Gotcha!

Flesh-eating pitcher plants are proving to be far more devious hunters than anyone imagined, as Stephanie Pain discovers

MONSTROUS man-eaters. Devourers of maidens. Flowers of evil. From the first hint that some plants had a taste for flesh, there have been horrible tales of what might be lurking unseen in far-off jungles.

Plenty of people were prepared to swallow reports of man-eating trees and bloodthirsty blooms, but naturalists had more trouble accepting the idea that plants could be carnivores. In the 1770s, the great Swedish naturalist Carl Linnaeus dismissed the idea as "against the order of nature". Even a century later, when Charles Darwin reported his meticulous observations of plants capturing and digesting insects, some still refused to believe it. One botanist found the notion so offensive he rejected Darwin's studies as "scientific garbage".

Since then biologists have got used to the idea that some plants eat animals, but now discoveries about one group, the *Nepenthes* pitcher plants, are upsetting some of their long-held beliefs. Careful experiments, high-speed video footage and some hard, sweaty work in the forests of Borneo are revealing that these plants are far more devious and more active in dispatching their prey than anyone suspected. There are even suggestions that some pitcher plants have "hunting strategies" akin to those of predatory animals.

"Pitcher plants have been studied for so long you would have thought everything would have been worked out by now," says Walter Federle, who studies insect biomechanics at the University of Cambridge. "But botanists worked mainly with herbarium specimens or plants in greenhouses. Studies carried out under natural conditions are showing a whole new side to the way pitcher plants capture and kill their prey."

With their gaping mouths and blood-red markings, *Nepenthes* put on a fearsome enough display and can capture a wide variety of prey – mostly insects but also spiders, scorpions and centipedes, the odd snail or frog and even an occasional rat. Yet their traps seem unsophisticated compared with those of other carnivorous plants. Venus flytraps snap shut on unwary insects in a fraction of a second, sundews actively embrace their victims with "flypaper" traps, while bladderworts sport explosive suction traps. For all their gruesome looks, *Nepenthes* appear to be equipped with only the most basic of traps – the passive "pitfall" trap. At least, that's what botanists thought.

Most of the 100 or so species of *Nepenthes* grow in the moist forests of south-east Asia, usually on poor soils or as epiphytes clinging to trees. To supplement their nutrient intake, they trap and digest animals in fluid-filled pitchers. The pitchers are highly modified leaves and although they vary greatly in size and shape, from finger-sized tubes to huge 3-litre jugs, they all conform to a basic pattern (see Diagram, page 37). The mouth has a pronounced rim, or "peristome", with nectar-producing glands just beneath the inside edge that lure prey into the pitcher. Inside, the walls are neatly zoned. The upper zone is smooth and waxy, while the walls below the surface of the pitcher fluid are dotted with glands that produce digestive enzymes which help break down the corpses of drowned prey.

Past studies concluded that the waxy zone was the crucial part of the trapping mechanism. Although insects' feet can usually grip the smoothest of surfaces, here they are defeated by wax crystals that slough off on contact. In lab experiments, neither ants nor flies could maintain a foothold on the waxy zone and fell into the fluid below (*New Phytologist*, vol 156, p 479). The wax also barred the exit of any that tried to climb out again. Yet Federle realised that this could not be the whole story. "Insects often fall straight over the rim and into the fluid without ever touching the waxy layer," he points out. What's more, some *Nepenthes* species lack a waxy zone altogether while others have some pitchers with it and some without – yet both are effective at capturing prey.

In search of an explanation, Federle and his colleague Holger Bohn travelled to the peat swamp forests of Brunei in north-west Borneo. Their plan was to observe the behaviour of different species of ants on the wax-free pitchers of *Nepenthes bicalcarata*, videoing their movements, monitoring the numbers falling into the pitchers and recording their efforts at climbing out.

