AARON M. ELLISON

18. THEY REALLY DO EAT INSECTS

Learning from Charles Darwin's Experiments with Carnivorous Plants

INTRODUCTION

Carnivorous plants. The idea of plants eating animals conjures up visions of giant Venus's flytraps making meals of humans in a Little Shop of Horrors or Triffids marauding across the English countryside. And indeed, these strange plants have inspired countless children's books and science-fiction movies. But carnivorous plants have their serious side as well, and botanists, zoologists, and ecologists have been studying them for nearly 500 years (Figure 1).

Figure 1. The first carnivorous plant to be illustrated in any flora was a sundew, Drosera cf. rotundifolia *(from Dodoens, 1554). We now know this to be a carnivorous plant, but there is no evidence that Dodoens thought it was carnivorous. Rather, he thought it was a type of moss and he called it a 'Ros solis' (Lat: dew of the sun)*

C.J. Boulter et al. (Eds.), Darwin-Inspired Learning, 243–256.

Linneaus (1753) named the majority of carnivorous plant genera, but neither he, nor other botanists of the 1500s, 1600s and early 1700s, seriously considered that the insects found associated with these plants were anything but nuisances to be avoided (Juniper, Robins & Joel, 1989). It was Charles Darwin, who in the mid-1800s used a series of keen observations and carefully designed experiments (Darwin, 1875), to demonstrate conclusively to his colleagues that these plants actively attract, trap, kill and digest insects and other small animals. Subsequent research has supported many of Darwin's conclusions about how carnivorous plants 'work' and shown how natural selection has led repeatedly to carnivory in a number of unrelated plant lineages.

THE IMPORTANCE OF EXPERIMENTS

Many other chapters in this book have emphasized the importance of observations: getting to know the world and the organisms around us. Darwin himself was a masterful observer. His observations of geological phenomena (see Chapter 13) and geographic variation among species led him inexorably through a series of deductions to the startling conclusions elaborated in *On the origin of species* (1859). Although Darwin himself did not do any conclusive experiments to support his hypothesis that evolution proceeded by natural selection, he pointed in *On the origin of species* to a type of experiment—artificial selection for plant and animal traits—practised routinely by farmers. But genetics was still far in the future, and farmers breeding new varieties of cattle, swine or wheat knew only that selective breeding worked, not how it worked. The conclusion that improved breeds could arise from artificial selection could be ascribed to a multitude of causes, ranging from particles of inheritance to divine intervention.

Experiments are the central tool used by scientists to identify cause-and-effect relationships and to separate true causes from false ones. In most cases, scientists first state a range of different, ideally mutually exclusive, *hypotheses*: proposed explanations for an observed phenomenon based on first principles (e.g. mathematical or physical axioms or theories) or other available information derived from observations or previous experiments (Chamberlain, 1890; Platt, 1964; Taper & Lele, 2004). The essential objective of any scientific experiment is to falsify (*not prove*!) one or more of these hypotheses. After several rounds of this process of observation → hypothesis generation → experimentation → hypothesis rejection, only one hypothesis should remain standing. Superficially, this process resembles the deductive method (and maxim) of Arthur Conan Doyle's famous detective, Sherlock Holmes: 'when you have eliminated the impossible, whatever remains, however improbable, must be the truth' (Conan Doyle, 1890 p. 111). But unlike detectives and courts of law, for whom or which 'beyond reasonable doubt' is sufficient to convict, scientists are ever-skeptical of the 'truth' and persist in trying to falsify even their seemingly most bullet-proof hypotheses (Popper, 1959). In other words, good scientists are always trying to *disprove* their pet hypotheses.

Scientific understanding advances most rapidly when existing explanations for observed phenomena are found wanting and new explanations are proposed and rigorously tested. The experiments described by Darwin in *Insectivorous plants* continue to provide an inspiring example of the inherent skepticism of science and of the power of such skepticism to lead to new knowledge and a deeper understanding of the world around us.[1]

CAN PLANTS REALLY BE CARNIVOROUS?

The Pre-Darwinian View

In the years before Darwin began studying carnivorous plants, botanists had routinely ignored or elided their observations that dead insects were found stuck to or inside the leaves of what we now know as carnivorous plants (Gerard, 1633, is a notable early exception to this otherwise general rule). On the other hand, they routinely put forth a wide range of reasons to explain why plants such as sundews *Drosera*, butterworts *Pinguicula*, bladderworts *Utricularia* and pitcher plants *Sarracenia* (in the Americas)*, Nepenthes* (in Southeast Asia), and *Cephalotus* (in Australia) all had strange sticky glands, elaborately shaped leaves or other mysterious structures (summarised in Juniper, Robins & Joel, 1989). For example, some had suggested that the gooey surfaces of butterwort leaves prevented insects that were too small to be effective pollinators from reaching the flower. It was also asserted that the water-filled pitchers of *Sarracenia* provided refuges for insects fleeing predation by frogs, and that flies would be released by the Venus's flytrap *Dionaea muscipula* after they ceased struggling.

