Carnivorous Plants: Physiology, ecology, and evolution

Aaron Ellison (ed.), Lubomír Adamec (ed.)

Search in this book

CHAPTER

15   Non-motile traps 🔓

Ulrike Bauer, Reinhard Jetter, Simon Poppinga

Abstract

Movement-independent trapping mechanisms are based on stickiness, slipperiness, and direction-dependent mechanical obstruction. Here, the implementation of these principles in flypaper, pitfall, and eel traps is discussed in the light of recent scientific advances. The chemical composition and rheological properties of trapping fluids, and the (micro-) morphology of trapping surfaces, are related to their functions. Recent discoveries including the role of surface wettability in prey capture by pitcher plants are presented, and the ecological implications of temporal variations of trap performance and promising directions for future research are discussed.

Keywords: Antiadhesive surfaces, direction dependence, eel traps, flypaper traps, pitfall traps, trap glues, trapping ecology, viscoelastic fluids, wax crystals, wettability

Subject: Plant Sciences and Forestry, Animal Pathology and Diseases

Collection: Oxford Scholarship Online

15.1 Introduction

Some carnivorous plants have trapping mechanisms that are not based on plant movement, but instead use stickiness, slipperiness, or direction-dependent mechanical obstructions to capture prey. Movement-independent trap types include adhesive "flypaper" traps, pitfall traps (but cf. Chapter 14, §14.3), and eel traps. Most non-motile traps employ complex mechanisms combining two or more movement-independent mechanisms (Rice 2007). For instance, the pitfall traps of *Nepenthes* often combine slippery surfaces with a sticky, viscoelastic trap fluid, and the flypaper traps of *Drosera* and *Pinguicula* regularly combine sticky mucilage and glues (Chapter 13) with prey-triggered leaf and tentacle movement (Chapter 14).

In general, successful trapping depends on a combination of prey attraction, capture, and retention. Juniper et al. (1989) distinguished between initial capture and prey retention as separate tasks, but also noted that

one trap component can serve both purposes. For example, both the sticky mucilage of *Drosera* and *Drosophyllum* and the waxy inner surface of many *Nepenthes* pitchers serve to catch prey and to retain it. The peristome of *Nepenthes* pitchers attracts insects by means of nectar secretion and contrasting UV reflectance patterns (Chapter 12) while also playing a key role for trapping (Chapter 13).

The simplest adaptations for prey capture affect trap orientation, shape, and geometry. Traps are often oriented to maximize their exposure to potential prey (Juniper et al. 1989). Examples include the broadened, flat leaves of many *Drosera* and *Pinguicula* species, the outward-oriented pitchers on *Cephalotus* rosettes, and the pitchers of *Darlingtonia* that are basally twisted so that each grows in a slightly different direction relative to others on the same rosette. Pitchers of *Sarracenia* and *Nepenthes* that specialize in capturing flying prey tend to be taller and more slender than those targeting crawling prey (Juniper et al. 1989). Moran (1996) found that the slender, funnel-shaped upper pitchers of *N. rafflesiana* capture more flying prey than its short, ovoid ground pitchers, but he also noted differences in how each pitcher type attracted prey. Furthermore, narrow tubular pitchers directly aid in the capture of flying prey. As prey try to fly out of the tubular section of the pitcher, they create downward air currents that hinder their escape. The magnitude of this effect depends on the shape and diameter of the pitcher tube, and on the location of the insect within it (Iosilevskii and Joel 2013).

In their review of trapping mechanisms, Juniper et al. (1989) emphasized micromorphology of pitcher and eel traps, and glue biochemistry of flypaper traps. Actual trapping mechanisms, if even discussed, largely were inferred from morphological data. The only systematic experimental study of trapping mechanisms done before 1989 was the series of simple but ingenious experiments by Knoll (1914) on wax crystal-covered surfaces of *Nepenthes* and of noncarnivorous *Iris germanica*. In contrast, a large number of detailed investigations into the trapping mechanisms of pitcher and flypaper traps have been done in the last twenty years. This increased interest in the function of non-motile traps has both helped to deepen our

p. 195   understanding of previously known trapping mechanisms and led to the discovery of ↳ entirely new ones (Bohn and Federle 2004, Bauer et al. 2012b, 2013). As a result, pitcher plants in particular have gained the attention of physicists and engineers, inspiring the development of novel biomimetic anti-adhesive and self-cleaning surfaces (Koch and Barthlott 2009, Wong et al. 2011; Chapter 20). In this chapter, we review how carnivorous plants use glues, slippery surfaces, and directional (anisotropic) features to trap and retain prey. Mechanisms of attraction are reviewed in Chapter 12; anatomy and morphology of traps are reviewed in Chapter 13.

15.2 Sticky traps and trap glues

Trap glues often are multifunctional and may contain digestive enzymes (Chapters 13, 16) or act as attractants (Chapter 12). In typical flypaper traps, prey (predominantly arthropods) are lured to the aerial trap leaves where mucilage-producing glands are located (Darwin 1875, Lloyd 1942, Juniper et al. 1989; Chapter 13); *Philcoxia* uniquely possesses below-ground trap leaves that capture nematodes (Pereira et al.

p. 196   2012; Figure 15.1a). The mucilage glands typically are raised on stalks—the ↳ "tentacles" of *Byblis, Drosera* (Figure 15.1b–d), *Roridula* (Figure 15.1f), and *Triphyophyllum* (Figure 15.1e) (Chapter 13, §13.4). Prey get stuck to the glue, struggle, and try to escape, thereby contacting even more glue, and eventually suffocate as their tracheae get plugged. However, some animals can move freely and live as commensals on adhesive traps (Chapters 1, 13, 23, 26).
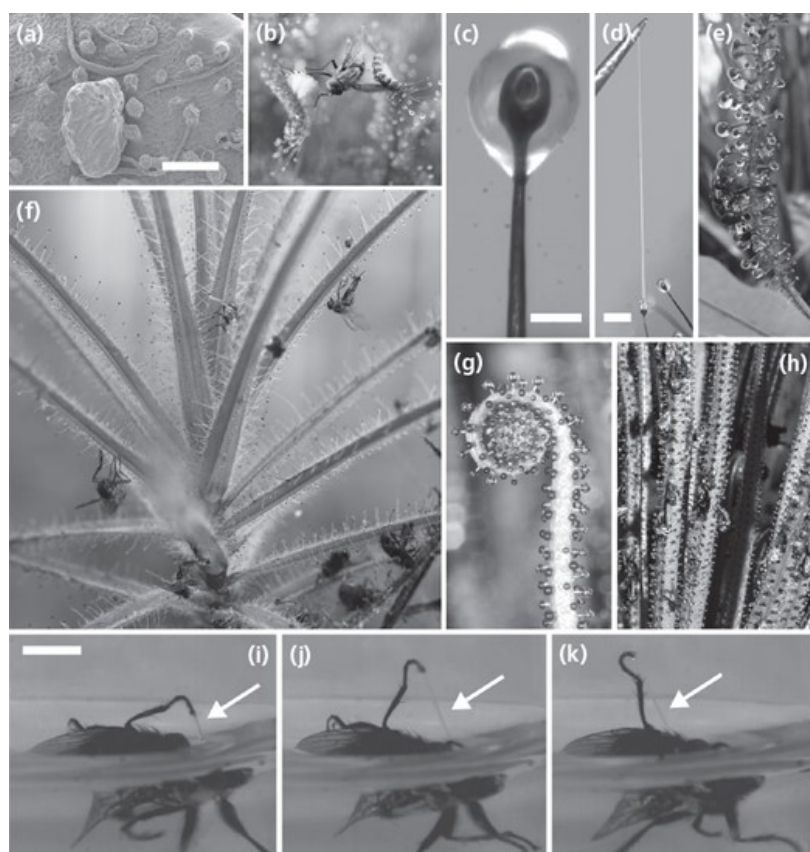
Figure 15.1 Sticky traps and trap glues. (**a**) Scanning electron micrograph showing details of the subterranean *Philcoxia minensis* trap leaf. Numerous stalked glands are visible on the surface. The image also shows several nematodes (the main prey of this species) and a sand grain; bar = 200 μm (Modified from Pereira et al. 2012 and reproduced with permission from *PNAS*). (**b**) *Drosera rotundifolia* trap leaves with prey. (**c**) *D. capensis* tentacle with mucilage drop; bar = 200 μm. (**d**) *Drosera* mucilage is characterized by high extensional viscoelasticity and forms long threads when touched and pulled away from the tentacle; bar = 500 μm. (**e**) *Triphyophyllum peltatum* trap leaf with glue-laden tentacles and a captured blowfly. (Image from Rembold et al. 2010b and reproduced with permission from the *Carnivorous Plant Newsletter*). (**f**) A *Roridula gorgonias* plant has captured multiple flies on its extremely sticky leaves. Three sizes of glandular trichomes occur along the leaf margins. (**g**) A young *Drosophyllum lusitanicum* leaf, showing outward circinate vernation, mushroom-shaped stalked glands, and trap mucilage. (Photograph by Anja and Holger Hennern, and used with permission). (**h**) Leaves of *D. lusitanicum* covered in small flying prey. (Photograph by Anja and Holger Hennern, and used with permission). (**i–k**) A fly on the surface of *Nepenthes rafflesiana* pitcher fluid wraps itself in long filaments of the highly viscoelastic liquid; bar = 3 mm.