Frustratingly, there was little to record: as others before them had pointed out, it was rare to witness the actual moment when a plant captured an insect. Most ants that wandered onto a pitcher wandered safely off again. Then Federle and Bohn had a breakthrough. Returning to their study site just after a downpour, they were astonished to see every ant that stepped onto a pitcher's rim slip helplessly into the fluid below. Inside the pitchers, many more ants were struggling to stay afloat, indicating a sudden and recent influx.

The reason was obvious, but completely unexpected. The peristomes of the pitchers were peculiarly glossy and wet. This is almost unheard of for plant surfaces, which usually repel water so that rain forms beads that roll off. Evidently there is something unusual about the peristome's surface chemistry that makes it highly wettable. When Federle dripped water onto a dry peristome, he found it spread out in seconds to form a film over the whole surface, an effect enhanced by the sculpturing of the epidermis into fine ridges running across the rim.

Back home, in lab experiments, he and Bohn found this film of water destroyed an ant's grip by preventing intimate contact between its footpads and the plant's epidermis. Like car tyres on a wet road, insects on the wet peristome were aquaplaning straight into the pitcher's mouth. The effect was so dramatic that Federle was amazed no one had noticed it before, yet previous investigators actually ruled out the rim as being part of the trapping mechanism. It is "not slippery… small insects (ants, etc) can walk freely on it", wrote one. "The peristome appears to offer a secure foothold for most visiting invertebrates," concluded another. Clearly they had not witnessed *Nepenthes* in wet conditions.

Further investigation showed that the wet peristome mechanism is just as important for those species of *Nepenthes* that have waxy inner walls. In *Nepenthes alata*, ▶

The nectar-loaded rim is only the first of the many tricks pitcher plants have up their sleeve

"The more an insect struggles, the faster it is caught - as if it is stuck in quicksand"

for instance, Bohn and Federle found the waxy walls helped in capturing prey when the peristome was dry, just as reported. When it was wet, however, most ants shot straight over the rim into the fluid – and the capture rate tripled. "We suspect that aquaplaning off the peristome is the fundamental trapping method. Other trapping structures are absent from some *Nepenthes*, but the rim is present in almost all species," says Federle.

The pitcher's skid-pan ploy is not the only one to have come to light recently. In France, ecologist Laurence Gaume at the University of Montpellier and physicist Yoël Forterre from the University of Provence in Marseille have discovered another previously unsuspected trapping mechanism that relies on the peculiar properties of pitcher fluid.

Like Federle, Gaume was puzzled by the fact that pitchers without waxy walls were as successful at capturing insects as those with them. She too had gone to Brunei to study pitchers, this time those of *Nepenthes rafflesiana*, a widespread species with a very broad spectrum of prey. Gaume noticed that the pitcher fluid was slimy to the touch, and that stringy filaments formed when she rubbed it between her fingers. She also noticed that insects falling into it found it impossible to get out: instead of repelling the fluid as they do water, they soon became wet and sank. Gaume began to suspect that the pitcher fluid had unusual properties which somehow helped trap and retain prey.

Back in France, Gaume teamed up with Forterre to investigate. They quickly ruled out any sort of rapid chemical attack: in the lab, insects plucked from the fluid dried themselves off and went on their way. Surface tension didn't come into it either: insects would sink faster if the fluid had especially low surface tension, but measurements showed it was little different from that of water. A third possibility was that the fluid was laced with surfactants, which would help to wet the prey. The researchers dismissed that too when they found that only moving insects became wet and drowned. If they dropped immobilised insects into the fluid they stayed dry. This suggested that whatever forces were at work were triggered by the insects themselves.

High-speed video footage of insects in the fluid confirmed that something unusual was happening. Dropped into a test tube of water, flies flew out within seconds and ants swam to the side and climbed out. Yet when thrown into tubes containing pitcher fluid, not a single insect escaped. "At first they struggled," says Gaume. "Soon they became coated by fluid and unable to move their wings or lift their legs." In one crucial piece of footage, a sticky filament visibly restrained a fly's leg. It was also evident that the more an insect struggled, the faster it was caught – as if it were stuck in quicksand (*PLoS One*, DOI: 10.1371/journal.pone.0001185).

