

Out of human sight

Plant evolves to escape being harvested

5 ANANTHANARAYANAN

Human activities have affected animal behaviour, and animal and plant populations the world over. They have affected the climate and the Earth itself. Could human actions have visibly affected the course of evolution?

Yang Niu, Martin Stevens and Hang Sun, from the School of Botany, Chinese Academy of Sciences, at Kunming, the capital of the Yunnan province, and the Centre for Ecology and Conservation, University of Exeter, describe, in the journal, *Current Biology*, an instance of a plant, which is sought after by humans, that has developed a variety with leaves that cannot be easily seen, and is hence less harvested. The researchers find that this variety appears more often in regions where there is heavy harvesting, which indicates that this is a case of evolution in action – a genetic adaptation to evade a predator – in this case, the human harvester of the plant.

Animals have evolved coats that merge with the background, either to avoid being spotted and hunted, or for concealment while hunting. Birds have evolved plumage to show the readiness to mate. Plants have adapted, along with conditions of soil and humidity, to supply the needs of pollinators, even of species that could feed on pests that affect the plants, apart from colours and appearance, to signal their identity and presence to pollinators.

Such adaptations, however, have come about over geological time scales, in the time it takes for the conflicting pressures of natural conditions or populations of species of predator or prey to settle into an ecological balance. A result of this gradual evolution of biodiversity in the natural world is that features of species are largely fixed and even specialised. The web of inter-relatedness makes changes that affect one population percolate to other species and ecological systems have evolved, over long time spans, to come to the aid of dwindling species and maintain the relative numbers.

While the pressures of geological changes and the pressures of predators have shaped ecology over millennia and millions of years, a question is whether natural systems have undergone genetic changes in response to human pressures. Humans, in the course of domestication of plants and grasses, and animals, as well as forming population concentrations, even urban centres, have clearly placed a heavy load on the environment. It has led to disturbance of habitats, the decline, even extinction of species and damage to the environment. But, has human activity, in the short time since human activity began, had an evolutionary impact on species?

To answer this question, the group writing



Fritillaria delavayi, which is conspicuous, on the right, may have evolved to be more discrete, to avoid being harvested

in *Current Biology* takes the case of *Fritillaria delavayi*, a small flowering plant found on high, rocky slopes in South-west China and Tibet. The bulb of the plant is valued in traditional Chinese medicine and has been actively harvested by humans over the last 2,000 years. The question to answer is: has this intense attack by humans led to any defensive changes in the appearance of *Fritillaria delavayi*?

"Leaf colour of *F. delavayi* varies among populations from grey to brown, to green. Grey or brown types appear well camouflaged, while green individuals are conspicuous," the paper says. Recent studies have revealed a number of instances of plants using camouflage, where the appearance of the plant matches the background of where the plant is found, for protections from being eaten by animals, the paper says. In the case of *Fritillaria delavayi*, however, over the last five years, no herbivore that is a natural enemy of the plant could be found in all accessible populations in the region of Yunnan, the paper says.

Even if there are no animal predators, the plant has been the target of harvesting by humans ever since medicinal properties of its bulb were discovered, some 2,000 years ago, the paper says. If there was a correlation between the distribution of the grey to brown variety, which is the less visible variety, and the conspicuous, green variety, and the intensity of harvesting, this could be an instance of human activity impacting the course of evolution.

The team hence carried out a survey of the variations in the colour, and luminosity, of leaves, and the colours of rock and soil in eight

locations in South-west China. It was found that there was significant divergence of colours and more in the case of camouflaged, that is, the grey-brown varieties among similarly coloured rock, than the green varieties. And the match of colours was best in the native background of the plants. This indicated that the variation of colours was not random, but "a result of population-specific selection," the paper says.

Next was the association between how closely the colour of the leaves matched the background and the intensity of the harvesting pressure. The harvest pressure was both how intensely the bulbs were collected and how easy or difficult it was to get the bulbs. It was found that the more intense the collection, the closer the match between the leaves and background, which is to say, the quality of the camouflage. As for difficulty of harvest, which was reflected by the time it took to reach the bulb, it was found that plants that were more difficult to harvest (when the bulbs were deeper, for instance) were less efficiently camouflaged.

