Cover Page

Short title: Digestive systems of carnivorous plants

Title: The digestive systems of carnivorous plants

One-sentence summary: A comparison of the forms and functions of digestive and absorptive glands in carnivorous plants sheds light on their convergent evolution.

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Author Contributions

D.G., M.F., and K.F. conceptualized the scope of the manuscript. D.G., M.F., A.F., and Q.L. acquired SEM images. M.F. performed methylene blue staining. D.G., M.F., A.F., Q.L., and K.F. provided plant photographs. C.S. supervised the SEM observation. D.G., M.F., K.J.G., Q.L., A.F., T.R., and K.F. wrote the manuscript with input from all authors. All authors revised and approved the manuscript. K.F. supervised the project. K.F. agrees to serve as the author responsible for contact and ensures communication.

Abstract

To survive in nutrient-poor habitats, carnivorous plants capture small organisms comprising complex substances not suitable for immediate reuse. The traps of carnivorous plants, which are analogous to the digestive systems of animals, are equipped with mechanisms for the breakdown and absorption of nutrients. Such capabilities have been acquired convergently over the past tens of millions of years in multiple angiosperm lineages by modifying plant-specific organs including leaves. The epidermis of carnivorous trap leaves bears groups of specialized cells called glands, which acquire substances from their prey via digestion and absorption. The digestive glands of carnivorous plants secrete mucilage, pitcher fluids, acids, and proteins, including digestive enzymes. The same (or morphologically distinct) glands then absorb the released compounds via various membrane transport proteins or endocytosis. Thus, these glands function in a manner similar to animal cells that are physiologically important in the digestive system, such as the parietal cells of the stomach and intestinal epithelial cells. Yet, carnivorous plants are equipped with strategies that deal with or incorporate plant-specific features, such as cell walls, epidermal cuticles, and phytohormones. In this review, we provide a systematic perspective on the digestive and absorptive capacity of convergently evolved carnivorous plants, with an emphasis on the forms and functions of glands.

Main text

The carnivorous plant leaf as an all-in-one organ analogous to the animal digestive tract. Like an animal's mouth, carnivorous plants use their trapping structures to "eat" their prey, primarily small arthropods. All carnivorous plants discovered to date capture their prey using modified leaves called "trap leaves", except for *Triantha* (false asphodel), which was recently shown to produce flypaper-type traps exclusively on its flower stalks (Lin et al., 2021). Although trap leaves share many functions with animal digestive tracts, there are striking differences in their spatial arrangements (Fig. 1). Most vertebrate digestive tracts are divided into functionally specialized organs such as the mouth, stomach, and intestines, where food is digested and absorbed in distinct compartments (Hedrich, 2015). In carnivorous plants, however, the prey does not travel through a digestive tract but instead remains in the same organ where it was captured for subsequent digestion and absorption (comparable to some animals with a blind-ended digestive tract, such as polyps (Steinmetz, 2019)). Therefore, in principle, trap leaves are all-in-one organs with multifaceted functions, regardless of trap type (Fig. 2). However, in certain trap types, a spatial split of functions may be observed within the organ (i.e., within a single leaf). A striking example is the eel traps of *Genlisea* (corkscrew plants), in which bifurcating arm-like trapping organs are well separated from the digestive chamber (Fig. 2).

Most carnivorous plants employ their leaf-derived traps (or parts of these structures) for both photosynthesis and prey capture, while a few plants develop specialized trap leaves in addition to conventional foliar leaves (*Cephalotus* [Albany pitcher plant], *Genlisea*, and some *Utricularia* [bladderworts] species) or compensate for the reduced photosynthetic function of the traps by generating modified shoots (most *Utricularia* species) (Fleischmann et al., 2018b; Fleischmann, 2018).

The primary function of the animal stomach is the chemical breakdown of food. The parietal cells of the human stomach secrete hydrochloric acid (Engevik et al., 2020), which creates a highly acidic environment with a pH of ~1.5 (Dressman et al., 1990; Russell et al., 1993). The acidic conditions serve as a barrier against food-borne pathogens and provide the optimal environment for digestive enzyme activity (Smith, 2003; Martinsen et al., 2005). Although typically not as acidic as the human stomach, the digestive fluids of carnivorous plants can be highly acidic, often reaching pH 2 to 3, which is more acidic on average than the gastric acids of insect-eating animals (Beasley et al., 2015) (Fig. 3 and Table S1). Akin to the animal stomach, this acidic environment is primarily generated by inorganic acids, mainly hydrochloric acid (Rea, 1982). The molecular machinery that generates hydrochloric acid is largely unknown in many carnivorous plants, but in *Dionaea* (Venus flytrap), active exocytosis coincides with the secretion of calcium, protons, and chloride, suggesting the involvement of vesicle-mediated transport that prevents disturbance of the membrane potentials of gland cells (Scherzer et al., 2017). Alternatively, membrane proteins such as ion channels may be involved in this process, as shown in animals (Fig. 1B).

One major proteolytic enzyme activated under acidic conditions in the human stomach is pepsin (Fruton, 2002). Since pepsin contains two aspartic acid residues in its active site, this enzyme belongs to the aspartic protease protein family. Carnivorous plants use enzymes similar to animal pepsin to break down animal proteins, as discovered by Charles Darwin (Darwin, 1875). More recently, carnivory-active proteolytic enzymes were isolated from *Nepenthes* (tropical pitcher plants), *Cephalotus*, and *Sarracenia* (North American pitcher plants) and were found to be aspartic proteases (Athauda et al., 2004; Hatano and Hamada, 2008; Rottloff et al., 2016; Fukushima et al., 2017). Although *Dionaea* also secretes aspartic proteases (Schulze et al., 2012; Paszota et al., 2014), cysteine proteases are likely the most abundant proteolytic enzymes in its digestive fluid (Takahashi et al., 2011; Libiaková et al., 2014). Many carnivorous plants possess several additional enzyme classes that degrade various high-molecular-weight compounds found in an insect's body. Examples include chitinases, which break down chitin, a component of the arthropod exoskeleton; ribonucleases, which break down nucleic acids; and other enzymes, such as amylases, esterases, and phosphatases (Ravee et al., 2018). This rich enzymatic repertoire parallels that of animal digestive systems (Lemaitre and Miguel-Aliaga, 2013; Janiak, 2016). Their evolutionary origin is often linked to defense mechanisms (discussed later), but some enzymes appear to have been coopted from other ancestral functions (Kocáb et al., 2020). The secretion of proteins such as digestive enzymes is assumed to occur via the conventional secretory pathway common to plants and animals (Wang et al., 2018), although other pathways may also be involved (see Supplemental Text 1). However, in several carnivorous plants, prey digestion is partly or fully performed by associated microorganisms that live in the trap—comparable to the intestinal microbiota in animals, which are also essential for digestion (Hanning and Diaz-Sanchez, 2015).

Digested food in the human stomach is transported to the intestine, where degraded products are absorbed. Numerous transporter proteins in animal intestines participate in the uptake of a variety of nutrients such as ions, sugars, amino acids, and peptides (Pácha, 2000; Jackson and Mclaughlin, 2006; Bröer, 2008; Boudry et al., 2010; Schmidt et al., 2010; Estudante et al., 2013; Bröer and Fairweather, 2018; Rajendran et al., 2018; Engevik and Engevik, 2021). Several transporter proteins involved in nutrient absorption have been identified in *Dionaea*, whose repertoire may be distinct from its human counterparts (Fig. 1B). Although transporters usually absorb only specific compounds, mammalian intestines, often during early postnatal life, can encapsulate extracellular macromolecules in vesicles and absorb them into cells intact (Pácha, 2000). This process, endocytosis, enables relatively non-selective nutrient uptake. This combination of membrane protein action and endocytosis is also found in carnivorous plant leaves (Adlassnig et al., 2012). Thanks to their variety of digestive enzymes and absorption pathways, carnivorous plants can utilize a wide range of prey-derived small and large molecules; the latter include proteins, nucleic acids, chitins, and glucans (Matušíková et al., 2018).

Digestive and absorptive glands. Glands are not unique to carnivorous plants, as many vascular plants possess glands for secreting various materials, including nectar, mucilage, resin, salts, aromatic

compounds, and physiological residues (Callow et al., 2000; Mehltreter et al., 2021). Such exudates often contain hydrolytic enzymes and other proteins (Shepherd and Wagner, 2007; Heil, 2011). Some of the most commonly secreted proteins are pathogenesis-related proteins, which prevent fungal and bacterial growth via hydrolytic activity or function in processes such as lipid transfer and defense signaling (Sels et al., 2008). As such, the glandular functions in trap leaves may be considered convergent exaptations of the various repertoires of structures and exudates found across angiosperm phylogeny (Juniper et al., 1989; Fleischmann et al., 2018a b). For example, in a study of 19 non-carnivorous plants, 15 species were found to have protease activity in their glandular trichome secretions (Spomer, 1999).

Like other secretory tissues, such as hydathodes, salt glands, and nectaries (Fahn, 1988; Vogel, 1998), the glands of carnivorous plants are distinguished by their physiological functions, which are related to prey digestion and nutrient absorption. Their morphology is often well differentiated from that of other epidermal cells (Juniper et al., 1989), but in Sarraceniaceae, epidermal cells that may differ only slightly in size from surrounding cells exhibit cuticular permeability and endocytotic activity, the hallmark features of carnivorous plant glands (Koller-Peroutka et al., 2019). Digestive glands secrete mucilage, ions, and proteins including digestive enzymes (Darwin, 1875; Juniper et al., 1989). The same or morphologically distinct glands then absorb the degraded compounds via the activities of membrane transport proteins and endocytosis. The occurrence of more than one type of gland is common in carnivorous plant groups (Juniper et al., 1989), but their functional differentiation is not clearly understood in many species. Although glands are defined based on their secretory or absorptive functions, they are often judged to be glands based on their morphology and localization. As such, it has been assumed that these morphological differences come with functional differences in terms of digestive and absorptive capabilities, but more recent evidence points towards at least partial overlap in functions between different types of glands in different lineages. For example, phosphatase activity could be detected in both sessile and stalked glands of *Pinguicula* (butterworts) (Płachno et al., 2006), suggesting both glands are capable of digestion. There is also evidence of endocytotic uptake in both types of glands of *Drosophyllum* (Adlassnig et al., 2012). However, a more comprehensive study comparing all relevant genera and glands will be necessary to dispel the initial dogma completely.

Evolution of different trap types from flypaper traps. The flypaper trap is the most frequently occurring type of trap in carnivorous plants, having independently evolved in at least six lineages, including three in the Lamiales alone (in *Pinguicula*, *Byblis* [rainbow plants], and *Philcoxia*) (Schäferhoff et al., 2010; Fleischmann et al., 2018a b), at least one each within the Caryophyllales and Ericales (Albert et al., 1992), as well as the recently discovered carnivorous inflorescences of *Triantha occidentalis* (Lin et al., 2021) (Fig. 2). Some plants are considered "para-carnivorous", that is, sticky plants that casually trap insects but do not make use of the trapped "prey", for example, *Ibicella* (Płachno et al., 2009) and *Stylidium* (Darnowski et al., 2006). Note that the features required for the

carnivorous syndrome are controversial and vary among researchers (Adamec et al., 2021); the term "para-carnivorous" is not clear-cut and does not imply a "transitional species" on the way to becoming a carnivorous plant. In any case, even more disparate species throughout the angiosperm phylogeny possess sticky trichomes (likely upwards of thousands of species), including ones that are unequivocally not currently considered carnivorous or para-carnivorous; instead, they entrap arthropods primarily for herbivore defense, as exemplified by several Lamiales and Solanaceae species (Adlassnig et al., 2010; Bar and Shtein, 2019; Adamec et al., 2021; Chase and Christenhusz, 2021).