Only the Australian pitcher plant *Cephalotus follicularis* was suspected of actually using insects for food. In December 1800, Robert Brown, a naturalist traveling with Matthew Flinders' expedition around Australia, observed and collected *Cephalotus* in south-west Australia. Brown observed that dead ants filled the plant's water-filled pitchers and Flinders wrote:

> Amongst the plants collected by Mr. Brown and his associates, was a small one of a novel kind, which we commonly call the pitcher plant. Around the root leaves are several little vases lined with spiny hairs, and these were generally found to contain a sweetish water, and also a number of dead ants. It cannot be asserted that the ants were attracted by the water, and prevented by the spiny hairs from making their escape; but it seemed not improbably, that this was a contrivance to obtain the means necessary either to the nourishment or preservation of the plant (Flinders, 1814, p. 64).

By the late 1700s and early 1800s, increasing evidence from careful observations of living specimens, such as those described above by Brown and Flinders in Australia, was leading to new thinking about many of these plants. For example, John Ellis, in his description of the Venus's flytrap wrote:

> Each leaf is a miniature figure of a Rat trap with teeth: closing on every fly or other insect that creeps between its lobes, and squeezing it to Death. (Ellis, 1770, caption Plate 1)

In the full description of *Dionaea*, Ellis further adds (italics in the original):

> ... that nature may have some view towards its *nourishment*, in forming the upper joint of its leaf like a *machine* to catch food. (Ellis, 1770, p. 37)

But Ellis also asserted (1770, p. 37) that *Dionaea* could not distinguish between live insects (prey) and 'a vegetable or mineral substance'. A century later, Darwin would use experiments to show otherwise.

In the intervening decades, the leaves and stalked glands ('tentacles') of sundews were clearly seen to move and 'imprison' insects (Sowerby, 1790, p. 867). Macbride (1818, p. 52) observed that flies walking unsteadily on the rim of the tube-shaped leaf of the yellow pitcher plant *Sarracenia flava* would lose their footing as an 'impalpable or loose powder' on the rim suddenly gave way, leaving only a surface of 'perfect smoothness' off which the fly slipped and fell into the pitcher.[2] Hooker (1858, p. 5080) noted that the pitcher of *Nepenthes villosa* is 'a great provision of nature for decoying and for the destruction of insects'. The observational stage was now set for Darwin's experiments.

DARWIN'S EXPERIMENTS WITH CARNIVOROUS PLANTS

Darwin's central achievement, described in *Insectivorous plants* (Darwin, 1875), was to use controlled, manipulative experiments to distinguish fiction from fact. The facts accumulated by Darwin's experiments with carnivorous plants eventually led to the development of new and testable theories of the evolutionary origin of carnivorous plants and how natural selection allows them to persist among their non-carnivorous relatives.[3]

Darwin's Experiments with Sundews

More than two-thirds of Insectivorous plants recounts Darwin's experiments with the round-leaf sundew *Drosera rotundifolia* (see Figure 2). This small plant, with leaves barely two centimetres across, grows throughout the northern hemisphere in bogs and fens. It can be nestled in and among Sphagnum mosses, its sticky, glistening leaves barely visible in the relatively giant forest of moss, or it can form dense, very visible aggregations on open mudflats.

Darwin opens *Insectivorous plants* with a short paragraph that is remarkable for its clarity, concision, and richness of data and hypotheses:

> During the summer of 1860, I was surprised by finding how large a number of insects were caught on the leaves of the common sun-dew (*Drosera rotundifolia*) on a heath in Sussex. I had heard that insects were thus caught, but knew nothing further on the subject. I gathered by chance a dozen plants,

> bearing fifty-six fully expanded leaves, and on thirty-one of these dead insects or remnants of them adhered; and, no doubt, many more would have been caught afterwards by these same leaves, and still more by those as yet not expanded. On one plant all six leaves had caught their prey; and on several plants very many leaves had caught more than a single insect. On one large leaf I found the remains of thirteen distinct insects. Flies (Diptera) are captured much oftener than other insects... . As this plant is extremely common in some districts, the number of insects thus annually slaughtered must be prodigious. Many plants cause the death of insects, for instance the sticky buds of the horse-chestnut (*Aesculus hippocastanum*), without thereby receiving, as far as we can perceive, any advantage; but it was soon evident that *Drosera* was excellently adapted for the special purpose of catching insects, so that the subject seemed well worthy of investigation. (Darwin, 1875, pp. 1-2)