(Images from Gaume and Forterre 2007).

The glues of *Drosera* and *Drosophyllum* (and probably those of less well-studied *Byblis, Nepenthes, Pinguicula, Philcoxia*, and *Triphyophyllum*) are sugar-based (Vintéjoux and Shoar-Ghafari 2000, Adlassnig et al. 2010). In all but very arid conditions (Volkova and Shipunov 2009), the *Drosera* glue drop is typically tens of μm in diameter (Figure 15.1c), corresponding to a nL fluid volume (Erni et al. 2011). The glue itself is a homogeneous, aqueous solution (pH 5) of an acidic polysaccharide composed of a D-glucurono-D-mannan backbone with alternating monosaccharide side groups (Rost and Schauer 1977, Gowda et al. 1982, 1983). It constitutes a natural hydrogel, consisting of a flexible, fibrous polysaccharide nano-network that allows for large stretching deformation (Huang et al. 2015) and is characterized by high viscosity, capillary thinning, and extensional viscoelasticity (Erni et al. 2011). These rheological properties are susceptible to pH changes and extreme temperatures. Freezing, thawing, and raising the temperature to 80 °C all lead to an irreversible loss of the glue's viscoelasticity (Rost and Schauer 1977). The fresh mucilage can be drawn out into long slender threads (Figure 15.1d). Elastic forces need to be overcome to stretch the long-chain

polymers, conferring high resistance to extensional flows. The movement of struggling prey is too fast for these elastic forces to relax, and the glue behaves like an elastic band.

The glue of *Drosophyllum lusitanicum* is similarly composed of the monomers arabinose, galactose, xylose, rhamnose, glucuronic acid, and ascorbic acid. However, it is more acidic (pH 2.5−3) than the *Drosera* glue (Juniper et al. 1989, Adlassnig et al. 2010), and emits a noticeable honey scent (Chapter 12). In further contrast to *Drosera*, the mushroom-shaped glands of *Drosophyllum* carry glue drops even when the relative humidity falls below 40% (Adlassnig et al. 2006, Figure 15.1g). The glue is highly hygroscopic (Darwin 1875), probably helping this xeromorphic plant to harvest water from fog (Adamec 2009a). Unlike the glue of *Drosera*, that of *Drosophyllum* cannot be drawn out into threads and is easily detached from the glands. Larger and heavier prey "slide" down the trap leaf, thereby coming into contact with even more glue, whereas small prey get firmly stuck to the trap (Rice 2007; Figure 15.1h).

The glandular tips of *Roridula* tentacles secrete a water-insoluble, viscoelastic resin (Bruce 1907; Chapter 13; Figure 15.1f) that consists of lipophilic aliphatic esters and carboxylic acids (Simoneit et al. 2008, Voigt and Gorb 2008, Frenzke et al. 2016). Its high desiccation resistance may be an adaptation to periodically dry environments (Voigt and Gorb 2010a). The resin also stays fully functional under wet conditions, even when submersed for 24 h (Voigt et al. 2015). It is extremely sticky, so that plants are often covered in trapped insects of considerable size and mass (Voigt and Gorb 2008). In addition to the adhesive properties of the glue, the hierarchical architecture of the tentacles plays a crucial role in prey capture (Voigt et al. 2009), with tentacles of three different lengths acting sequentially. First, long, slender, and very flexible tentacles make comparably weak adhesive contact with the prey. The still mobile animal, in its struggle to escape, touches more of the long tentacles, until it also comes into contact with a second type of tentacles. These are thicker, shorter, and four times stiffer than the long ones, and produce an adhesive that is 50% stickier. Finally, the animal sticks to the short and thick tentacles that are almost 50 times stiffer than the longest ones and produce the stickiest glue. The animal exhausts itself in its attempts to escape and eventually dies.

Roridula does not produce its own digestive enzymes (Marloth 1925), but relies on a mutualistic relationship with mirid bugs for prey digestion (Chapter 26). The bugs feed on the trapped insects and then defecate on the leaves. The mineral nutrients contained in the feces are absorbed through the thin, porous cuticle of the leaves (Ellis and Midgley 1996). A combination of locomotive adaptations and a thick coating of anti-adhesive epicuticular grease enables the bugs to walk on the trapping leaves without getting stuck (Voigt and Gorb 2008, 2010b).

p. 197 Large amounts of water-insoluble mucilage also are secreted on the outer surfaces of leaves, inflorescences, and traps of some species of *Genlisea* and *Utricularia*. The biological function of these mucilages has not yet been investigated in detail (Fleischmann 2012a, Poppinga et al. 2016b).

Finally, the viscoelastic fluid in the pitchers of some *Nepenthes* (Nepenthaceae) species must also be considered a trap glue, despite its predominantly retentive function. Initial trapping is largely based on anti-adhesive surfaces (§15.3). Prey slip and fall into a fluid pool with both retentive and digestive properties. Gaume and Forterre (2007) showed that the viscoelastic relaxation time of the trap fluid of *N. rafflesiana* exceeds the typical timescale of the leg movements of struggling prey. As a consequence, prey become entangled in multiple sticky fluid threads, and eventually drown and die (Figure 15.1i−k). Gaume and Forterre (2007) found no effect of surface tension for prey retention in *N. rafflesiana*, but Armitage (2016a) showed in laboratory experiments that pitcher-dwelling bacteria reduce the surface tension of *Darlingtonia californica* pitcher fluid, leading to improved prey retention. Bazile et al. (2015) investigated the effects of fluid viscoelasticity and pH on prey capture and retention in four *Nepenthes* species. Fluid pH affected mainly survival times, with more acidic fluid killing prey more quickly. Retention efficiency increased exponentially with viscoelasticity, higher levels of which were necessary for effective retention of ants relative to flies.