While plant surfaces usually repel water, the rims of *Nepenthes* are slippery when wet

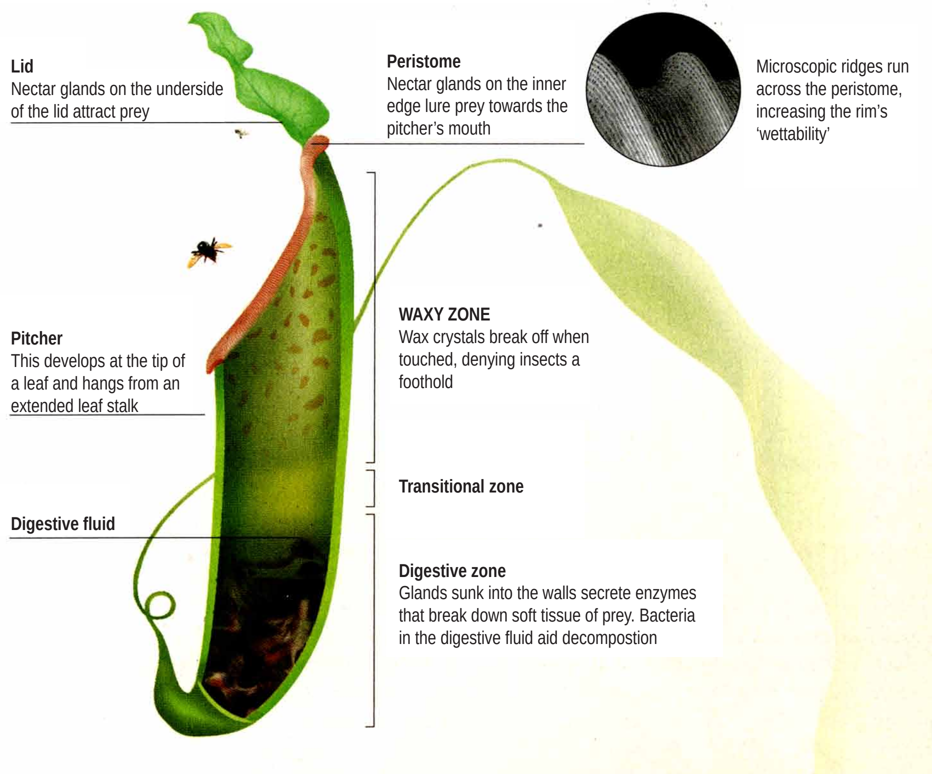
Elastic trickery

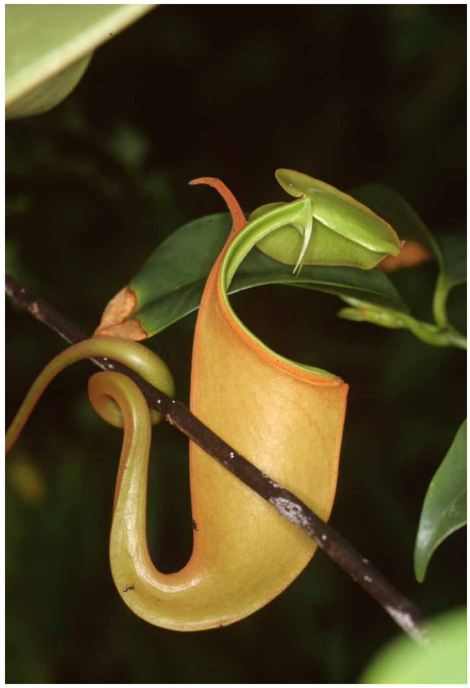
Forterre realised that the fluid's behaviour was typical of complex liquids containing long-chain polymers, known as "viscoelastic fluids". Any movement within the fluid deforms the polymers, stretching them like little springs and generating elastic forces in the fluid. If the movement ceases, the polymers eventually relax: "Particular polymer solutions have a characteristic relaxation time," explains Forterre. "If the movement is slow the elastic forces have time to relax, but if it's faster than the relaxation time then they can build up to enormous values."