The 'surprise' in the opening sentence points out how few reliable facts were known about sundews in spite of its widespread distribution and abundance.[4] From the description, the reader can derive an estimate of the probability of insect capture by leaves (31/56 = 0.55; cf. Dixon, Ellison & Gotelli, 2005; Ellison & Gotelli, 2009), an estimate of the upper bound of the maximum number of insects caught per leaf (13), and an hypothesis that flies are the most frequently captured insect. Finally, Darwin compares sundews to horse-chestnuts. The latter, like many other plants armoured with spines, bristles or sticky hairs, kills insects but do not derive benefits from them. In contrast, he hypothesises that sundews appear to be 'excellently adapted' to capture insects, and presumably derive some benefit from doing so.[5]

Darwin then proceeds to describe in detail the range of experiments he used to determine: whether *Drosera* is responsive to different kinds of stimuli; if the

Figure 2. The round-leaf sundew Drosera rotundifolia *with an entrapped ant. (© Aaron M. Ellison)*

response is sensitive to temperature, the kind of substance stuck to the leaf or various poisons; and whether and how it digests and absorbs nutrients from material stuck to the leaves. Throughout, Darwin works with a variety of artificial prey—bits of meat and other animal parts, liquids (including human urine) and salts containing nitrogen (ammonia), phosphorus from chemical salts or infused from leaves, as well as glass, cinders, his wife's hair, chalk—caused to land on and stimulate the sundew's sticky leaf pad once or repetitively. Unlike with real insect prey, Darwin was able to control carefully the precise chemical composition and exact amounts of each of these substances—in one case as little as one twenty-millionth of a grain (3.3 nanograms) of ammonium phosphate [$(NH_4)_3PO_4$]—and their precise placement on the leaf. Such precision and control is now seen as the *sine qua non* of a scientific experiment, and permits rigorous testing and evaluation of scientific hypotheses.

At the same time, it is important that the artificial conditions of the garden or a laboratory experiment are relevant to the messier conditions of the 'real world' (for further discussion, see Chapter 2). Darwin was certainly aware of this need. For example, when reporting his results of how 'motor impulses' appeared to be transmitted from one part of the leaf to another, he wrote:

> I will give here a case not included in the above thirty-five experiments [on transmission of motor impulses]. A small fly was found adhering by its feet to the left side of the [leaf] disc. The tentacles on this side soon closed in and killed the fly; and owing probably to its struggle whilst alive, the leaf was so much excited that in about 24 hrs. all the tentacles on the opposite side became inflected; but as they found no prey, for their glands did not reach the fly, they re-expanded in the course of 15 hrs.; the tentacles on the left side remaining clasped for several days. (Darwin, 1875, p. 237)

But after this (and several other specific and unique examples), Darwin returns to the 'general results' from the controlled experiments.

After conducting literally hundreds of experiments on *Drosera rotundifolia*, and observing half a dozen other species of sundews he had growing in his greenhouse, Darwin was able to draw a number of key conclusions. The leaves capture insects using a sticky fluid poised at the ends of the tentacles densely arrayed on each leaf's surface. These tentacles move inward and envelop the prey. Movement is stimulated more by animal substances than by inert ones, and only when the glands are touched more than twice. Thus, a raindrop or a passing breeze does not trigger the prey-capture response. The sensitive parts of the leaves are the glands, tentacles and cells immediately beneath them. The movement of tentacles spreads across the leaf surface in a manner similar to a reflex or a motor impulse seen in animal neurons. Finally, the leaves truly dissolve insect prey and the glands absorb the digested nutrients. Meat is more readily digested and absorbed than cartilage, and the plants are especially sensitive to direct additions of nitrogen and phosphorus.

Darwin's experiments did not provide direct proof that sundews grew better when fed additional prey (see also Note 6). He had done an experiment in which

200 sundews were transplanted from the field into small dishes. All of the plants were covered with gauze so that insects could not be captured by any of the plants. Then, half of the plants were fed additional roast meat, and half were left unfed ('starved'). All of the plants died, however; Darwin's son Francis wrote that 'the experiments intended to decide the question [i.e. provide direct proof that sundews or other carnivorous plants get a substantial benefit from capturing, digesting, and absorbing prey] only failed through an accident' (F. Darwin, 1878a, pp. 222-223). Francis Darwin repeated the experiment in 1877, with better success (F. Darwin, 1878a, 1878b). In the season in which they were fed, the fed plants grew somewhat better—exclusive of flowers, fruits, and seeds they were just over 20% heavier than the starved plants—but more dramatically, the fed plants produced more than twice as many seeds as the starved plants, and the seeds of the fed plants weighed nearly twice as much as the seeds of the starved plants (F. Darwin, 1878a). Francis only harvested half of the plants, however; the remainder were left to overwinter (as dormant winter buds, or hibernacula). When they re-sprouted in the spring, they were not fed at all, but they continued to grow, using nutrients stored in the hibernacula. After about 10 weeks (from mid-January to 3 April 1878), the plants were harvested, dried, and weighed. The plants that had been fed the previous season were just over twice as heavy as the plants that had been starved the previous season.[6] Unknown to Francis Darwin at the time he did his experiment, Kellermann & von Raumer had done a similar experiment with *Drosera rotundifolia* fed aphids (Kellermann & von Raumer, 1878); the results, compared explicitly in F. Darwin (1878b) were qualitatively identical.