Flypaper traps may have given rise to all other trap types (Albert et al., 1992; Fleischmann et al., 2018b). In the carnivorous Caryophyllales, the most parsimonious hypothesis is that the flypaper trap type is plesiomorphic, with snap traps and pitfall traps derived from ancestors with sticky traps (Heubl et al., 2006; Renner and Specht, 2011; Fleischmann et al., 2018b; Fleischmann et al., 2018a). Similarly, the flypaper trap of *Pinguicula* is sister to the two other trap types in Lentibulariaceae in carnivorous Lamiales (Müller et al., 2006). Although possibly not an immediate phylogenetic sister (Löfstrand and Schönenberger, 2015), the pitfall traps in Ericales are also closely related to those of a flypaper trap lineage (*Roridula*).

Evidence suggests that mucilage production in ancestral flypaper traps has been retained in some of these other trap types. For instance, both *Utricularia* and *Genlisea* (suction and eel traps, respectively; Lentibulariaceae) produce bifid trichomes with mucilage secretions on their traps and globose glands that secrete mucilage on their leaves (Taylor, 1989; Płachno et al., 2006; Adlassnig et al., 2010; Fleischmann, 2012). Interestingly, certain species of the pitfall-trapping *Nepenthes* genus produce a mucilage-derived, highly viscoelastic digestive fluid (Gaume and Forterre, 2007; Bauer et al., 2011; Bonhomme et al., 2011; Renner and Specht, 2011) that aids in prey retention (Di Giusto et al., 2008; Moran et al., 2013; Bazile et al., 2015; Gaume et al., 2019; Kang et al., 2021), representing a type of hybrid trapping strategy reminiscent of their close relatives *Drosera* (sundews). Exploring mucilage-mediated interactions with other organisms could shed light on the evolution of carnivorous plants (Box 1).

Mucilage production and secretion mechanisms. Little is known about the production and secretion of mucilage across the various carnivorous plant lineages, although limited evidence is available for members of the Caryophyllales (Droseraceae and Drosophyllaceae) and Lamiales (Lentibulariaceae). The mucilage of *Drosera binata* contains an acidic polysaccharide comprising arabinose, galactose, glucuronic acid, mannose, and xylose (Gowda et al., 1982; Erni et al., 2008), while the acidic polysaccharide of *D. capensis* is slightly modified and is composed of ester sulfate, galactose, glucuronic acid, mannose, and xylose (Rost and Schauer, 1977). Across *Drosera*, however, the Golgi apparatus appears to be responsible for both mucilage production and secretion (Schnepf, 1961a; Dexheimer, 1978; Outenreath and Dauwalder, 1986; Lichtscheidl et al., 2021). The glands of the Caryophyllales carnivores *Triphyophyllum* and *Drosophyllum* also produce acidic secretions. The

constituents of these secretions in *Triphyophyllum* are unknown, but those in *Drosophyllum* contain carbohydrates produced by the Golgi apparatus (Schnepf, 1961b; Schnepf, 1963a; Schnepf, 1972; Marburger, 1979). Interestingly, the polysaccharide found in *Drosophyllum* mucilage differs from that of *Drosera* and includes the monomers arabinose, galactose, glucuronic acid, rhamnose, and xylose, as well as ascorbic acid (Schnepf, 1963b). Similarly, in *Pinguicula* of the Lentibulariaceae, polysaccharides are prevalent in the sticky mucilage and are likely transported intracellularly by vesicles derived from the Golgi apparatus, as observed in *Drosera* and *Drosophyllum* (Heslop-Harrison and Knox, 1971; Vassilyev and Muravnik, 1988). In *P. vulgaris*, the mucilage itself is stored within vacuoles, as well as the periplasmic space, before being released to the gland surface (Vassilyev and Muravnik, 1988; Adlassnig et al., 2010). In closely related *Genlisea*, mucilage is also stored in the periplasmic space of secretory glands (Płachno, 2008). A notable exception to the polysaccharide-rich mucilages of carnivorous plants is the genus *Roridula*, which secretes resinous compounds and will be discussed further below.

Convergent co-option of digestive enzymes. The highly repeated convergent evolution of plant carnivory (Fig. 2) suggests that the transition from the non-carnivorous to carnivorous state was broadly genetically accessible to a wide range of angiosperm lineages. In agreement with this idea, all known digestive enzymes of carnivorous plants are not unique but originated from ubiquitous gene families found throughout flowering plants (Fukushima et al., 2017). In particular, defense-related genes tend to be repurposed for digestive physiology (Bemm et al., 2016), with possible changes in biochemical properties occurring through positively selected convergent amino acid substitutions (Fukushima et al., 2017). Several proteins involved in plant defense, including hydrolytic enzymes, are secreted to the extracellular space (Lee et al., 2004). Pathogenic microbes, fungi, and both phytoparasitic and herbivorous (and sometimes prey) insects share many biological components (e.g., chitin), perhaps providing a ready basis for the evolutionary co-option of enzyme-encoding genes.

Secretion of digestive enzymes. Various digestive enzymes have been identified in the digestive fluid of carnivorous plants and are thought to be secreted from glands (Heslop-Harrison, 1975; Juniper et al., 1989; Ravee et al., 2018; Hedrich and Fukushima, 2021). In particular, extracellular phosphatase activity is a widely detected, key characteristic of the glands of carnivorous plants (Płachno et al., 2006; Płachno et al., 2009; Lin et al., 2021). However, thus far, genes encoding secreted phosphatases have only been isolated in *Nepenthes* and *Cephalotus* (Fukushima et al., 2017). Additionally, commonly used dye-based method appears to label both intracellular and extracellular phosphatase activity following intensive endocytosis (Płachno et al., 2006), which may confound the extracellular signal with the intracellular noise of housekeeping phosphatases. Not much is known about the tissue-specific secretion and localization of digestive enzymes, except for the phosphatases and the aspartic protease Nepenthesin I expressed in the parenchyma around the glands of *Nepenthes* (Athauda et al., 2004). In

Cephalotus, which conditionally produces distinct trapping leaves (Fukushima et al., 2017; Fukushima et al., 2021), approximately half of the genes encoding digestive fluid proteins are specifically expressed in pitcher leaves, but the other half are also expressed in the photosynthetic, non-trapping leaves (Fukushima et al., 2017). Trap-preferential gene expression has been reported in other species as well, with a few exceptions (Rottloff et al., 2011; Nishimura et al., 2013; Rottloff et al., 2013; Arai et al., 2021). Perhaps these digestive enzymes exist in a bifunctional state for defense and digestion, or perhaps they are encoded by sub-/neofunctionalized duplicates specialized for digestive physiology, which might influence the tissues and cell types that secrete the enzymes.

Proton transport. The acidity of digestive fluid is a hallmark of carnivorous plants. Although the pH varies among carnivorous plant genera (Fig. 3B), the digestive fluids of carnivorous plants are often more acidic than the gastric juices of animals with specialized feeding habits, including insect-eating carnivores (Fig. 3A). This strong acidity has several potential benefits, including the capacity for (1) killing prey (Bazile et al., 2015); (2) suppressing microbial growth (Buch et al., 2013); (3) acid-mediated auto-activation of aspartic proteases, a process similar to pepsin activation in the animal stomach (Runeberg-Roos et al., 1991; Fruton, 2002; Buch et al., 2015); (4) efficient degradation of proteins and other substrates by digestive enzymes with acidic pH optima (An et al., 2001; Saganová et al., 2018); and (5) nutrient absorption driven by proton gradients. Protons and potassium ions are thought to be the primary cations in some carnivorous plant species due to their abundance and the scarcity of other cations (Nemček et al., 1966; Juniper et al., 1989; Scherzer et al., 2013; Gao et al., 2015; Scherzer et al., 2015; Fasbender et al., 2017) (Box 2). Although the pH of digestive fluid varies among species, its acidity is usually higher than the apoplastic pH in other plants (Fig. 3A). Compared to other pitcher plants, many Sarraceniaceae species rely more on microbes than their own digestive enzymes (Luciano and Newell, 2017), likely explaining why the liquid in their pitchers tends to be less acidic than that of other carnivorous plants (Fig. 3). In many carnivorous plant groups, the digestive fluid is acidic even in the resting state and becomes more acidic upon prey capture (Table S1).

The strong acidity of digestive fluid can be attributed to the activity of proton pumps (Rea, 1984). This view was supported by pharmacological treatment of *Nepenthes* with H^+-pump inhibitors and an activator that especially affected plasma membrane H^+-ATPases (An et al., 2001). Noninvasive microelectrode ion flux measurements confirmed that the gland cells in *Nepenthes* and *Dionaea* release protons into the pitcher or snap-trap lumen (Moran et al., 2010; Scherzer et al., 2017). In *Nepenthes*, the putative plasma membrane proton pump gene *NaPHA1* is expressed in glands (An et al., 2001). In *Dionaea*, the levels of vacuolar AHA10-type proton pump transcripts changed in response to coronatine, which mimics bioactive jasmonic acid and induces some prey-capture responses in carnivorous Caryophyllales, a process likely related to acid secretion by exocytotic vesicles (Scherzer et al., 2017) (Supplemental Text 1). Future research should address which proton pumps are responsible for fluid acidification and how they differ among carnivorous species.

Anion transport. To generate hydrochloric acid, both chloride and protons must be excreted into the digestive fluid. Classical pharmacological analyses with metabolic inhibitors demonstrated that the ionic gradients between digestive fluid and gland cells are actively modulated in carnivorous plants (Juniper et al., 1989). Chloride ions are a principal anion in the digestive fluids of some carnivorous species, such as *Nepenthes* spp. (Morrissey, 1955; Nemček et al., 1966). In these pitcher plants, the release of chloride ions coincides with the secretion of proteases (Lüttge, 1966), as in *Dionaea* (Rea et al., 1983; Scherzer et al., 2017) and *Pinguicula* (Heslop-Harrison and Heslop-Harrison, 1980). In *Dionaea*, the vacuolar voltage-dependent chloride channel CLC (stands for ChLoride Channel) is implicated in chloride transport during prey digestion (Scherzer et al., 2017). Since digestive fluid contains only trace amounts of organic acids (Voelcker, 1849; Morrissey, 1955), it appears that organic anions such as malate (which functions in osmotic regulation in certain plant cells) do not play major roles in this process (Fernie and Martinoia, 2009; Araújo et al., 2011; López-Arredondo et al., 2014). However, organic acids are relatively abundant in the traps of *Utricularia*, even though the fluid pH is close to neutral (Sirová et al., 2011).