His measurements showed that insects that fall into pitcher fluid move their limbs so fast the elastic forces keep on building. "The whole liquid becomes very stiff. It's as if they are swimming in jelly," says Gaume. To make matters worse, as the victim tries to lift a limb out of the liquid, it drags with it a trail of fluid that, instead of scattering as droplets,

VIEW TO A KILL

A *Nepenthes* pitcher deploys an array of features that help it trap and retain prey





The trap only works intermittently, giving *Nepenthes* an element of surprise

stretches like a rubber band, creating an elastic filament. "The filaments are like elastic ropes, which are difficult for an insect to break," says Forterre. The harder the insect kicks, the tighter the fluid's grip becomes. "The only chance of escape would be to move more slowly. But once an insect has fallen in, it usually panics and thrashes about."

For plants that grow in rainy habitats, a trap based on elastic fluid has another advantage: it keeps on working even when it becomes very dilute. In this case it was just as effective at trapping insects when diluted to just 5 per cent of its original concentration. "You need only a very low concentration of polymers to have an elastic effect," says Forterre.

The researchers are now investigating the chemistry of pitcher fluid and suspect that it will contain polysaccharides, just like other viscoelastic plant mucilages. They also want to know how widespread viscoelastic fluids are in pitcher plants. "We have preliminary data that suggest many species of *Nepenthes* might use an elastic trap," says Gaume. "It's a rather cryptic feature so it could be more common than anyone realises."

For Federle, these findings suggest that there may well be pitcher plants with other novel means of dispatching prey waiting to be discovered. "We think the wet peristome is probably universal but that there are a range of mechanisms for retaining prey," he says. For Gaume and Forterre the discoveries raise the intriguing possibility that pitchers are not quite the passive traps everyone had thought. "*Nepenthes* may not show dramatic movement like Venus flytraps, but the trapping mechanism is also based on elastic forces activated by the movement of an insect," says Gaume.

Federle's latest studies add weight to this idea. In 2005 and 2006, he returned to Brunei with Bohn and their colleague Ulrike Bauer, and attached sensors to the peristomes of *Nepenthes rafflesiana*. Round-the-clock monitoring revealed that there was a distinct daily cycle of wetness, regardless of whether it had rained. For most of the day pitchers had dry rims and captured nothing, but from early evening to early morning they were wet and highly effective at trapping prey.

The dramatic daily shift in wetness was not simply the result of water condensing from the humid air as the evening cooled. The team also observed an increase in the amount of nectar in the evening, and their experiments showed that the nectar is hygroscopic – it absorbs moisture from the air – and that this prolongs the period when the rim is wet (*Proceedings of the Royal Society B*, vol 275, p 259). "This is a novel function for nectar," says Federle. "It is possible that *Nepenthes* regulates the degree of wetting by adjusting the amount of nectar it secretes."

These findings left Federle rather puzzled. Why, he wondered, would a carnivorous plant have evolved a trapping mechanism that works only intermittently? Federle thinks that this apparent drawback could be part of a strategy to increase the number of insects it captures. For most *Nepenthes*, ants form the bulk of prey. When food supplies are patchy, ants send out scouts, and if they find a good source of food they fetch their nestmates. Lone scouts that encounter a wet pitcher will die, but those that find a dry pitcher could well return with many others. "Overall, this strategy could yield more prey than a continuous low rate of capture," says Federle.

The implications of this are intriguing. Botanists had assumed that carnivorous plants were on automatic – traps always at the ready. Federle has shown this isn't the case for *Nepenthes*. The exact time when the trap is activated varies, not just between night and day but also according to the weather, where the plant grows and nectar secretions. Federle argues that this unpredictability may have evolved for the same reason that predatory animals hunt only intermittently – to make it difficult for their prey to evolve countermeasures to avoid capture.

"The element of unpredictability may be important for *Nepenthes*," says Federle. "Ants visiting the pitcher may step onto a rim that's harmless – or slippery. They can't tell before they step onto the pitcher." So much for the idea of the simple pitfall trap hanging there waiting for insects to fall in. ●