Despite the revolutionary nature of his findings—the experiments described in *Insectivorous plants* overturned nearly a century of botanical dogma[7]—Darwin is characteristically modest at the close of his general summary:

> I have now given a brief recapitulation of the chief points observed by me, with respect to the structure, movements, constitution, and habits of *Drosera rotundifolia* [ed]; and *we see how little has been made out in comparison with what remains unexplained and unknown* [ed]. (Darwin, 1875, p. 277)

In fact, our scientific understanding of the mechanisms by which sundews attract, capture, kill and digest prey has changed little since 1875. On the other hand, we now know much more about how carnivorous plants evolved and how the nutrients from the prey are partitioned among growth, respiration and reproduction.

Darwin's Experiments with Other Carnivorous Plants

Darwin repeated on a range of other carnivorous plants many of the experiments that he had conducted on *Drosera rotundifolia*. For example, he showed that only repeated stimulation in short succession of the trigger hairs of the Venus's flytrap would cause the leaves to close over their prey. As rain did not stimulate the inflection of the sundew's tentacles, the flytrap was similarly 'indifferent to the heaviest shower of rain' (Darwin, 1875, p. 291). Darwin explored digestion and absorption of a wide range of nutrients and other chemicals not only in *Dionaea* but also in the waterwheel plant *Aldrovanda vesiculosa*, the dewy-pine *Drosophyllum lusitanicum*, the rainbow plant *Byblis gigantea*, the flycatcher bush or vlieëbos *Roridula dentata* and many species of butterworts *Pinguicula*, bladderworts *Utricularia* and lobster-pot plants *Genlisea*.[8] As importantly, returning to his hypothesis about the difference between carnivorous plants and other plants with sticky leaves or buds, Darwin explored the ability of other plants to digest and absorb nutrients. In two saxifrages *Saxifraga umbrosa* and *S*. cf. *rotundifolia*, a white-edged cultivar of the Chinese primrose *Primula sinensis*, a pink *Pelargonium zonale,* the cross-leaved heath *Erica tetralix,* sweet four o'clock *Mirabilis longiflora* and cultivated tobacco *Nicotiana tabacum*, Darwin found repeatedly that their glandular hairs or other structures were immobile and unable to absorb nutrients.

WHERE DARWIN WENT WRONG: THE EVOLUTION OF CARNIVOROUS PLANTS

Although *Insectivorous plants* was published sixteen years after *On the origin of species*, Darwin does not dwell extensively on how carnivorous plants might have evolved. He does refer, albeit obliquely, to relationships among carnivorous plants and suggests homologies among their key structures.[9] These references suggest that he was at least developing a theory as to their evolutionary origin. Several lines of evidence pertain.

First, Darwin considered the sticky glands of all the Droseraceae (*Drosera, Dionaea, Aldrovanda, Drosophyllum, Roridula, Byblis*; but see Note 9) to have the homologous trait of being able to absorb nutrients:

> These octofid [eight-part] projections [on the leaves of *Dionaea*] are no doubt homologous with the papillae on the leaves of *Drosera rotundifolia.* (Darwin, 1875, p. 288)

> By comparing the structure of the leaves, their degree of complication, and their rudimentary parts in the six genera, we are led to infer that their common parent form partook of the characters of *Drosophyllum, Roridula*, and *Byblis*. The leaves of this ancient form were almost certainly linear, perhaps divided, and bore on their upper and lower surfaces glands which had the power of secreting and absorbing. (Darwin, 1875, p. 358)

> The above-named three genera, namely *Drosophyllum*, *Roridula*, and *Byblis*, which appear to have retained a primordial condition, still bear glandular hairs on both surfaces of their leaves; but those on the lower surface have since disappeared in the more highly developed genera, with the partial exception of one species, *Drosera binata*. The small sessile glands have also disappeared in some of the genera, being replaced in *Roridula* by hairs, and in most species of *Drosera* by absorbent papillae. *Drosera binata*, with its linear and bifurcating leaves, is in an intermediate condition... . A further slight change would