15.3 Anti-adhesive surfaces

The pitfall traps of *Brocchinia, Catopsis, Cephalotus, Darlingtonia, Heliamphora, Nepenthes*, and *Sarracenia* all rely predominantly on slippery surfaces for prey capture and retention, often aided by directional surface features (Chapter 13, §13.3). Whether a surface is more effective for initial capture or prey retention depends on two factors: its position within the trap and the presence or absence of attractive features, particularly nectar (Chapter 12). Generally, surfaces at or close to the trap opening tend to play a more important role in initial trapping, whereas features deep inside the trap are more heavily involved in retention. However, attractive components such as nectaries often extend deep into the pitcher interior, e.g. in *Heliamphora* (Juniper et al. 1989, Bauer et al. 2013; Chapter 12), and insects such as mosquitoes are attracted to the pitcher fluid for breeding (Beaver 1983) and may get trapped in viscoelastic *Nepenthes* fluid during oviposition.

The anti-adhesive properties of pitfall trap surfaces are based on four general principles. First, microscopic roughness greatly reduces the available contact area for adhesive pads on the tarsi of insects (Scholz et al. 2010). The same effect prevents adhesive tape from sticking to fine-grained sandpaper. At the same time, the tarsal claws cannot interlock with the plant surface if its surface roughness is sufficiently fine-scale. This principle has been experimentally validated for *Nepenthes* and is likely to also play a crucial role on the trapping surfaces of *Cephalotus, Darlingtonia*, and *Sarracenia*. Second, easily detachable wax crystals (Chapter 13, §13.3.1) may contaminate the adhesive pads of insects walking on the surface, making the insects more likely to fall (Federle et al. 1997, Markstädter et al. 2000). This has been reported for *Brocchinia, Catopsis*, and *Nepenthes* (Gaume et al. 2004), and is likely to work in a similar way in other species with trap surfaces covered in epicuticular wax crystals. Third, directional features such as the downward-pointing hairs or papillae of the Sarraceniaceae and *Cephalotus*, and the overlapping peristome cells and downward-oriented lunate cells of *Nepenthes*, allow claws to interlock on the way into the pitcher, but not in the opposite direction (Gaume et al. 2002, Gorb et al. 2004, Bauer and Federle 2009, Bauer et al. 2013; Chapter 13, §13.3.3). And fourth, highly wettable (superhydrophilic) surfaces lead to the formation of stable water films under humid or rainy conditions (Bohn and Federle 2004, Bauer and Federle 2009, Bauer et al. 2013; Chapter 13, §13.2.3). Experimental studies on the detailed trapping mechanisms to date have focused almost exclusively on the genus *Nepenthes.*

15.3.1 Wax blooms

Epicuticular wax crystal coatings fulfill multiple ecological functions in all major plant groups (Barthlott 1989) and are often extremely slippery for insects (Jeffree 1986). They also occur in all genera, but not all species, of carnivorous bromeliads and pitcher ↳ plants, excepting *Cephalotus*. Wax crystal layers usually are located on interior surfaces of the trap above the digestive fluid. Juniper et al. (1989) described a thick coating of filamentous wax crystals in *Brocchinia reducta*, noting that insects not only slip on these surfaces, but also get entangled in the fine waxy threads. Gaume et al. (2004) and Poppinga et al. (2010) showed a similar wax crystal structure on the adaxial leaf surface of *Catopsis berteroniana* (Figure 15.2a). Platelet-shaped wax crystals are found in *Sarracenia* (Figure 15.2b), *Darlingtonia*, and *Nepenthes* (Figure 15.2c, 15.2d), but they are produced by *Darlingtonia* only when it grows under high-light conditions (Juniper et al. 1989, Poppinga et al. 2010). Wax blooms also are apparent in some *Heliamphora* species (notably *H. ceracea* and *H. macdonaldae*; ↳ McPherson et al. 2011), but the crystalline ultrastructure has not yet been examined in this genus.
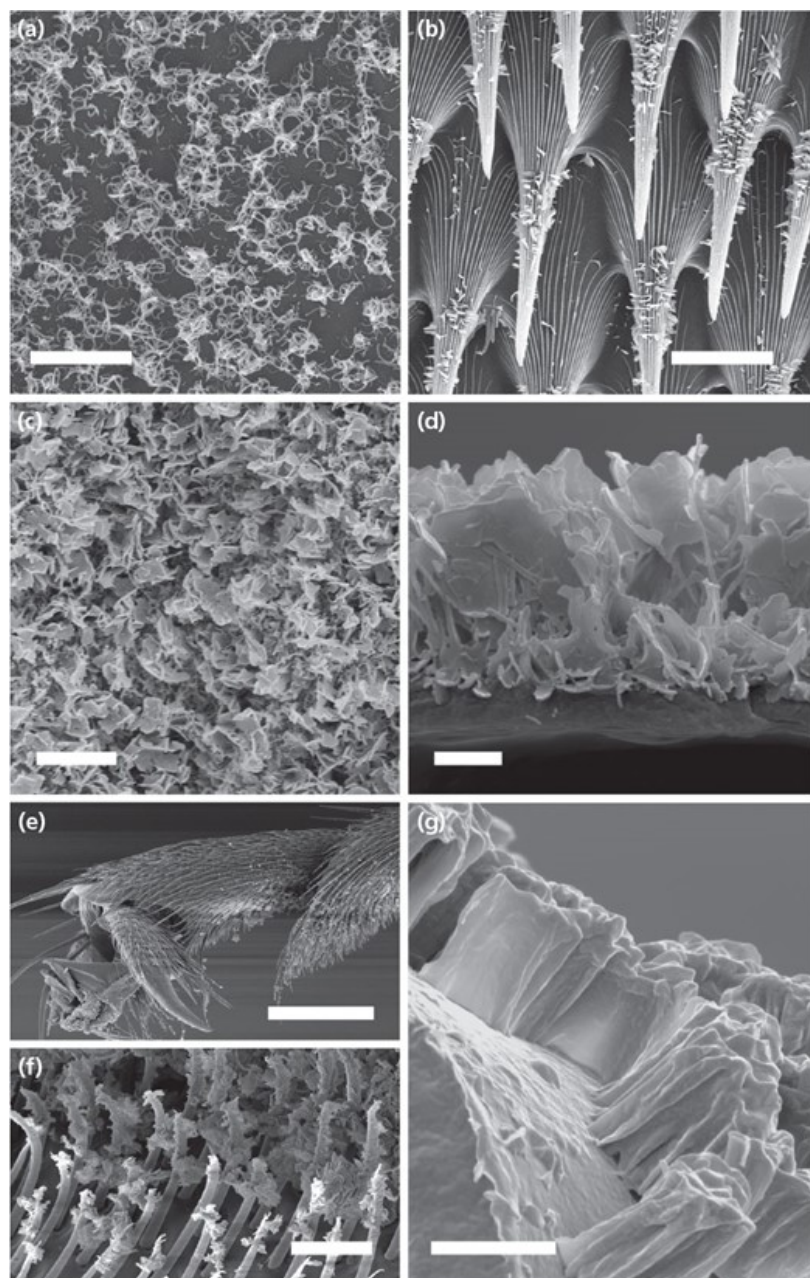
Figure 15.2 Wax blooms and their effect on insect locomotion. (**a**) Thread-like wax crystals on the inner surface of a *Catopsis berteroniana* leaf; bar = 20 μm (modified from Poppinga et al. 2010 and reproduced with permission from CSIRO Publishing). (**b**) Wax scales and cuticular folds (see also Figure 15.3a) on the imbricate cells of a *Sarracenia leucophylla* pitcher; bar = 20 μm (modified from Poppinga et al. 2010 and reproduced with permission from CSIRO Publishing). (**c–d**) Upright-standing wax platelets on the inner pitcher wall of *Nepenthes gracilis* in top (**c**) and side (**d**) views; bars = 5 μm (**c**) and 1 μm (**d**). (**e–f**) Severe wax contamination on adhesive pads and pretarsal hairs of a weaver ant after trying to climb the waxy inner wall of a *Nepenthes* pitcher; bars = 100 μm (**e**) and 10 μm (**f**); (Micrographs by Walter Federle, and used with permission). (**g**) Pillar-like wax crystals on the lower pitcher lid surface of *Nepenthes gracilis*; bar = 2 μm.

