reducta*. Reasons for these differences include size, the life span of the pitcher as well as its fluid.
- **Mutualistic Activities** Inquilines closely interact with their host. Some live as parasites, but the vast majority are mutualists. Beneficial activities include secretion of enzymes, feeding on the plant's prey and successive excretion of inorganic nutrients, mechanical break up of the prey, removal of excessive prey and assimilation of atmospheric N₂.
- **Conclusions** There is strong evidence that CPPs influence their phytotelm. Two strategies can be distinguished: (1) *Nepenthes* and *Cephalotus* produce acidic, toxic or digestive fluids and host a limited diversity of inquilines. (2) Genera without efficient enzymes such as *Sarracenia* or *Heliamphora* host diverse organisms and depend to a large extent on their symbionts for prey utilization.

Key words: *Brocchinia*, carnivorous plants, *Cephalotus*, *Heliamphora*, pitcher inquilines, mutualism, *Nepenthes*, phytotelm, *Sarracenia*, symbiosis.

INTRODUCTION

Carnivorous pitcher plants (CPPs) use cone-shaped leaves filled with fluid to trap and digest animals and to absorb their soluble ingredients. However, no species of pitcher plants kills all organisms entering the traps. At least some organisms, ranging from bacteria to vertebrates, are able to survive and propagate in the traps. For some inquilines, pitcher traps are the only habitat the species occupies. This study presents a synopsis of 130 years of research, focusing on (a) the pitcher fluid as the environment of inquilines, (b) the diversity of inquilines on the natural site and (c) the contribution of inquilines in prey degradation and the benefit for the plant.

Carnivorous pitcher plants

About 500 species of vascular plants trap and digest animals in order to gain additional inorganic nutrients (Juniper *et al.*, 1989; Barthlott *et al.*, 2004). The retention of animals is performed by different mechanisms such as sticky adhesive traps, moveable snap traps, pitchers, suction bladders or eel traps (Lloyd, 1942). The trapped animal usually dies within

a short time from drowning or O₂ deficiency (Adamec, 2007). The corpse is dissolved by digestive enzymes produced either by the plant itself (carnivorous plants *sensu stricto*) or by mutualistic organisms (protocarnivorous plants) (Peroutka *et al.*, 2008). The epidermis of the trap is equipped with glands or a porous cuticle where dissolved nutrients are absorbed. For a complete survey, compare Barthlott *et al.* (2004), Juniper *et al.* (1989) and Peroutka *et al.* (2008).

The aim of prey capture is the acquisition of inorganic nutrients, especially N and P; the uptake of organic compounds is negligible in most species (Adamec, 1997; Schulze *et al.*, 1997, 2001). Carnivorous plants are able to colonize habitats with highly oligotrophic soils but are restricted to very specific ecological niches; detailed descriptions of their ecology are given by Benzing (1987), Ellison (2006), Ellison and Gotelli (2001) and Givnish (1989). The net benefit of carnivory seems to be marginal (Karagatzides and Ellison, 2009).

CPPs independently developed five times in geographically separated regions. However, the traps are remarkably similar in all species. The trapping process is driven by gravity: the prey falls into a hollow leaf and is unable to climb out. A typical pitcher trap consists of four zones (Fig. 1). (A) The uppermost part of the pitcher is an appendix carrying attraction glands

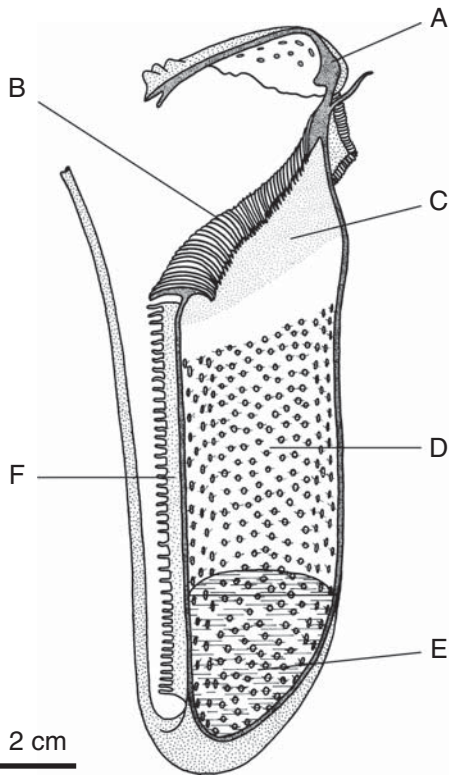


FIG. 1. Longitudinal section through a typical carnivorous pitcher plant, *Nepenthes × coccinea*. (A) Pitcher hood with attractive glands, (B) smooth pitcher margin, (C) retention zone with loose wax crystals, (D) absorption zone with digestive glands, (E) digestive fluid, (F) hairy longitudinal ledge. Glands are drawn disproportionately large for better visibility.

producing nectar and volatiles. The appendix may form a hood to protect the pitcher against precipitation (other functions are discussed by Bauer *et al.*, 2008). (B) The pitcher margin (peristome) may also be equipped with attractive glands. A wettable, slippery surface or inward-pointing hairs facilitate the stumbling of animals and let them fall into the pitcher. (C) The upper part of the pitcher serves for prey retention. The inner surface is covered with downward-pointing hairs or loose wax crystals that make climbing impossible. (D) The bottom of the pitcher is equipped with a permeable cuticle and absorbs dissolved nutrients. Glands for enzyme production are also located in this zone. The lower part of this zone is

covered by the pitcher fluid (E). The outside of the traps is usually rough and hairy and equipped with longitudinal ridges (F) in order to facilitate the access to the pitcher by animals.

About 110 species of CPPs have been described from the plant families shown in Table 1.

The phytotelm concept

Phytotelmata are defined as water bodies held and enclosed by living terrestrial plants (Kitching, 2000). Phytotelmata are usually grouped according to the part of the plant hosting the water body. Kitching (2000) distinguishes the following types: (a) tree holes; (b) water-filled cavities in stumps of bamboo and similar grasses; (c) leaf axils filled with water; (d) Bromelian tanks; and (e) traps of CPPs. Additional types include hyalocytes of *Sphagnum* mosses or fluid-filled flower buds in *Aconitum variegatum* (Molisch, 1921).

Phytotelmata are restricted to humid climates, since no species is able to compensate for extensive evaporation. As the chemistry of ponds is influenced by the bedrock, the water of phytotelmata is altered by the surrounding plant tissue. Influences of the plant include (a) the dissolution of tannins from bark in tree holes; (b) secretion of mucilage in some leaf axes and buds; (c) removal of inorganic ions in Bromelian tanks and CPPs; and (d) the secretion of digestive enzymes by CPPs. There can be little doubt that CPPs exert the strongest influence on their enclosed water bodies.

Phytotelmata differ greatly in size and life span. Tree holes may exist for decades and contain >1000 L (Kitching, 2000), whereas fluid-filled flower buds exist for some days and contain <1 μ L (Molisch, 1921). CPPs are found in an intermediate position, containing up to 1.5 L (McPherson, 2009) and existing for one or two vegetation periods (Bradshaw, 1983; Clarke, 1988). All phytotelmata seem to be inhabited by organisms, the so-called inquilines. Most major taxa of fresh water organisms have been found in phytotelmata, including bacteria, algae, fungi, protozoa, aquatic plants, lower metazoans, arthropods and vertebrates. Phytotelm inquilines are usually characterized by small size, the ability to survive long periods of desiccation and either high motility or easy distribution by wind or zoochory (Williams, 1996; Schwartz and Jenkins, 2000).