> convert the linear leaves of this latter species into the oblong leaves of *Drosera anglica*, and these might easily pass into orbicular ones with footstalks like those of *Drosera rotundifolia.* (Darwin, 1875, p. 360)
>
> The parent form of *Dionaea* and *Aldrovanda* seems to have been closely allied to *Drosera*.. ... (Darwin, 1875, p. 360)

Darwin similarly considered the production of digestive enzymes by these six genera of the Droseraceae to be homologous, although evidence for digestion by the unrelated (but see Note 9) *Pinguicula* and *Nepenthes* presented a 'remarkable problem' (Darwin, 1875, p. 361). Conversely, the third characteristic of the Droseraceae—the ability of leaves, hairs, and glands to move when stimulated[10]—was not seen as a homologous trait:

> It should, however, be borne in mind that leaves and their homologues... have gained this power [of movement when stimulated], in innumerable instances, independently of inheritance from any common parent form... . We may therefore infer that the power of movement can be by some means readily acquired. (Darwin, 1875, pp. 363-364)

Darwin's hypotheses regarding homologies and the evolution of carnivorous plants have been supported only partially by subsequent data (reviewed by Ellison & Gotelli, 2009; see also Note 9). In part, this reflects the fact that strong selection in nutrient-poor environments has led repeatedly to the evolution of carnivory in a wide range of plant lineages (Albert, Williams & Chase, 1992; Adamec, 1997). That there are only a few ways that plants have evolved carnivory—sticky traps, pitfall traps, bladders and lobster-pots—led Darwin erroneously to identify homologies in homoplasies (similar traits arising in unrelated species as a result of similar selective pressures). But perhaps more importantly (in the context of this chapter), it was impossible for Darwin in the nineteenth century, just as it is for us today, to use controlled experiments to distinguish among hypotheses for the origin of different species, genera and higher taxa.

CARNIVOROUS PLANTS SINCE DARWIN AND THE CONTINUING IMPORTANCE OF EXPERIMENTS

Changes in our understanding of how carnivorous plants 'work' have proceeded in fits and starts. Darwin's work, summarized in *Insectivorous plants*, overturned several centuries of botanical 'truths' about carnivorous plants. His detailed descriptions of how carnivorous plants capture and digest insects and other small invertebrates, as well as how they absorb nutrients, but not carbon, from their prey have, by and large, stood the test of time.[11] Darwin's emphasis on experimental demonstration of the ability of truly carnivorous plants to actively capture, entrap, kill and digest prey, and then to absorb the nutrients of the digested prey (characteristics which, along with a mechanism for prey attraction, constitute the 'carnivorous syndrome' [Juniper, Robins

& Joel, 1989, p. 3]) has consigned many other suggested ‘carnivorous’ plants to the dustbin of hopeful fantasies. Although we now recognise many more carnivorous plant species, only two (or perhaps three) truly carnivorous plant genera have been discovered since *Insectivorous plants* was published: the carnivorous bromeliad *Brocchinia* (and possibly *Catopsis*; Frank & O’Meara, 1984; Givnish et al., 1984) and the liana *Triphyophyllum peltatum* (Green, Green & Heslop-Harrison, 1979).

Intensive experimental research on carnivorous plants re-emerged in the 1940s and again in the 1980s, supporting some of Darwin’s hypotheses and overturning others. The new sets of hypotheses and theories developed in the 1980s (Givnish et al., 1984) have been re-examined critically in the last 15-20 years, and again some of the hypotheses have been supported but others have not (e.g. Ellison & Farnsworth, 2005; Ellison & Gotelli, 2009; Ellison & Adamec, 2011). At each of these times, and in all of these cases, experiments have been the critical tool used to advance scientific understanding.

Carnivorous Plants as Educational Tools

The fascination that carnivorous plants hold for children of all ages, the general ready availability of these plants from commercial growers and biological supply companies and the ease with which they can be grown both in glasshouses and in classroom terraria[12] create opportunities for a wide range of enquiry-based projects (see also discussions in Chapters 10, 13 & 27). The questions that Darwin asked about carnivorous plants, and the hypotheses that he tested, continue to be relevant to ecologists and evolutionary biologists today. For example, what is the range of adaptations shown by plants and animals? How do plants obtain nutrients when they are otherwise scarce? How does competition for these scarce nutrients lead to natural selection, new adaptations and evolutionary change? How do particular species fit into broader assemblages, food webs and ecosystems? What can we do to conserve these botanical curiosities as more and more land is used extensively and intensively for a growing human population, and the climate continues to change?