The majority of *Nepenthes* species employ wax crystals during at least some stages of their ontogeny, and a recent comparative analysis suggested that the presence of epicuticular wax crystals on the entire pitcher wall from fluid to rim is the ancestral character state within the genus (Bauer et al. 2012a). Ontogenetically early ground pitchers often show wax crystals throughout the pitcher wall, whereas they are reduced or absent in upper pitchers of the same species (Gaume and Di Giusto 2009). The ultrastructure of inner–wall

surface waxes in *Nepenthes* is very uniform across all species examined to date, further supporting a common evolutionary origin.

Generally, the epicuticular wax layer consists of a sponge-like lower matrix with a superimposed dense array of very thin, upright-standing platelets with variable orientation (Figure 15.2d). Juniper et al. (1989) proposed that the wax platelets would be oriented downward, more or less parallel to the inner pitcher wall, but both SEM and AFM images consistently show platelets protruding perpendicularly from the underlying matrix (Riedel et al. 2003, Gaume et al. 2004, Scholz et al. 2010). The platelets are connected to the matrix via thin stalks that break easily. Detached platelets contaminate adhesive pads of insect tarsi in experimental trials (Juniper and Burras 1962, Gorb et al. 2005; Figure 15.2e, 15.2f). However, the extent to which wax crystals may break under natural trapping conditions remains controversial. Scholz et al. (2010) observed that the wax platelets of *N. alata* pitchers were remarkably stable, and did not detect significant wax contamination on the tarsal pads of ants or stick insects after they had slipped off the surface naturally. These authors also showed experimentally that a reduction of the available contact area because of microscopic roughness, at a scale similar to the *Nepenthes* wax, is by itself sufficient to impede insect adhesion.

Nepenthes gracilis is the only species known to produce morphologically distinct wax crystals on the surfaces of two different pitcher parts (Bauer et al. 2012b). The inner wall is coated with wax platelets similar to those of other *Nepenthes* species, whereas the underside of the pitcher lid carries clusters of coarser, pillar-shaped wax blocks (Figure 15.2g). This surface is far less slippery than the inner wall, and insects can walk upside down under the lid and harvest the generously secreted nectar. However, when the lid is perturbed, for example by the impact of a rain drop, the insect loses its footing and falls into the pitcher (Bauer et al. 2015b; Chapter 14, §14.3). When the wax crystal layer is removed experimentally, the *N. gracilis* lid loses its trapping function (Bauer et al. 2012b).

The ultrastructure of epicuticular wax crystals is largely determined by their chemical composition, although environmental influences during crystal formation may affect their structure to a lesser extent (Koch and Ensikat 2008). Riedel et al. (2003) found that the epicuticular wax crystals on the inner wall of *N. alata* consist of a mixture of aliphatic compounds: very long-chain (C_{22+}) aldehydes account for approximately 60% of the mixture; triacontanal (43%) is the most abundant constituent. The remaining 40% of the wax mixture include alcohols, fatty acids, esters, and a small amount of alkanes. The slippery epicuticular crystals are formed by accumulation of particularly high amounts of triacontanal (together with other wax aldehydes) near the pitcher wall surface, likely leading to spontaneous crystal formation. In contrast, the underlying intracuticular wax contains higher proportions of alcohols (31%), esters, and pentacyclic triterpenoids, but only 28% aldehydes and 9% triacontanal.

The chemical composition of epicuticular waxes on inner pitcher walls of *N. albomarginata, N. khasiana*, and three further hybrids is nearly identical to that of *N. alata* (Riedel et al. 2007), suggesting very similar mechanisms of crystal formation and hence wax biosynthesis in most, if not all, *Nepenthes* species that rely on slippery waxes to capture prey. Aldehyde-based crystals of similar morphology also have been described in noncarnivorous plants, including rice (Haas et al. 2001), suggesting convergent evolution of slippery surfaces in very distantly related plant lineages.

15.3.2 Cuticular folds

Microscopic surface roughness can be achieved not only by epicuticular wax blooms, but also by fine-scale folds of the cuticle itself. Such cuticular folds, ↳ usually arranged parallel to each other, are found ubiquitously across all genera of pitcher plants except the carnivorous bromeliads (Chapter 13, §13.3.2). The scale, density, and location of folds on the pitcher vary greatly between genera. In *Sarracenia, Darlingtonia*, and *Cephalotus*, strikingly similar downward-pointing imbricate cells line the upper sections of the pitchers. Each of these shark tooth-shaped cells is delicately striated by fine, ridge-like cuticular folds (Figure 15.3a). Their function has not yet been studied experimentally in pitcher plants, but cuticular folds hamper attachment of beetles in several noncarnivorous plants (Prüm et al. 2012). We hypothesize that these folds reduce the available contact area for tarsal adhesive pads of insects in a manner similar to the wax platelets in *Nepenthes* pitchers.
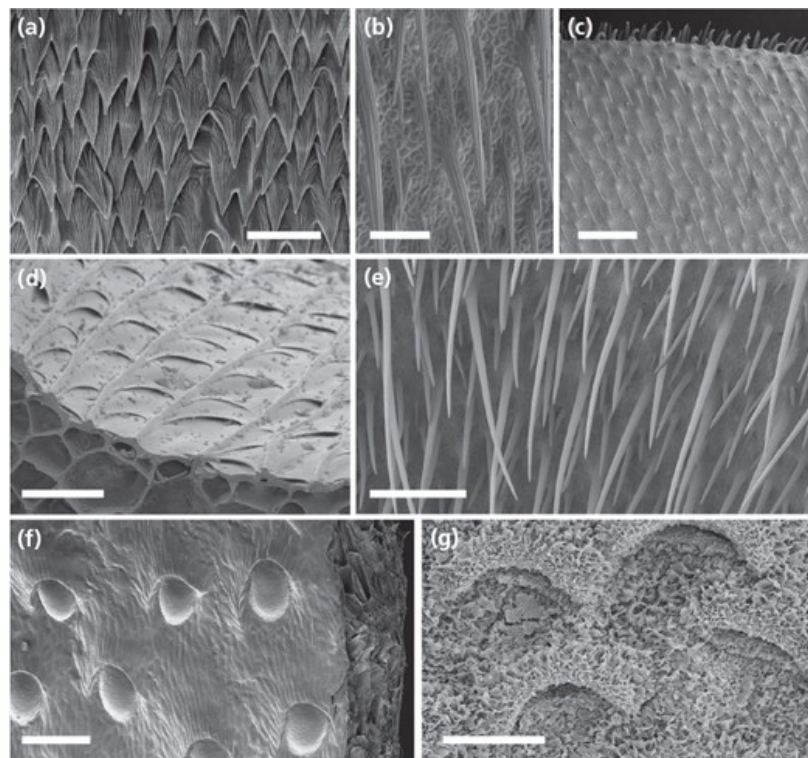