Ammonium absorption. In contrast to the digestive tracts of animals (Romero-Gómez et al., 2009), ammonium likely serves as the preferred form of nitrogen for uptake in carnivorous plants (Fig. 1B). After prey capture, ammonium is released into the digestive fluid in *Dionaea* (Scherzer et al., 2013). The addition of pure protein also resulted in ammonium accumulation, and the relative abundance of released amino acids indicates that the enzymatic deamination of glutamine, in particular, produces ammonium in the digestive fluid of *Dionaea* (Scherzer et al., 2013). Tracer experiments supported the notion that nitrogen, likely in the form of ammonium, is separated from the carbon skeleton of glutamate in digestive fluid (Fasbender et al., 2017). In multiple carnivorous plants, ammonium transporters (AMTs) appear to play pivotal roles in ammonium uptake. Transporters for nitrogenous compounds in *Nepenthes* often show negligible expression in glands, except for AMT1 (Schulze et al., 1999). *AMT1* transcripts are localized exclusively to the head cells of the gland, pointing to the involvement of AMT1 in ammonium uptake. Likewise, in *Dionaea*, *AMT1* shows gland-specific expression, with further upregulation following coronatine treatment (Scherzer et al., 2013). *Cephalotus* also has an *AMT1* gene that shows preferential expression in pitcher leaves (Fukushima et al., 2017). Interestingly, some *AMT1* genes in *Arabidopsis thaliana* (thale cress) are highly expressed in roots and are thought to be involved in the uptake of ammonium ions from the soil (Gazzarini et al., 1999; Rawat et al., 1999), suggesting possible co-option of this gene from roots to traps in multiple lineages.

Membrane trafficking. The direct transport of nutrients via membrane proteins is not the only way substances are absorbed and distributed by cells. Large molecules, such as whole proteins and degraded peptides, can be taken up and released via endocytosis and exocytosis, respectively (Battey et al., 1999;

Doherty and McMahon, 2009; Paez Valencia et al., 2016). Active endocytosis is observed in the glands of many carnivorous lineages (Adlassnig et al., 2012). In *Nepenthes*, for example, a few small vesicles were observed within gland cells one hour after the application of a fluorescent tracer, and by 30 hours they combined into one or a few large vesicles that occupied most of the cell volume (Adlassnig et al., 2012).

Membrane trafficking must also be involved in the export of digestive enzymes. Newly synthesized digestive enzymes could follow the classical pathway of protein secretion, in which proteins are synthesized in the endoplasmic reticulum and modified in the Golgi apparatus to be packaged into vesicles in the trans-Golgi network and shuttled out via the plasma membrane (Battey et al., 1999; Cui et al., 2020). Indeed, exosome formation was observed in the glands of coronatine-stimulated *Dionaea* (Hawes et al., 1991; Thiel and Battey, 1998; Scherzer et al., 2017) and other species (Juniper et al., 1989).

Cuticular permeability. To exchange substances efficiently, the plasma membranes of gland cells must be accessible to the external environment. The plant epidermis is usually protected by a continuous cuticle, but gland cells of carnivorous plants often show cuticular pores or gaps that allow the passage of small molecules. The presence of such cuticular discontinuities has been revealed in many carnivorous plants using electron microscopy and staining with dyes such as methylene blue, which cannot penetrate intact cuticles (Juniper et al., 1989; Płachno et al., 2007; Adlassnig et al., 2012; Koller-Peroutka et al., 2019; Lichtscheidl et al., 2021). While the glands of many species exhibit cuticular permeability, there are some inter-species differences (Adlassnig et al., 2012). In *Drosera*, both stalked and sessile glands show cuticular permeability. *Cephalotus* produces small and large glands, but only small glands show clear cuticular permeability. Dye staining appears to correspond well with functional maturity; in *Dionaea*, immature glands do not stain, and only mature glands show clear permeability. Using fluorescent tracers, endocytotic activity was detected in cells exhibiting cuticular permeability (Adlassnig et al., 2012). In carnivorous Ericales (Sarraceniaceae and Roridulaceae), nutrient uptake is achieved through cuticular pores and an underlying digestive epithelium (Juniper et al., 1989; Anderson, 2005; Płachno et al., 2006) that functions as a gland. The genetics underlying cuticular discontinuity remain unknown.

Hormonal regulation of gland cell physiology. The digestive systems of carnivorous plants have a likely origin in defense mechanisms against herbivores (Hedrich and Fukushima, 2021). Considering that phytohormones regulate diverse physiological processes, such as plant growth, abiotic stress resistance, and defense against pathogens and insects, it is highly likely that their roles extend to digestive physiology (Pavlovič and Mithöfer, 2019). Jasmonate accumulation during prey capture has been directly observed in *Drosera*, *Aldrovanda* (waterwheel plant), and *Nepenthes* (Nakamura et al., 2013; Yilamujiang et al., 2016; Krausko et al., 2017; Jakšová et al., 2021). In *Dionaea*, jasmonates

induce trap closure and digestive fluid secretion (Escalante-Pérez et al., 2011; Libiaková et al., 2014; Pavlovič and Mithöfer, 2019), coupled with proton efflux (Scherzer et al., 2017). While jasmonate induced a carnivory-related response in Caryophyllales species, no effect was detected in *Pinguicula* and *Utricularia* (Kocáb et al., 2020; Jakšová et al., 2021). Although several other phytohormones are also important in plant defense (Berens et al., 2017), the application of abscisic acid, salicylic acid, gibberellin, and indole-3-acetic acid had no detectable effect on the trapping and digestive physiology of *Dionaea*, *Drosera*, or *Pinguicula* (Escalante-Pérez et al., 2011; Libiaková et al., 2014; Krausko et al., 2017; Pavlovič et al., 2017; Kocáb et al., 2020). By contrast, salicylic acid induced trap closure in *Aldrovanda*, although the observed pharmacological damage questions its physiological interpretation (Jakšová et al., 2021). The roles of these and other phytohormones, including ethylene, cytokinins, and brassinosteroids, remain largely unexplored.

Gland morphology in Oxalidales. Oxalidales has only one carnivorous member, *Cephalotus follicularis* of the monotypic family Cephalotaceae, which remains quite isolated phylogenetically and morphologically in this angiosperm order (Fleischmann et al., 2018b). Although *Cephalotus* uses pitcher-shaped leaves as pitfall traps similar to those of the independently evolved carnivorous lineages *Nepenthes* and Sarraceniaceae, the arrangement and types of glands are lineage specific. Unlike in *Nepenthes*, the lower part of the inner pitcher wall is not evenly endowed with glands in *Cephalotus* (Moran et al., 2010); instead it has two opposing areas where the glands are densely localized (Fig. 2). Within these gland patches, both small and large glands are embedded in the epidermis and are easily distinguished. From a purely visual point of view, small gland cells can be described as immobile stomatal guard cells whose aperture is plugged with a "wall plug" comprising a thickened cell wall (Juniper et al., 1989). Large glands consist of multiple (25 to 200) cells arranged in a dome-like pattern forming clusters of different sizes (Vogel, 1998) (Supplemental Text 2). Large clusters are found in the glandular patch, and the glands gradually become smaller from the pitcher wall up to its peristome (Juniper et al., 1989; Vogel, 1998). The small glands have permeable cuticles (Adlassnig et al., 2012) and various enzyme activities such as esterase, protease, and phosphatase activity (Juniper et al., 1989; Płachno et al., 2006). The large glands have impermeable cuticles (Adlassnig et al., 2012), and only acid phosphatase activity (Płachno et al., 2006) has been demonstrated. These differences gave rise to the idea that *Cephalotus* developed a division of labor in its secretory systems: large glands for fluid production and small glands for digestive enzyme production (Juniper et al., 1989). Whether such a strict division of labor exists or whether these activities overlap remains a question for future research. Analysis of gland morphology pointed to a likely evolutionary connection between somatic guard cells and small glands (Lloyd, 1942) but not large glands (Parkes and Hallam, 1984), although such morphological (dis-)similarity does not provide conclusive evidence for their evolutionary (un)relatedness (Juniper et al., 1989).

Gland morphology in Caryophyllales. Some of the most well-known carnivorous plants are found in the non-core group of the order Caryophyllales (a.k.a., Nepenthales), ranging from the sundew and Venus flytrap (both Droseraceae) to the pitcher plants of the Nepenthaceae; this order includes genera with a variety of trap types (Supplemental Text 3; Fig. 2). Glandular trichomes are prevalent in the lineages sister to the carnivorous group, such as *Plumbago* (Supplemental Text 3), and these trichomes may be homologous to those in caryophyllid carnivores. A carnivorous common ancestor of Caryophyllales might have already developed two types of glands, stalked and sessile (Heubl et al., 2006), although a stochastic character mapping analysis did not necessarily support such a scenario (Renner and Specht, 2011). Only a single type of digestive gland maintains the pitcher fluid of *Nepenthes* by releasing enzymes and absorbing nutrients (An et al., 2002; Adlassnig et al., 2012). A piece of epidermis arches above each digestive gland (Owen, Jr., 1999; Wang et al., 2009). These structures are morphologically similar to the lunate cells of the upper parts of the pitcher, which are thought to provide difficult locomotive terrain for trapped insects (Wang et al., 2009; Wang et al., 2016; Wang et al., 2018a). A continuous layer of epidermal cells curves underneath the gland, with vascular cells in close proximity (Owen, Jr., 1999). The stalked glands in the other carnivorous Caryophyllales are vascularized whereas the glands of all other carnivorous plants are non-vascularized (Fenner, 1904; Lloyd, 1942; Juniper et al., 1989; Fleischmann et al., 2018b). In *Drosera*, these glands are called tentacles due to their exceptional anatomical and physiological characteristics. Nitschke (Nitschke, 1861) suggested that these organs represent modified leaf pinnae or outgrowths of the lamina margin, a theory that has since been refuted (Lloyd, 1942). In approximately 90% of *Drosera* species (Fleischmann et al., 2018a), increasing numbers of tentacles move toward the captured prey, likely to increase the contact surface area with the prey (Juniper et al., 1989). It was originally believed that the site of mechanosensation was the neck of the stalked cells, directly under the gland head, where the stalk is most bendable (Williams, 1976). However, transcripts of the stretch-activated ion channel gene *FLYCATCHER1* (*FLYC1.1* and *FLYC1.2*) were recently found to be localized specifically to the outer secretory cells of the glandular head, whereas in *Dionaea*, *FLYC1* transcripts were specifically detected in sensory cells (in which most trigger hair flexure occurs) (Procko et al., 2021), pointing to the evolutionary connection between digestive glands and Venus flytrap trigger hairs. These trigger hairs invoke rapid trap closure via action potentials, but little is known about the associated channels (Böhm and Scherzer, 2021), except for FLYC1, which functions in mechanosensing (Procko et al., 2021), and the Shaker-type channel K^+ channel *Dionaea muscipula* 1 (KDM1), which functions in K^+ re-uptake during the hyperpolarization phase (Iosip et al., 2020). The X-shaped quadrifid digestive glands of the aquatic plant *Aldrovanda* (Droseraceae) show remarkably similar morphology to those of the non-related Lamialean genus *Utricularia* (Lentibulariaceae). This gland shape increases the surface area of the expanded gland head cells in plants with an aquatic lifestyle.

Gland morphology in Lamiales. Among Lamiales, *Byblis* and *Philcoxia* are passive flypaper-type carnivorous plants with relatively few species, whereas Lentibulariaceae is a large family comprising three genera with different trapping mechanisms: *Pinguicula* with flypaper traps, *Genlisea* with eel traps, and *Utricularia* with suction traps (Supplemental Text 4). As in other flypaper-type carnivorous plants, *Byblis*, *Pinguicula*, and at least some species of *Philcoxia* show dimorphism, with stalked and sessile glands (Fig. 2). The terminal cells of their glands form head-like structures, except in *Utricularia*, where they develop arm-like elongations, like those in the glands located at the trap margins of *Aldrovanda* (Droseraceae). The type of cuticular discontinuity varies among Lentibulariaceae genera (Płachno et al., 2007). The non-vascularized stalked glands of *Pinguicula* produce mucilage via a unique mechanism among carnivorous plants. It has been suggested for three *Pinguicula* species that during maturation, the gland fills with digestive fluid and undergoes autolysis, leaving dead cells full of mucilage (Heslop-Harrison and Heslop-Harrison, 1981). Thus, *Pinguicula* might be incapable of regenerating the gland after excretion. However, a study of another species provided compelling evidence that the glands remain active during digestion (Vassilyev and Muravnik, 1988). This discrepancy, which may stem from interspecies differences, should be reexamined in the future. In Lentibulariaceae, like in most Lamiales, gland cells are polyploid, which likely aids in their increased physiological activity (Fleischmann et al., 2018b).

