

Evolution of carnivory in angiosperms

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3.1 Introduction

Carnivorous plants are an ecologically defined group of organisms (Darwin 1875), not a single lineage marked by common descent. To date, > 800 species of angiosperms—in five orders, 12 families, and 19 genera—are broadly recognized as carnivorous plants (Table 3.1, Figure 3.1; Appendix).

3.1.1 Evolution of carnivory

Charles Darwin (1875) provided the first conclusive evidence that some plants could trap and digest animals. Ever since, evolutionary biologists have asked how and when carnivory evolved among plants. Darwin (1875) himself was convinced that there had been a number of different, independent origins of plant carnivory, but establishing the relationships between various carnivorous genera was, until the advent of molecular systematics, often hampered by convergent, parallel, or divergent evolution of morphological traits associated with carnivory (Ellison and Gotelli 2009).

By definition, carnivorous plants must have some unequivocal adaptation(s) to attract, trap, or digest prey, be capable of absorbing nutrients from killed animals next to their surfaces, and obtain some benefit thereby in terms of increased growth, survival, or reproduction (Givnish et al. 1984, Givnish 1989, Juniper et al. 1989, Adamec 1997a; Chapter 1). The “carnivorous syndrome” (Chapter 1) manifests itself through numerous morphological and physiological adaptations, from gross morphology and

leaf form (Chapters 5–10), through glandular structure and function, to gene expression and evolution (Chapters 11–16). Many species have specific ecological associations (digestive mutualism, myrmecophily, coprophagy) with other organisms that contribute to prey capture and digestion or plant protection against herbivores (Givnish 1989, Ellis and Midgley 1996, Anderson and Midgley 2003, Alcaraz et al. 2016; Chapters 23–26). Several of the morphological adaptations of carnivorous plants (such as secretory glands in Nepenthales and Lamiales), and many of the genes activated in the trap leaves (e.g., Bemm et al. 2016) appear to have evolved or been repurposed from herbivore defense mechanisms (Darwin 1875, Kerner von Marilaun 1878, Müller et al. 2004, Alcalá et al. 2010; Chapters 13, 16).

Five general types of traps have evolved in carnivorous plants: adhesive (“flypaper”) traps, consisting of sticky glandular leaves; pitcher (“pitfall”) traps, formed by tubular leaves or, in the case of tank-forming monocots, rosettes of leaves; “snap-traps”, formed by rapidly closing laminar lobes; specialized eel (or “lobster-pot”) traps, formed by narrow, tubular leaves that are internally lined with retrorse hairs; and suction (“bladder”) traps, which are highly modified, sac-like leaves. Some of these trapping methods have evolved only once—e.g., suction traps in *Utricularia*—whereas others have evolved convergently in several different clades—e.g., epiascidiate pitchers in Cephalotaceae, Nepenthaceae, and Sarraceniaceae, in which the adaxial (upper) side of the leaf forms the pitcher interior (Arber 1941, Lloyd 1942, Franck

Table 3.1 Carnivorous plant taxa. For each family, the number of total genera and the number of carnivorous genera (if different) are given in parentheses. Estimated phylogenetic (stem) age is in millions of years before present (Mya). For each genus, the total number of species and the number of carnivorous species (if different) are given in parentheses. Modified from Fleischmann (2010), species numbers based on and updated from McPherson (2011), McPherson et al. (2011), Fleischmann (2012a, 2012b, 2015a), Lowrie (2013), Givnish et al. (2014a), Gonella et al. (2016), Scatigna et al. (2017), and APG IV (2016). Age estimations based on Figure 3.1 (S. Smith and T. Givnish *unpublished data*), except for carnivorous Ericales (Ellison et al. 2012) and for *Genlisea* and *Utricularia* (Ibarra-Laclette et al. 2013).

Order	Family	Phylogenetic age (estimate)	Genus	Phylogenetic age (estimate)	Trap type	Distribution
Poales	Bromeliaceae (58; 2)	21.2	<i>Brocchinia</i> (20; 2)	1.9 (<i>B. reducta</i>)	Pitfall	Guyana Highlands
			<i>Catopsis</i> (≈20; 1)	2.6 (<i>C. berteroniana</i>)	Pitfall	Neotropics
	Eriocaulaceae (6; 1)	89.5	<i>Paepalanthus</i> (≈450; 1)	2.7 (<i>P. bromelioides</i>)	Pitfall	Brazil
Nepentales	Droseraceae (3)	84.8	<i>Drosera</i> (≈250)	53.4	Adhesive	Cosmopolitan
			<i>Dionaea</i> (1)	48.0	Snap	Eastern USA
			<i>Aldrovanda</i> (1)	48.0	Snap	Old World
	Nepenthaceae (1)	84.8	<i>Nepenthes</i> (≈130–160)	84.8	Pitfall	Southeast Asia, India, Australia, Madagascar, Seychelles
	Drosophyllaceae (1)	70.4	<i>Drosophyllum</i> (1)	70.4	Adhesive	Western Mediterranean
	Dioncophyllaceae (3; 1)	54.2	<i>Triphyophyllum</i> (1)	6.9	Adhesive	Tropical western Africa
Ericales	Sarraceniaceae (3)	48.6	<i>Sarracenia</i> (11)	22.8	Pitfall	Eastern USA + Canada
			<i>Darlingtonia</i> (1)	35.0	Pitfall	Western USA
			<i>Heliophora</i> (23)	22.8	Pitfall	Guyana Highlands
	Roridulaceae (1)	38.1	<i>Roridula</i> (2)	38.1	Adhesive	Cape of South Africa
Oxalidales	Cephalotaceae (1)	32.2	<i>Cephalotus</i> (1)	32.2	Pitfall	Southwest Western Australia
Lamiales	Byblidaceae (1)	44.5	<i>Byblis</i> (8)	44.5	Adhesive	Australia
	Lentibulariaceae (3)	43.4	<i>Pinguicula</i> (≈96)	33.5	Adhesive	Cosmopolitan, excluding Australia
			<i>Genlisea</i> (30)	31.0	Eel	Tropical Africa, Neotropics
			<i>Utricularia</i> (≈240)	31.0	Suction	Cosmopolitan
Plantaginaceae (≈90/1)	44.2	<i>Philcoxia</i> (7)	19.3	Adhesive	Brazil	

1976, Froebe and Baur 1988, Juniper et al. 1989, Fukushima et al. 2015; Figure 3.2). In Droseraceae and Lentibulariaceae, divergent trap types have evolved in closely related genera. Adhesive traps can be active (with mobile glands or leaves: *Drosera*, *Pinguicula*; Chapter 14) or passive (not capable

of movement upon prey capture: *Byblis*, *Drosophyllum*, *Philcoxia*, *Roridula*, *Triphyophyllum*; Figure 3.3; Chapter 15). Some species of *Drosera* (e.g., *D. glanduligera*) have traps that combine functional properties of adhesive traps and snap-traps (Poppinga et al. 2012; Chapter 14).

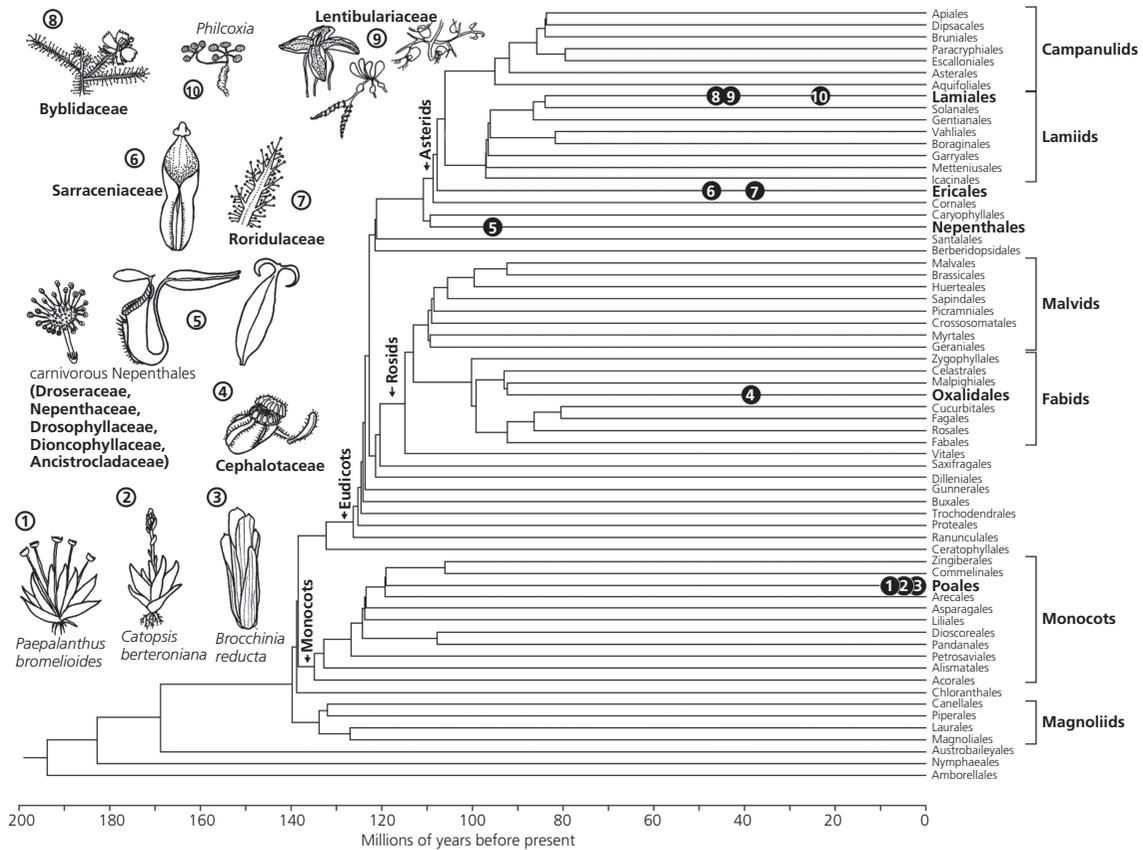


Figure 3.1 Distribution and dates of origin of the ten carnivorous clades of flowering plants. Ordinal classification follows APG (2016) except that Caryophyllales was split into Nepenthales plus Caryophyllales s.s. Ages of nodes (including stem ages of orders) were estimated from a maximum-likelihood analysis of multiple plastid loci, constrained by the topology of the tree presented by APG (2016). For noncarnivorous taxa, all families were represented by a single placeholder; for carnivorous clades, all species with appropriate sequences in GenBank were included. Twenty-three primary calibration points were obtained from Magallón et al. (2015); four secondary calibration points for the crown ages of monocots, Asparagales, Poales, and Bromeliaceae were obtained from Givnish et al. (2015) and S. Smith and T.J. Givnish (*unpublished data*). Dots indicate stem ages of carnivorous clades: Byblidaceae, Lentibulariaceae, and *Philcoxia* in Lamiales; Roridulaceae and Sarraceniaceae in Ericales; the Droseraceae-Nepenthaceae-Drosophyllaceae-Dioncophyllaceae-Ancistrocladaceae clade in Nepenthales; Cephalotaceae in Oxalidales; and *Paepalanthus bromelioides*, *Brocchinia reducta*, and *Catopsis berteroniana* in Poales. Age of *Paepalanthus bromelioides* calculated from data of Tróvó et al. (2013) run on RAxML; ultrametric tree formed using chronoPL assuming stem age of *Paepalanthus* = 48.5 Mya. The age of *Catopsis berteroniana* is based on the stem age of *Catopsis* estimated here and branch lengths within the genus given by Gonsiska (2010). Illustration by Andreas Fleischmann.