TABLE 1. Diversity of pitcher plants

| Family | Genus | Species | Distribution | Reference |
|----------------|---------------------|--------------------------------------|---------------------------------------------------------------|--------------------------------|
| Nepenthaceae | <i>Nepenthes</i> | 87 | Southeast Asia, Australia, Madagascar, Indian Ocean | Cheek and Jebb (2001) |
| Sarraceniaceae | <i>Sarracenia</i> | 8 (plus many subspecies and hybrids) | Eastern North America, Western Europe | McPherson (2007b) |
| | <i>Heliamphora</i> | >15 | North-eastern South America | McPherson (2007b) |
| | <i>Darlingtonia</i> | 1 | Western North America | McPherson (2007b) |
| Cephalotaceae | <i>Cephalotus</i> | 1 | South-western Australia | Lloyd (1942) |
| Bromeliaceae | <i>Brocchinia</i> | 2 | North-eastern South America | McPherson (2007b) |
| | <i>Catopsis</i> | 1 | South-eastern North America, Caribbean, Eastern South America | Barthlott <i>et al.</i> (2004) |
| Eriocaulaceae | <i>Paepalanthus</i> | 1? | Brazil | Figueira <i>et al.</i> (1994) |

THE PITCHER FLUID: ENVIRONMENT OF THE PHYTOTELM INQUILINES

Biodiversity and ecology of any water body are strongly determined by water chemistry. However, little is known about a possible selective influence on the inquilines. Kitching (2000) complains that most authors only determine volume and pH. Information on other variables is scarce and often fragmented, but there is evidence that pitcher fluids are highly different with regard to their origin, pH and digestive capabilities (see below). Some pitcher fluids contain narcotics, antibiotics or detergents. Finally, the availability of prey is of crucial importance as a source of food for pitcher inquilines.

Origin and physical factors of the fluid

In most CPPs, the traps are usually filled with fluid. In empty traps, the capability to retain prey is reduced (Jaffe *et al.*, 1992; Newell and Nastase, 1998). The pitcher fluid is either produced by the plant itself or consists of collected rain water. An excessive amount of fluid, however, would result in flooding, leading to a wash out of nutrients, prey animals and phytotelm inquilines.

In *Nepenthes* and *Cephalotus*, closed immature traps already contain fluid (Lloyd, 1942; Clarke, 1988; Bauer *et al.*, 2009). The fluid is produced by glands covering the inner surface of the traps (Vines, 1897; Goebel, 1932). Secretion of fluid by plants is known as guttation (Lösch, 2001). Water is transferred from the xylem into the parenchyma and excreted via pores or glands (hydathodes). In non-carnivorous plants, guttation initiates a water flow without transpiration and serves for the excretion of various compounds, especially Ca^{2+} . Cheek *et al.* (2001) found hydathodes at the leaf base and the stem of *Nepenthes*. The amounts of fluid secreted in the pitchers are extraordinarily large. In the non-carnivorous *Holcus lanatus* (Poaceae), 3 μL of fluid are secreted monthly per leaf (Hughes and Brimblecombe, 1994); in *Colocasia* (Araceae), a few millilitres were observed (Lösch, 2001). Large *Nepenthes* traps may contain >1 L of fluid (Barthlott *et al.*, 2004). In the tiny traps of *Cephalotus* up to 0.78 mL can be found, especially if evaporation of the fluid is reduced by shading (Clarke, 1988). Though flooding of the pitcher is avoided by a hood in most species, the hood does not cover the pitcher in *N. dubia*, *N. eustachya*, *N. ampullaria* or *N. inermis* (Clarke *et al.*, 2001). No information is available regarding if and how flooding is avoided in these species.

In *Sarracenia purpurea*, a few droplets of fluid are produced by the plant itself (Batalin, 1880; Hepburn *et al.*, 1927). The water household of the pitchers was studied in detail by Kingsolver (1979, 1981): typical traps contain about 30 mL of rainwater. Pitchers ran dry after 5–30 d without precipitation. The desiccation risk of a pitcher depended on its size as well as on shading. Sixty-three per cent of the small pitchers (diameter 15–19 mm) and 89 % of the large pitchers (diameter 35–39 mm) growing in the shade contained fluid, compared with 34 % and 71 %, respectively, of those growing in the full sun. Under controlled conditions [20 °C, 50 % relative humidity (RH)], young pitchers evaporated 0.35 ± 0.12 mL of water per day; in old pitchers, evaporation rates were slightly

higher. The temperature of the fluid exhibits daily and seasonal changes. In a population in Michigan, temperatures of 8–32 °C were found in July. In October, temperatures of 7–18 °C were measured. Macroclimatic data explained up to 30 % of the water balance of the pitchers. By comparing real pitchers with models of slightly varied shape, it was demonstrated that the pitcher is optimized for minimal transpiration and collecting a maximum of precipitation. Furthermore, freezing of the fluid occurs only slowly and requires more time than a typical night frost (Swales, 1972). Variable temperatures and fluid levels provide a highly unpredictable habitat for the pitcher inquilines. Not all inquilines of *S. purpurea* are adapted to this kind of stress. Larvae of *Wyeomyia smithii*, for example, are killed by desiccation (Kingsolver, 1979).

Rain water is also found in most traps of *Sarracenia rubra*. Pitchers of *S. flava*, *S. leucophylla* and *S. alata* are covered by a hood keeping off rainwater and are therefore usually dry (Bradshaw, 1983). In *S. psittacina*, traps become filled with water due to the frequent flooding of its habitat (Braem, 2002). The opening of the *Darlingtonia* pitcher points downwards, thus no rain water can be collected. Pitcher fluid is found only occasionally (Treat, 1875). The formation of droplets at the inner pitcher surface shows that *Darlingtonia* is capable of secreting at least tiny amounts of fluid (authors' own observation).

In *Heliamphora nutans*, traps contain 3.9 ± 3.3 mL (W. Adlassnig and K. Pranjić, unpubl. obs.). Secretion was not observed. A hood is lacking; precipitations are the only source of fluid. Flooding of the pitchers is prevented by an overflow (Lloyd, 1942). Fluid temperature in *H. nutans* oscillates between 9.5 ± 2.5 °C in the morning and 19.8 ± 2.6 °C at noon (W. Adlassnig and K. Pranjić, unpubl. obs.). The same is true for *H. tatei* (Jaffe *et al.*, 1992).

In *Brocchinia*, *Catopsis* and *Paepalanthus*, no studies on the origin of fluid are available. Since all these genera possess hoodless pitchers and grow in a humid climate, the collection of rain water seems probable. In *C. berteroniana*, Jabiol *et al.* (2009) found 9–85 mL of fluid. Flooding of the pitchers occurs regularly in the natural habitat in *B. reducta* (authors' own observation). The water level in *B. reducta* tanks is lowered when the plant sets flowers, since the growing inflorescence causes a gap between the leaves forming the tank (M. Edlinger, pers. comm.). It is unknown if empty tanks are able to trap.

Chemical composition

The pH of most pitcher fluids is acidic and rarely neutral (Fig. 2). Prey capture may induce strong fluctuations (Lloyd, 1942; Barthlott *et al.*, 2004), e.g. from pH 5.5 to 3.0 in *Nepenthes*. Morrissey (1960) suggested active and energy-dependent acidification of the fluid, which was proven by An *et al.* (2001) in *N. alata*: changes in pH are caused by H^+ secretion from living epidermis cells. Once the pitcher fluid is isolated, the pH does not change any more (Bauer *et al.*, 2009).