Gland morphology in Ericales. Sarraceniaceae comprises three extant taxa: *Heliamphora* (sun pitchers), *Darlingtonia* (cobra lily), and *Sarracenia*. Their pitfall traps share an elongated, funnel-shaped silhouette that in some species collects rainwater, while in other species, an enlarged pitcher lid prevents the pitchers from being flooded (Chen et al., 2018). In all of these pitcher plants, the prey falls into the pitcher, where it is then digested. For glands, Sarraceniaceae utilize morphologically unremarkable epidermal cells called digestive epithelia (Fig. 2), wherein endocytosis occurs (Koller-Peroutka et al., 2019). Dye staining of digestive zones revealed regions of these epidermal cells with permeable cuticles (Koller-Peroutka et al., 2019).

Ericales contains an additional carnivorous genus, *Roridula*, with flypaper-type traps. In addition to digestive epithelia, it has morphologically distinctive glands that sit on top of a multicellular trichome. Each globular gland contains an indentation at its pole for increased surface area (Fig. 2). The longest trichomes are thought to be responsible for prey entanglement, the shortest ones for immobilization, and the medium-sized ones for slowing down prey movements (Voigt et al., 2009). The adhesive power of the glue, which in *Roridula* is resinous (in all other sticky carnivorous plants, it is aqueous), is derived from triterpenoid compounds (Simoneit et al., 2008), making it a lipophilic resin that is sticky even underwater (Voigt et al., 2015). Due to this lipophilic secretory nature, *Roridula* exhibits unique features, such as digestive mutualism with symbiotic hemipterans (Ellis and Midgley, 1996) and a lack of digestive enzymes in the fluid (Lloyd, 1934) (Box 1 and Supplemental Text 5). However, even in the absence of symbionts, *Roridula* seems to be capable of nutrient uptake from prey

to some extent (Płachno et al., 2009). Digestive epithelia seem to be the likely site of nutrient uptake, since phosphatase activity was only found in the epidermis of the leaves, rather than stalked glands (Płachno et al., 2009).

Gland morphology in Poales. Many epiphytic bromeliads collect water in a "tank" formed by tightly arranged rosette leaves (Ladino et al., 2019). Insects and other organic material can accumulate in these small bodies of water, termed phytotelmata. Among bromeliads, *Brocchinia reducta, B. hechtioides* and *Catopsis berteroniana* are recognized as carnivorous (Fish, 1976; Frank and O'Meara, 1984; Givnish et al., 1984; Givnish et al., 1997; Fleischmann et al., 2018b). *Brocchinia reducta* actively utilizes dead matter by absorbing free nutrients, earning the species a spot among carnivorous plants (Givnish et al., 1984; Benzing et al., 1985). *Brocchinia hechtioides* is less well studied, but it shares many carnivory-associated traits with *B. reducta*, such as overall morphology and habit, acidic tank water, emission of nectar-like scent, presence of insect carcasses in the tank and similar trichome structure (Givnish et al., 1997). The glandular trichomes of *B. reducta* have very weak phosphatase activity, but it remains unclear if they produce digestive enzymes themselves (Płachno et al., 2006): Digestion is likely handled by bacteria and inquilines (Leroy et al., 2016). In *B. reducta*, glands are scattered across the entire leaf surface instead of being restricted to specific zones as in other pitcher plants (Juniper et al., 1989). These glandular trichomes are embedded in epidermal cavities, with the heads even with the inner tank surface (Benzing et al., 1985). The gland cap is radially organized, but it lacks the central disc cells typically observed in Tillandsioideae species such as *Catopsis* (Benzing et al., 1985). In that genus, four central disc cells are surrounded by multiple layers of cells, with each layer increasing in cell number (Benzing, 1976).

Paepalanthus bromelioides belongs to the Eriocaulaceae and even though not directly related to the bromeliads, its habitus is very similar to them: A rosette of leaves forms a water tank, the leaves are covered in wax possibly slippery to insects and produce UV-reflecting powder (Figueira et al., 1994). Although its carnivorous nature is under debate among scientists (Fleischmann et al., 2018b), some evidence points towards the plant being able to partially utilise nitrogen from insect carcasses and faeces of inquiline predators falling into the water tank (Nishi et al., 2013). This species may be considered carnivorous under the confines of digestive mutualism but remains severely understudied. While there are mentions of hydrophilous trichomes near the leaf bases, a detailed description of any digestive glandular structure has yet to be provided (Figueira et al., 1994).

Gland morphology in Alismatales. *Triantha* is the only carnivorous lineage in the monocot order Alismatales. Carnivory has only been demonstrated in *Triantha occidentalis* (Lin et al., 2021), but it may also exist in the three other species of the genus. *Triantha* is unique among carnivorous plants in that it captures prey (small insects) solely on its sticky flowering stems and thus only during the flowering season, perhaps to enhance reproductive fitness (Lin et al., 2021). Unlike other genera in

Tofieldiaceae, *Triantha* contains glandular hairs along its inflorescences (Packer, 2003), with fewer, smaller glands along the lower part of the stem, which is less sticky. The cylindrical glands are multicellular and typically concave at the top. The internal structure of the gland remains to be studied. The flowering stem of *T. occidentalis* secretes phosphatase (Lin et al., 2021), and phosphatase substrate hydrolysis is strongest on the glands, which appear to specifically secrete this digestive enzyme. The other digestive enzymes that *Triantha* may produce and the mechanism by which the plant absorbs nutrients remain to be demonstrated.

Concluding remarks. Studies of multiple carnivorous plant lineages revealed that various properties of glands have been acquired in parallel, such as gland dimorphism, cuticular permeability, acid secretion, endocytotic activity, and digestive enzyme secretion. However, the underlying molecular mechanisms are often unknown; thus, it is not clear whether these similar traits are brought about by the functions of common genes (see Outstanding Questions). The exception is the genes encoding digestive enzymes, in which multiple cases of convergent co-options are well documented. By contrast, the actions of phytohormones and gland morphology tend to be lineage specific. The glands in *Dionaea* have been particularly well characterized, mainly in terms of enzyme secretion and nutrient absorption (Hedrich and Neher, 2018; Hedrich and Fukushima, 2021). To understand the evolutionary trends of carnivorous plant glands, it is important to study multiple lineages and to apply knowledge about a well-studied species to other species. In addition to studying glands, further research is needed to integrate our fragmentary knowledge about other carnivory-related traits, such as prey attraction and trap development. The convergent evolution of carnivorous plants provides an opportunity to study both common, convergent trends and unique traits in the establishment of glands and other specialized tissues.

Advances Box

- Glandular structures are common among vascular plants, but many carnivorous plant glands show a distinct, common set of features for digestion and absorption.
- The glands of carnivorous plants secrete mucilage, acids, and proteins, including digestive enzymes, and absorb degraded products using membrane proteins and endocytosis.
- Many genetic components underlying carnivory are tightly linked to defense mechanisms, such as pathogenesis-related proteins and jasmonate-mediated gene regulation.

Outstanding Questions Box

- How do the digestive fluids of carnivorous plants achieve the same level of acidity as the gastric juices of some animals?
- Are there convergent evolutionary trends in gland functions among independently evolved carnivorous plants, as well as between carnivorous plants and animals?
- Which cells of non-carnivorous ancestors of a given lineage served as the evolutionary origin of carnivorous glandular cells?
- How were ancestral cellular functions overwritten, repurposed, or reconciled with the new carnivorous functions of glands?
- Which molecular evolutionary mechanisms (e.g., gene duplication with sub-/neofunctionalization and/or new regulatory relationships) led to the convergent co-option of multiple protein families involved in gland functions?

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Text Boxes

Box 1. Sticky mucilage provides biotic interactions. Mucilage production has implications for other biotic interactions in carnivorous plants. *Roridula* relies on symbiotic hemipterans living on their traps to digest their prey (Ellis and Midgley, 1996), and similar interactions might also occur in *Byblis* (China and Carvalho, 1951; Hartmeyer, 1998; Lowrie, 1998). A possibly mutualistic, fungivorous mite species was found living in the sticky leaves of *Pinguicula longifolia* (Antor and Garcia, 1995). These symbiotic arthropods require particular biomechanical adaptations to overcome the adhesive forces of these sticky glands and maintain mobility (Voigt and Gorb, 2010). Caterpillars (Fletcher, 1908; Osaki and Tagawa, 2020) and a hoverfly larva (Fleischmann et al., 2016) have also evolved behavioral and physical adaptations to overcome mucilage adhesion to consume the leaves and tentacles or entrapped prey of *Drosera*. Almost nothing is known about the effects of viscoelastic fluid on the aquatic symbionts living in *Nepenthes* pitchers, but one study (Gilbert et al., 2020) revealed little difference in the microbial community composition between species with and without sticky fluid in a greenhouse setting. The nature of the potential microbial and arthropod communities in highly viscoelastic fluid in pitcher plant phytotelmata remains largely unexplored.

Box 2. Transporters enable cation uptake. Like other plants, carnivorous plants require nitrogen and phosphate, but other elements such as potassium, iron, and manganese are also essential (Adlassnig et al., 2009). The task of potassium uptake in *Dionaea muscipula* is divided between two membrane proteins: the K^+ transporter 1-like (KT1-like), Shaker-type potassium channel DmKT1 and the high affinity K^+ transporter-type (HAK-type) transporter DmHAK5 (Scherzer et al., 2015). The low-affinity, high-capacity channel DmKT1 absorbs the K^+ released by digestion of prey using the steep K^+ gradient between the gland cell and the digestive fluid. To avoid the turning point of K^+-flowback, these channels close in response to low K^+ concentrations, and the proton-driven transporter DmHAK5 prevents unused K^+ from being wasted: This transporter has high-potassium affinity but weak selectivity. Sodium absorption is likely to be mediated by the sodium channel DmHKT1, whose transcript level is up-regulated by mechanical stimulations and the application of coronatine (Böhm et al., 2016a; Böhm et al., 2016b).

Supplemental Data

Supplemental Methods S1.

Supplemental Text S1. Potential roles of the vacuole in gland physiology.

Supplemental Text S2. Possible link between large glands and extrafloral nectaries.

Supplemental Text S3. Glands of Caryophyllales carnivores.

Supplemental Text S4. Glands of Lamiales carnivores.

Supplemental Text S5. Glands of Ericales carnivores.

Supplemental Table S1. The pH levels of digestive fluids of different species.