3.1.2 Origins of carnivory

Although Croizat (1960) proposed that carnivorous plants had a single origin, contemporary researchers have used molecular systematics to demonstrate that carnivory evolved independently among flowering plants at least ten times (Albert et al. 1992, Chase et al. 1993, Givnish et al. 1997, 2011, 2014a, Cameron et al. 2002, Müller et al. 2004, 2006, Heubl

et al. 2006, Ellison and Gotelli 2009, Fleischmann 2010, Schäferhoff et al. 2010, Ellison et al. 2012, Pereira et al. 2012, Givnish 2015; Schwallier et al. 2016, Stephens et al. 2015b; Figure 3.1, Table 3.1). Carnivory likely arose once in the Nepenthales (“non-core Caryophyllales” *sensu* APG IV 2016; §3.2) and Oxalidales, twice in the Ericales, and three times each in the Lamiales and the Poales (Figure 3.1). Nearly 98% of all carnivorous plant species are found in just

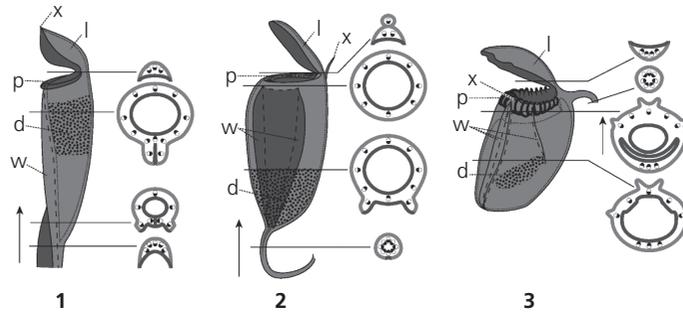


Figure 3.2 (Plate 1 on page P1) Convergent evolution of foliar pitchers in unrelated taxa. External pitcher appearance, surfaces, and schematic cross sections of **1.** *Sarracenia*; **2.** *Nepenthes*; and **3.** *Cephalotus*. All have pitcher leaves of episciadate ontogeny but of fundamentally different morphology and anatomy. Arrows: position and growth direction of shoot axis, axis in cross sections located below; *d*: digestive (glandular) zone inside pitcher; *w*: wing (ala) of pitcher outer surface; *p*: peristome; *l*: lid; *x*: true leaf apex; light gray (green in color plate): abaxial surface; dark gray (red in color plate): adaxial surface; partially black-and-white filled ellipses: main vascular bundles in cross section, black: xylem, white: phloem. Pitchers not shown in correct size relations. Pitfall traps of the carnivorous monocots (Chapter 10) are made up of the entire rosette, while in the taxa illustrated here, each pitcher is derived from a single leaf. Illustration by Jan Schlauer.

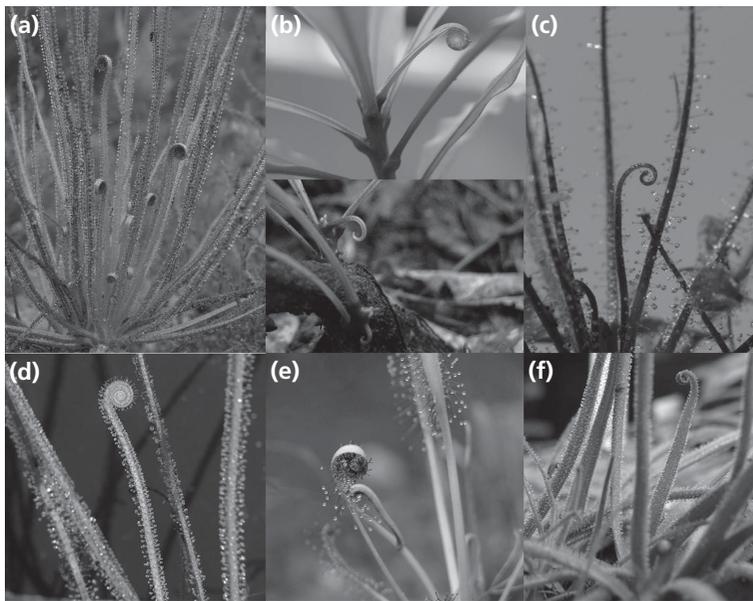


Figure 3.3 (Plate 2 on page P1) Convergent and homologous evolution of adhesive traps in carnivorous plants. The peculiar outwardly circinate venation of **(a)** *Drosophyllum* also is observed in the related *Triphyophyllum* **(b)**, both in the emerging carnivorous (top) and noncarnivorous leaves (bottom; both *Nepenthes*). **(c)** This is paralleled in the unrelated *Byblis* (illustrated by *B. aquatica*; Lamiales). *Drosera* (*Droseraceae*, *Nepenthes*); illustrated by **(d)** *Drosera tracyi* and **(e)** *D. capensis* and **(f)** *Pinguicula* (early-branching *Lentibulariaceae*, illustrated by *Pinguicula heterophylla*), have similar active flypaper traps, but with inward circination. In both, the leaves of several species are motile upon stimulation by prey. Photographs by Andreas Fleischmann.

the *Nepenthes*, *Lentibulariaceae*, and *Ericales*, 93% in the first two clades alone (Ellison and Gotelli 2009; Table 3.1). None of these five angiosperm orders are exclusively carnivorous, but nine of the 12 families in which carnivorous plants occur are exclusively carnivorous or very nearly so.

Four families—*Bromeliaceae*, *Eriocaulaceae*, *Dioncophyllaceae*, *Plantaginaceae*—comprise predominantly noncarnivorous genera and species

(Table 3.1) and apparently involve quite recent transitions to or losses of the carnivorous habit. Carnivory also has been lost a few times in the *Nepenthes*—in the *Ancistrocladaceae* and *Dioncophyllaceae* (*Habropetalum* and *Dioncophyllum*; §3.7). Carnivorous lineages are especially numerous in regions with open, nutrient-poor sites and abundant rainfall relative to evaporation, where the economics of nutrient capture and plant growth are

likely to favor carnivory as an ecological adaptation (Givnish 1989; Chapter 18).

More than one trap type has evolved in two unrelated clades—Lentibulariaceae and Nepenthes (Figure 3.1)—but in all cases, they appear to have originated from adhesive traps (Müller et al. 2004, Heubl et al. 2006, Fleischmann 2010). This implies a minimum of one or two transitions between trap types within these lineages, and raises questions about the homology among diverse trap types (e.g., sticky leaves in *Pinguicula*, below-ground eel traps in *Genlisea*, and suction traps in *Utricularia* in Lentibulariaceae; adhesive traps in *Drosera*, *Drosophyllum*, and *Triphyophyllum*, snap-traps in *Aldrovanda* and *Dionaea*, and pitcher traps in *Nepenthes* within carnivorous Nepenthes).

Carnivorous Ericales also have two different trap types (pitcher traps in Sarraceniaceae, sticky traps in Roridulaceae), but in this lineage, independent origins of the traps is more likely (§3.4.1). Resin-secreting glands are widespread among Ericales (e.g., several species of *Rhododendron*, Ericaceae), and many species are associated with capsid bugs that feed opportunistically on casually caught arthropods (e.g., Sugiura and Yamazaki 2006). Carnivory could have evolved easily from such ancestors and the digestive mutualism of *Roridula* (Chapters 10, 26) could represent simply a continuation and intensification (via obligate mutualism of plant and animal partner) of an Ericalean exaptation of sticky plants and associated, scavenging plant bugs. In contrast, the fundamentally different trap type of Sarraceniaceae, an episcidate pitcher, appears to have evolved de novo as a carnivorous trap from noncarnivorous foliar leaves (e.g., Arber 1941, Franck 1976, Fleischmann 2010, Fukushima et al. 2015; Chapter 18).

Carnivory appears to have evolved at least three times in the Lamiales: in Byblidaceae, Lentibulariaceae, and *Philcoxia* of Plantaginaceae (Schäferhoff et al. 2010, Pereira et al. 2012). Many members of Lamiales are strongly glandular with high secretory potential, which appears to constitute a certain exaptation for carnivory (Müller et al. 2004), and several other glandular members of Lamiales repeatedly have been suspected as being carnivorous or “proto-carnivorous” (*sensu* Givnish et al. 1984). These include *Ibicella* and *Proboscidea*

(Martyniaceae) (e.g., Beal 1875, Mameli 1916), and *Lathraea*, *Tozzia*, and *Bartsia* (Orobanchaceae) (e.g., Kerner von Marilaun and Wettstein 1886, Groom 1897, Heslop-Harrison 1976). However, nutrient uptake from casually or intentionally caught animals has not been detected in any of these genera (Schmidt and Weber 1983, Juniper et al. 1989, Rice 1999, Płachno et al. 2009a).

Carnivory also appears to have arisen at least three times in the monocot order Poales, twice in Bromeliaceae (two species of *Brocchinia* [Givnish et al. 1984, 1997] and *Catopsis berteroniana* [Fish 1976]), and once in the otherwise noncarnivorous Eriocaulaceae (*Paepalanthus bromelioides*; Nishi et al. 2013). In all three lineages, a pitfall trap evolved from a rosette of leaves with tightly overlapping bases that impound rainwater. This trap is very different in design from those in the three eudicot pitcher-plant families, and is quite long-lived; the plant’s single rosette persists as individual leaves are borne and die. Digestive glands are unknown in these monocot carnivores, but absorptive hairs on the bases of individual leaves take up nutrients.