Pitcher fluids are usually rich in O_2 (Juniper *et al.*, 1989). This feature distinguishes CPPs from tank bromeliads where O_2 saturation may be very low (Laessle, 1961). Under greenhouse conditions, the following saturations were measured in

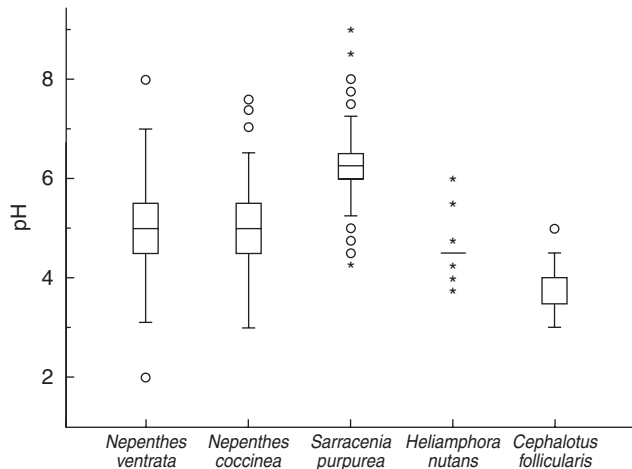


FIG. 2. pH values of fluids from different CPPs; plants grown in the greenhouse (authors' own observation), $P < 0.01$ (Kruskal–Wallis H -test). Outliers are marked as open circles and extreme values as asterisks.

randomly selected traps (E. Mayer and W. Adlassnig, unpubl. obs.): *S. purpurea*, $88.2 \pm 10.1\%$ ($n = 126$); *Nepenthes* \times *ventrata*, $90.2 \pm 22.3\%$ ($n = 14$). More information on the O_2 balance of the fluid is given in context with the mutualistic activities of the pitcher inquilines.

The fluid of *Nepenthes rafflesiana* is highly viscoelastic, suggesting the presence of a linear polysaccharide (Gaume and Forterre, 2007). Immediately after opening, the fluid can be stretched out to >30 cm; within 2 weeks, the extensional viscoelasticity decreases (Bauer et al., 2009). The high viscoelasticity enables the plant to increase its prey spectrum (Di Giusto et al., 2008). The extensional viscosity of the fluid can be maintained even after strong dilution, possibly an adaptation to high precipitations (Gaume and Forterre, 2007). In *N. inermis* the pitcher fluid directly contributes to the trapping process, as it is sticky and retains animals by adhesion (Rice, 2007).

The pitcher fluid of *Nepenthes* contains about $17\text{--}25$ mg L^{-1} Cl^{-} (Morrissey, 1955). Cl^{-} is secreted as well as absorbed by the digestive glands, apparently in order to maintain the optimal concentration for the function of digestive enzymes (Lüttge, 1966a, b). Furthermore, malate, citrate, oxalate, Ca^{2+} and Mg^{2+} have been found (Smith, 1893). The total dry mass content of the fluid is $0.27\text{--}0.98\%$, of which about two-thirds are non-volatile (Lloyd, 1942). In *S. purpurea*, a constant concentration of 10^{-5} M \times L^{-1} Ca^{2+} was found (Meir et al., 1991). Furthermore, the fluid contains organic substances soluble in water and in hydrophobic solvents (Istock et al., 1983).

Hatano and Hamada (2008) found antimicrobial peptides in *N. alata*. Surfactants seem to occur in several CPPs in order to facilitate drowning of prey by reducing the surface tension and increasing the likelihood that the prey will enter the fluid, though none have been isolated so far. Ants drown in the fluid of *Heliophora* whereas they are able to run on the surface of pure water (Jaffe et al., 1992). The same is true for *S. flava* (Hepburn et al., 1927; Plummer and Jackson, 1963) and probably many other CPPs.

In *Nepenthes madagascariensis* (Ratsirarson and Silander, 1996), *N. inermis*, *N. talangensis* \times *ventricosa* (Devečka,

2007) and *S. purpurea* (James, 1883), prey animals stop struggling for escape within a few minutes, which is much faster than in pure water. In *S. purpurea*, animals become motionless even if drowning is prevented (James, 1883). The odour of the pitcher fluid of *N. madagascariensis* may cause headache in humans (Ratsirarson and Silander, 1996). As some of these effects do not require direct contact with the fluid, the presence of a narcotic volatile is indicated.

The odour of the pitcher fluid seems to attract prey. Jürgens et al. (2009) provide a list of possible attractants. Moran (1996) demonstrated the attractive capacity of the fluid in *N. rafflesiana* var. *typica*. With time, the intensity of the odour increases (Bauer et al., 2009). The scent is not necessarily produced by the plant itself – the scent of decaying carcasses of prey animals was shown to have an attractive effect in *Sarracenia* spp. (Bhattarai and Horner, 2009; Müllner, 2009).

Digestive enzymes

The presence of digestive enzymes in the fluid of several carnivorous pitcher plants provides a contrast to other types of phytotelmata. However, not all CPPs produce digestive enzymes by themselves (Supplementary Data Table S1, available online). Though prey is broken down anyway, the origin of enzymes is of crucial importance: in carnivorous plants *sensu stricto*, enzyme production is an additional investment of the plant. In protocarnivorous species, the plant relies on a functioning phytotelm community for prey utilization. Digestive enzymes can be expected to form a stress factor for trap inquilines.

In *Nepenthes*, the capability to degrade proteins was already known to Hooker (1874). Jentsch (1972), Steckelberg et al. (1967) and Tökés et al. (1974) isolated and purified endo- and exopeptidases which were classified as nepenthesin I and nepenthesin II. The optimum temperature is $50\text{--}60$ °C and the optimum pH is about 2 (Smith, 1893; Steckelberg et al., 1967). Both values are rarely found at the natural site. An anonymous author (H.M.R., 1899) suggested that these features may reflect a general high level of resistance in these enzymes, which guarantee a prolonged time of activity. Indeed, the activity of RNases from *Nepenthes* spp. lasts for several months (Matthews, 1960). The efficiency of digestion seems to differ between various species of *Nepenthes* but is not evidently correlated with pH (Takahashi, 2007). In two species (*N. rafflesiana* and *N. alata* \times *merrilliana*), no digestion of proteins was detected. In other species, protease activity was present; *N. thorelii* and its hybrids proved to be most aggressive. An et al. (2002) found evidence for the expression of aspartic proteinases in the pitchers of *N. alata*. Four genes representing two subgroups of basic chitinases, denoted as Nkchit1b and Nkchit2b, were found in *N. khasiana* (Eilenberg et al., 2006). Reviews on this topic are provided by Frazier (2000) and Eilenberg and Zilberstein (2008). In *N. gracilis*, Chia et al. (2004) described digestion without enzymes: reactive oxygen species are found in the pitcher fluid which damage both proteins and biomembranes of the prey. This mechanism is able to overcome protease inhibitors formed by the victim and can be expected to cause stress for inquilines.

In Sarraceniaceae, the situation concerning digestive enzymes is unclear. Early researchers noted that degradation of meat takes place with the same speed in *Sarracenia* pitcher fluid and in sterile distilled water (reviewed by Smith, 1893). Thus, digestive enzymes, if present, are of limited efficiency. Gallie and Chang (1997), however, report nucleases, proteases and phosphatases in pitcher fluid of *S. purpurea* sterilized with germicides. Enzyme production seems to be limited to the first weeks after the opening of the pitcher. In *Heliamphora*, Jaffe et al. (1992) found enzymes in *H. tatei* but not in *H. nutans*, *H. minor* and *H. ionasii* ssp. *maguirei*.