Darwin’s observations and experiments themselves—enumerating and quantifying the types of insects captured and consumed by carnivorous plants, determining what nutrients are absorbed by individual leaves and what environmental stimuli cause the plant to move and capture its prey—can be encouraged and repeated using simple tools. Technology unavailable to Darwin, but now seen increasingly in secondary schools, such as high-speed web-cams, isotope mass spectrometers and DNA sequencers, can yield new insights into the physiological ecology and evolution of carnivorous plants (e.g. Forterre et al., 2005; Butler & Ellison, 2007; Butler, Gotelli & Ellison, 2008; Ellison et al., 2012). Carnivorous plants are also being used to address questions such as how to identify and forecast tipping points in ecological systems (Sirota et al., 2013). Such experiments require only some pitcher plants, a ready supply of prey (e.g. ground-up ants or wasps) and a probe for measuring dissolved oxygen; these experiments are already being adapted for classroom use.[13] The information garnered from these experiments is likely to be useful in determining how to prevent catastrophic 'regime shifts' in ecosystems.

CONCLUSION

The key feature of any well-designed experiment is to identify a small number of hypothesised critical processes which, when carefully examined, allow the testing and (potential) falsification of one or more plausible hypotheses. All well-designed experiments have 'treatments', in which the process of interest is excluded or manipulated, and 'controls', in which the same process is unmodified. Of course, there is some variability in each individual replicate to which a treatment is applied or a control is assigned (and so all good experiments have replicates in all treatment and control groups). In botanical experiments, such variability may be caused by genetic differences among individuals; unappreciated environmental variation within a controlled environment chamber or greenhouse, such as light quantity or temperature in the centre of a bench or at its edge; or uncontrollable processes in the field. Nonetheless, the hallmark of a successful experiment is that the 'signal' (the effects of the experimental manipulation) adequately exceeds the 'noise' caused by small-scale differences in genotype, growth chambers, greenhouses or site characteristics in the field. Experiments also provide a degree of control over when, where and how a biological process is activated or manipulated, and they enable repeatability in both time and space that can never be achieved with observational studies. As a consequence of all of these attributes, from long before Darwin's time until today, experimental results provide the 'gold standard' of scientific evidence.

There are mechanical 'rules' for good experimental design: the most important is adequate numbers of independent replicates of both treatment and control individuals. But effective application of the scientific method—repeated hypothesis development, testing and rejection—still requires a lot of creativity and new thinking. Darwin's research with carnivorous plants remains an inspiring example of how to test hypotheses effectively and skeptically and generate new theories of how the world works.

NOTES

[1] The scientific method of falsification and the inherent skepticism of scientists is the fundamental point of contrast between science and religion; unlike science, religion requires faith and a suspension of disbelief.

[2] A similar phenomenon, termed 'aquaplaning' (Bohn & Federle, 2004, p. 14138) was experimentally demonstrated for the unrelated Asian pitcher plant *Nepenthes rafflesiana* by Bauer, Bohn and Federle (2008), but Macbride's observations have not yet been tested experimentally for any species of *Sarracenia*.

[3] Within a year of the publication of *On the origin of species*, Darwin had already moved on to the problem of the evolution of carnivory in plants. As he wrote to Charles Lyell in 1860 (F. Darwin 1911, p. 492):

> … at the present moment, I care more about *Drosera* than the origin of all the species in the world.

[4] Darwin references an 1860 bibliography on prior works on *Drosera*, but notes (*Insectivorous plants*, p. 1) that '[m]ost of the notices published before 1860 are brief and unimportant'.

[5] In the second edition of *Insectivorous plants* (1893), Darwin adds a section on pp. 15-16 describing subsequent experiments of the benefits, in terms of growth and especially reproduction that *Drosera* obtains from additional prey. These latter experiments were actually done by Charles Darwin's son Francis (F. Darwin, 1878a, 1878b), who, unlike his father, was able to successfully demonstrate experimentally that *Drosera rotundifolia* plants 'profit by their carnivorous habitats' (F. Darwin 1878a, p. 222). The lack of attribution of these experiments to Francis Darwin apparently resulted from a proof-reading error (Randal Keynes, personal communication, 13 November 2013).

[6] Francis Darwin's experiment (F. Darwin, 1878a, 1878b) is an example of what we now call a 'Before-After-Control-Impact' (or BACI) experiment. The plants themselves were first collected in the field, and then divided into two groups. Half were starved (the 'control') and half were fed (the 'impact'). The measure of effect is the change from the initial to the final state (hence 'before' versus 'after'). The same 'before' versus 'after' effects were tested on the plants that were allowed to overwinter (F. Darwin, 1878b). For additional details on BACI designs, see Gotelli & Ellison (2012).

[7] Mainstream botanists from the 1700s on had followed Linnaeus's lead in denying that plants could either deliberately entrap insects or use the nutrients obtained from captured prey. In the second (revised) edition of *Insectivorous plants* (1893), Francis Darwin wrote (p. 243):

> Linnaeus was unable to believe that the plant could profit by the captured insects. .. he consequently regarded the capture of the disturbing insect as something merely accidental and of no importance to the plant. Linnaeus' authority overbore criticism if any was offered; his statement about the behaviour of the leaves [in this case, of the Fly-trap] was copied from book to book.