3.1.3 Phylogeography and timing of origin

Dating the origins of carnivorous plants is challenging. Zanne et al. (2014) provide a recent but controversial (Edwards et al. 2015) fossil-calibrated phylogeny for all angiosperms, and Givnish et al. (2011, 2014a) for bromeliads and monocots. Maximum ages for the origins of carnivory can, at least in principle, be estimated from the stem (root) ages of carnivorous lineages on those trees, assuming that the last common ancestor of the lineage already had been carnivorous. However, carnivory may have evolved at any point between the stem and crown age of a carnivorous lineage; the latter is the first date at which extant species or genera within a lineage began diverging from each other. Further, several studies of deep-node angiosperm phylogenies (e.g., Soltis et al. 2011, Zanne et al. 2014, Tank et al. 2015) either do not resolve the closest relatives of some clades of carnivorous plants, or underrepresent carnivorous lineages and their relatives in the sampling, so that phylogenetic relationships reconstructed often are not very meaningful (e.g.,

the phylogenetic position of Lentibulariaceae as sister to Schlegeliaceae in Refulio-Rodriguez and Olmstead 2014 and APG IV 2016). Thus, the ages of certain groups, especially in Lamiales, and their precise closest relatives remain conjectural.

With these provisos, the maximum (stem) age of carnivorous lineages appears to range from 1.9 Mya for *Brocchinia reducta* (Bromeliaceae) to 95.1 Mya for the carnivorous clade of Nepenthes (Table 3.1, Figure 3.1). Within the Nepenthes, the species-rich genus *Drosera* is nearly cosmopolitan, and *Nepenthes* is widespread in the Austral-Asian tropics. Among the remaining, monotypic genera, *Aldrovanda* is widespread but sparsely distributed in the Old World, whereas *Dionaea*, *Drosophyllum*, *Triphyophyllum* have more restricted ranges (Table 3.1). Within the Ericales, *Darlingtonia* and *Sarracenia* (Sarraceniaceae) are endemic to western and eastern North America, respectively, whereas *Heliamphora* is restricted to the Guyana Shield of northern South America. *Roridula* (Roridulaceae) today is found only in the Cape region of South Africa but also is known from ≈ 35 – 47 Mya fossils from the margin of the Baltic Sea in northwestern Europe (Sadowski et al. 2015), supporting age estimations of ≈ 38 Mya for Roridulaceae (Ellison et al. 2012).

Both Nepenthes (stem age of the order ≈ 109 Mya, and of the carnivorous lineage, ≈ 95 Mya) and the Sarraceniaceae + Roridulaceae clade (stem age ≈ 65 Mya; ≈ 51 Mya according to Ellison et al. 2012) are phylogenetically old enough to have rafted via continental drift to several of the southern continents and subcontinental fragments as Gondwana broke up. The apparently younger (≈ 43 Mya) Lentibulariaceae also have a nearly cosmopolitan distribution, that principally reflects the range of widespread species that have evolved most recently (hibernacula-forming temperate *Pinguicula*, the aquatic *Utricularia* subg. *Utricularia*) and that have in many cases been involved in postglacial range extensions. In contrast, the similarly aged *Cephalotus* (32.2 Mya) and Byblidaceae (44.5 Mya) have two of the narrowest distributions. *Cephalotus* is restricted to a small portion of southwest Australia, whereas *Byblis* grows in southwest Australia, northern Australia, and southern New Guinea (Chapter 10). The four occurrences of carnivory in genera or families that are not themselves wholly

carnivorous involve the most recent origins of carnivory: *Philcoxia* (19.3 Mya in our reconstructions; 3.4–8.4 Mya in Zanne et al. 2014); *Brocchinia* (1.9 Mya) and *Catopsis* (2.6 Mya); and *Paepalanthus* (2.7 Mya) (Table 3.1, Figure 3.1).

Phylogenetic ages of carnivorous clades do not correlate with diversification. The similarly species-rich genera *Drosera* and *Utricularia* are among the oldest and youngest clades, respectively, of carnivorous plants. Extant species diversity is always a result of speciation and extinction events, and especially in the phylogenetically old carnivorous plant lineages, we find many species-poor or even monotypic genera or families, indicating extinction events (Table 3.1; Figure 3.4). Most of the monotypic genera—*Dionaea*, *Aldrovanda*, *Drosophyllum*, *Cephalotus*—are considered paleoendemics; their extant species likely are the sole survivors of formerly more diverse and species-rich lineages. The evolution of carnivory is not associated with subsequent diversification in any lineage, and does not appear to be a “key innovation” that boosted speciation. Not even the evolution of a novel trap type can be linked to rapid speciation in most cases. For example, one might expect that the evolution of pitcher traps in *Nepenthes* from sticky ancestors could have driven speciation. This is not the case. All early-branching lineages of *Nepenthes* are species-poor, whereas the majority of extant species diversity occurs in derived lineages with limited molecular divergence or short branch lengths (Meimberg et al. 2001, Merckx et al. 2015, Schwallier et al. 2016). This pattern implies geographic radiation (*sensu* Simões et al. 2016), including relatively recent speciation events and reticulate evolution following colonization of and adaptation to highland habitats on Malesian islands.

Early-branching lineages of *Drosera* also are species-poor and geographically isolated, whereas most extant diversity in this genus is found in derived clades with very short branch lengths (Rivadavia et al. 2003, 2012, Fleischmann et al. *unpublished data*), reflecting recent and likely sympatric speciation and reticulation. A similar pattern occurs in *Utricularia*—most species diversity is found in the derived lineages (Jobson et al. 2003, Müller and Borsch 2005)—but in this case transition

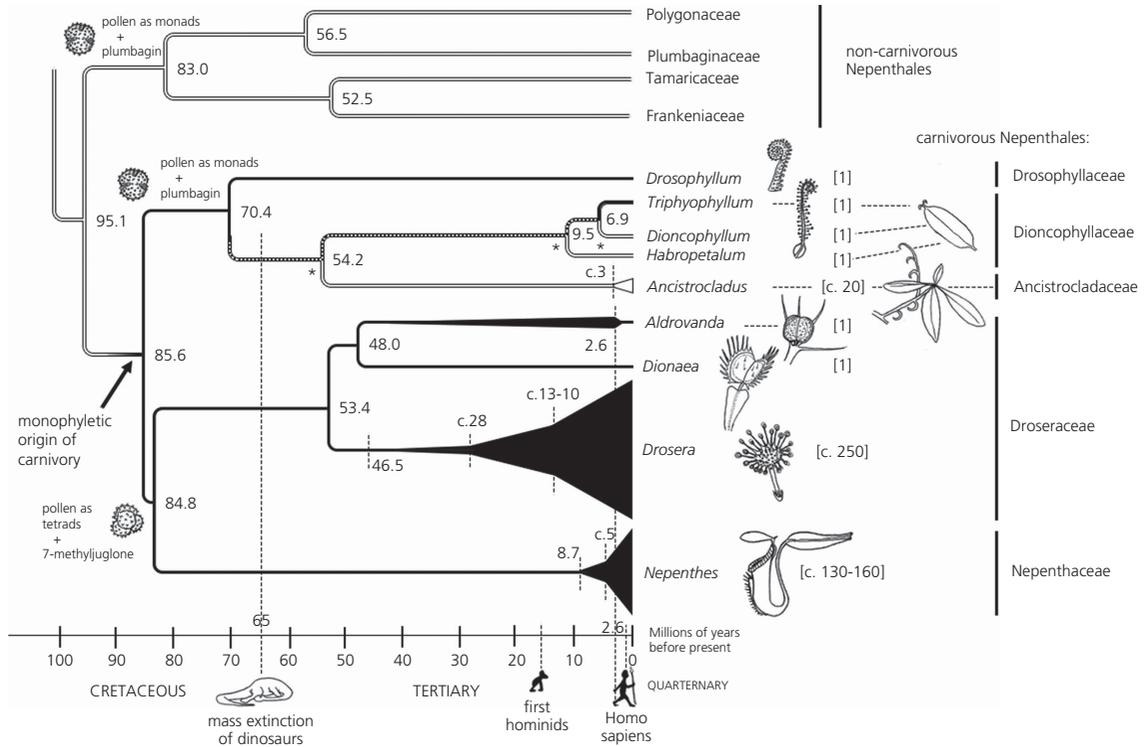


Figure 3.4 Dated phylogenetic tree of Nepenthes; topology based on APG (2016) and underlying data, divergence times based on relaxed clock estimations (S. Smith and T. Givnish *unpublished data*). Carnivorous lineages shown in black, noncarnivorous lineages in white; dotted lines = both character states possible. Divergence times given for each branch. Asterisks mark nodes with loss of carnivory. Diversification of *Ancistrocladus*, *Drosera*, and *Nepenthes* are based on divergence times of species-rich clades. Possible extinction events were not considered except for *Aldrovanda*; data on fossil *Aldrovanda* taxa from Degreef (1997). Numbers of extant species for each genus given in square brackets. Illustration by Andreas Fleischmann.

from apparently passive traps of the first-branching *U. sect. Polypompholyx* (three species) to active suction traps in all subsequently branching lineages (the other ≈ 240 species; Chapter 8) could have been a key innovation promoting diversification (Westmeier et al. 2017).

3.2 Nepenthes

In Nepenthes, carnivory appears to have evolved only once (Albert et al. 1992, Meimberg et al. 2000, Rivadavia et al. 2003, Heubl et al. 2006; Figure 3.4). The carnivorous lineage (“carnivorous Nepenthes”: Droseraceae, Nepenthaceae, Drosophyllaceae, Dioncophyllaceae, Ancistrocladaceae) is sister to an entirely noncarnivorous clade.

Nepenthes were treated by APG IV (2016) as the non-core group of Caryophyllales. The members of this monophyletic group differ from their sister core-Caryophyllales (APG IV 2016) in lacking betalains and frequently containing acetogenic naphtho- and anthraquinones (Hegnauer 1990, Schlauer 1997b). Relaxed molecular clock estimations (S. Smith and T. Givnish *unpublished*; Figures 3.1, 3.4) date the stem age of carnivorous Nepenthes to 95.1 Mya (Magallón et al. 2015: 83 Mya); doubtlessly this is the phylogenetically oldest lineage of carnivorous plants, of Late Cretaceous (Cenomanian) and putatively Gondwanan origin.

Sister to the carnivorous Nepenthes is a clade composed of Frankeniaceae, Tamaricaceae, Plumbaginaceae, and Polygonaceae (Meimberg

et al. 2000, Heubl et al. 2006; Figure 3.4), most of which also have glandular hairs or active secretory tissues, strongly suggesting their presence in the common ancestor of both nepenthalean groups. Special multicellular, vascularized glands that excrete chalk or salts occur in Frankeniaceae, Plumbaginaceae, and Tamaricaceae (Wilson 1890, Cuénoud et al. 2002, Heubl et al. 2006) and have excretory functions; members of these families often occur in extreme, saline, sulfur-rich, or calcareous soils in which N or P are likely to be limiting. Darwin (1875) felt that such sticky glandular hairs—presumably serving as defensive or excretory functions—provided a natural first step (exaptation) in the evolution of carnivory.

In carnivorous Nepenthes, leaf glands secrete digestive fluids; those species with adhesive traps also secrete aqueous mucilage for trapping. The glands themselves respond to various tactile and chemical stimuli in all of the species, but only in Droseraceae do the glands and leaf laminae respond with nastic and tropic movements upon stimulation by prey. These movements can be very fast, as in the active snap-traps of *Aldrovanda* and *Dionaea*, and certain *Drosera* species (Chapters 4, 14).