In *Cephalotus*, proteases, phosphatases and nucleases were detected (Barthlott et al., 2004; Takahashi et al., 2009). In *Brocchinia*, *Catopsis* and *Paepalanthus*, most authors agree that enzyme production is lacking (Givnish et al., 1984; Barthlott et al., 2004) but provide little experimental evidence.

Prey: the source of energy for the pitcher inquilines

The vast majority of trap inquilines consists of heterotrophic organisms – pitcher phytotelmata are ‘brown’ ecosystems (Williams, 2006; Butler et al., 2008). Prey is the most important source of organic compounds. Experimental manipulation of the trapping success shows a strong influence of prey availability on many (Trzcinski et al., 2005) but not all (Miller et al., 1994) trap inquilines in *S. purpurea*. In *Nepenthes bicalcarata* the abundance but not the diversity of trap inquilines is positively correlated with the number of prey objects (Cresswell, 2000). For more details on the dependence of CPP phytotelmata on their resources, compare Kitching (2000, 2001).

Though carnivorous pitcher plants arose independently five times and exhibit a wide range of different trap shapes, the prey spectra are surprisingly uniform: Tan (1997) studied the prey of six species of *Nepenthes* in Singapore. All pitchers containing prey had trapped ants. About 70 % of the traps contained other animals as well, including other Hymenoptera, Coleoptera, Isoptera, Orthoptera, Hemiptera, Arachnida and snails. In *N. bicalcarata*, ants and termites were the most abundant prey; an average of 35 animals were caught per trap (Cresswell, 2000). In *N. rafflesiana*, lower pitchers trapped exclusively ants, whereas the prey of upper pitchers consisted of ants and flying insects (Moran, 1996). As an exception, *N. albomarginata* was specialized on termites (Moran et al., 2001; Merbach et al., 2002).

In *S. purpurea*, ants account for 75 % of the prey (Newell and Nastase, 1998). Other Hymenoptera, Coleoptera, Myriopoda and snails are also trapped (Heard, 1997). Ants are dominant as well in *S. rubra*, *S. minor* (Givnish, 1989), *Darlingtonia* (Ellison, 2005), *Heliamphora* (Gonzalez et al., 1991; Jaffe et al., 1992) and *Cephalotus* (Dakin, 1917; Lloyd, 1942). *Brocchinia* has a broader prey spectrum compared with sympatric *Heliamphora*, comprising Hymenoptera, Coleoptera, Neuroptera and Acari; however, ants are most important (Gonzalez et al., 1991).

Vertebrates are trapped occasionally. *Nepenthes rajah* sometimes traps small, climbing mammals (Stehli, 1934). For both *N. rafflesiana* (Brodie and Lee, 2000) and *N. truncata* (Anonymous, 2006), one report was found that a

single mouse was trapped. *Sarracenia purpurea* was described to catch newts (*Notophthalmus viridescens*) (Butler et al., 2005). Most authors describe an unpleasant odour after the trapping of vertebrates whereas the fluid is scentless under normal conditions. This indicates that innate strategies of the plant against putrefaction (Clarke and Kitching, 1995; Hatano and Hamada, 2008) are overstrained by large animals. In overfed pitchers, aerobic inquilines usually die (Bradshaw and Creelman, 1984).

Some pitcher plants use their traps to collect detritus. In the pitchers of *Nepenthes ampullaria*, Moran et al. (2003) found few animals but abundant dead foliage; isotope measurements provided evidence that most nutrients are gained from plants instead of animals; mechanisms for prey retention are reduced. Upper pitchers of *N. lowii*, *N. macrophylla* and *N. rajah* collect faeces of animals that feed on the nectar produced by the peristome without trapping them (Clarke, 1997; Clarke et al., 2009; Chin et al., 2010). In *H. nutans*, <50 % of the traps contain animals (Jaffe et al., 1992), but dead foliage is frequently found (Studnička, 2003).

As a summary, most carnivorous pitcher plants trap mainly social insects, especially ants. This specialization is explained by Tan (1997): large numbers of social insects constantly visit the pitchers and feed on the nectar. In spite of a low trapping efficiency [<1 % in *S. purpurea* (Newell and Nastase, 1998) and *Darlingtonia californica* (Ellison, 2005)], many individuals can be trapped during the life span of a pitcher [e.g. 18 animals, six of them Hymenoptera, by each pitcher of *S. purpurea* (Heard, 1997)]. Strong fluctuations in the trapping efficiency of the pitchers may help to overcome learning effects by social prey animals (Bauer et al., 2008). For the pitcher phytotelm, the specialization on ants may be of importance, as a constant import of formic acid will lower the pH of the pitcher fluid (Plummer and Jackson, 1963) and possibly raise the redox potential.

DIVERSITY OF PITCHER INQUILINES

Overview

Pitcher plants occur in different climatic zones, plant communities and floristic realms. Their pitchers are differently shaped, of variable size and their fluids exhibit different chemical compositions. As a consequence, they host different inquilines.

Numbers of published inquiline species range from 165 for *S. purpurea* to only three in *S. minor* (Fig. 3). Several studies indicate that differences in inquiline diversity are due to the composition of the pitcher fluid (Clarke and Kitching, 1993; Mayer, 2005; Müllner et al., 2008). Concerning metazoa, a sufficient level of knowledge has been achieved for *S. purpurea*, *N. ampullaria*, *N. bicalcarata*, *N. mirabilis*, *N. albomarginata*, *N. rafflesiana* and *N. gracilis* which all were the subject of numerous studies, part of which were carried out using standardized sampling techniques (e.g. Bradshaw, 1983; Bradshaw and Creelman, 1984; Clarke and Kitching, 1993). In other CPPs, the finding of new species can be expected. Most phyla of fresh water organisms can be found in pitcher plants (Fig. 4, Supplementary Data Tables S2–S4, available online). Micro-organisms have been widely

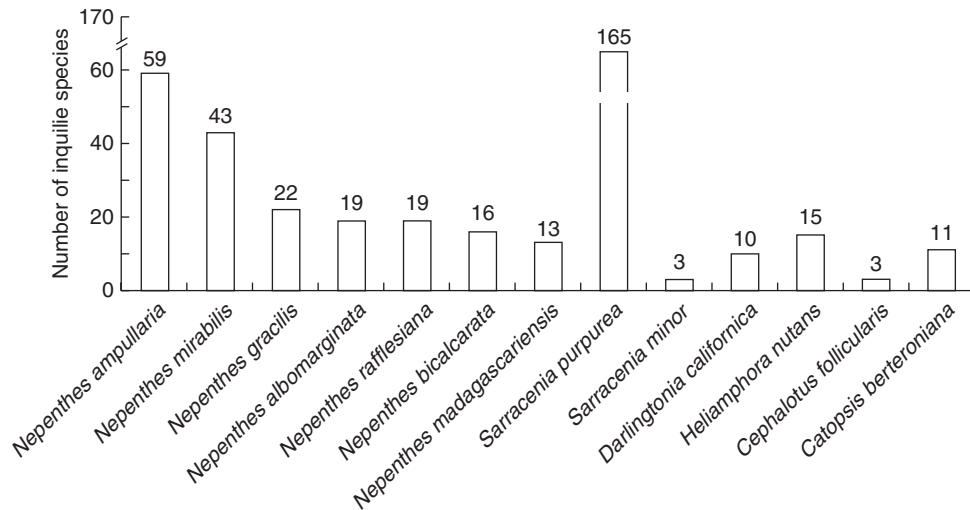


FIG. 3. Diversity of phytotelmata for different species of pitcher plants.