[8] Darwin, like other botanists of the time, considered these plants to be members of the sundew family (Droseraceae), in which were placed all of the sticky-trapping carnivorous plants. Analyses done in the last 20 years have shown not only that most of these non-carnivorous plants are unrelated to the Droseraceae, but also that *Byblis* and *Roridula* are neither related to the Droseraceae nor to each other. Furthermore, Darwin, in discussing Hooker's observations on digestion of insects by the Asian pitcher plants (*Nepenthes*), wrote (pp. 361-362):

> The six genera of the Droseraceae have probably inherited this power [of digestion] from a common progenitor, but this cannot apply to *Pinguicula* or *Nepenthes*, for these plants are not at all closely related to the Droseraceae.

In fact, there is now strong support for asserting that the Droseraceae (which includes only *Drosera, Dionaea, and Aldrovanda*) is ancestral to, and the sister family of, the clade that includes *Nepenthes* (Nepenthaceae) and *Drosophyllum* (Drosophyllaceae). See Ellison & Gotelli (2009) for a detailed discussion of convergent evolution among, and current hypotheses for, phylogenetic positions of carnivorous plants

[9] The concept of homology, or the correspondence of (morphological) traits of different organisms resulting from common evolutionary history, is another of Darwin's fundamental contributions to evolutionary biology (Ghiselin, 2005).

[10] Five years later, Darwin published an entire book on movement in plants (Darwin, 1880).

[11] Darwin's understanding of the mechanism by which *Utricularia* bladders trap their prey is a notable exception. Darwin thought that small aquatic crustaceans pushed their way into the bladder, but this turns out not to be even close to an accurate description of the actual mechanism, in which Treat observed the role of 'trigger hairs' (Treat, 1875-see Chapter 2) and which Lloyd (1942) described as a nearly ideal mousetrap. The bladderwort's trap is a purely mechanical, vacuum trap. Tripping the 'trigger hairs' opens the vacuum seal, and the animal that hit them is sucked into the bladder, which rapidly (within 10 milliseconds) reseals and resets Lloyd's (1942, pp. 266-267) 'better mousetrap'.

[12] It is important to note, however, that carnivorous plants such as *Drosera rotundifolia* and other sundews, *Sarracenia* species, and many bladderworts *Utricularia* and butterworts *Pinguicula* native to temperate climatic zones go dormant for at least six weeks, and often as much as four-six months,

in the winter. Careful planning of classroom experiments is required to ensure that experiments are conducted when the plant is actively growing. D'Amato (2013) provides detailed guidelines on carnivorous plant cultivation.

[13] See the London-based INQUIRE project. See http://www.inquirebotany.org/en/discussions/pitcher-plants-as-ecosystems-ibse-626.html.

REFERENCES

Adamec, L. (1997). Mineral nutrition of carnivorous plants: A review. *Botanical Review, 63*(3), 273–299.

Albert, V. A., Williams, S. E., & Chase, M. W. (1992). Carnivorous plants: Phylogeny and structural evolution. *Science, 257*(5076), 1491–1495.

Bauer, U., Bohn, H. F., & Federle, W. (2008). Harmless nectar source or deadly trap: *Nepenthes* pitchers are activated by rain, condensation and nectar. *Proceedings of the Royal Society B: Biological Sciences, 275*(1632), 259–265.

Bohn, H. F., & Federle, W. (2004). Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. *Proceedings of the National Academy of Sciences, USA*, *101*(39), 14138–14143.

Butler, J. L., & Ellison, A. M. (2007). Nitrogen cycling dynamics in the carnivorous northern pitcher plant. *Sarracenia purpurea*. *Functional Ecology, 21*(5), 835–843.

Butler, J. L., Gotelli, N. J., & Ellison, A. M. (2008). Linking the brown and green: Nutrient transformation and fate in the *Sarracenia* microecosystem. *Ecology, 89*(4), 898–904.

Chamberlin, T. C. (1890). The method of multiple working hypotheses. *Science* (Old Series), *15*(366), 92–96.

Conan Doyle, A. (1890). *The sign of four*. London: Spencer Blackett.

D'Amato, P. (2013). *The savage garden, revised: Cultivating carnivorous plants*. Berkeley, CA: Ten Speed Press.

Darwin, C. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* London: John Murray.

Darwin, C. (1875). *Insectivorous plants.* London: John Murray.

Darwin, C. (1880). *The power of movement in plants.* London: John Murray.

Darwin, F. (1878a). Insectivorous plants. *Nature, 17*(429), 222–223.