The immobile traps found in the other carnivorous Nepenthes conventionally are called “passive,” reflecting only their lack of mobility, not their physiological activity (Chapters 12, 15). For example, the traps of some *Nepenthes* species that grow in areas of heavy rainfall use a wettable peristome and viscoelastic pitcher fluid to capture prey, whereas some of those in drier areas use epicuticular waxes to precipitate prey into the pitcher (Moran et al. 2013; Chapters 5, 12, 15). Several species of *Nepenthes* are specialized on capturing ants or termites (Chapters 15, 21) and some acquire nutrients from vertebrate excreta (Clarke et al. 2009, Chin et al. 2010; Chapters 5, 15, 26).

Molecular data indicate unequivocally that *Dionaea* and *Aldrovanda* are sister to each other, and jointly sister to *Drosera*; these three genera of Droseraceae (Chapter 4), in turn, are sister to *Nepenthes* (Nepenthaceae; Chapter 5). Sister to this clade are *Drosophyllum* (Drosophyllaceae; Chapter 10) and Dioncophyllaceae + Ancistrocladaceae (Schäferhoff et al. 2009, Brockington et al. 2009, Zanne et al. 2014, Magallón et al. 2015, APG IV 2016; Figure 3.4).

The position of *Nepenthes* in these recent reconstructions differs from that in earlier phylogenies (Meimberg et al. 2000, 2001, Cameron et al. 2002, Heubl et al. 2006, Renner and Specht 2011), that did not place it sister to Droseraceae, but in a grade Drosophyllaceae [Dioncophyllaceae + Ancistrocladaceae]. However, the sister relationship Droseraceae + Nepenthaceae is well-supported by morphological and phytochemical synapomorphies, including echinate pollen tetrads (Takahashi and Sohma 1982), the presence of 7-methyljuglone and its presumed precursor shinanolone, and the respective isomers plumbagin and isoshinanolone (Schlauer et al. 2005). In contrast, all members of the Drosophyllaceae-Dioncophyllaceae-Ancistrocladaceae clade share pollen monads and exclusively plumbagin or isoshinanolone. Both features also are found in Plumbaginaceae and thus can be considered plesiomorphic in Nepenthes (Figure 3.4).

Within Dioncophyllaceae, noncarnivorous *Habropetalum* and *Dioncophyllum* are consecutive sisters to the part-time carnivorous *Triphyophyllum*. Dioncophyllaceae in turn is sister to noncarnivorous Ancistrocladaceae, a monogeneric family of ≈20 species of paleotropical lianas (Figure 3.4). Heubl et al. (2006) assume that carnivory was lost in early branching Dioncophyllaceae and subsequently regained in *Triphyophyllum*. It seems more plausible, however, that continuous loss of carnivory happened in all derived members of Dioncophyllaceae + Ancistrocladaceae that live in wet tropical rainforest habitats where sticky traps with water-based glue are ineffective (Fleischmann 2010, 2011b). *Triphyophyllum* only produces carnivorous leaves during the less rainy part of the year (Green et al. 1979) and might be in an evolutionary transition away from carnivory. For the same ecological reason, pitcher traps might have evolved in *Nepenthes* from sticky trap ancestors to cope with rainforest habitats in tropical latitudes.

Heubl et al. (2006) and Renner and Specht (2011) outline different evolutionary pathways to the diversity of traps seen in carnivorous Nepenthes from closely related outgroup taxa (Plumbaginaceae, Polygonaceae, Frankeniaceae). Renner and Specht (2011) envision one of two initial paths—leaf pinnation or emargination—leading from a leaf

with sessile glands to one with stalked glands on the leaf perimeter and sessile glands studding the surface of the leaf interior. It is not clear why these routes should be necessary, given that a stalked gland simply could evolve from a sessile one through elongation of the basal cells and vascularization (Heubl et al. 2006). In the noncarnivorous, sister Plumbaginaceae, stalked, vascularized glands are present e.g., on the calyx lobes, where they are not confined to the margins.

Once a gland becomes stalked, selection should favor vascularization if secretion of substantial amounts of fluids must be maintained far from the veins of the lamina. In Lamiales, however, higher secretory activity is a result of polyploid tissues and special reservoir cells at the base of the stalked gland (§3.4.2). Heubl et al. (2006) and Gibson and Waller (2009) propose that the stalked glands evolved into trigger hairs or marginal teeth in both *Dionaea* and *Aldrovanda*. *Nepenthes* apparently lost stalked glands, and evolved some pitted glands, presumably from ancestral sessile glands or as a sunken version of stalked glands (Heubl et al. 2006, Fleischmann 2010). Renner and Specht (2011) assume an independent origin of stalked glands in Droseraceae, *Drosophyllum*, and *Triphyophyllum*, whereas Heubl et al. (2006) and Fleischmann (2010) propose a common origin from noncarnivorous ancestors and subsequent loss in several taxa (or reversal into pitted glands in Nepenthaceae).

3.2.1 Drosophyllaceae

Drosophyllum is isolated both geographically and systematically. It was described originally as a species of *Drosera*, but based on morphology (Chrtek et al. 1989) and molecular sequence data (Meimberg et al. 2000), this genus is placed in its own, monogeneric family. Almost every character beyond carnivory (i.e. woody habit, glandular trichomes on the abaxial leaf surface, reverse circinate vernation, pantoporate pollen in monads, axial placentation) contradicts inclusion of *Drosophyllum* in Droseraceae. Many different lines of evidence place it in a clade containing Dioncophyllaceae and Ancistrocladaceae. With the only carnivorous representative of the former family, *Triphyophyllum*, it shares passive adhesive traps and reversely circinate leaf vernation, which is

paralleled in the angiosperms only in the Lamialean *Byblis* (Figure 3.2). Phylogenetic age estimations date the lineage to 70.4 Mya (Magallón et al. 2015: 57.9 Mya), and the single extant species *Drosophyllum lusitanicum* most likely is a paleoendemic of a once more diverse and widespread lineage.

3.2.2 Dioncophyllaceae

This family contains two noncarnivorous genera (*Dioncophyllum* and *Habropetalum*) and carnivorous *Triphyophyllum*. All are monotypic and endemic to tropical West Africa. *Triphyophyllum* only produces carnivorous leaves during a short part of its juvenile phase (Green et al. 1979), probably to acquire extra nutrients to reach maturity and flowering. Carnivory is expressed during the rainy season but before its peak, when heavy downpours would likely wash away secretions (Green et al. 1979). However, a carnivorous stage is not essential to complete its life cycle (Bringmann et al. 2002), and for the largest part of its life the species is noncarnivorous (Fleischmann 2011a). *Habropetalum* and *Triphyophyllum* are sympatric, but the latter apparently is more closely related to the disjunct *Dioncophyllum* (Meimberg et al. 2000). Its fruits open before the seeds mature, and, uniquely among vascular plants, the seeds surpass the ovary in size.

The taxonomic affinity of Dioncophyllaceae long has been discussed, and a position near carnivorous Nepenthaceae (among other noncarnivorous families erroneously assigned) was discussed first by Airy-Shaw (1951). Molecular phylogenetic evidence (Cameron et al. 1995, Meimberg et al. 2000, 2001), convincing similarities in anatomy and pollen morphology, and the presence of acetogenic naphthylisoquinoline alkaloids provides strong support for Ancistrocladaceae being the most closely related sister group of Dioncophyllaceae (Dahlgren 1980). These characters also suggest a close relationship of Dioncophyllaceae to *Drosophyllum*.

Fossils from the Eocene of Raychikha in the Amur district have been interpreted as seeds of Dioncophyllaceae (Fedotov 1982), but these fossils, which are larger than the seeds of extant Dioncophyllaceae, also could belong to quite different families, and could represent, for example, fossil fruits near *Paliurus* (Rhamnaceae). If these fossils

were, however, assigned accurately to Dioncophyllaceae, a West African diversification of a formerly much more widespread family must have taken place relatively recently. Divergence time of Dioncophyllaceae is estimated at ≈ 54 Mya (S. Smith and T. Givnish *unpublished data*; Magallón et al. 2015: 36.2 Mya) but the extant species are much younger (9.5–6.9 Mya; Figure 3.4). The same holds true for the entirely noncarnivorous sister Ancistrocladaceae, where diversification of extant species is dated to ≈ 3 Mya (i.e., to a major split of the African and Asian lineages of *Ancistrocladus*; Figure 3.4).

3.2.3 Nepenthaceae

Nepenthaceae contains only the extant genus *Nepenthes*. Although the family is phylogenetically old, dated to the Late Cretaceous (84.8 Mya based on divergence time from Droseraceae; Table 3.1; Magallón et al. 2015 estimated it to be 76.8 Mya), diversification of the extant species of *Nepenthes* is much more recent (≈ 8.7 Mya for the earliest-branching lineages and ≈ 6 –4 Mya for the species-rich Malesian clades; Figure 3.4). This likely represents an adaptive radiation in newly formed montane habitats of the Malay Archipelago (Merckx et al. 2015, Schwallier et al. 2016; Chapter 5). Pollen originally assigned to Droseraceae (*Droseridites*) from the Kerguelen Islands tentatively has been transferred to *Nepenthes* (Krutzsch 1985). In this context *Droseridites parvus* from the Mid-Palaeocene of Assam (Sah and Dutta 1974) should be considered as possible *Nepenthes* pollen (*Nepenthidites*; Kumar 1995). Fossil pollen assigned to *Nepenthes* also has been discovered in the mid-Miocene of north Borneo (Anderson and Müller 1975), and its presence here in a center of recent diversity (Merckx et al. 2015, Schlauer 2000, Schwallier et al. 2016) is unsurprising. But the assignment of European Tertiary pollen to the same genus (Krutzsch 1985) is at least as puzzling as the dubious *Triphyophyllum* seed from Siberia (Fedotov 1982; §3.2.2).

The combination of apparently plesiomorphic and apomorphic characters within Nepenthaceae—dioecious, four petaloid perianth segments, stamina fused in a column, pollen in tetrads (a synapomorphy with Droseraceae), and axial placentation—isolates the family far from any hypothetical ally.

These isolated characters, besides chorology, could also suggest an older phylogenetic age for the family as a whole. The affinity of Nepenthaceae to Dioncophyllaceae (e.g., Dahlgren 1980) is weakly supported, and the divergence between them must have taken place at an early stage in the evolution of Nepenthesales.