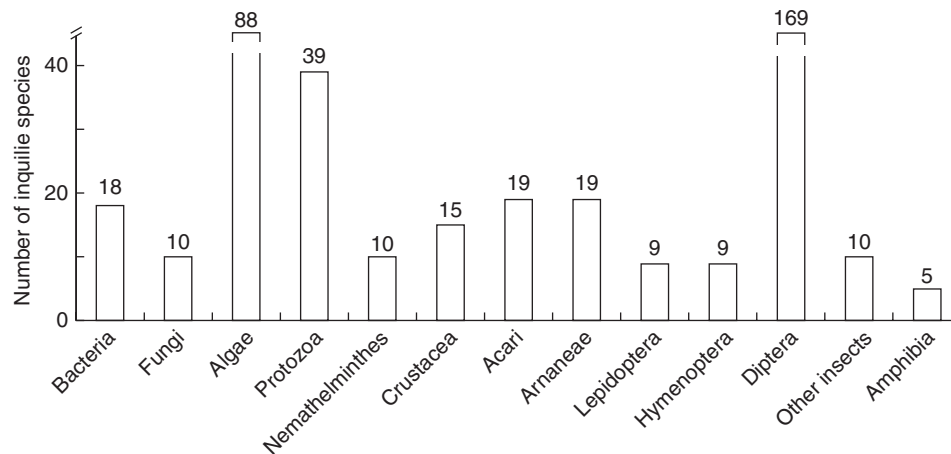


FIG. 4. Diversity of the most important taxa of inquilines in CPPs.

neglected so far and are probably highly under-represented. Among animals, Diptera are by far the most diverse order. Their high mobility, combined with well developed eyes and sense of smell, enables them to find pitchers for oviposition, which seems to be the reason for their eminent success.

Some organisms visit but do not constantly inhabit the pitchers. These visitors are not retained, and they enter the traps sporadically for shelter or to feed on the plant's prey. Geckoes (Ratsirarson and Silander, 1996), monkeys (Barthlott *et al.*, 2004) and mantises (Carrow *et al.*, 1997) frequently steal dead prey. Several species of frogs also overcome retention by traps of *Nepenthes* and *Heliamphora* and hide and bathe in the fluid (Ratsirarson and Silander, 1996; D'Amato, 1998; Hua, 2004; Hua and Kuizheng, 2004). Since the relationship between trap visitors and plants is loose, they will not be described in more detail.

Inquilines of *Nepenthes*

At their natural sites, *Nepenthes* pitchers are colonized by metazoa virtually without exception (Fuller, 1912). If the animals are experimentally removed, the pitchers are

recolonized within 2 weeks (Cresswell, 1998). See Supplementary Data Table S2 for details of inquilines described in the literature.

The diversity of bacteria, fungi, protozoa and algae seems to be low, possibly due to the small number of studies dealing with this topic (van Oye, 1921; Okahara, 1933). A search for other micro-organisms seems to be highly promising.

Among Crustacea, Copepoda inhabit the fluid (Reid, 2001) whereas the crab *Geosesarma malayanum* visits the traps only searching for food (Ng and Lim, 1987). Rumphius (1750, cited after Beekmann, 2004) described a shrimp-like animal living in *Nepenthes* pitchers. Though the rest of Rumphius' record proved to be highly accurate, this species was never found again.

Among Arachnida, Acari live in the fluid (Fashing, 2008) whereas Araneae inhabit the dry upper part of the pitcher. *Misumenops thienemanni* and other Thomisidae use a strand to dive into the pitcher fluid (Carrow *et al.*, 1997). The high viscoelasticity of the fluid of *N. rafflesiana* is overcome by extremely slow motions (Gaume and Forterre, 2007). Other spiders seal the pitcher with a net and catch animals falling into the pitcher (Ratsirarson and Silander, 1996).

Dipteran larvae occupy diverse ecological niches as filter feeders, detritivores or predators. This adaptability results in a diversity of >140 species. After metamorphosis, imagines leave the pitcher. Detailed information on their nutrition and behaviour is given by Kitching (2000).

The frog *Kalophrynus pleurostigma* spawns into lower pitchers of *N. ampullaria*, where up to 100 tadpoles develop simultaneously within one pitcher (Ming, 1997).

Clarke and Kitching (1993) showed that differences in inquiline diversity between sympatric *Nepenthes* species are not due to sampling artefacts but can be verified using standardized techniques. The highest diversity is found in *N. ampullaria*. Relationships between the inquilines can be rather complex. Three consistent food webs have been published by Kitching (2000), Ratsirason and Silander (1996) and Sota et al. (1998), respectively. Up to four trophic levels are found, including micro-organisms.

Pitcher inquilines of *Sarracenia*

The phytotelm of *S. purpurea* has been studied in more detail than in any other CPP. The species described are shown in Supplementary Data Table S3. Information on bacteria in *S. purpurea* is more comprehensive when compared with *Nepenthes* but still far from complete. Miller et al. (w. y.) estimated that at least 30 cultivable species occur regularly. As cultivation detects only a small percentage of all micro-organisms (Wagner et al., 1994), the real diversity is unknown. Most bacteria grow on decaying prey, others are phototrophic (Lindquist, 1975), or assimilate atmospheric N₂ and contribute to the plant's nutrition even if no prey is trapped (Prankevicus and Cameron, 1991).

Protozoa, especially ciliates and flagellates, are diverse and abundant in *Sarracenia* pitchers (Hegner, 1926). Most genera are ubiquitous and tolerate low water qualities (Streble and Krauter, 2002) as can be expected due to the presence of dead prey. The occurrence of algae in *Sarracenia* is enigmatic: at the natural site in North America, algae show low abundance and diversity in the pitchers (Cochran-Stafira and von Ende, 1998). The same is true for greenhouse plants (Mayer, 2005). Neophytic populations in Germany, however, are colonized by a great diversity of Chlorophyta and other algae, possibly due to the lack of a predator such as *Wyeomyia* larvae (Gebühr et al., 2006). The diversity and abundance of fungi are very low. Lindquist (1975) and Mayer (2005) found only isolated hyphae in the fluid. The outside of the pitcher, however, is frequently infected by fungi (MacMillan, 1891).

The rotifer *Habrotricha rosea* inhabits about 70 % of all pitchers (Petersen et al., 1997). Other Rotatoria are rare; colonization of new pitchers is performed via zoochory on females of the pitcher plant mosquito *Wyeomyia smithii* (Bateman, 1987).

Like in *Nepenthes*, Arthropoda are the most diverse group in *Sarracenia* pitchers. Nine species of Crustaceae have been described; most of them generally prefer phytotelmata (Reid, 2001). Acari live submerged in the fluid, whereas Araneae seal pitchers with their nets (Cresswell, 1991). The same trapping strategy was acquired by European spiders inhabiting *S. purpurea* populations introduced to Switzerland (Hartmeyer, 1996).

The caterpillar *Exyra fax* (Jones, 1907, 1921) feeds on the pitchers and bites a hole at the pitcher base to get rid of the fluid. The opening of the pitcher is sealed by silk. *Exyra* feeds on the inner leaf epidermis and the mesophyll but not on the vascular tissue and the outer epidermis which continues to form a protective shelter (Atwater et al., 2006). In *S. flava*, the caterpillar *Archips parallela* is poorly adapted to the pitchers and sometimes is trapped and killed (Jones, 1908).