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643 **Figure Legends**

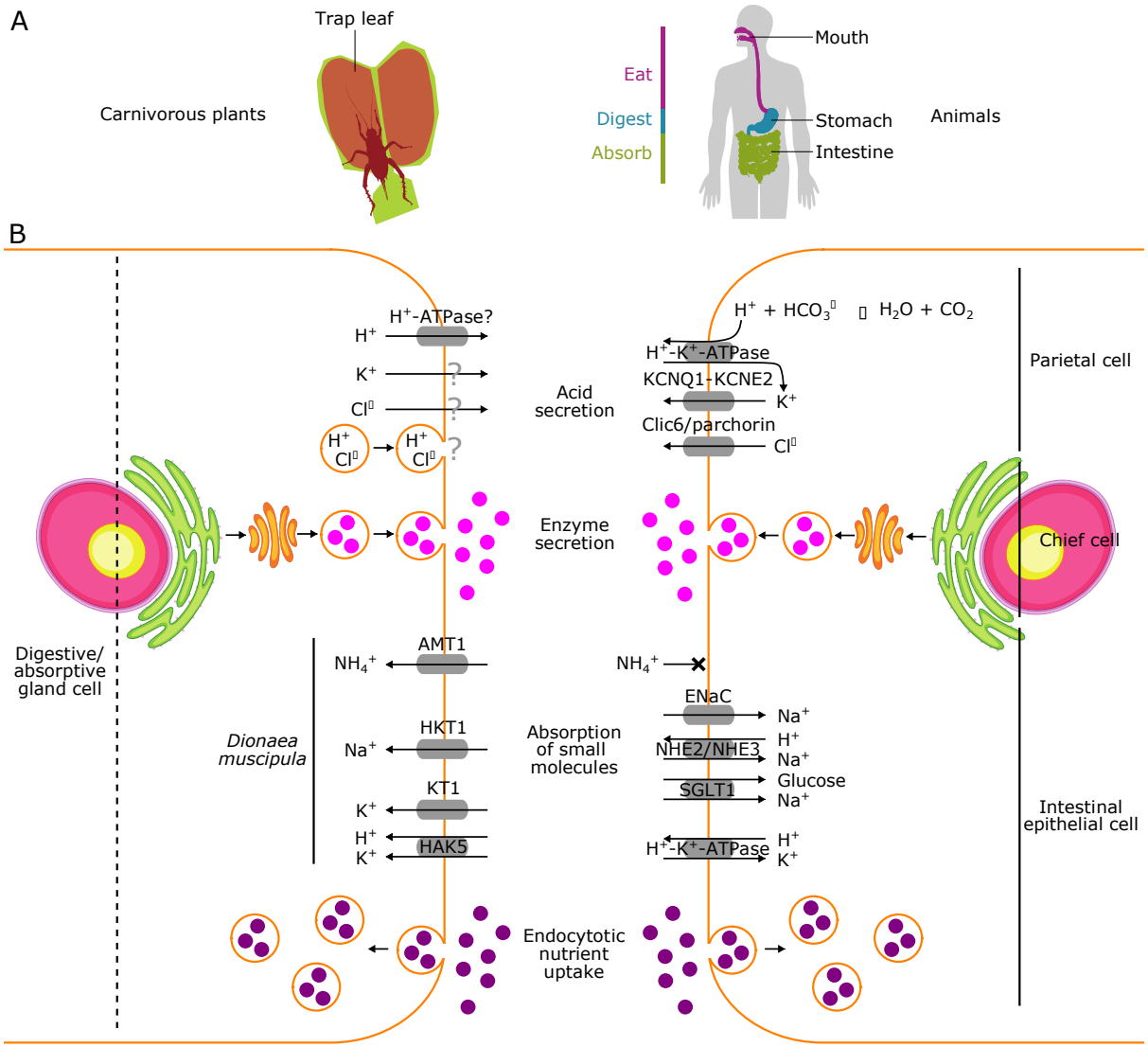

Figure 1. Functional similarities between a trap leaf and a digestive tract. (A) The spatial differentiation of the digestive system. The sites for eating, digestion, and absorption are spatially separated in the animal system (symbolized by colors), but not in carnivorous plants (overlapping colors). (B) Secretory and absorptive pathways that are discussed in the main text and Box 2. Note that the figure shows an imaginary synthetic cell because interspecies and gland-type-specific differences in these processes are often unknown in carnivorous plants. Among the many secretory and absorptive pathways and membrane proteins identified in parietal cells (Yao and Forte, 2003; Engevik et al., 2020), chief cells (Hirschowitz, 1967), and intestinal epithelial cells (Pácha, 2000; Rajendran et al., 2018; Engevik and Engevik, 2021) in animals, only the counterparts of those characterized in carnivorous plants are shown. The cell wall and cuticle are not shown. The organelles are not shown to scale.

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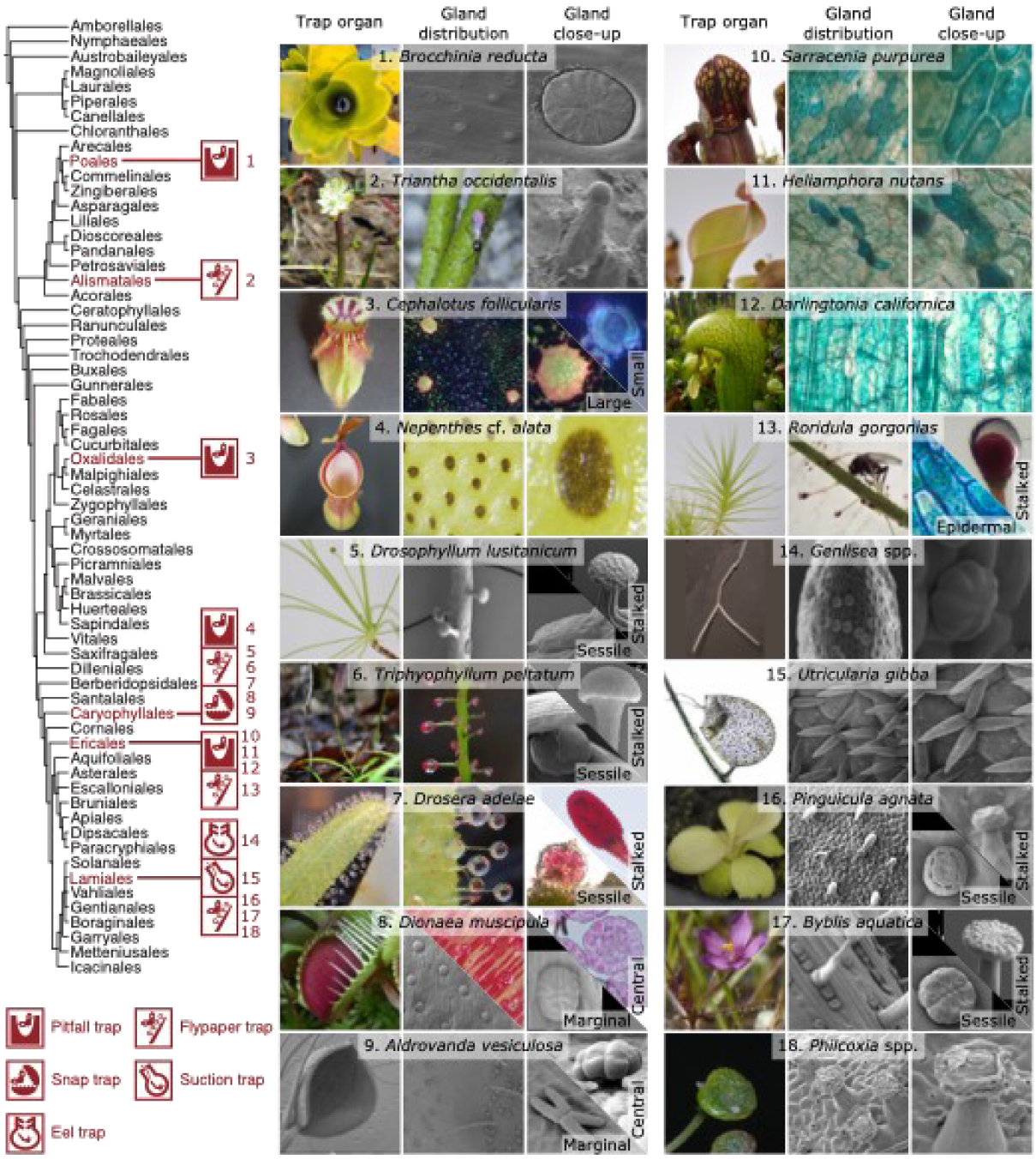

Figure 2. Evolution of glandular cells in carnivorous plants. The order-level phylogeny of flowering plants (The Angiosperm Phylogeny Group et al., 2016) is shown on the left, with lineages containing carnivorous plants and their trap types highlighted in red. Trap leaves and glands of representative species are shown on the right. To increase visibility, methylene blue staining was applied to the glands of Cephalotus, Sarracenia, Heliamphora, Darlingtonia, and Roridula. Whole or parts of the photographs of Genlisea, Utricularia, and Philcoxia were reproduced from the literature (Yang et al., 2009; Pereira et al., 2012; Fleischmann, 2018). The photographs of Aldrovanda were provided by Dirk Becker. Original pictures (including scale bars for microscopic pictures) are available in figshare (https://doi.org/10.6084/m9.figshare.18271529) under CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/).

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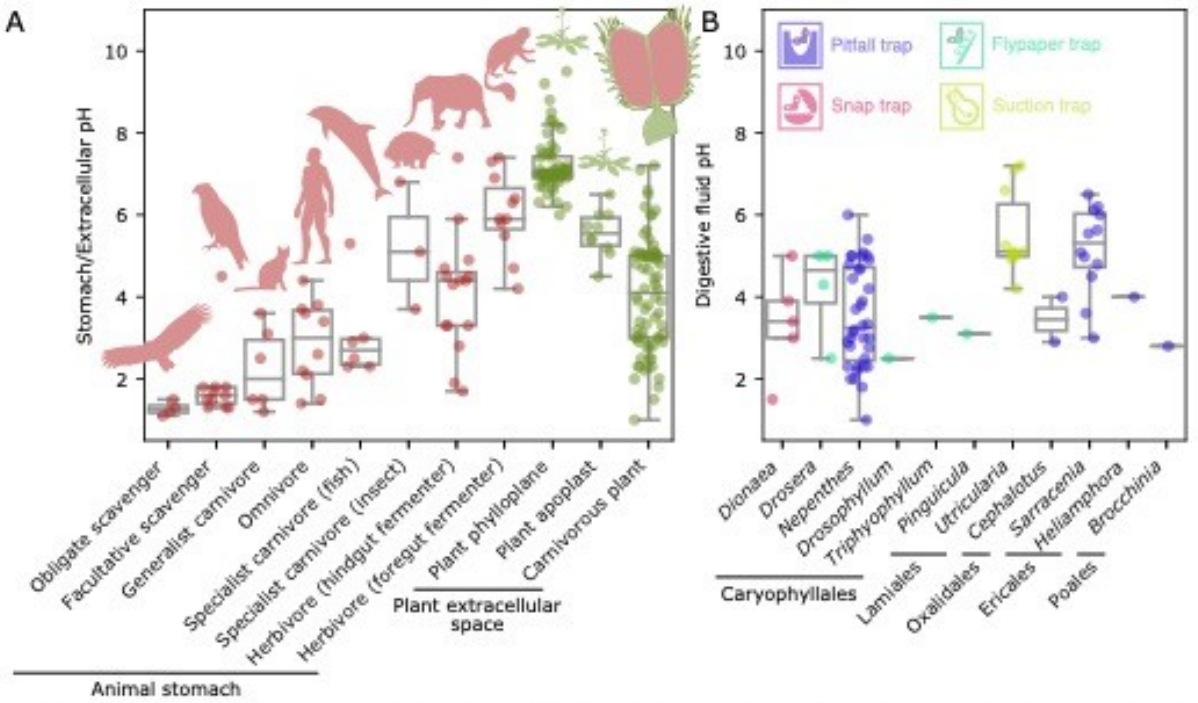

Figure 3. Digestive fluid acidity across the tree of life. (A) Extracellular pH in the digestive organs of plants and animals. The plant apoplast and phylloplane (i.e., leaf surface) were included for comparison with the digestive fluid of carnivorous plants. The datasets for animal stomachs and plant phylloplane were obtained from the literature (Beasley et al., 2015; Gilbert and Renner, 2021). The source data for the others are available in Table S1. When pH was measured at multiple time points or under multiple conditions, only the lowest value was included. The silhouettes of representative organisms were obtained from PhyloPic (http://phylopic.org). The silhouette of Cathartes aura is licensed under CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0/) by Sevcik et al. (B) pH of the digestive fluids of different carnivorous plant genera.