In all *Nepenthes*, the leaves of mature plants are composed of a basal blade (expanded, photosynthetic part, not necessarily the lamina) that terminates in a tendril that supports a pitcher with two ventral wings and a rim at its orifice that is formed by a slippery, radially ribbed peristome, a dorsal spur, and a covering lid. Tendrils, wings, or peristome ribs may be reduced in some species but their position and frequently some rudiments usually are apparent. Despite this morphological uniformity, there are at least eight hypotheses for the ontogeny of the pitchers and their various appendages (Franck 1976, Fukushima and Hasebe 2014; Chapter 18).

As none of the close relatives of *Nepenthes* have structures even nearly resembling pitchers, the evolution of the genus remains somewhat conjectural, but all metamorphoses that have led to its general pitcher morphology must have occurred before its phylogenetic divergence. That all of its carnivorous relatives derive their traps exclusively from the leaf lamina similarly implies that the *Nepenthes* pitchers likewise are derived from the lamina. As their most recent carnivorous relatives have a sticky adaxial leaf surface, and various forms of invagination or even peltation can be observed in *Drosera* leaves, it is reasonable to assume that this adaxial surface evolved to become the interior, glandular surface of the pitcher. The boundary between adaxial and abaxial surfaces at the orifice of the *Nepenthes* pitcher corresponds to the leaf margin; it is located just below the inner border of the peristome where glands terminate vascular bundles. The peristome itself is formed by asymmetric divisions of epidermal cells (Owen and Lennon 1999). The pitcher lid is a unique structure without a close parallel in any related genus. It is formed by fusion of two lateral lobes just beneath the spur that is the original leaf apex (Schmid-Hollinger 1970), and the pair of subapical hooks of the climbing leaves in Dioncophyllaceae may be an ontogenetic homologue. Similarly,

some species in *Drosera* subg. *Ergaleium* have a pair of auricles or crescentic outgrowths of the lamina (e.g., *D. peltata*) or dichotomously divided leaves (*D. binata*).

3.2.4 Droseraceae

Considerable differences in floral morphology (stamina many vs. equal in number to petals and sepals; placentation basal or axial vs. parietal) separate *Dionaea* from *Aldrovanda* and *Drosera*, but the striking similarity and monophyletic origin of the traps of *Dionaea* and *Aldrovanda* supports the inclusion of *Dionaea* into Droseraceae. This placement is fully corroborated by pollen morphology (Takahashi and Sohma 1982) and molecular phylogenetic reconstructions (Williams et al. 1994, Meimberg et al. 2000, Rivadavia et al. 2003, Heubl et al. 2006, Renner and Specht 2011; Figure 3.4). The subdivision of Droseraceae into several families (Chrtek et al. 1989) seems unjustified beyond the exclusion of *Drosophyllum*. Phylogenetic reconstructions (Cameron et al. 2002, Heubl et al. 2006, Renner and Specht 2011) show a sister relationship of the snap-trap genera *Aldrovanda* and *Dionaea*, with *Drosera* being sister to both (Chapter 4).

Senonian fossils that were described initially as seeds under the name *Palaeoaldrovanda splendens* (Knobloch and Mai 1984) have been re-identified as insect eggs (Heřmanová and Kvaček 2010) and thus cannot contribute to fossils constraining the origin of Droseraceae. Another series of Eocene fossil seeds and possibly traps of *Aldrovanda* (Degreef 1997, Schlauer 1997a), including several different and now extinct species and genera (*Saxonipollis*) occurs through large parts of temperate Eurasia. These fossils are congruent with the widely scattered Palearctic distribution of *Aldrovanda vesiculosa* (Chapter 4).

Pollen of Droseraceae are highly diagnostic: their endoaperturate and echinate pollen tetrads are unique among extant angiosperms, albeit with superficial similarities to Annonaceae pollen. Nepenthaceae have very similar, but much smaller, echinate, and inaperturate tetrads (Takahashi and Sohma 1982, Fleischmann et al. *unpublished data*).

Droseraceae has been widespread since the Early Tertiary; its pollen is represented in Eocene strata

of central Australia (*Fischeripollis halensis*; Truswell and Marchant 1986) and in Eocene to earliest Oligocene strata of Antarctica (*Fischeripollis*; Macphail and Truswell 2004). *Drosera* pollen also has been recorded from the Lower Miocene from New Zealand (Mildenhall 1980), and Miocene pollen (as *Droserapollis* and *Droserapites*) of uncertain affinity within the Droseraceae has been found in Taiwan (Huang 1978).

It is unlikely that recent species of *Drosera* existed in Europe before the Pliocene, although several finds of mid-Miocene pollen from Europe have been assigned either to *Drosera* (*Droserapollis*) or *Nepenthes* (*Droseridites*; Krutzsch 1985). A single record from central European mid-Miocene (*Fischeripollis*) has been assigned to *Dionaea* (Krutzsch 1970). The earlier European fossils may be attributed to now extinct lines of Droseraceae or even to other families. Regardless, the fossil record of Droseraceae is the richest of any carnivorous plant lineage, and it testifies a wide distribution of the family since the Early Tertiary.

Molecular clock estimates date the stem age of Droseraceae to the Late Cretaceous (84.8 Mya; 76.8 Mya in Magallón et al. 2015); the split of the snap-trap *Dionaea* + *Aldrovanda* clade from *Drosera* at 53.4 Mya; and the divergence of the earliest-branching lineage of *Drosera* to 46.5 Mya (Table 3.1; Figure 3.4). The species-rich lineages of *Drosera* are comparatively older (~10–13 Mya) than those of sister genus *Nepenthes*, implying a much earlier diversification of the former (Figure 3.4).

3.3. Oxalidales

3.3.1 Cephalotaceae

The monotypic family Cephalotaceae is endemic to a small area in southwest Australia. The narrow-endemic Albany pitcher plant, *Cephalotus follicularis*, is its only species. No fossils have been attributed to Cephalotaceae, and morphology provides no convincing clues for a related and recent plant genus (based on apparent floral similarities, it has been thought to be close to Crassulaceae and Saxifragaceae). Its unique floral morphology (Chapter 10) and pitcher development isolate the Cephalotaceae from all other carnivorous plant families; it has frequently been considered a paleoendemic.

The first molecular phylogenetic reconstructions of carnivorous angiosperms placed *Cephalotus* close to *Oxalis* (Albert et al. 1992). Although these two genera have only a few morphological synapomorphies, subsequent DNA sequence analyses confirmed Cephalotaceae as being deeply nested within Oxalidales (Figure 3.5). More recent phylogenetic reconstructions identified the monotypic Brunelliaceae (the Neotropical *Brunellia*, ≈60 species of evergreen trees in the Andes, Mexican highlands, and the Caribbean) as the closest relative of

Cephalotus (e.g., Bradford and Barnes 2001, Soltis et al. 2011, Sun et al. 2016). This grouping had been suggested earlier by Engler (1897) on morphological grounds.

Heibl and Renner (2012) retrieved a slightly different topology, with Elaeocarpaceae (≈600 species of (sub)tropical trees and shrubs) as immediate sister to Cephalotaceae, and Brunelliaceae as common sister to both. This result could be explained by limited taxon sampling, as this part of the Oxalidales tree was not focus of their study (C. Heibl *personal*

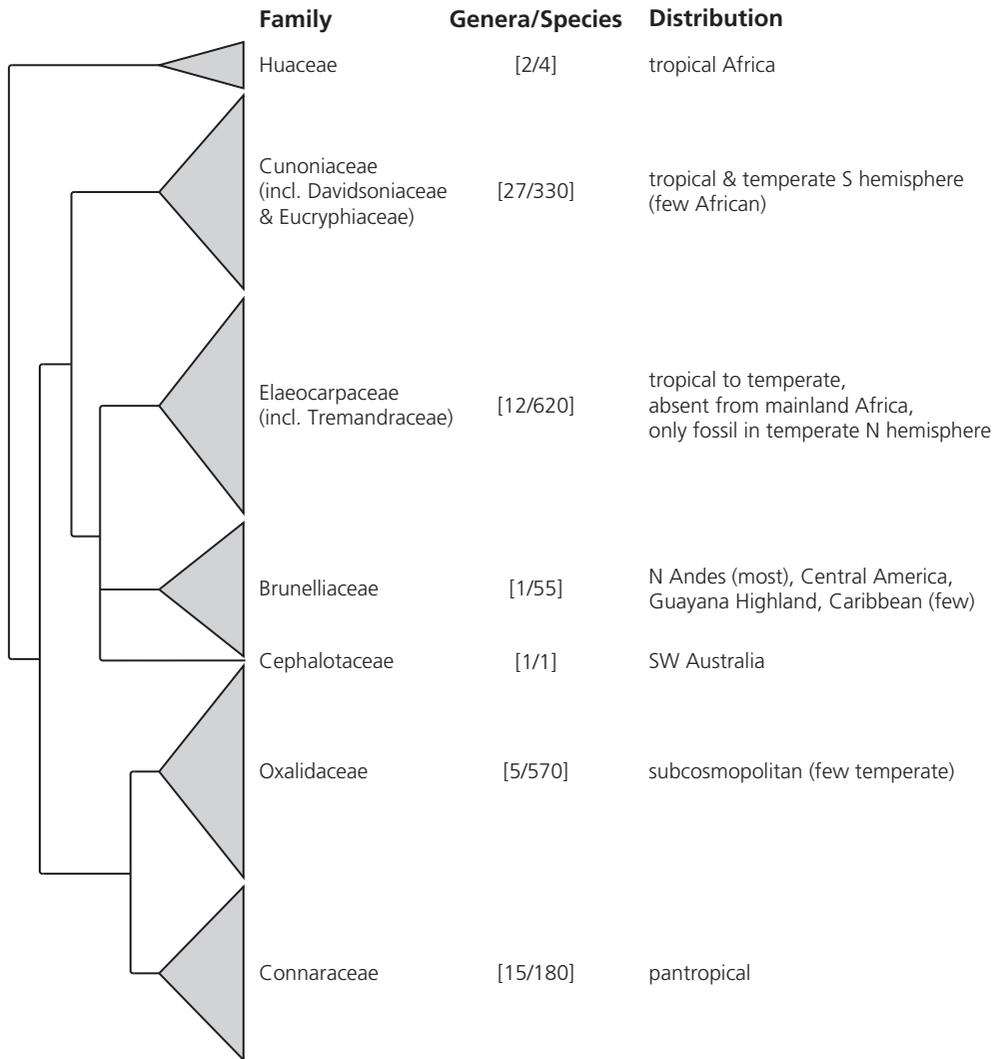


Figure 3.5 Phylogenetic tree of Oxalidales and Cephalotaceae. Illustration by Jan Schlauer.

communication). Nevertheless, Cephalotaceae can be expected to have a position in crown group Oxalidales (these are ≈ 110 Mya; Tank et al. 2015) within an early branch of the rosid eudicots (APG IV 2016) originating from an Early Tertiary or Late Cretaceous lineage.