Fifteen species of Diptera occur in *Sarracenia*. Genera such as *Aedes*, *Metriocnemus* or *Toxorhynchites* occur in *Nepenthes* as well, in spite of the huge geographic distance. The mosquito *W. smithii* strictly depends on *S. purpurea* to complete its larval development. If eggs are occasionally deposited in *S. flava* or *S. purpurea* × *Sarracenia* sp., larvae die before metamorphosis (Bradshaw, 1983). The larvae live as filtrators next to the surface of the fluid and feed on small particles of decaying prey and organisms such as protozoa and Rotatoria (Addicott, 1974). In the food web of the pitcher, *Wyeomyia* serves as the top predator; the population size of *Wyeomyia* is negatively correlated with inquiline diversity (Buckley et al., 2003). *Metriocnemus knabi* lives at the bottom of the pitcher and feeds on drowned prey. By breaking up the carcasses, *Metriocnemus* supports the filter feeder *Wyeomyia* (Heard, 1994b); females of *Wyeomyia* even prefer traps containing *Metriocnemus* for oviposition (Heard, 1994a). The larvae of *Sarcophaga sarraceniae* live in the masses of dead prey animals, especially if they protrude over the level of fluid. The feet of the imago are equipped with enlarged claws that enable the animal to leave the pitcher after metamorphosis (Riley, 1874). In European populations, larvae of other *Sarcophaga* species have been found (Adlassnig et al., 2010).

All other species of *Sarracenia* host significantly fewer species of inquilines, including *S. flava*, *S. leucophylla* and *S. rubra* whose habitats overlap with *S. purpurea*. Thus, the lower diversity is due to properties of the pitcher (Bradshaw, 1983). Three hypotheses were suggested. (1) Pitchers of *S. purpurea* survive 2 years instead of only 1 year in all other species, resulting in more time for the immigration of species and the development of complex ecosystems (Bradshaw, 1983; Heard, 1997). (2) The lack of a hood increases the capture of rain water and therefore the amount of fluid in *S. purpurea*, providing a more extensive habitat. (3) Information on enzyme production in *Sarracenia* is incomplete. The production of toxic or narcotic compounds was suggested by James (1883) but never tested. Thus, a selective effect of the fluid is possible as well.

The food webs within *Sarracenia* traps seem to be quite similar to those of *Nepenthes*. Due to the lack of aggressive digestive enzymes, several authors stress the role of sarcophagus micro-organisms for prey degradation (Bradshaw, 1983; Trzcinski et al., 2005; Butler et al., 2008).

Inquilines of other pitcher plants

Inquilines for all other genera are enumerated in Supplementary Data Table S4 (available online). The phytotelmata of *Darlingtonia* and *Heliophora* (Sarraceniaceae) show similarities to *Sarracenia*. In *Darlingtonia*, only two species are abundant (Austin, 1878; Naem, 1988). The Dipteran

Metriocnemus edwardsi is very frequent; the mite *Sarraceniopus darlingtoniae* can only compete if the trapping success of the plant is fluctuating.

On *Heliophora*, only few observations are available since this genus is restricted to inaccessible table mountains. Compared with *S. purpurea*, the bacterial flora is similar in *Heliophora* but fungi are more abundant (Pranjić *et al.*, 2007). No information on the digestive capabilities of bacterial inquilines has been published. Among insects, only two species of *Wyeomyia* are regularly found (Zavortink, 1985; Barrera *et al.*, 1989). Like in *Nepenthes* and *Sarracenia*, spiders spin their webs at the pitcher opening (Jaffe *et al.*, 1992). The food webs within the pitcher are unknown.

For *Cephalotus follicularis*, diverse insect communities were described by Kitching (2000). *Pseudomonas* is the only known bacterium (Juniper *et al.*, 1989). In the greenhouse, algae (*Chlorella* sp.) and mosses may grow in the pitchers (authors' own observation).

Little is known regarding carnivorous Bromeliaceae. Spiders utilize *Brocchinia* traps as they do in *Nepenthes* and *Sarracenia*. In the fluid, Dipteran larvae obviously play an important role and at least the two genera *Wyeomyia* and *Metriocnemus* occur in Sarraceniaceae as well. *Utricularia humboldti* (Lentibulariaceae) was reported to colonize tanks of *Brocchinia* spp. and possibly *B. reducta* (Taylor, 1994). Thus, one carnivorous plant would host another one, and *U. humboldti* would be the only vascular plant inhabiting pitcher phytotelmata. Studnička (2003) and McPherson (2007a), however, found *Utricularia* only in the tanks of the non-carnivorous *Vriesera atra* and *B. tatei* but not in *B. reducta*, probably due its acidic pH. The confusion may be caused by the similarity of various *Brocchinia* species.

In the Bromeliad *Catopsis berteroniana*, 11 inquiline species have been described. *Catopsis berteroniana* hosts a distinctive set of inquilines compared with sympatric non-carnivorous bromeliads, probably due to the chemical composition of the fluid (Jabiol *et al.*, 2009). No inquilines have been published for *Paepalanthus bromelioides*.

ORIGIN OF PITCHER DIVERSITY

So far, the internal relationships of the pitcher inquilines have been the main focus of research. Only in certain species such as *S. purpurea* and a few species of *Nepenthes* does the present level of knowledge seem satisfactory. Two detailed reviews on the structure of phytotelm communities were provided by Kitching (2000, 2001). Related topics, such as temporal fresh waters in general, have been discussed by Williams (1996) and Schwartz and Jenkins (2000). In the present review, only selected observations are described and no discussion of phytotelm theory is intended.

Five prerequisites for the establishment of a population in a pitcher trap can be identified.

(1) The species must occur in the same habitat as the CPP. Many bacteria, protists and fungi are distributed worldwide. Most metazoa are more limited. Some phytotelm inquilines exhibit a more limited geographic distribution than their host. For example, in the centre of the geographical distribution of *Nepenthes*, more Nepenthobiontes are observed than in the periphery (Williams, 2006). The reason may be

the better chance to find a new pitcher for colonization (Clarke and Kitching, 1993; Kitching, 2000). In *S. purpurea*, *W. smithii* has a very limited ability to fly. At new habitats of its host, *Wyeomyia* establishes only after decades (Hamilton and Duffield, 2002). The same is true for *E. fax* (Atwater *et al.*, 2006). Thus, all pitcher plants seem to have equal chances to be colonized by micro-organisms whereas many higher organisms are only available in limited areas.

(2) The organisms have to find a pitcher. Micro-organisms enter the traps by chance, i.e. by rain washing cells and spores from the air into the traps, or micro-organisms may be attached to prey. *Paramecium caudatum* lacks suitable diaspores and does not occur in the traps of *S. purpurea* but survives if introduced into the fluid (Hegner, 1926). The same is true for some other protists (Kneitel and Miller, 2003). Among insects, pregnant females of *Wyeomyia* actively search for traps that provide a habitat for their larvae. Unoccupied traps are preferred, leading to an even distribution of larvae (Harvey and Miller, 1996). In *N. ampullaria*, the biodiversity of one pitcher correlates with the number of other pitchers in the close vicinity (Mogi and Yong, 1992). Here, the chances to be found by a trap inquiline searching for a new habitat are best.