Darwin, F. (1878b). The nutrition of *Drosera rotundifolia. Nature, 18*(449), 153–154.

Darwin, F. (1911). *The life and letters of Charles Darwin, including an autobiographical chapter*. New York, NY: D. Appleton & Co.

Dixon, P. M., Ellison, A. M., & Gotelli, N. J. (2005). Improving the precision of estimates of the frequency of rare events. *Ecology, 86*(5), 1114–1123.

Dodoens, R. (1554). *Cruijdeboeck.* Leyden, The Netherlands.

Ellis, J. (1770). *Directions for bringing over seeds and plants from the East Indies, and other distant countries in a state of vegetation. To which is added, the figure and botanical description of a new plant, Dionaea muscipula or Venus's Flytrap.* London: L. Davis.

Ellison, A. M., & Adamec, L. (2011). Ecophysiological traits of terrestrial and aquatic carnivorous plants: Are the costs and benefits the same? *Oikos, 120*(11), 1721–1731.

Ellison, A. M., & Farnsworth, E. J. (2005). The cost of carnivory for *Darlingtonia Californica* (Sarraceniaceae): Evidence from relationships among leaf traits. *American Journal of Botany, 92*(7), 1085–1093.

Ellison, A. M., & Gotelli, N. J. (2009). Energetics and the evolution of carnivorous plants: Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany, 60*(1), 19–42.

Ellison, A. M., Butler, E. D., Hicks, E. J., Naczi, R. F. C., Calie, P. J., Bell, C. D., & Davis, C. C. (2012). Phylogeny and biogeography of the carnivorous plant family Sarraceniaceae. *PLoS ONE, 7*(6), e39291.

Flinders, M. (1814). *A voyage to Terra Australis; undertaken for the purpose of completing the discovery of that vast country* (Vol 1). London, UK: W. Bulmer & Co.

Forterre, Y., Skotheim, J. M., Dumais, J., & Mahadevan, L. (2005). How the Venus flytrap snaps. *Nature, 433*(7204), 421–425.

Frank, J. H., & O'Meara, G. F. (1984). The bromeliad *catopsis berteroniana* traps terrestrial arthropods but harbors *Wyeomyia* larvae (Diptera: Culicidae). *Florida Entomologist, 67*(3), 418–424.

Gerard, J. (1633). *The herbal or general history of plants, revised and enlarged by Thomas Johnson*. London: Norton & Whittakers.

Ghiselin, M. T. (2005). Homology as a relation of correspondence between parts of individuals. *Theory in Biosciences, 124*(2), 91–103.

Givnish, T. J., Burkhardt, E. L., Happel, R. E., & Weintraub, J. D. (1984). Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. *American Naturalist, 124*(4), 479–497.

Gotelli, N. J., & Ellison, A. M. (2012). *A primer of ecological statistics* (2nd ed.). Sunderland, MA: Sinauer Associates.

Green, S., Green, T. L., & Heslop-Harrison, Y. (1979). Seasonal heterophylly and leaf gland features in *Triphyophyllum* (Dioncophyllaceae), a new carnivorous plant genus. *Botanical Journal of the Linnean Society, 78*(2), 99–116.

Hooker, W. J. (1858). *Nepenthes villosa* from Kina-Baloo, Borneo. *Curtis's Botanica Magazine, 14*(3rd series), 5080.

Juniper, B. E., Robins, R. J., & Joel, D. M. (1989). *The carnivorous plants*. London: Academic Press.

Kellermann, C., & von Raumer, E. (1878). Vegetationsversuche an *Drosera rotundifolia*, mit und ohne Fleischfutterung. *Botanische Zeitung, 36*(14), 209–218, 225–229.

Linnaeus, C. (1753). *Species plantarum, exhibentes plantas rite cognitas, ad genera relatas*. Stockholm, Sweden: Laurentius Salvius.

Lloyd, F. E. (1942). *The carnivorous plants*. New York, NY: Ronald Press.

Macbride, J. (1818). On the power of *Sarracenia adunca* to entrap insects. *Transactions of the Linnean Societyof London*, *12*, 48–52.

Platt, J. R. (1964). Strong inference. *Science, 146*(3642), 347–353.

Popper, K. (1959). *The logic of scientific discovery*. London: Routledge.

Sirota, J., Baiser, B., Gotelli, N. J., & Ellison, A. M. (2013). Organic-matter loading determines regime shifts and alternative states in an aquatic ecosystem. *Proceedings of the National Academy of Sciences, USA*, *110*(19), 7742–7747.

Sowerby, J. (1790). *English botany*. London: R. Hardwicke.

Taper, M. L., & Lele, S. R. (Eds). (2004). *The nature of scientific evidence*. Chicago, IL: University of Chicago.

Aaron M. Ellison
Harvard Forest,
Harvard University,
USA