The development of the *Cephalotus* pitcher is unique among the episcidiate carnivorous pitchers. In *Cephalotus*, the lid is an excrescence of the lamina base (or the transversal zone of a hypothetical ancestral peltate or pinnate leaf, according to Froebe and Baur 1988) and the rim of the peristome is homologous to the apical leaf margin (Figure 3.3). That is, the lid of the *Cephalotus* pitcher constitutes what is the pitcher body in *Nepenthes* and Sarraceniaceae. A *Cephalotus* pitcher hence may be regarded as an “upside-down pitcher.”

The pitchers of the three pitcher-plant families, although of similar shape and identical function, are analogous, not homologous (Figure 3.3). Pitchers of *Cephalotus* and Sarraceniaceae consist of the entire leaf blade, whereas the leaf blade of *Nepenthes* contributes the foliar part, tendril, and pitcher. In Sarraceniaceae and *Nepenthes*, the pitcher lid is the terminal part of the leaf (it still increases size from growth after the pitcher has opened), whereas in *Cephalotus*, the pitcher body is the terminal leaf part, and the lid is the basal part. The *Cephalotus* pitcher grows from the lid to the base; the lid reaches its final size and position first, while the pitcher bottom continues to inflate and increase in size.

Froebe and Baur (1988) were not convinced that the three alae of *Cephalotus* pitchers were simple epidermal outgrowths of the outer pitcher wall (assuming an episcidiate, peltate leaf). Instead, they hypothesized a “rhachis pitcher” formed from a modified pinnate leaf (twice paired, tetramerous): the basal pair of pinnae would fuse congenitally to become the lid, the subsequent pair of pinnae would equal the two lateral alae, and the median wing would be formed by a terminal pair of pinnae. Their theory largely has been neglected or even rejected (except by Conran 2004a) but it merits reconsideration in the light of phylogeny. Most members of crown group Oxalidales—the majority of species of the immediate sister Brunelliaceae, all Oxalidaceae, all Connaraceae, and most Cunoniaceae—possess compound or pinnate laminae.

3.4 Asteridae: Ericales

3.4.1 Roridulaceae

Roridula has long been considered a paleoendemic of the Cape Flora (Warren and Hawkins 2006), but findings of 35–47 Mya old Eocene amber inclusions from the Baltic (Sadowski et al. 2015) demonstrate that the genus once was more widespread. The *Roridula* lineage has established a symbiotic relationship—digestive mutualism—with carnivorous capsid bugs (Miridae: Hemiptera) of the genus *Pameridea* (Ellis and Midgley 1996, Anderson and Midgley 2003, Anderson 2005; Chapter 26) to overcome the lack of its own digestive enzymes in the resinous glands.

Resinous glands are quite common in various genera of Ericaceae (e.g., *Rhododendron*, *Erica*) many of which casually trap insects (Darwin 1875, Sugiyama and Yamazaki 2006). Hydrolytic enzymes cannot operate in hydrophobic resin in which water is unavailable, so active digestive enzymes are not found in the sticky resin droplets secreted by the glands of Ericales, including *Roridula* (Lloyd 1934, Ellis and Midgley 1996, Plachno et al. 2006, 2009a). This seems to be an evolutionary dead end for a sticky trap. However, through a digestive mutualism, the *Roridula* lineage established an alternative carnivorous pathway of gaining nutrients from captured prey (Anderson and Midgley 2003).

Capsid bugs and other arthropods frequently are found on numerous glandular, albeit noncarnivorous, plants, feeding on the adhering insects and depositing their feces directly onto the plant surface. Most plants can take up dissolved nutrients applied directly to their leaves, and any nutrients in the feces similarly could be absorbed. Unlike glandular noncarnivorous plants that trap insects only haphazardly, the carnivorous *Roridula* appears to attract prey to its sticky, scented leaves (Fleischmann 2010) and obtain a substantial fraction of its nitrogen budget ($\approx 70\%$) indirectly from its insect prey (Anderson and Midgley 2002). In contrast, a careful study of *Rhododendron macrosepalum*—an example of a plant in Ericales with sticky leaves, buds, and sepals that entrap large numbers of insects, which in turn are consumed by associated mirid bugs—showed no uptake of nitrogen via those bugs (Anderson et al. 2012).

The leaves of *Roridula* are highly absorptive of ultraviolet light (Midgley and Stock 1998), a feature *Roridula* shares convergently with sticky traps of *Drosophyllum* and *Drosera* (Joel et al. 1985, Juniper et al. 1989). The cuticle of the leaf surface of *Roridula* contains cuticular gaps, and the epidermal layer underneath consists of highly absorptive cells (Anderson 2005), very similar to those observed in the glands and digestive surfaces of other carnivorous plants, including the digestive epithelium of the related Sarraceniaceae (Joel and Juniper 1982, Juniper et al. 1989). The pores and absorptive cells of *Roridula* rapidly take up nutrients from the bug feces (Anderson 2005), and to a lesser degree directly from caught prey that contacts the leaf surface (Piłachno et al. 2009a).

3.4.2 Sarraceniaceae

No reliable fossils of Sarraceniaceae have been collected. The compression/impression fossil *Archaeamphora longicerva* from the Early Cretaceous of China, was assigned erroneously to Sarraceniaceae by Li (2005). These fossils do not represent early pitcher leaves, but are leaf galls of gymnosperm leaves (Wong et al. 2015).

Unlike the pitchers of *Nepenthes* or the bladders of *Utricularia*, the pitchers of the Sarraceniaceae do not seem to be derived from sticky ancestors (Figure 3.6). All extant members of Sarraceniaceae have pitcher leaves, thus the most reasonable assumption is their common ancestor also had ascidiate pitcher leaves (§3.2.3, §3.3.1; Chapter 9). Sarraceniaceae may have evolved from plants with leaves that formed natural (water-filled) ascidiate phytotelmata (Fleischmann 2010).

3.5 Asteridae: Lamiales

Lamiales includes the majority of extant carnivorous plant species (Ellison and Gotelli 2009, Fleischmann 2010), and represent the only angiosperm order in which entirely carnivorous lineages evolved several times in distantly related families (the entirely carnivorous Byblidaceae and Lentibulariaceae, and *Philcoxia* of Plantaginaceae; Fritsch et al. 2007, Schäferhoff et al. 2010, Pereira et al. 2012). The multiple origins of carnivory in Lamiales may be

related to the widespread occurrence of glandular hairs in this order; the exaptation of such hairs may have been important in the evolution of carnivory (§3.2). The presence of such hairs would also make the evolution of carnivory more likely from a cost/benefit viewpoint (Chapter 18).

3.5.1 Byblidaceae

Byblis had been considered to be closely related to Lentibulariaceae (Albert et al. 1992, Jobson et al. 2003), but morphological characters including floral symmetry, corolla morphology, and gland anatomy imply an independent origin of Byblidaceae. More recent phylogenies (Müller et al. 2004, 2006, Schäferhoff et al. 2010) that are based on more comprehensive sampling of Lamiales taxa clearly support *Byblis* as a distinct lineage in the basal group of this asterid order, not closely related to Lentibulariaceae of crown Lamiales.

3.5.2 Plantaginaceae

Carnivory appears only in a single genus in Plantaginaceae. *Philcoxia* is a morphologically isolated lineage in the family (e.g., peltate leaves), and its phylogenetic position within Plantaginaceae remains somewhat unclear because of limited taxon sampling of New World Plantaginaceae in most published phylogenetic reconstructions (A. Scatigna *unpublished data*), although Fritsch et al. (2007) had a comparatively good taxon sampling and revealed it to be phylogenetically close to *Gratiola* and *Bacopa*. Any theories about evolution of carnivory in this genus without knowing its closest relatives remain purely speculative, but carnivory of *Philcoxia* is linked to its nutrient-poor quartzitic sand habitats (Fritsch et al. 2007, Pereira et al. 2012; Chapter 10).

3.5.3 Lentibulariaceae

The phylogenetic affinities of Lentibulariaceae are still unknown, and the family has repeatedly fallen into an unresolved clade within the crown-group of Lamiales (Müller et al. 2006, Schäferhoff et al. 2010). A questionable (and only weakly supported) sister relationship with Schlegeliaceae

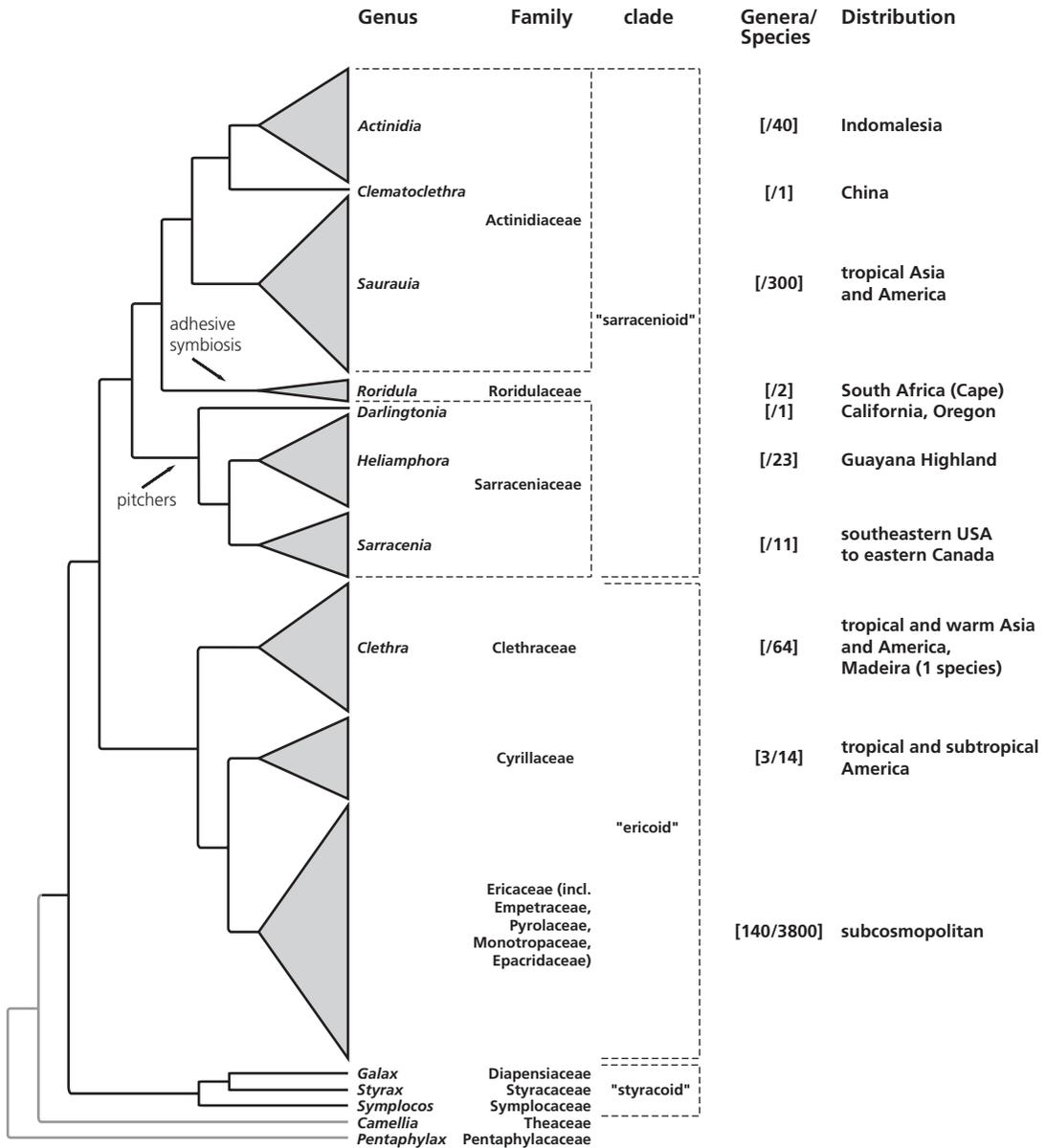


Figure 3.6 Phylogenetic tree of carnivorous Ericales. Illustration by Jan Schlauer.