(3) Survival in the pitcher must be possible. As pitcher traps are designed to catch and drown animals, most organisms are not able to survive in the fluid. Only a few studies, however, actually tested the ability of aquatic organisms to survive in the pitcher fluid. Fuller (1912) tested whether specific adaptations are required for survival in *Nepenthes* traps. He identified Dipteran larvae in *Nepenthes*, collected taxonomically related but free-living larvae and introduced them into the pitchers where they died within a short time. The selective effect of the fluid is confirmed by Tan and Ng (1997) who found extensive similarities between phytotelmata at the natural site and in the garden, and by Mayer (2005) who compared plants from the greenhouse with data from the natural site. In peat bogs with *S. purpurea*, algal communities in the pitchers and in nearby hollows differ significantly (Gebühr *et al.*, 2005, 2006). Okahara (1933) mentions similarities between the bacterial fauna of *Nepenthes* and of the human intestines, which are caused by the common need to resist digestion. The selective influence of the pitcher fluid offers an opportunity for the plant to select certain symbionts. In *Nepenthes* sp., for example, the pH of the pitcher fluid decreases a few days after prey capture, when the number of bacteria has reached its maximum, in order to inhibit further bacterial growth (Higashi *et al.*, 1993).

(4) Resources must be available. The main source of energy for inquilines is the plant's prey; autotrophs are rare (Supplementary Data Tables S2–S4). In *Sarracenia* and *Nepenthes*, protists and bacteria are more abundant if the fluid contains more organic nutrients (Higashi *et al.*, 1993); in more detail, bacteria are limited by C_{org} and, to a lesser extent, P_i (Gray *et al.*, 2006). [In non-carnivorous tank bromeliads, where drowned animals are rare, bacterial growth is rather limited by the lack of NH_4^+ (Haugrich *et al.*, 2009).] In *S. purpurea*, *W. smithii* and *Metriocnemus knabi* are both limited by the availability of drowned prey. Amphibian tadpoles do not rely on external nutrients but obtain nourishment from their yolk until metamorphosis (Lim and Ng, 1991).

(5) Predators and competitors must allow for long-term survival. Bacterivorous rotifers and protozoa are limited by the top predator *W. smithii* and not by the availability of bacteria (Miller *et al.*, 2002; Gray *et al.*, 2006). The presence of *W. smithii* seems to lower the biodiversity (Addicott, 1974; Buckley *et al.*, 2003). Several species of insects do not survive in *S. purpurea*, only because they are immediately killed by *M. knabi* (Petersen *et al.*, 2000). Competition between the inquilines seems to be limited, e.g. the respective abundances of *W. smithii* and microcrustaceans are not correlated (Miller *et al.*, 1994). High inquiline diversity seems to enhance the stability of phytotelm communities (Trzcinski *et al.*, 2005). Long-term survival is also determined by the life span of the pitcher and by desiccation or freezing of the fluid (Bradshaw, 1983).

RELATIONSHIPS BETWEEN INQUILINES AND THEIR HOST

Four types of interaction between a CPP and its inquilines are possible: (1) the inquilines may damage the pitcher or extract nutrients; (2) the inquilines may contribute to the digestion of prey; (3) there may be some other benefit for the plant; or (4) there may be no interaction at all. For types 1–3, examples have been found; the existence of type 4 was never verified.

Inquilines as parasites

Autotrophic algae are more or less abundant in most CPPs. They consume inorganic nutrients dissolved in the pitcher fluid, unless they die and decay before the pitcher becomes senescent.

In both *Nepenthes* and *Sarracenia*, caterpillars inhabit the pitchers and feed on their inner wall, which leads to the destruction of the pitcher and to the loss of its trapping ability (Juniper *et al.*, 1989; Chen and Chan, 1997; Atwater *et al.*, 2006). In *C. berteroniana*, the weevil *Metamasius callizona* feeds on the meristematic tissue at the bottom of the pitcher and kills the plant (Frank and Fish, 2008). These herbivores are a small minority among all pitcher inquilines. The presence of tadpoles in the pitchers is probably neutral for *N. ampullaria* but the mating parents damage the pitchers (Ming, 1997).

The trapping success is also reduced by visitors that feed on the plant's prey. The spider *Misumenops nepenthicola* (Beaver, 1983) and the ant *Camponotus schmitzi* (Clarke and Kitching, 1995) dive into *Nepenthes* pitchers and catch prey or inquilines. Terrestrial spiders sealing pitchers in almost all CPPs strongly reduce their trapping efficiency (Cresswell, 1992). Other visitors consuming the plant's prey include apes, geckoes, crabs, mantises, etc. It can be assumed that they significantly reduce the trapping success and therefore harm the plant; Moran and Moran (1998) showed that lacking prey is a severe stress factor for *N. rafflesiana*. In *Sarracenia* and *Nepenthes*, however, trap visitors catch caterpillars feeding on the pitchers (McPherson, 2007b; Merbach *et al.*, 2007). Furthermore, some nutrients are removed if inquilines leave the trap for dispersal.

Inquilines contribute to prey digestion

Nepenthes and *Cephalotus* produce their own digestive enzymes; in Sarraceniaceae, Bromeliaceae and Eriocaulaceae, enzyme production is dubious or improbable (Peroutka *et al.*, 2008). Besides plant-derived enzymes, Plummer and Jackson (1963) suggest digestion by the metabolic activity of the pitcher inquilines. In species without their own enzyme production, inquilines can be expected to play a key role. The ultimate aim of plant carnivory is the uptake of inorganic nutrients (Plummer and Kethley, 1964; Rischer *et al.*, 2002); thus, degradation and oxidation of prey-derived macromolecules by inquilines will rather improve their usability for the plant and not reduce their nutrient value.

Two mechanisms of prey degradation can be distinguished. (1) Bacteria, fungi and some algae secrete digestive enzymes. Dissolved nutrients may be absorbed by the CPP, or by the inquilines. In this case, they again become available for the plant if the inquilines die within the lifetime of the pitcher. (2) Animal pitcher inquilines incorporate particular organic matter and excrete soluble compounds such as NH_4^+ , PO_4^{3-} , urea, etc.

Although these considerations are in accordance with current biological knowledge, few aspects have been tested so far. Bacteria are frequently mentioned in schemes of prey degradation, but their exact role is usually neither specified nor quantified (Bradshaw, 1983; Ratsirarson and Silander, 1996; Sota *et al.*, 1998; Trzcinski *et al.*, 2005; Eilenberg and Zilberstein, 2008). Moran *et al.* (2010), however, point out that *Nepenthes* species with poor digestive capabilities avoid extreme pH values of the fluid that may inhibit bacterial growth. Some data on bacterial counts are available: *N. alata* hosts up to 3.6×10^8 cultivable units per mL (CUs mL^{-1}) (Sota *et al.*, 1998), in *S. purpurea*, 10^6 – 10^7 CUs mL^{-1} were found (Cochran-Stafira and von Ende, 1998).

Protease formation by bacteria and fungi was detected in the traps of *S. purpurea* (Bay, 1893), *S. flava* (Plummer and Jackson, 1963), *Nepenthes mastersi* (Bay, 1893), *N. mirabilis* (Okahara, 1933) and *Nepenthes* sp. (Lüttge, 1964). The failure of Hepburn (1918) to find bacterial protease in *Nepenthes* is probably due to the lack of an appropriate cultivation protocol. Bacteria and fungi isolated from *N. mirabilis* were able to degrade amino acids to NH_4^+ (Okahara, 1933), the preferred N species for *Nepenthes* (Schulze *et al.*, 1999). Since arthropods are the most important prey organisms, chitinolysis is an important step in prey utilization. Lindquist (1975) detected chitinolytic bacteria in *S. purpurea*. Bacteria can also be expected to release PO_4^{3-} from nucleotides or phospholipids. Non-sterile pitcher fluid of a *Nepenthes* hybrid contained phosphatases as well as phosphoamidase (Higashi *et al.*, 1993), which may have been produced by inquilines.