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Competing Interests

The authors declare no competing interests.

Supplemental Data

for

The digestive systems of carnivorous plants

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List of Supplemental Materials:

Supplemental Methods S1–S2

Supplemental Texts S1–S5

Supplemental Tables S1 (separate file)

Supplemental References

Additional online resources (available from https://doi.org/10.6084/m9.figshare.18271529)

Supplemental Methods

Supplemental Method S1. Scanning electron microscopy. Samples were fixed overnight in glutaraldehyde solution (6.25% [w/v] glutaraldehyde in 75 mM Sørensen phosphate buffer, pH 7.4) and washed five times with Sørensen phosphate buffer (100 mM, pH 7.4) in 5-minute intervals. Dehydration was performed via a nine-step process using an acetone gradient (30%/50%/75%/90%/5×100%[v/v] acetone for 15/20/30/45/5×30 min). Finally, critical-point-dried (BAL-TEC CPD 030 Critical Point Dryer) samples were sputter-coated in a gold-palladium alloy (BAL-TEC SCD 005 Sputter Coater). Images were taken using a JEOL JSM-7500F field emission scanning electron microscope.

Supplemental Method S2. Methylene blue staining. Samples were stained with an aqueous 0.1% [w/v] methylene blue solution. *Cephalotus follicularis*, *Sarracenia purpurea*, and *Heliamphora nutans* pitchers were filled with the methylene blue solution. *Darlingtonia californica* received the same treatment, but the hood of the trap was cut open to allow access to the pitcher. *Roridula gorgonias* leaves were removed at the base and completely submerged in the solution. After 10 minutes, the pitchers were removed from the plant and thoroughly rinsed with water to remove excess staining solution. *Roridula* leaves were removed from the bath and rinsed with water as well. Microscopy sections were taken from the digestive zone of the pitcher (lower 15%) and along the midrib of the *Roridula* leaf.

Supplemental Texts

Supplemental Text S1. Potential roles of the vacuole in gland physiology. Although its involvement in gland cell physiology is unknown, in non-carnivorous plants, the vacuole builds vesicles that fuse with the plasma membrane (or the vacuole directly fuses with this structure) and release their contents into the apoplast (Echeverría, 2000; Hatsugai et al., 2009; Yun and Kwon, 2017; Shimada et al., 2018). Thus, proteins are safely stored inside the vacuole and are rapidly released in large quantities when needed. In *Dionaea muscipula* gland cells, *AHA10* (encoding a vacuolar proton pump) and *CLC* genes (encoding vacuolar anion transporters) were upregulated in response to coronatine, pointing to a role for the vacuole in digestive physiology (Scherzer et al., 2017).

The vacuole plays a major role in fluid secretion in *Pinguicula* (Heslop-Harrison and Heslop-Harrison, 1981). During the last stage of glandular cell maturation, a controlled type of autolysis takes place, which has been described as interrupted holocrine secretion. During this process, the vacuolar membrane dissolves, and its entire contents are released (Heslop-Harrison and Heslop-Harrison, 1981).

Supplemental Text S2. Possible link between large glands and extrafloral nectaries. *Cephalotus* utilizes extrafloral nectaries to attract its prey (Vogel, 1998; Ellison and Adamec, 2018). The structure of the nectaries resembles that of large glands, but they contain only 25 to 30 cells (Vogel, 1998). The extrafloral nectaries are located on the inner side of the lid, on the teeth of the peristome, and on the outer side of the pitcher wall, whereas most large glands are located in the glandular region inside the trap (Juniper et al., 1989; Vogel, 1998; Ellison and Adamec, 2018).

Supplemental Text S3. Glands of Caryophyllales carnivores. Arguably the most famous and well-studied carnivorous plant is *Dionaea muscipula*, the Venus flytrap. The trapping organ comprises two leaf lobes with teeth-like extensions at their rims, invoking the image of a foothold trap. The inner surfaces of the lobes are covered with digestive glands. The upper, exposed part of each gland is ~100 µm in diameter, comprising secretory cells of a slightly convex shape (Scala et al., 1968). Basal cells anchor the gland to the epidermis and are connected to the secretory cells by a layer of endodermoid cells (Juniper et al., 1989). Upon prey capture, the trap closes and starts releasing digestive fluid through the secretory cells. Additional sessile glands on the trap rim are tasked with the secretion of carbohydrate-rich mucilage (Joel et al., 1985). These glands lie in small indentures, which protect them from physical damage when the trap closes (Juniper et al., 1989).

Aldrovanda vesiculosa is the aquatic sister species to *Dionaea muscipula*. Their traps look very similar, with two lobes connected via a midrib and teeth-like structures sitting at the rim. *Aldrovanda* traps are much smaller (only a few millimeters in size). Digestive glands are densely distributed in the center of the trap. The rim region bears quadrifid glands (sometimes called absorptive hairs (Atsuzawa et al., 2020)): These X-shaped structures closely resemble those of *Utricularia*, another aquatic

carnivore of the distantly related Lentibulariaceae whose quadrifid glands in X-shaped structures likely aid in water removal and digestion (Lloyd, 1942; Fineran and Lee, 1975; Fineran, 1985; Płachno et al., 2007). The idea that *Aldrovanda* glands possess the convergent function of water removal is attractive due to their morphological similarity, but this needs to be confirmed. The glands in *Aldrovanda* likely play a role in digestion, as they show phosphatase activity (Płachno et al., 2006). The teeth-like structures of the snap traps might be modified stalked glands that have lost their secretory function (Heubl et al., 2006).

The genus *Drosera* is a diverse taxon with ca. 260 recognized species (Fleischmann et al., 2018a; Cross et al., 2020). *Drosera* traps come in various shapes and sizes, but they all share common elements. All known *Drosera* species have sticky flypaper traps. The stickiness is provided by stalked glands covering the leaf surface. These glands vary in size, with the longest tentacles sitting at the outer regions and gradually smaller ones toward the center. The glandular head is roughly oval and is usually covered by mucilage droplets, which earned *Drosera* its common name "sundew". When viewed under the microscope, distinct structures inside the glandular head become visible: The outermost cells are epidermal cells with a very thin cuticle (Fenner, 1904; Lloyd, 1942), allowing for easier mucilage excretion. The next layer comprises inner gland cells, which are parenchymal. Below this is a bell-shaped area of endodermoid cells separated by Casparian strips (Fenner, 1904): These lignified cell walls are usually associated with roots, where they function in water transport (Lersten, 1997). Casparian strips are impermeable to water, effectively forcing water through the symplastic pathway (Stöckle and Vermeer, 2020). At the core of the glandular head is the vascular system, a tracheid body, which narrows into a single canal through the stalk (Ragetli et al., 1972). Unlike the flypaper traps of other lineages, the stalked glands move slowly in ca. 90% of the known *Drosera* species (Fleischmann et al., 2018a). Once an insect is stuck on the leaf, tentacles around the insect begin to move towards it, involving more glands to increase prey retention. An exceptionally fast movement occurs in *D. glanduligera*, *D. burmannii*, and some of the Australian pygmy *Drosera* (*D.* section *Bryastrum*), where the outermost tentacles catapult prey directly onto the leaf center (Poppinga et al., 2012). Directly on the leaf surface, among the forest of tentacles, sit much smaller, sessile glands. These glands lack a stalk and typically consist of four head cells, a single-celled neck, and two basal cells. These sessile glands lack vasculature at maturity (Fenner, 1904; Juniper et al., 1989). An additional type of sessile gland with just two head cells has been reported in *D. capensis* (Naidoo and Heneidak, 2013). Sessile glands may aid in digestion by releasing digestive enzymes (Matušíková et al., 2005; Naidoo and Heneidak, 2013), but their role in absorption was put into question by fluorescent tracer experiments (Adlassnig et al., 2012). Their fluid is more liquid than that of stalked glands, providing better solubility of digestive enzymes (Von Byern and Grunwald, 2010).

More distantly related to the Droseraceae, but still part of the Caryophyllales, are two other plants with flypaper traps: *Drosophyllum lusitanicum* and *Triphyophyllum peltatum*. Rather atypical for most carnivorous plants, the monotypic *D. lusitanicum* grows in relatively dry environments (Lloyd,

1942; Adlassnig et al., 2006). Its leaves are elongated and very thin. They are curled up when young and straighten out gradually over time, similar to ferns. On the leaf surface, as well as on the flower pedicle and calyx, are relatively large stalked glands and sessile glands that are visible to the naked eye (Ojeda et al., 2021). The stalked glands are mushroom-shaped and exude mucilage. They do not move in response to prey capture. The sessile glands function in digestion and remain dry until stimulated (Green et al., 1979; Vassilyev, 2005). The stalked and sessile glands of *Drosophyllum* contain xylem and phloem.

Triphyophyllum peltatum is a special case among carnivorous plants in that it is only carnivorous during a short period of its lifetime. While the closely related Ancistrocladaceae lost their carnivory entirely, *T. peltatum* can produce trap leaves when the plant is still young, usually during the rainy season (Green et al., 1979; Fleischmann et al., 2018b). The traps are morphologically similar to those of *Drosophyllum* and grow and uncurl in the same manner. These traps also have both stalked mucilage glands and sessile digestive glands with xylem and phloem (Green et al., 1979; Marburger, 1979). Unlike both *Drosera* and *Drosophyllum*, tracheids branch out extensively inside the stalked glands of *T. peltatum* (Juniper et al., 1989). *Triphyophyllum* glands are the largest among the known flypaper carnivores (and among angiosperms in general, as they are only outsized by the resinous glands of some Velloziaceae), with heads reaching up to 1 mm in diameter (Green et al., 1979).

The last carnivorous group of the Caryophyllales (regarding trap type) is the Nepenthaceae. *Nepenthes* species typically produce large, elaborate pitcher traps. A short, flexuous tendril sprouts off its flattened, photosynthetic petiole, which serves as the connection between the pitcher proper and the rest of the plant. The pitchers themselves are often funnel-shaped, as they are more bulbous at the bottom (depending on the species) and open up toward the top. The entrance to the pitcher is marked by a thick collar—the peristome—with short tooth-like protrusions and a leaf hanging above as a lid. Nectary glands in small cavities between the teeth attract prey (Joel, 1988; Moran, 1996; Merbach et al., 2001). At the very bottom of the *Nepenthes* pitcher is the digestive zone. Here, large digestive glands maintain the digestive fluid by releasing enzymes and absorbing nutrients (An et al., 2002; Adlassnig et al., 2012). Cells in the topmost layer of the digestive glands have thick walls and a thin cuticle facing the inside of the pitcher. A continuous layer of epidermal cells curves underneath the gland, with vascular cells in close proximity. A piece of epidermis arches above each digestive gland (Owen, Jr., 1999; Wang et al., 2009); these lunate ridges resemble stomatal cells. More lunate cells can be found near the peristome, albeit without glands beneath them (Wang et al., 2018). Due to their morphology, lunate cells are thought to originate from guard cells and to interfere with insect movement by providing a difficult surface in conjunction with particular wax crystals (Wang et al., 2009; Wang et al., 2016; Wang et al., 2018).