was obtained by Refulio-Rodriguez and Olmstead (2014) and adopted by APG IV (2016), but this was almost surely the result of limited taxon sampling. Plausible sister groups are those with glandular hairs, which are widespread among the Lamiales. Small arthropods frequently have been reported as adhering to such glands, especially on the

flowers. The main purpose of such “defensive killing” (Juniper et al. 1989) is probably to exclude non-pollinating insects from the flowers, and to protect the generative organs from herbivores (Kerner von Marilaun 1878, Fleischmann 2010). There is some experimental evidence that the carnivorous glands of *Pinguicula* also play a defensive role against

herbivores (Alcalá et al. 2010), and the sticky foliage may have evolved first for defensive purposes, only later becoming modified into a successful flypaper trap (Darwin 1875).

One candidate relative is the Martyniaceae, which includes many strongly glandular genera. Two of these, *Ibicella* and *Proboscidea*, have been hypothesized to be carnivorous (Beal 1875, Mameli 1916) or “proto-carnivorous” (Rice 1999). Both genera have very sticky, glandular leaves of the flypaper trap type, and catch numerous arthropods (Rice 1999), but they are unable to absorb any nutrients from their putative prey (Płachno et al. 2009a). The glandular hairs of Martyniaceae also are not specialized, but have a generalized anatomy found ubiquitously in glandular Lamiales genera (Müller et al. 2004).

The dense glandular hairs in several other genera of the Lamiales often excrete water (Groom 1897). These secretory glands show a remarkable similarity in design and function to the digestive glands of carnivorous Lamiales and could represent an exaptation for carnivory in Lamiales (Müller et al. 2004). In both Lentibulariaceae and Byblidaceae, we find a gland specialization to stalked secretory glands and sessile digestive glands. This parallels the gland dimorphism observed in the carnivorous Nepenthales (Juniper et al. 1989, Heubl et al. 2006, Renner and Specht 2011), and represents a further specialization toward carnivory.

Unlike the glands of the sticky-trap genera in carnivorous Nepenthales (*Drosophyllum*, *Triphyophyllum*, and *Drosera*), which are vascularized to exchange digestive fluid and nutrients (Heslop-Harrison 1975, Juniper et al. 1989, Heubl et al. 2006), the unicellular or pluricellular stalks of the glands of carnivorous and noncarnivorous Lamiales are not lined with vascular bundles. Therefore, the attachment of the digestive glands to vascular tracheid elements must have been a key innovation for the evolution of carnivory in the Lentibulariaceae (Müller et al. 2004). This anatomical change is accomplished by a single, large, basal cell that is embedded in the epidermis of the leaf (Chapter 13). This prominent “reservoir cell” of lentibulariacean glands is physiologically connected to the subjacent tracheid cells by plasmodesmata and has storage functions related to prey digestion and nutrient

uptake (Heslop-Harrison 1975, 1976). The cuticle of the gland head cells also has become modified in the Lentibulariaceae: it bears several cuticular gaps that secrete mucus, release enzymes, and take up nutrients from dissolved prey (Juniper et al. 1989).

Although the shape of the glandular hairs found in the three genera of Lentibulariaceae is very different, even differing among members of the same genus in the case of *Utricularia* (Taylor 1989), their functional anatomy is identical (Heslop-Harrison 1975, 1976, Juniper et al. 1989, Płachno et al. 2007a). Their different structures are adaptations to different trap types and ecosystems. The quadrifid glands in aquatic traps of *Utricularia* also are convergent with those in snap-traps of *Aldrovanda* (Nepenthales).

Genlisea and *Utricularia* are immediate sister genera, and *Pinguicula* is sister to both (Jobson et al. 2003, Müller et al. 2004, 2006; Chapter 6). The rhizophylls of *Genlisea* and the bladder traps of *Utricularia* are homologous to one another, and to the adhesive foliar leaves of *Pinguicula* (Müller et al. 2004, Fleischmann 2012a; Figure 3.7; Chapter 7). The traps of *Genlisea* and *Utricularia* are episcidiate in ontogeny (Juniper et al. 1989), and so a likely scenario for their evolution is a continued inward folding and final fusion of the lateral margins of adhesive leaves of the presumed common ancestor (Fleischmann 2012a).

Such involute folding happens temporarily in the motile leaves of many extant *Pinguicula* species after they capture prey, mainly to prevent loss of prey by rain or kleptoparasites (Chapters 6, 14). In two small butterwort species, one that may grow partially submerged under water in some habitats (*Pinguicula lusitanica*) and one whose leaves are buried in *Sphagnum* (*P. villosa*), the leaf margins always are highly involute. The margins of these leaves nearly touch and create almost tubular leaves whose glandular adhesive upper laminae are protected inside the tube. Such an ecological scenario may have occurred in the (possibly aquatic) environment where the trapping organs of the common ancestor of *Genlisea* and *Utricularia* evolved (Fleischmann 2012a).

Whereas it is unlikely that all the rosette leaves were transformed through involution, heterophylly—one type for photosynthesis, one for prey capture—appears to have taken shape early

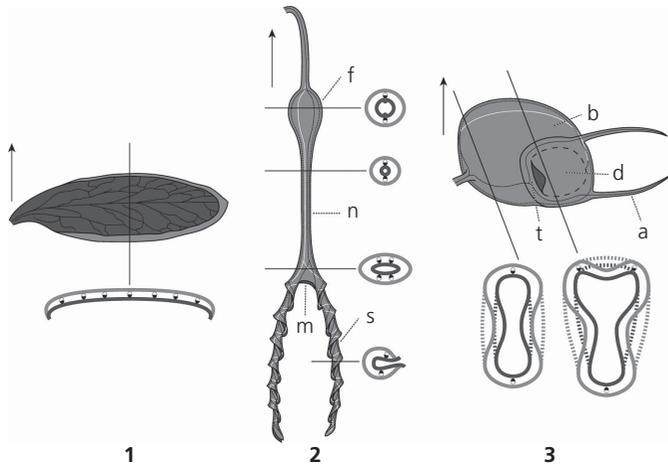


Figure 3.7 (Plate 3 on page P2) Homology of tissues among traps in Lentibulariaceae. External appearance, surfaces and schematic cross sections of **1.** *Pinguicula*; **2.** *Genlisea*; and **3.** *Utricularia* (before suction and after door opening, dashed lines in cross sections indicate shape after suction). Arrows: position and growth direction of shoot axis, axis in cross sections located below; *f*: flask; *n*: neck; *m*: mouth; *s*: spiral arm; *b*: bladder; *t*: threshold; *d*: door; *a*: antenna; light gray (green in color plate): abaxial surface; dark gray (red in color plate): adaxial surface; partially black-and-white filled ellipses: vascular bundles in cross section, black: xylem, white: phloem. Traps not shown with true size relations. Illustration by Jan Schlauer.

in the evolution of the *Genlisea–Utricularia* lineage; all species of *Genlisea* and all of the phylogenetically early branching species of *Utricularia* are heterophyllous, rosette-forming plants (Fleischmann 2012a). Heterophylly could have resulted in ancestral plants that formed some rolled, tubular leaves from a rosette of conventional foliar leaves. These tubular leaves can be envisaged as having had an apical opening and interior surfaces covered with (carnivorous) glands. Any small prey entering these tubular leaves would have become stuck to the glands and subsequently digested. Such a trap system is comparatively resistant against loss of prey to rain or kleptoparasitism and the narrow tubular traps also would have worked under water. The tubular, sticky leaves of the hypothesized ancestor of the *Genlisea–Utricularia* lineage would have had a small cross-sectional diameter, limiting the size of prey to very small organisms. To increase the numbers of trapped small prey, or to exploit different substrates, these tubular leaves might have formed below the soil or water surface.

3.6 Poales

Carnivory has been demonstrated for three tank-forming species of Bromeliaceae: two species of *Brocchinia* (Givnish et al. 1984, 1997, 2014a, Benzing 1987, Płachno et al. 2006) and one species of *Catopsis* (Fish 1976, Frank and O’Meara 1984, Givnish et al. 2014a; Chapter 10). Carnivory also has been proposed for one member

of the Eriocaulaceae, *Paepalanthus bromelioides* (Nishi et al. 2013; Chapter 10). There is no monocot family comprising entirely carnivorous members, and the four species of carnivorous monocots known today each are isolated within genera of noncarnivorous species (Table 3.1), indicating a very recent transition to carnivory.

Carnivory has evolved more recently in the Poales (within the last ≈ 3 Mya) than in any eudicot lineage. Their trap architecture is also completely different: simple pitfall traps made from the entire plant body, which does not differ morphologically from that of their noncarnivorous relatives.

3.6.1 Bromeliaceae

Within the Bromeliaceae, the documented carnivorous plants are *Brocchinia hechtoides* and *B. reducta* (Brocchiniaceae) (Givnish et al. 1984, 1997, 2014a, Benzing 1987, Płachno et al. 2006) and *Catopsis berteroniana* (Tillandsioideae) (Fish 1976, Frank and O’Meara 1984, Givnish et al. 2014a; Chapter 10). In all three species, the evolution of the tank habit and of absorptive leaf trichomes are key innovations for the evolution of carnivory. In other species in these genera, these innovations are applied to other specialized nutrient acquisition strategies, including myrmecophily, nitrogen fixation, and epiphytism with inputs from fallen vegetable detritus or frog excrement; epiphytism is the only alternative strategy in *Catopsis* (Givnish et al. 1997, 2011, 2014a,

Givnish 2017). Across the family, the tank habit appears to have evolved three times, always in tropical montane conditions and often among epiphytes, (Givnish et al. 2011, 2014a).