Figure 15.3 Cuticular folds and anisotropic surfaces. (**a**) Imbricate cells on the inner pitcher surface of a *Sarracenia* hybrid are finely striated by cuticular folds (see also Figure 15.2b); bar = 50 μm. (**b**) Cuticular folds on the downward-pointing trichomes of *Heliamphora nutans*; bar = 200 μm. (**c**) The upper part of the *H. nutans* inner pitcher wall is densely covered in short, downward-pointing, highly wettable hairs; bar = 500 μm. (**d**) Cuticular folds forming the small-scale ridges on the *Nepenthes* peristome. This image also shows the tile-like steps formed by overlapping epidermal cells. Both steps and ridges are crucial for the high wettability of the surface; bar = 50 μm. (**e**) Long trichomes in the lower region of the hairy zone of a *H. nutans* pitcher; bar = 500 μm. (**f**) Hooded digestive glands on the inner wall of a *Nepenthes* pitcher, providing a foothold for claws only in downward direction; bar = 200 μm. (**g**) Lunate cells in the waxy zone of a *N. gracilis* pitcher; bar = 20 μm.

Broader, more widely spaced cuticular folds also are found on the surface of downward-pointing trichomes in *Heliamphora* (Figure 15.3b) and *Darlingtonia*. For *Heliamphora*, it has been proposed that these ridges help to increase the wettability of the hairs (Bauer et al. 2013). Even larger-scale cuticular ridges mark the centerlines of the elongated epidermal cells on the *Nepenthes* peristome (Figure 15.3d), creating a microscopic radial pattern of parallel ridges and grooves that enhances the wettability of the surface (§15.3.4).

15.3.3 Directional (anisotropic) surfaces

All pitcher plants other than carnivorous bromeliads have various types of directional surface structures. Frequently, different anisotropic features are found in different parts of the trap, or even in combination on the same tissue. All have in common that they provide a secure grip for an insect's tarsal claws in only one direction. Examples of such directional structures include the tooth-like imbricate cells of *Sarracenia* (Figure 15.3a), *Darlingtonia*, and *Cephalotus*, and the trichomes of *Heliamphora* (Figure 15.3b, 15.3c, 15.3e) and *Darlingtonia*. Similar downward-pointing trichomes are also found on the inside of the hood of many *Sarracenia* pitchers (Juniper et al. 1989). In *H. nutans*, the trichomes densely cover the inner pitcher wall,

from its top ↳ down to just above the maximum fluid level that is determined by a small drainage hole in the pitcher wall. The trichome length gradually increases from ≈0.2 mm at the top (Figure 15.3c) to >1.5 mm at the bottom of the hairy section (Figure 15.3e). At the same time, the incline of the wall steepens from top to bottom, approaching vertical in the lower half of the hairy section. Bauer et al. (2013) found that ants could walk relatively safely on the short trichomes of the upper region, but the surface became increasingly treacherous as they moved deeper into the pitcher. Ants invariably fell once they reached the lower half of the hairy section. Experimental studies on the anti-adhesive function of the trichomes of other species of Sarraceniaceae as yet are lacking.

Downward-pointing trichomes with slightly different morphology also are found in the lower part of *Sarracenia* and *Heliamphora* pitchers, where they are permanently submerged in the digestive liquid. The exact function of these hairs is unknown, but they probably play a role in prey retention, either by directly obstructing attempts to climb the pitcher wall or by encouraging disoriented prey to move deeper into the pitcher. The latter effect can commonly be observed in *Nepenthes* pitchers, where the hooded glands of the digestive zone provide unidirectional footholds for claws, causing struggling insects to drag themselves deeper into the pitcher (U. Bauer *unpublished data*).

Nepenthes pitchers not only possess hooded glands (Figure 15.3f)—usually located below the fluid surface but in some species extending all the way up to the peristome—but also characteristic lunate cells within the upper zone of the inner wall where the slippery epicuticular wax crystals are found (Figure 15.3g). The function of these cells is not currently known, but they do not appear to be secretory. The anisotropic structure of the lunate cells allows insect tarsal claws to grip only in a downward direction (Gaume et al. 2002, 2004, Gorb et al. 2004). The unidirectional orientation of the lunate cells may be an adaption for prey retention, but it is unlikely to contribute much to initial prey capture. Knoll (1914) proposed that the projections of the lunate cells may have a jolting effect on insects, causing them to fall away from the wall instead of sliding down the surface. This could make prey more likely to fall backwards onto the fluid surface, thereby improving the retention efficiency of the trap. Systematic experimental studies are needed to confirm this hypothesis.

The secretions of the glandular zone also may aid in retention of at least some prey taxa. Gaume et al. (2002) found that the fresh glandular surface of *N. alata* impeded the locomotion of ants. The surface appeared to be sticky, making it difficult for ants to detach their feet while prompting extensive cleaning behavior. Gorb et al. (2004) measured significantly lower friction forces for *Pyrrhocoris apterus* bugs on fresh relative to dried glandular surfaces of *N. ×ventrata*, but found no measurable effect for *Calliphora vicina* blowflies. The glandular surface also has been described as readily wettable by the same authors. More research is needed to investigate whether aquaplaning effects (§15.3.4; Chapter 13, §13.2.3) also play a role on the glandular surface.