The flowers of *Plumbago* (Plumbaginaceae), a non-carnivorous taxon from the sister group to the carnivorous lineage in Caryophyllales, bear sticky glandular trichomes, superficially resembling those of *Drosophyllum*, but with little or no vascularization (Stoltzfus et al., 2002). Although these

trichomes can trap insects and secrete digestive enzymes, the plant does not appear to make use of the digested nutrients (Stoltzfus et al., 2002). Unlike in *Triantha*, whose glands on flower stalks are utilized for carnivory (Lin et al., 2021), the glandular trichomes on floral organs of other plants are thought to function in defense against herbivory (Kerner von Marilaun, 1878). The flower is an expensive and critical organ for plants, making it advantageous to invest in its protection ("optimal defense theory") (McKey, 1974; McKey, 1979; Rhoades, 1979).

Supplemental Text S4. **Glands of Lamiales carnivores.** The sticky leaves of ca. 110 species of *Pinguicula* are usually arranged in a rosette, with each leaf heavily covered in stalked and sessile glands. Below each stalked gland sits a basal reservoir cell, which is connected to four to eight surrounding cells via plasmodesmata (Heslop-Harrison and Heslop-Harrison, 1981). The stalks of these glandular trichomes are single-celled and shaped like a bowling pin: thicker near the base and narrowing towards the head. The head itself is composed of a single cell surrounded by 16 roughly symmetrically distributed cells (Fenner, 1904; Juniper et al., 1989). The sessile glands are arranged in a similar manner, but without the stalk and surrounded by just eight cells (Fenner, 1904; Lloyd, 1942; Heslop-Harrison and Heslop-Harrison, 1981). Underneath the secretory head of the sessile gland is a single endodermoid cell, followed by another single reservoir cell (Heslop-Harrison and Heslop-Harrison, 1981).

Genlisea is a unique genus. Instead of roots, its 30 species bear subterranean leaves (Fleischmann, 2012; Fleischmann, 2018). These leaves lack chlorophyll and form a tube, which branches off into two "arms". Each arm is twisted like a corkscrew, providing an entrance for potential prey into the trap. Inward-pointing trichomes allow the prey to only move further inside. The prey eventually ends up inside a thicker part of the tube: a digestive chamber. On the inner surface of the digestive chamber are digestive glands, which are thought to continuously release digestive fluid into the chamber (Płachno et al., 2007). An ancestor to *Genlisea* might have actively transported water (Jobson et al., 2004), and the positioning of digestive glands could be a remnant of this (Płachno et al., 2007; Fleischmann, 2012). The glands are made of three types of cells: terminal cells in radial arrangement, one endodermoid cell, and one basal cell (Lloyd, 1942; Płachno et al., 2007). These cells take on both secretory and absorptive functions (Fleischmann, 2012).

Utricularia is an immediate sister genus to *Genlisea* containing both terrestrial and aquatic species. These species lack roots, instead bearing subterranean or aquatic trap leaves. These traps form a bladder whose entrance contains sensory trigger hairs and is sealed off by a trap door (Lloyd, 1942; Reifenrath et al., 2006; Westermeier et al., 2017). To arm the trap, water inside must be evacuated to reduce the water pressure inside (Lloyd, 1942). When the prey disturbs the trigger hairs at the trap door entrance, it becomes sucked inside the trap via the rapid bulging of bladder walls (Lloyd, 1942). The trap door is covered with stalked mucilage glands, which are thought to seal the trap door following prey capture (Westermeier et al., 2017; Płachno et al., 2019). Along the inside of the trap walls are bifid or quadrifid glands. Their terminal cells exhibit a highly complex cell wall labyrinth, which might

enable vigorous water transport (Fineran and Lee, 1975; Sasago and Sibaoka, 1985; Płachno et al., 2007). *Utricularia* might use these glands to rapidly remove water from the trap (Lloyd, 1942; Fineran and Lee, 1980; Fineran, 1985) and for absorptive and secretory processes (Lloyd, 1942; Fineran and Lee, 1975; Fineran, 1985; Sirová et al., 2003; Juang et al., 2011).

In *Byblis*, like most species of *Pinguicula*, most of the aerial parts of the plant are densely covered in mucilage glands, up to and including the floral scapes and sepals (Juniper et al., 1989; Kocáb et al., 2020). Its stalked glands are highly similar to those of *Pinguicula* as well, with a single-celled stalk and a radial arrangement of head cells (Fenner, 1904; Juniper et al., 1989). However, rather than sitting on top of a singular basal cell, *Byblis* stalked glands are surrounded by smaller epidermal cells (Fenner, 1904; Juniper et al., 1989).

A relatively recent addition to the cadre of lamialean carnivorous plants is *Philcoxia*. This genus currently comprises seven species endemic to Brazil (Scatigna et al., 2018). *Philcoxia* plants have minute, green leaves, which are usually buried beneath a thin layer of sand and prey upon nematodes with their sticky traps (Pereira et al., 2012). While early studies could not confirm phosphatase activity in the glandular trichomes of these plants, more recent reports of phosphatase activity in *P. minensis* and the presence of nematodes stuck on the leaves of *P. rhizomatosa* categorize *Philcoxia* as a carnivorous plant (Pereira et al., 2012; Scatigna et al., 2015; Scatigna et al., 2017; Fleischmann et al., 2018b). In addition to stalked glands that secrete mucilage, sessile glands have been described (Taylor et al., 2000), although whether they are universally present in this genus is not clear.

Supplemental Text S5. **Glands of Ericales carnivores.** There is little evidence for the presence of digestive enzymes in *Roridula* glands and their resinous exudate (Lloyd, 1934). Płachno et al. studied phosphatase activity in different carnivorous lineages and did not observe fluorescence in *Roridula* stalked glands, but rather in the epidermis of the leaf surface (Płachno et al., 2006), which constitutes a physiologically highly active "digestive epithelium" for nutrient uptake from the feces of associated mutualistic arthropods (Anderson, 2005). A follow-up study found that despite the absence of phosphatase activity in the glands (and the absence of a symbiotic insect), phosphate uptake was comparable to that of *Drosophyllum lusitanicum* (Płachno et al., 2009). There may be an evolutionary connection between this phenomenon and digestion in the Sarraceniaceae. Both Roridulaceae and Sarraceniaceae appear to absorb nutrients using relatively simple glands comprising specialized epidermal cells bearing thin cuticles for easier transport, whereas other carnivorous lineages utilize more complex structures for the same task.

One hypothesis on how carnivory in plants could evolve is via "foliar feeding" (Fernández and Eichert, 2009; Fernández and Brown, 2013) on hairy, non-carnivorous leaves. Dense trichomes retain sticky droplets that catch insect parts and other organic matter. The released nutrients could then be absorbed through the epidermis. Under this scenario, carnivory in the Ericales would represent a step between foliar feeding and advanced carnivory. The extrafloral glands of Ericales secrete resin, not

aqueous mucilage, but enzymes cannot dissolve prey in lipophilic resin; this may explain the origin of digestive mutualism with capsid bug partners in *Roridula* (Anderson, 2005; Fleischmann et al., 2018b).

Supplemental Tables

Supplemental Table S1. The pH levels of digestive fluids of different species. (separate file)

Category	Species	Trap type	Tissue	Treatment/ Conditions	pH	Reference	Reference URL
plant apoplast	*Arabidopsis thaliana*	NA	root meristem	no	5.4	Barbez et al. (2017)	https://doi.org/10.1073/pnas.1613499114
plant apoplast	*Arabidopsis thaliana*	NA	root trichoblast	no	4.5–6.0	Bibikova et al. (1998)	https://doi.org/10.1242/dev.125.15.2925
plant apoplast	*Arabidopsis thaliana*	NA	root to shoot transition zone	no	5.4	Kašík et al. (2013)	https://doi.org/10.1016/j.msec.2013.07.045
plant apoplast	*Arabidopsis thaliana*	NA	top zone of hypocotyls	no	5.07	Kašík et al. (2013)	https://doi.org/10.1016/j.msec.2013.07.045
plant apoplast	*Arabidopsis thaliana*	NA	root	no	6.0–6.4	Martinière et al. (2018)	https://doi.org/10.1073/pnas.1721769115
plant apoplast	*Brassica napus*	NA	leaf	no	5.73 – 5.83	Husted et al. (1995)	https://doi.org/10.1104/pp.109.4.1453
carnivorous plant	*Brocchinia reducta*	pitcher	trap	Field conditions, Insects found in trap	2.8-3.0	Givnish et al. (1984)	https://doi.org/10.1086/284289
carnivorous plant	*Cephalotus follicularis*	pitcher	trap	no	2.9	Takahashi et al. (2009)	Comparative studies on the acid proteinase activities in the digestive fluids of Nepenthes, Cephalotus, Dionaea, and Drosera
carnivorous plant	*Cephalotus follicularis*	pitcher	trap	no feeding	4	Adlassnig et al. (2011)	https://doi.org/10.1093/aob/mcq238
plant apoplast	*Commelina communis*	NA	stem base xylem sap	no	5.2	Jia et al. (2007)	https://doi.org/10.110

plant apoplast	*Commelina communis*	NA	leaf blade xylem sap	no	6.2	Jia et al. (2007)	4/pp.106.089110 https://doi.org/10.1104/pp.106.089110 Comparative studies on the acid proteinase activities in the digestive fluids of Nepenthes, Cephalotus, Dionaea, and Drosera
carnivorous plant	*Dionaea muscipula*	snap	trap	feeding	3.9	Takahashi et al. (2009)	https://doi.org/10.1016/S0044-328X(82)80125-6
carnivorous plant	*Dionaea muscipula*	snap	trap	mechanical stimulation	3	Rea (1982)	https://ora.ox.ac.uk/objects/uuid:a45794bc-2d23-4226-aa1f-87b1520c2a00
carnivorous plant	*Dionaea muscipula*	snap	trap		5	Robins (1978)	https://doi.org/10.1002/j.1537-2197.1977.tb11931.x
carnivorous plant	*Dionaea muscipula*	snap	trap		1.5	Lichtner and Williams (1977)	https://doi.org/10.1073/pnas.1112535108
carnivorous plant	*Dionaea muscipula*	snap	trap	coronatine treated	3.4-4.4	Escalante-Pérez et al. (2011)	https://doi.org/10.1016/j.febslet.2005.09.043
carnivorous plant	*Drosera adelae*	flypaper	trap	no	4.3	Okabe et al. (2005)	Comparative studies on the acid proteinase activities in the digestive fluids of Nepenthes, Cephalotus,
carnivorous plant	*Drosera capensis*	flypaper	trap	no	2.5	Takahashi et al. (2009)	

carnivorous plant	*Drosera capensis*	flypaper	trap		5	Rost and Schauer (1977)	Dionaea, and Drosera Physical and chemical properties of the mucin secreted by Drosera capensis https://doi.org/10.1039/C1SM05815K
carnivorous plant	*Drosera spathulata*	flypaper	trap	no	5	Erni et al. (2011)	Enzyme secretion and digest uptake in carnivorous plants https://doi.org/10.1093/aob/mcq238
carnivorous plant	*Drosophyllum lusitanicum*	flypaper	trap		2.5–3.0	Heslop-Harrison (1976)	https://doi.org/10.1111/j.1399-3054.1995.tb00846.x
plant apoplast	*Helianthus annuus*	NA	leaf	no	5.7	Hoffmann and Kosegarten (1995)	https://doi.org/10.1104/pp.106.089110
plant apoplast	*Helianthus annuus*	NA	stem base xylem sap	no	5.4	Jia et al. (2007)	https://doi.org/10.1104/pp.106.089110
plant apoplast	*Helianthus annuus*	NA	leaf blade xylem sap	no	6.8	Jia et al. (2007)	ISBN 978-0-12-392170-3
carnivorous plant	*Nepenthes*	pitcher	trap		2.5–3.3	Juniper et al. (1989)	https://doi.org/10.1038/176122 0b0
carnivorous plant	*Nepenthes*	pitcher	trap	recently opened	2.3–4.6	Morrisey (1955)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes* × *"Bill Bailey" (N. singalana* × *ventricosa)*	pitcher	trap	no	2.3	Gilbert et al. (2020a)	https://doi.org/10.1007/s00425-018-2917-7
carnivorous plant	*Nepenthes* × *Mixta*	pitcher	trap	no	7	Saganová et al. (2018)	https://doi.org/10.1007/s00425-
carnivorous plant	*Nepenthes* × *Mixta*	pitcher	trap	NH4+ feeded	1	Saganová et al. (2018)	