Nutrients absorbed by bacteria are partly recycled via bacterivorous metazoa. Błędzki and Ellison (1998) provide a rough calculation on the metabolism of the rotifer *Habrotrocha rosea*, inhabiting about 70 % of the traps of *S. purpurea* (Petersen *et al.*, 1997): an average pitcher hosts 388 ± 924 (range 0–960) rotifers feeding mainly on bacteria. P is exclusively excreted as PO_4^{3-} , namely 2.0 ng h^{-1} . Seventy per cent of the excreted N is NH_4^+ , namely 0.5 ng h^{-1} . An average plant producing six leaves per year receives

18–88 mg of P and 9–43 mg of N from rotifers. Dipteran larvae fulfil a similar function, as they feed on the plant's prey and excrete excessive N mainly as NH_4^+ (Bradshaw and Creelman, 1984).

Large insect larvae frequently have another beneficial effect by mechanically breaking up the prey and therefore improving the accessibility for digestive enzymes and smaller inquilines. In *S. purpurea*, this task is performed by *M. knabi* (Heard, 1994b), and in *Nepenthes* by *Megaselia* spp., *Tripteroides* spp., *Dasyhelea* spp. and others (Ratsirarson and Silander, 1996; Sota et al., 1998; Kitching, 2000). In *S. purpurea*, however, N uptake was not significantly enhanced by the presence of large arthropods, indicating that their role is negligible compared with microbes (Karagatzides et al., 2009). Furthermore, insects leave the pitchers as imagines and take incorporated N and P with them.

Other effects of inquilines on the plant

Prankevičius and Cameron (1991) found Azotobacteriaceae in *S. purpurea* that assimilate atmospheric N_2 . These authors as well as Ellison et al. (2003) estimate that bacterial N fixation may exceed the plant's need even without prey capture. Cyanobacteria inhabit the fluid of *H. nutans* in the natural habitat and may contribute to the plant's nutrition via N fixation (W. Adlassnig and K. Pranjić, unpubl. obs.).

The vast majority of pitcher inquilines are aerobic. In spite of the intensive metabolomic activities in the fluid, a high degree of O_2 saturation is maintained (Juniper et al., 1989; authors' own observation). Gas exchange via the small surface of the pitcher fluid is insufficient for this aim: if the fluid of *S. purpurea* is transferred to a container with a similar surface, the inquilines die within a short time period (Bradshaw and Creelman, 1984). Thus, the fluid is obviously supplied with O_2 through the pitcher walls via cuticular gaps (Bradshaw and Creelman, 1984; Napp-Zinn, 1984; Joel and Heide-Jørgenson, 1985; Juniper et al., 1989). Epidermal chloroplasts are a common feature in carnivorous plants (reviewed by Adlassnig, 2007); CO_2 produced by the inquilines can be easily consumed by the leaf whereas photosynthetic O_2 will readily diffuse into the fluid. This process is beneficial for both sites: the inquilines maintain a high level of metabolic activity and intensively degrade the plant's prey; the plant avoids photorespiration under high light and low CO_2 conditions. Rarely, a surplus of prey leads to the complete consumption of O_2 , followed by the breakdown of the pitcher community and the mass development of the anaerobic photoheterotrophic bacterium *Rhodopseudomonas palustris* in *S. purpurea* (Bradshaw and Creelman, 1984). It is unclear if prey degradation takes place under anaerobic conditions. In *N. bicalcarata*, the ant *Camponotus schmitzi* prevents anaerobic conditions by removing large prey objects and reducing the risk of putrefaction; otherwise, the pitcher may die (Clarke and Kitching, 1995). The filter feeder *W. smithii* prevents the colonization of the pitchers of *S. purpurea* by microalgae. *Sarracenia purpurea* introduced to Europe do not host *Wyeomyia* and are therefore inhabited by up to 65 species of algae which may compete with the plant for N and P (Gebühr et al., 2006).

Terrestrial animals visiting the traps usually reduce the fitness of the plant. *Nepenthes lowii*, however, seems to depend on such visitors for nutrient supply. The upper traps lack mechanisms for prey retention but still produce nectar. The nectar is consumed by tree shrews (*Tupaia montana*) which defecate into the pitchers and therefore supplement the plant with nutrients (Clarke, 1997; Clarke et al., 2009; Chin et al., 2010).

CONCLUSIONS AND FUTURE PERSPECTIVES

Host or environment?

Various species of CPPs host highly different sets of inquilines. The reasons for these differences comprise (based on Clarke, 1998) (a) the occurrence of different organisms in the habitat of the plant; (b) the selective influence of the pitcher and its fluid; (c) competition and predation among the inquilines; and (d) accidental observations. Concerning (a) and (c), much information is available and a high level of understanding has been achieved; however, these data provide no satisfactory explanation for the differences between various host species. Isolated data have been published concerning (b) as well, but there were few efforts to test the selective influence of the fluid. The importance of (d) is difficult to estimate, since several species are known only from single observations. The most important studies comparing pitcher phytotelmata from different species (Clarke and Kitching, 1993; Clarke, 1998; Jabiol et al., 2009) postulate an influence of the plant or its fluid on the species composition of the phytotelm. The growing evidence for the presence of toxic substances, digestive enzymes, radicals, detergents, narcotics, gelling agents and acids in some pitcher fluids indicates that the influence of the plant has been underestimated thus far.

The influence of physical factors is dubious. CPPs with large pitchers such as *Nepenthes* spp. and *S. purpurea* definitely host more species than *Heliophora*, *C. follicularis* or most other species of *Sarracenia*. Within *S. purpurea*, on the other hand, the correlation between the volume of fluid and inquiline diversity is weak (Gotelli and Ellison, 2006). Desiccation, extreme heating or freezing of the pitchers is detrimental for the inquilines. At least in *H. nutans* and *S. purpurea*, the pitchers are designed to minimize such events.

Future studies on pitcher fluids and inquilines

The present data on the chemistry of pitcher fluid are numerous but too fragmentary to enable a real understanding. Modern analytical techniques for routine analyses of complex samples are required to clarify this topic. Studies on the pitcher fluid may be of surprising relevance, especially in *Nepenthes*, where several compounds can be used in pharmacy: Digestive enzymes of *N. alata* remove antigens from red blood cells (Kajii et al., 1988; Kamesaki et al., 1989). In the same species, Hatano and Hamada (2008) found proteins with a distinctive activity against micro-organisms. In traditional Indonesian and Madagascan medicine, *Nepenthes*

fluid is used as an eyewash as well as a cure for headaches, asthma and burns (D'Amato, 1998; Schoenwetter *et al.*, 2006).

The microbiology of the pitchers is another topic which has been neglected so far. Information on the biodiversity, and the abundance and the metabolic rates of bacteria and fungi is required, as micro-organisms can be expected to play a key role in pitcher phytotelmata. The fast growth and propagation of micro-organisms and their ubiquitous diaspores make them ideally adapted to the short-lived phytotelmata. Micro-organisms possibly carry out all digestive reactions that may be required to break down prey. Additional reactions include the fixation of atmospheric N₂ in *S. purpurea* (Prankevicus and Cameron, 1991) or the acidification of the pitcher fluid (Lindquist, 1975; Mayer, 2005). All these reactions have never been quantified; their relevance for the plant is unknown. The occurrence and importance of viruses in the pitcher fluid is completely unknown. Only Cochran-Stafira and Yakimova (2003) studied the occurrence of potential vectors in *S. purpurea* but the ecological role of viruses within the phytotelm is undefined.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following tables. Table S1. Digestive enzymes reported from CPPs. Table S2. Species of pitcher inquilines described from *Nepenthes*. Table S3. Species of pitcher inquilines described from *Sarracenia*. Table S4. Pitcher inquilines of other pitcher plants.

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