15.3.4 Wettable (superhydrophilic) surfaces

The trapping function of fully lubricated, superhydrophilic surfaces is a relatively recent discovery (Bohn and Federle 2004). It is astonishingly effective and has been found in all *Nepenthes* species examined to date and in one species of *Heliamphora* (Bauer et al. 2013). When the *Nepenthes* peristome is dry, it is completely safe for insects to walk on (Lloyd 1942, Gaume et al. 2002), but it becomes extremely slippery as soon as it gets wet. The extraordinary wettability of the peristome (Figure 15.4a) probably results from a combination of a hydrophilic surface composition and an enhancing effect of the complex hierarchical surface topography. Microscopic roughness amplifies the chemical properties of a surface; a familiar example of this effect is the water-repellent, self-cleaning lotus leaf whose properties are based on surface hydrophobicity combined with a hierarchical micropattern (Barthlott and Neinhuis 1997). The opposite effect occurs on the *Nepenthes* peristome: an applied water droplet spreads instantly and forms a thin stable film on the surface. This water film prevents the tarsal adhesive pads of insects from making contact with the underlying surface and causes them to slip, leading Bohn and Federle (2004) to term the ↳

p. 202   phenomenon "insect aquaplaning" when they first discovered it in *N. bicalcarata.*
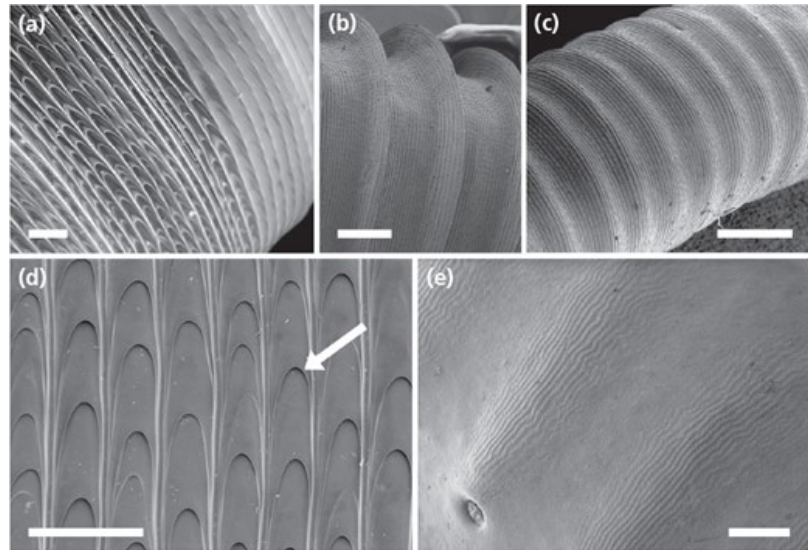

Figure 15.4 Microtopography of the *Nepenthes* peristome. Arrows point toward the interior of the pitcher. (**a**) Environmental scanning electron micrograph of a *N. alata* peristome. The two macroscopic grooves on the far right are covered by a continuous water film; bar = 100 μm. (**b–c**) Typical hierarchical ridge structure. The lower pitcher of *N. lowii* (**b**) shows more acute macroscopic ridges and deeper macroscopic grooves than the *N. muluensis* peristome (**c**); bars = 500 μm. (**d**) Detail of the microscopic ridges with steps (arrow) in between. The steps promote directional water transport from the inside to the outside of the peristome; bar = 50 μm. (**e**) Detail of the peristome of an *N. lowii* upper pitcher, showing only faint remnants of the typical ridge structure. The upper pitchers of this species are not effective insect traps and collect mammalian feces instead; bar = 500 μm.

The microstructure of the *Nepenthes* peristome, although to some degree variable between species, is universally characterized by a radial ridge pattern. Most species show hierarchical ridges on two distinct length scales (Figure 15.4b, 15.4c). The larger, macroscopic ridges are visible with the naked eye. Water spreads along these ridges, filling grooves between them before spreading to neighboring grooves. This confines water spreading and may result in partially slippery peristomes when only a few individual drops are applied. Further, it limits the exposed surface area of a given volume of water on the peristome, reducing evaporation rates and prolonging periods of slipperiness. The grooves vary in depth among species and can be shallow and indistinct (e.g., *N. muluensis*; Figure 15.4c) or semicircular in cross-section and delineated by acute ridges (e.g., lower pitchers of *N. lowii*; Figure 15.4b). The effect of different groove cross-sections on

wettability and water-spreading speed has not yet been studied, but it is widely accepted that the grooves aid water spreading via capillary effects (Bauer and Federle 2009).

Superimposed on this macroscopic ridge structure is a pattern of microscopic ridges and grooves. The latter are shallow in cross-section and are formed by parallel rows of elongated epidermal cells. The cells overlap like tiles on a roof, creating a series of sloped steps toward the interior of the trap (Figure 15.4d). Similar to lunate cells, these steps render the peristome surface strongly anisotropic, providing good footholds for tarsal claws only when an insect is traveling into the pitcher. Using a series of experimental manipulations of ant feet and subsequent friction force measurements, Bohn and Federle (2004) showed that the directional topography is effective against the claws, whereas the lubricating water layer incapacitates the adhesive pads of the insects. In addition, the steps promote directional wetting, aiding water to spread outward and upward, against gravity, on the peristome. The tile-like arrangement of cells within each groove creates a microcavity at the top end of each cell, overhung by a sharp edge formed by the bottom end of the ↳ next cell. In top view, this edge runs roughly semicircularly across the groove before tapering downwards along the adjacent ridges. Water gets pinned at sharp edges, preventing it from flowing to the other side, an effect similar to that seen when overfilling a glass. The same effect hampers downward spreading of water on the peristome. On the way up, however, the water gradually rises to and above the level of the next step while filling the groove and cavity below, allowing it to overflow easily into the next compartment (Chen et al. 2016). In the natural setting of a more or less vertically oriented peristome, this effect is likely to be counterbalanced by gravity and capillary forces because the grooves narrow toward the inner peristome edge. The combination of all three effects probably ensures that the peristome is evenly wetted, independent of where water is deposited.

The microscopic groove pattern appears to be highly conserved across *Nepenthes* species, except for *N. lowii*, for which only the lower pitchers possess a peristome with this hierarchical ridge pattern. The upper pitchers have a much reduced peristome with a largely smooth surface and only faint remnants of a microscopic ridge structure locally above each nectar gland (Figure 15.4e). This reduced peristome is neither wettable nor slippery for insects, and upper pitchers of *N. lowii* have been shown to be specialized for collecting feces of tree shrews (*Tupaia montana*) instead (Clarke et al. 2009; Chapter 13, §,13.6; Chapter 21).

The wetness-induced slipperiness of the *Nepenthes* peristome is paralleled in pitchers of the South American *Heliamphora nutans.* Bauer et al. (2013) showed that its hairy inner pitcher wall also is highly wettable, and water spreads easily on the surface. In contrast to *Nepenthes*, the water appears to spread uniformly in all directions. Wetting markedly increases the trapping efficiency of *H. nutans*, particularly in the upper section of the pitcher that provides a secure foothold when dry, but becomes extremely slippery when wet. Superhydrophilicity is an unusual trait for densely hairy surfaces which are often strongly water-repellent. The parallel cuticular folds on the trichomes (Figure 15.3b) of *H. nutans* are similar in dimension to the microgrooves on the *Nepenthes* peristome and are thought to be crucial for its wetting properties.