							018-2917-7 Comparative studies on the acid proteinase activities in the digestive fluids of Nepenthes, Cephalotus, Dionaea, and Drosera
carnivorous plant	*Nepenthes alata*	pitcher	trap	no	2.9	Takahashi et al. (2009)	https://doi.org/10.1016/j.syapm.2015.05.006
carnivorous plant	*Nepenthes albomarginata*	pitcher	trap		5–7	Takeuchi et al. (2015)	https://doi.org/10.1016/j.syapm.2015.05.006
carnivorous plant	*Nepenthes ampullaria*	pitcher	trap		5–7	Takeuchi et al. (2015)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes boschiana*	pitcher	trap	no	3.9	Gilbert et al. (2020a)	https://doi.org/10.1093/aob/mcq238
carnivorous plant	*Nepenthes coccinea*	pitcher	trap	no feeding	5	Adlassnig et al. (2011)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes copelandii*	pitcher	trap	no	3.8	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes dubia*	pitcher	trap	no	1.8	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes eymae*	pitcher	trap	no	3.7	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes fusca*	pitcher	trap	no	4.9	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes hamata*	pitcher	trap	no	3	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-

							020-61193-x
carnivorous plant	*Nepenthes hirsuta*	pitcher	trap		6	Takeuchi et al. (2015)	https://doi.org/10.1016/j.syapm.2015.05.006
carnivorous plant	*Nepenthes hybrida*	pitcher	trap	no and feeding	3.14 – 4.11	Higashi et al. (1993)	https://doi.org/10.1007/BF02344372
carnivorous plant	*Nepenthes inermis*	pitcher	trap	no	2.2	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes jacquelineae*	pitcher	trap	no	2	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes khasiana*	pitcher	trap	no	3.3	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes maxima*	pitcher	trap	no	3	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes ramispina*	pitcher	trap	no	2.3	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes sanguinea*	pitcher	trap	no	2	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes singalana*	pitcher	trap	no	2.3	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes tentaculata*	pitcher	trap	no	4.2	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes truncata*	pitcher	trap	no	3	Gilbert et al. (2020a)	https://doi.org/10.1038/s41598-020-61193-x
carnivorous plant	*Nepenthes ventrata*	pitcher	trap	no feeding	5	Adlassnig et al. (2011)	https://doi.org/10.1093/aob/mcq238

plant apoplast	*Nicotiana tabacum*	NA	leaf epidermis	no	6.5	Martinière et al. (2018)	https://doi.org/10.1073/pnas.1721769115
carnivorous plant	*Pinguicula glandiflora*	flypaper	trap	no	5.0–5.4	Heslop-Harrison and Knox (1971)	https://doi.org/10.1007/BF00387439
carnivorous plant	*Pinguicula glandiflora*	flypaper	trap	feeding	3.1–3.4	Heslop-Harrison and Knox (1971)	https://doi.org/10.1007/BF00387439
carnivorous plant	*Sarracenia*	pitcher	trap		3–9	Juniper et al. (1989)	ISBN 978-0-12-392170-3
carnivorous plant	*Sarracenia purpurea*	pitcher	trap	leaf age	3.6–6.3	Fish and Hall (1978)	https://doi.org/10.2307/2424941
carnivorous plant	*Sarracenia purpurea*	pitcher	trap	no	6.5–7.5	Galli and Chang (1997)	https://doi.org/10.1104/pp.115.4.1461
carnivorous plant	*Sarracenia purpurea*	pitcher	trap	no and feeding	4.5–7.2	Plummer and Jackson (1963)	https://doi.org/10.2307/2422922
carnivorous plant	*Sarracenia purpurea*	pitcher	trap	feeding	6.2	Wakefield et al. (2005)	https://doi.org/10.1890/04-1673
carnivorous plant	*Sarracenia purpurea*	pitcher	trap	no feeding	6	Adlassnig et al. (2011)	https://doi.org/10.1093/aob/mcq238
plant apoplast	*Solanum lycopersicum*	NA	stem base xylem sap	no	6	Jia et al. (2007)	https://doi.org/10.1104/pp.106.089110
plant apoplast	*Solanum lycopersicum*	NA	leaf blade xylem sap	no	6.2	Jia et al. (2007)	https://doi.org/10.1104/pp.106.089110
carnivorous plant	*Triphyophyllum peltatum*	flypaper	trap		3.5–4.5	Green et al. (1979)	https://doi.org/10.1111/j.1095-8339.1979.tb02188.x
carnivorous plant	*Utricularia aurea*	suction	trap	no	5.25	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x
carnivorous plant	*Utricularia aurea*	suction	trap	feeding	n/a	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x
carnivorous plant	*Utricularia australis*	suction	trap	no	4.95	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x

carnivorous plant	*Utricularia australis*	suction	trap	feeding	4.93	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x
carnivorous plant	*Utricularia australis*	suction	trap	no	5.1	Adamec et al. (2010)	https://doi.org/10.2478/s11756-010-0002-1
carnivorous plant	*Utricularia australis*	suction	trap	control	7.2	Sirová et al. (2011)	https://doi.org/10.1071/FP11023
carnivorous plant	*Utricularia australis*	suction	trap	P addition	7.3	Sirová et al. (2011)	https://doi.org/10.1071/FP11023
carnivorous plant	*Utricularia australis*	suction	trap	N addition	7.3	Sirová et al. (2011)	https://doi.org/10.1071/FP11023
carnivorous plant	*Utricularia foliosa*	suction	trap	no	5.06	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x
carnivorous plant	*Utricularia foliosa*	suction	trap	feeding	5.05	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x
carnivorous plant	*Utricularia foliosa*	suction	trap	no	6.6	Adamec et al. (2010)	https://doi.org/10.2478/s11756-010-0002-1
carnivorous plant	*Utricularia foliosa*	suction	trap	age: young	7.1	Sirová et al. (2009)	https://doi.org/10.1016/j.aquabot.2008.07.007
carnivorous plant	*Utricularia foliosa*	suction	trap	age: intermediate	7.2	Sirová et al. (2009)	https://doi.org/10.1016/j.aquabot.2008.07.007
carnivorous plant	*Utricularia vulgaris*	suction	trap	no	4.97	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x
carnivorous plant	*Utricularia vulgaris*	suction	trap	feeding	4.99	Sirová et al. (2003)	https://doi.org/10.1046/j.1469-8137.2003.00834.x
carnivorous plant	*Utricularia vulgaris*	suction	trap	no	5.1	Adamec et al. (2010)	https://doi.org/10.2478/s11756-010-0002-1

carnivorous plant	*Utricularia vulgaris*	suction	trap	age: young	5.1	Sirová et al. (2009)	https://doi.org/10.1016/j.aquabot.2008.07.007
carnivorous plant	*Utricularia vulgaris*	suction	trap	age: intermediate	5.1	Sirová et al. (2009)	https://doi.org/10.1016/j.aquabot.2008.07.007
carnivorous plant	*Utricularia vulgaris*	suction	trap	age: old	4.2	Sirová et al. (2009)	https://doi.org/10.1016/j.aquabot.2008.07.007
carnivorous plant	*Nepenthes ampullaria*	pitcher	trap	no	4.696429	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes gracilis*	pitcher	trap	no	3.190476	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes hirsuta*	pitcher	trap	no	5.078125	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes rafflesiana*	pitcher	trap	no	4.5625	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes reinwardtiana*	pitcher	trap	no	4.666667	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes stenophylla*	pitcher	trap	no	2.760417	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes tentaculata*	pitcher	trap	no	5.40625	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes veitchii*	pitcher	trap	no	4.447368	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Sarracenia alata*	pitcher	trap	no	4.979167	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Sarracenia flava*	pitcher	trap	no	5.083333	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Sarracenia leucophylla*	pitcher	trap	no	5.542857	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001

carnivorous plant	*Sarracenia purpurea*	pitcher	trap	no	4.8	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Sarracenia rosea*	pitcher	trap	no	5.630435	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Sarracenia rubra*	pitcher	trap	no	6.113636	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes ampullaria*	pitcher	trap	common garden transplant (potted plant), allowed to capture prey naturally	4.958333	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes bicalcarata*	pitcher	trap	common garden transplant (potted plant), allowed to capture prey naturally	4.791667	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes gracilis*	pitcher	trap	common garden transplant (potted plant), allowed to capture prey naturally	3.53125	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes rafflesiana*	pitcher	trap	common garden transplant (potted plant), allowed to capture prey naturally	2.8	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Sarracenia purpurea*	pitcher	trap	no	4.823529	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Sarracenia purpurea*	pitcher	trap	common garden transplant (potted plant), allowed to capture prey naturally	5.03125	Bittleston et al. (2018)	https://doi.org/10.7554/eLife.36741.001
carnivorous plant	*Nepenthes mindanaoensis*	pitcher	trap	no	3.339286	Gilbert et al. (2020b)	https://doi.org/10.1007/s00248-

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carnivorous plant	*Nepenthes albomarginata*	pitcher	trap	no	3.5	Gaume et al. (2016)	https://doi.org/10.1002/ece3.1920
carnivorous plant	*Nepenthes ampullaria*	pitcher	trap	no	4.7	Gaume et al. (2016)	https://doi.org/10.1002/ece3.1920
carnivorous plant	*Nepenthes bicalcarata*	pitcher	trap	no	4.9	Gaume et al. (2016)	https://doi.org/10.1002/ece3.1920
carnivorous plant	*Nepenthes rafflesiana var. gigantea*	pitcher	trap	no	3.3	Gaume et al. (2016)	https://doi.org/10.1002/ece3.1920
carnivorous plant	*Nepenthes gracilis*	pitcher	trap	no	2.2	Gaume et al. (2016)	https://doi.org/10.1002/ece3.1920
carnivorous plant	*Nepenthes hemsleyana*	pitcher	trap	no	2.8	Gaume et al. (2016)	https://doi.org/10.1002/ece3.1920
carnivorous plant	*Nepenthes rafflesiana var. typica*	pitcher	trap	no	2.1	Gaume et al. (2016)	https://doi.org/10.1002/ece3.1920

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