In *Brocchinia*, the earliest diverging lineages—the so-called “Prismatica clade” and “Melanacra clade”—lack tanks, have a very small total surface area of trichomes, and often grow in sandy lowland areas or well-drained upland sites in the Guyana Shield. The two late-diverging lineages—the “Micrantha clade” and the “Reducta clade”—are sister to one another, have tanks and relatively large areas of leaf trichomes, and are found at moderate to high elevations on *tepui*s of the Guyana Shield (Givnish et al. 1997). *Brocchinia* species appear to have begun diverging from each other ≈ 13 Mya; impounding and non-impounding lineages diverged ≈ 9 Mya and carnivorous taxa evolved ≤ 5 Mya (Givnish et al. 2011; cf. ≈ 12 Mya for *B. reducta* based on Zanne et al. 2014 vs. 1.2 Mya for one of the carnivorous species based on our data presented here; Figure 3.1). Carnivory in *Brocchinia* evolved in association with the wet, extremely infertile habitats on the slopes and summits of the Guyanan *tepui*s.

Both carnivorous species of *Brocchinia* have a nearly cylindrical rosette of steeply inclined, bright yellow-green leaves, with a fine waxy dust on the inner leaf surface, and substantial areas of relatively large, live trichomes on the leaf bases (Givnish et al. 1984, 1997, Gaume et al. 2004). The tank fluid emits a sweet nectar-like odor, is highly acidic, and collects ants and other insects that do not otherwise live in pools of water.

Brocchinia reducta is the shorter of the two carnivorous species, and specializes on ants, whereas *B. hechtioides* has a rosette roughly twice as tall, and appears to be specialized on bees and wasps (Chapter 10). Nutrient inputs via carnivory are so substantial that both species can grow on bare sandstone. On Chimantá *tepui*, the nocturnal treefrog *Tepuihyla obscura* (Hylidae) takes shelter in the tanks of *B. hechtioides* and *B. reducta* by day (Kok et al. 2015), suggesting that these species also obtain nutrients from frog excrement. The tillandsioid bromeliad *Vriesea bituminosa* obtains roughly 25% of its nitrogen budget from frog excrement (Romero et al. 2010). Spiders living above bromelioid

bromeliads (both tank- and non-tank-forming species) contributed to their growth and nitrogen budget (Romero et al. 2006, Goncalves et al. 2011).

Catopsis berteroniana is an epiphyte with bright yellow leaves that collects large numbers of dead flying insects in its central tank. A number of other species of *Catopsis* have a similar growth form and may also be carnivorous or approaching carnivory. The initial argument for carnivory in *C. berteroniana* (Fish 1976) was relatively weak. Subsequent experiments by Frank and O’Meara (1984) showed that *C. berteroniana* trapped prey 12 times faster than several control tank-bromeliads, and isotope data revealed enrichment of ^{15}N -nitrogen in plant tissue, as expected if a substantial amount of its nitrogen budget is of animal origin (Gonsiska 2010).

3.6.2 Eriocaulaceae

Paepalanthus (≈ 450 species) is one of the largest genera in the Eriocaulaceae. The leaf rosette of *Paepalanthus bromelioides* is massive, several cm wide, and analogous to the tanks of many bromeliads (Chapter 10). Jolivet and Vasconcellos-Neto (1993) and Figueira et al. (1994) proposed that this species is carnivorous. Its unusually large rosette impounds rainwater, its leaves are covered with a slippery wax, and its leaf bases bear absorptive trichomes. Many micro-predators live in or above the rosettes and help capture prey and deliver nutrients to the plants via excrement or carcasses. This carnivorous pathway accounts for 27% of N inputs, whereas 67% comes from the termite nests that envelop its roots (Nishi et al. 2013).

Paepalanthus bromelioides grows only in the Serra do Cipó highlands of the Serra do Espinhaço mountain range in Minas Gerais, Brazil, where it grows in open, fire-swept *campos rupestres* (“rocky fields”) vegetation over nutrient-poor sandstone. Its growth form is somewhat similar to that of *Bonnetia maguireorum* (Bonnetiaceae) from the tallest of the *tepui*s, the Serra de la Neblina (T.J. Givnish personal observation). Givnish et al. (1986) argue that the massive rosette and largely unbranched habit of *B. maguireorum* arose as an adaptation to fire that occurs on rocky, highly unproductive surfaces. One of us (T.J. Givnish) therefore hypothesizes that this growth form evolved in *Paepalanthus* subg.

Platycaulon, which is endemic to the fire-swept Serra do Espinhaço. This evolution proceeded from relatively small individuals (the earliest diverging *P. macropodus*) to much more massive, derived forms including *P. bromelioides*, *P. velozoides*, and *P. planifolia* (phylogenetic relationships established by Trovó et al. 2013). These large rosettes form phytotelmata that likely would serve as pre-adaptation for carnivory.

3.7 Loss of carnivory

There are at least a few evolutionary reversals from carnivorous to noncarnivorous lineages (Fleischmann 2010, 2011a) that may be caused by adaptation to new habitats where carnivory is selected against (e.g., adhesive traps in very wet environments). In tropical wet forests, part-time and

facultative (*Triphyophyllum*; Figure 3.8) or complete loss of carnivorous traits (in *Dioncophyllum*, *Habropetalum*, and *Ancistrocladus*; §3.2.2) has occurred in the otherwise carnivorous clade of Nepenthales. Similarly, *Drosera schizandra* grows in relatively rich rainforest soil in understory habitats in rainy montane forests of Queensland (A. Fleischmann *personal observation*). Its large leaves bear comparatively few, scattered glands that, unlike most other *Drosera* spp., do not regenerate mucilage after it has been washed away by rain (Bourke 2009, Fleischmann 2011a). Occasional spontaneous mutants of *Drosera* species bear no tentacles (e.g., *D. erythrorhiza* with fully eglandular laminae from Western Australia; Dixon et al. 1980, K. Dixon and A. Cross *personal observations*; Figure 3.8). *Drosera caduca* from tropical northern Australia grows on the nutrient-poor sandy soils (Lowrie 2013). Unlike



Figure 3.8 (Plate 4 on page P3) Loss of carnivory in carnivorous Nepenthales (a–e) and Lamiales (f). (a) Normally developed *Drosera caduca* (Droseraceae) leaves consist of an enlarged petiole, the lamina greatly reduced to often a single, apical tentacle, or fully absent. This *Drosera* produces carnivorous foliage only in juvenile plants and after dormancy, but for the largest part of its life, it is a noncarnivorous sundew. (b) An almost entirely eglandular, noncarnivorous, naturally occurring mutant of *Drosera erythrorhiza*. (c) The predominant habit of the part-time carnivorous liana *Triphyophyllum peltatum* is noncarnivorous (shoot with the double-hooked climbing leaves shown). (d) The post-carnivorous Ancistrocladaceae (illustrated by *Ancistrocladus abbreviatus* from Sierra Leone); the inset shows the typical Mettenian glands that link it to Nepenthaceae. (e) *Nepenthes lowii*, a coprophagous rather than carnivorous pitcher plant (Chapter 26). (f) Shoots of the aquatic rheophyte *Utricularia neottioides* from Brazil usually lack traps almost entirely. Photograph (b) by Kingsley Dixon, all others by Andreas Fleischmann.

its fully carnivorous congeners, *D. caduca* has leaves with carnivorous laminae only as a juvenile plant or when freshly emerging from dry dormancy. For the rest of its life cycle (and during anthesis), the leaves consist only of a greatly enlarged, noncarnivorous petiole (Fleischmann 2011a, Lowrie 2013; Figure 3.8). The causes of this seasonal heterophylly is not known.

Exploitation of alternative nutrient sources is another mechanism by which carnivory may be lost. The rainwater-impounding pitchers of *Nepenthes* have allowed a few species to evolve mutualisms with tree shrews, bats, or rodents that use them as latrines or roosts and provide abundant nutrients in excrement (Clarke et al. 2009, Chin et al. 2010, Grafe et al. 2011, Greenwood et al. 2011, Schöner et al. 2013; Chapters 5, 26; Figure 3.8).

Some *Utricularia* species, especially in soft-water lakes, appear to depend less on carnivory and more on consumption of algae or pollen (Richards 2001, Peroutka et al. 2008, Koller-Peroutka et al. 2015). At least two aquatic rheophyte species of *Utricularia* are largely noncarnivorous. The Brazilian *U. neottioides* and the African *U. rigida* rarely develop any traps along their foliar shoots (Taylor 1989, Fleischmann 2011a, Adamec et al. 2015a; Figure 3.8). In the swiftly floating water of their natural habitats, suction traps likely would not work, and these species may be in the process of evolving away from carnivory. Populations of *U. neottioides* from red soil streams of coastal Brazil do produce traps (V. Miranda *personal communication*), whereas populations from blackwater streams in the *campos rupestres* of central Brazil do not (Adamec et al. 2015a, A. Fleischmann *personal observation*). These might represent two different evolutionary lineages employing different carnivorous strategies. Last, some species of tank-forming *Brocchinia* obtain nutrients from myrmecophily, leaf-fed epiphytism, N₂-fixation via cyanobacterial plugs, or possibly mutualisms with frogs (Givnish et al. 1997, Givnish 2017). However, these examples represent transitions within the tank-forming lineage itself, not losses of carnivory.

3.8 Future research

Over the past two decades, molecular phylogenetics have identified the relationships among different groups of carnivorous plants and their noncarnivorous ancestors. As a result, we are now in a far better position to understand the evolution of carnivorous plants, based on our knowledge of time and location of divergence from noncarnivorous ancestors; inferences about exaptations; and identification of evolutionary drivers of carnivory that acted on ecological differences between carnivorous plants and their noncarnivorous relatives.

Genomics and evolutionary developmental biology provide new opportunities and methods to explore the evolution of carnivorous plants (e.g., Bemm et al. 2016, Fukushima et al. 2017). We can gather a more detailed understanding of genetic shifts that led to carnivory by investigating evolutionary relationships of the genes involved in carnivorous structures and functions among close relatives of carnivorous plants and common ancestors of carnivorous and noncarnivorous sister groups. We could explore developmental, physiological, and ecological consequences of modified genes or highly amplified families of genes (e.g., using CRISPR) in noncarnivorous relatives.

Genomics may provide the most compelling data for deciding whether carnivory arose once or twice in Ericales by examining whether the same genes or the same orthologous copies of those genes are involved in Sarraceniaceae and *Roridula*, and possibly also in the sister-group, Actinidiaceae.

Last, *Brocchinia* offers opportunities for tracing the evolution of carnivory using sequence- and genomic-level data together with accurate time-calibrated phylogenetic trees. This genus includes both carnivorous and noncarnivorous species; species with a series of other highly specialized means of nutrient capture; and the evolution of carnivory is comparatively recent, which might allow for the use of comparative genomics, development, and morphology to study the first stages of carnivory and other specialized means of nutrient capture.