15.4 Mechanical obstructions

Beside microscopic surface features (§15.3.3), larger-scale mechanical barriers for prey capture and retention also are common in pitfall or eel traps. Examples include the overhanging funnel region of *Cephalotus* (Figure 15.5a), the peristome of *Nepenthes* (Figure 15.5b), and the long trichomes inside *S. psittacina* pitchers (Figure 15.5c). The overhanging geometry and downward-pointing teeth of the *Nepenthes* peristome may aid in the retention of captured prey (e.g., Lloyd 1942, Juniper et al. 1989), but at least ants can cross this barrier freely in both directions if the peristome surface is dry (U. Bauer *unpublished data*).
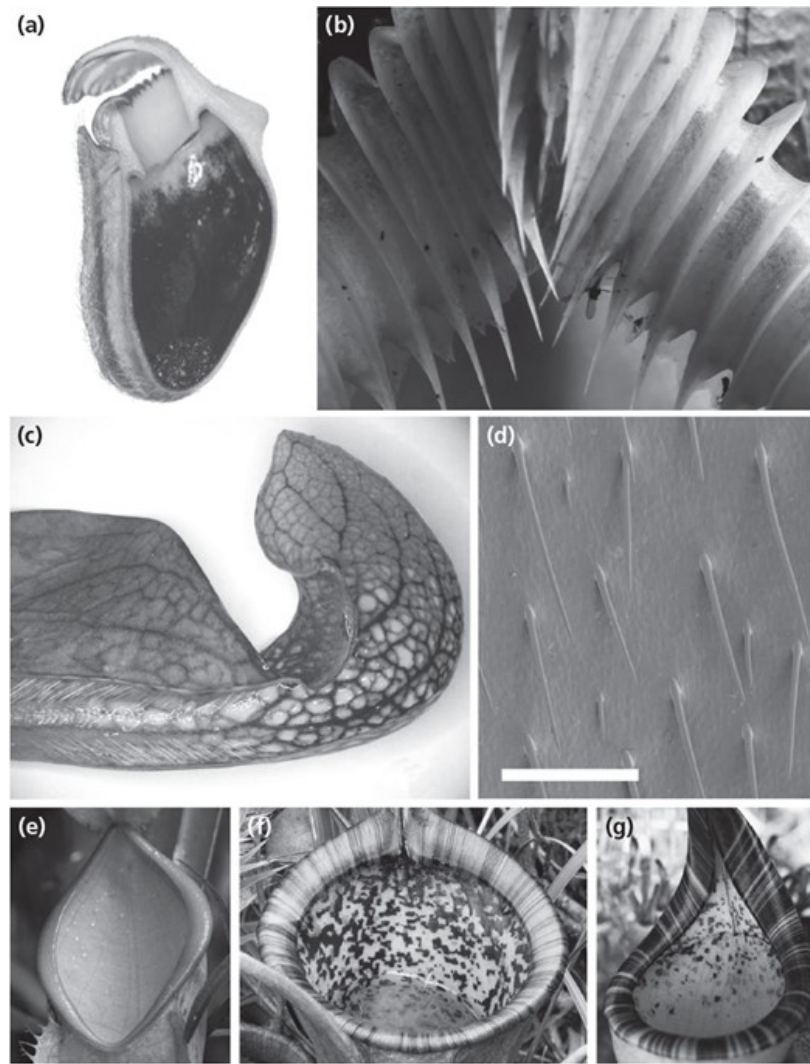
Figure 15.5 Diversity of physical barriers in pitcher traps. (**a**) Cross-section of a *Cephalotus follicularis* pitcher showing the peristome, funnel, and main pitcher body with accumulated prey. (**b**) The overhanging architecture and downward-pointing teeth of many *Nepenthes* peristomes are thought to present an escape barrier for captured prey, as well as preventing theft. (**c**) Eel trap of *Sarracenia psittacina*, with long, inward-pointing hairs inside the narrow tubular pitcher. (**d**) Downward-pointing trichomes in the permanently submerged retentive zone of a *Heliamphora nutans* pitcher; bar = 500 μm. (**e–g**) Variations in the extent of the waxy zone in three species of *Nepenthes*. (**e**) *N. tentaculata* with a waxy layer covering the entire inner pitcher wall above the fluid. (**f**) *N. attenboroughii* free of wax crystals. (**g**) Upper pitcher of *N. fusca* showing a largely wax-free inner wall with a small remnant wax crystal patch in the upper rear section. In lower pitchers of the same species, the entire inner wall above the fluid is covered in wax crystals.

Long inward–pointing trichomes, commonly present in the retention zones of *Cephalotus, Darlingtonia, Heliamphora* (Figure 15.5d), and *Sarracenia* pitchers, also contribute to prey retention. The pitchers of *Sarracenia psittacina* grow horizontally, and their interior surface is densely covered with such trichomes (Figure 15.5c). These traps have been described as lobster or eel traps (Lloyd 1942, Cheek 1988). Prey can enter the trap by pushing through the hairs, but are unable to reverse direction against their incline. Eel traps are also found in *Genlisea* species. Their subterranean leaves are achlorophyllous and, like roots, provide both anchorage and nutrient absorption from the soil (Adamec 2008c). They also function as traps for attracting, capturing, and digesting prey (Darwin 1875). The general architecture of the trap is homogeneous among the species: an inverted Y–shape, with a terminal digestive vesicle, and a tubular neck and helical arms equipped with rows or funnel–like rings of stiff, unidirectional bristles (Fleischmann 2012a; Chapter 13, §13.5.2). Prey enter the trap through openings at the branching region and along both

helical arms. Like the *S. psittacina* hairs, the bristles inside the trap only allow prey to move further inwards, toward the digestive vesicle.

15.5 Ecological implications of wetness-activated trapping mechanisms

p. 204 The discovery of wetness- or rain-activated trapping mechanisms in *Nepenthes* and *Heliamphora* fundamentally changed our understanding of how these traps work and interact with their ↳ environment. Previously, non-motile traps were believed to be static not only with regards to the absence of movement (but see Chapter 14, §14.3), but also in effectiveness over time. Only long-term changes of capture rates with trap age had been reported. Fish and Hall (1978) observed peak capture rates in *Sarracenia purpurea* pitchers approximately two weeks after pitcher opening. After that, prey capture gradually decreased over the remaining lifespan of the pitcher, but it was unclear whether these changes were caused by changes in attractiveness, trapping efficiency, or both. Bauer et al. (2009) also observed age-dependent changes in prey capture in *Nepenthes rafflesiana.* Prey-capture rates during the first week after pitcher opening coincided not only with an increase in nectar production (attractiveness), but also with increased trapping efficiency due to changes in peristome wettability and resulting slipperiness.

In addition to these gradual changes, however, the wetness-activated slipperiness of both the *Nepenthes* peristome and the *Heliamphora* inner pitcher wall also leads to dramatic short-term variability in trapping efficiency. This has been studied for *N. rafflesiana*, but similar effects likely occur in other, mainly peristome-trapping, *Nepenthes* species, and in other pitcher plants with wettable slippery surfaces that occupy habitats with pronounced short-term variability in ambient humidity.

p. 205 *Nepenthes rafflesiana* in Northern Borneo typically inhabits open shrublands and forest edges at low elevations. These habitats experience large diurnal ↳ variations of temperature and humidity, and pitcher peristomes generally are dry during the hotter hours of the day. Traps become wet when it rains or when rising humidity leads to condensation on the peristome. Nectar secreted onto the peristome facilitates condensation because its high sugar content renders it strongly hygroscopic (Bauer et al. 2008; Chapter 12, §12.3). During dry periods, the peristome is safe for insects to visit, and provides a rich source of nectar (Bauer et al. 2008). This enables ants, the predominant prey of *N. rafflesiana* (Moran 1996, Adam 1997), to establish foraging trails to the pitchers. Many ant species show a pronounced division of labor with dedicated scouts constantly searching for new food sources. The temporary ineffectiveness of the *N. rafflesiana* peristome ensures that scouts visiting during dry periods can safely sample nectar from the peristome, and return to recruit nest-mates to the trap. When the conditions turn wet later, the established foraging trail ensures that more ants fall prey to the now slippery pitcher (Bauer et al. 2015a; Chapter 12, §12.7).

A similar pattern is expected for the rain-driven lid trapping mechanism of *N. gracilis* (Chapter 14, §14.3) because the prey of *N. gracilis* is as ant-dominated as that of *N. rafflesiana.* In addition, *Nepenthes gracilis* also may benefit from the habit of small and delicate insects to seek shelter from heavy rain on the underside of leaves. A generally poor trap performance (Tan 1997), or the formation of localized slippery patches on partially wetted peristomes (§15.3.4) also may promote scout survival and ant recruitment.

Only the genus *Nepenthes* is known to have considerable interspecific variation in functional trap features. Wax crystals may cover the entire inner pitcher wall above the digestive fluid (Figure 15.5e), the wax layer may be confined to the uppermost section of the pitcher wall (Figure 15.5g), or it may be absent altogether (Figure 15.5f). Peristome size and geometry, and fluid viscoelasticity also are variable. Differences occur not only between species, but often also between upper and lower pitchers of the same species (Gaume and Di

Giusto 2009, Bonhomme et al. 2011b, Bauer et al. 2012a, Benz et al. 2012). Moreover, Bauer et al. (2012a) and Bonhomme et al. (2011b) both noted the existence of distinct trapping syndromes with mutually exclusive combinations of morphological or physiological adaptations. For example, enlarged peristomes and viscoelastic fluids are typically found in the absence of wax crystals. Moran et al. (2013) found strong correlations between the trapping syndromes of 94 *Nepenthes* species and climate variables linked to their geographical distribution. Peristome- and viscoelasticity-dependent trapping strategies were confined to perhumid regions, whereas wax crystals dominated in drier and more seasonal areas.

15.6 Future research

Much progress has been made in unraveling trapping mechanisms of non-motile traps in the past decades, but large knowledge gaps still remain. Most research to date on adhesive, flypaper traps has focused on the physiology of mucilage production and the chemical composition of the mucilage. Comparatively few studies have examined the detailed biomechanics of prey capture, or the physical properties of the trap glues. The development of new portable devices for measuring fluid viscoelasticity in the field (Collett et al. 2015) should provide further insights into the rheology of trapping secretions, and force measurements and high-speed video recordings could be used to quantify effects of viscoelastic secretions on insect locomotion and prey retention.

Prey capture mechanisms by plants with eel traps remain speculative. For example, it is still unclear whether *Genlisea* traps are completely passive or whether the plants generate physiologically costly water currents to actively suck up prey (as does *Utricularia*; Chapter 14, §14.2.3). Fleischmann (2012a) comprehensively summarized arguments for both scenarios, visualized trap water currents with ink, and highlighted immobile trap contents such as soil debris and non-ciliate algae. Future experiments, including careful observations of water displacement inside the traps and the targeted inhibition of active water transport processes, are necessary to determine how *Genlisea* traps really work.

A great deal of knowledge has accrued over the past two decades about pitcher-plant traps, but studies have focused almost exclusively on *Nepenthes* (but see Bauer et al. 2013 for comparable work on *Heliamphora nutans*). The hairy surfaces found in pitchers of *Heliamphora, Sarracenia*, and *Darlingtonia* are

morphologically similar, but circumstantial observations suggest that not all of ↳ them are equally wettable. Systematic investigations of surface microstructures and wettability in Sarraceniaceae are needed to fully understand possibly divergent functions. Conversely, striking morphological similarities of *Cephalotus* pitchers, when compared with both *Nepenthes* and *Sarracenia,* strongly suggest evolutionary convergence of trapping mechanisms across all three families of pitcher plants. However, the trapping mechanism of *Cephalotus* pitchers has not yet been studied in any detail, so evolutionary convergence remains but an intriguing hypothesis.

We now have some understanding of various trapping mechanisms, but very little is known about either the evolution or the ontogeny of the underlying structures. The taxonomic and morphological diversity of *Nepenthes* provides an ideal opportunity for comparative morphological and phylogenetic studies, and the few studies of the evolution of trapping mechanisms have focused on this genus. Bonhomme et al. (2011b) investigated the occurrence of wax layers and viscoelastic trap fluids in 23 species and related these traits to the altitudinal distribution of the species. They also performed retention experiments with insects, but unfortunately did not analyze their results in a phylogenetic context. Further studies are needed to establish whether differences in viscoelasticity are partially responsible for variations of prey spectra between ground and aerial pitchers, or between sympatric *Nepenthes* species. The chemical composition underlying the viscoelastic properties of the *Nepenthes* pitcher fluid also remains to be investigated.

Benz et al. (2012) and Bauer et al. (2012a) independently did comparative phylogenetic analyses of *Nepenthes* pitcher traits, including peristome size and shape, and presence and extent of the wax crystal layer on the inner wall. The latter study comprised a total of 60 species and presented strong evidence for the divergent evolution of distinct trapping strategies within the genus. Ancestral state reconstruction suggested that the common ancestor of modern *Nepenthes* species had pitchers with narrow peristomes that were symmetrical in cross-section, and a full-length wax crystal layer. Further comparative studies of the trapping mechanisms and prey spectra of diverse *Nepenthes* species, and also across the Sarraceniaceae, are needed to refine these initial conclusions. Updated comprehensive molecular phylogenies are needed to place the results in a phylogenetic context.

Hardly anything is known about the ontogeny of trapping surfaces. The few ontogenetic studies on *Cephalotus* and *Nepenthes* focused on macroscopic pitcher development (Arber 1941, Froebe and Baur 1988, Owen and Lennon 1999) or physiological and trapping-related traits of already opened pitchers (Bauer et al. 2009). More studies are needed to unravel the development of the complex directional surface structures such as the peristome micropattern inside developing pitcher buds, and the genetic underpinnings controlling these developmental processes. Our understanding of the development of the epicuticular wax crystal layers is similarly limited. The mechanisms of wax biosynthesis in epidermal cells are reasonably well understood for the genetic model plant species *Arabidopsis thaliana* (Samuels et al. 2008), but the function and regulation of the biochemical machinery that produces the specific wax compounds involved in trapping mechanisms of carnivorous plants remain unknown.

The study of diverse interactions between carnivorous plants and their prey organisms, and with the environment, is fascinating and has prompted a number of astonishing discoveries over the past two decades. In recent years, engineers have increasingly taken interest in anti-adhesive plant surfaces as an inspiration for the development of self-cleaning, self-repairing, or non-sticky surfaces and paints (e.g., Wong et al. 2011, Barthlott et al. 2017; Chapter 20). Future advances in the study of surface development and genetic determination of surface patterns could open up novel approaches to pesticide-free, mechanical crop protection, and the viscoelastic trap glues of Droseraceae, Lentibulariaceae, *Triphyophyllum*, and *Roridula* may inspire the development of environmentally friendly, sugar- or resin-based elastic adhesives. The increased scientific interest in plant biomechanics and the continuing discovery of new trapping mechanisms and new species of carnivorous plants likely will provide further exciting opportunities for cross-disciplinary research and tantalizing practical applications.