The notion was that a closer colour match resulted in longer detection time, and hence in less harvest. To make sure that this was a fact, there was an online citizen science experiment, where people were asked to "locate a fritillary target as quickly as possible in each of 14 randomly allocated photo slides, simulating the herb collection process by collectors." As expected, better camouflaged plants took longer to be detected.

The images used were both based on three colours – red, green, blue – or on two colours – blue and yellow. This allowed for a comparison between the sensitivity of human eyes and possi-

ble natural herbivores, which see in only two colours. It was seen that the plants in three colour images were spotted faster. As the plants are intensely harvested by humans, having the right colour would hence greatly improve a plant's chances of avoiding detection, the paper says.

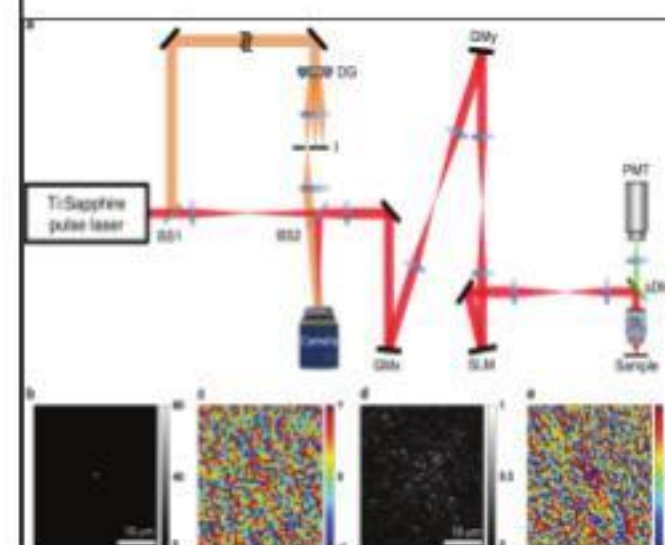
In principle, camouflage could have arisen as a result of selection against detection by animal herbivores, if any, even before human harvesters became significant. This possibility, however, is remote, the paper says, as no animals that targeted the plants or the bulbs were seen in any of the eight populations studied. In any case, the paper says, the bulbs were rich in alkaloids, chemicals that keep animals, like rodents away. While it is the alkaloid content that makes for the use of the bulbs in traditional medicine, it is clear that the colour-specific distribution of the plants is an evolutionary response to human predation.

There have not been many studies of how the plants have evolved under harvesting pressure, the paper says. Given the complexity of the visual environment, the subject is one with several aspects to examine. As a reverse of camouflage, there is the incidence of mimicry, by weeds, of cultivated plants, an unintentional result of selection by humans, the paper says. "Given that humans have long collected animals and plants for a variety of traits, we expect there to be many other analogous examples of humans driving changes in colouration in the wild," the paper concludes.

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PLUS POINTS

New microscope



Scientists have developed a new type of microscope that is able to see through skulls.

A research team led by professor Choi Wonshik at the Centre for Molecular Spectroscopy and Dynamics in Seoul, South Korea developed the tool, which has been able to create a microscopic map of neural networks in a mouse's brain through the animal's skull.

Such an act is difficult to achieve without damaging flesh and bone. Skulls are thick, and inconsistent, which means that light shone on them scatters easily. The deeper that scientists wish to see, the more difficult it becomes. Even when it is achievable, it is challenging. When light travels through biological tissue, two types of photons (the massless elementary particle that makes up light) are generated: ballistic photons and multiply scattered photons.

Ballistic photons can travel straight through the object without deflection,



Choi Wonshik

but the scattered photons show up as noise on the image. The further through the light must travel, the worse this ratio becomes, as scattered photons become more numerous than ballistic photons. Moreover, optical aberration of ballistic photons can reduce the contrast and blur the image when the picture is reconstructed. In neuroscience research, this has meant that optical imaging a mouse's brain has meant the skull has to be removed or thinned.

This new microscope, however, might provide the answer. Called a reflection matrix microscope, it uses both its own hardware and a computational adaptive optics algorithm to correct faults in the image.

Conventional imaging microscopes discard all out of focus light when they are used, only focusing on those at the point of illumination; this reflection matrix microscope, by contrast, records all the scattered photons at their positions. The algorithm then corrects the scattered photons, selectively extracts ballistic light, and corrects severe optical aberrations. The scientists claim that the number of aberrations that can be corrected is 10 times greater than that of standard systems.

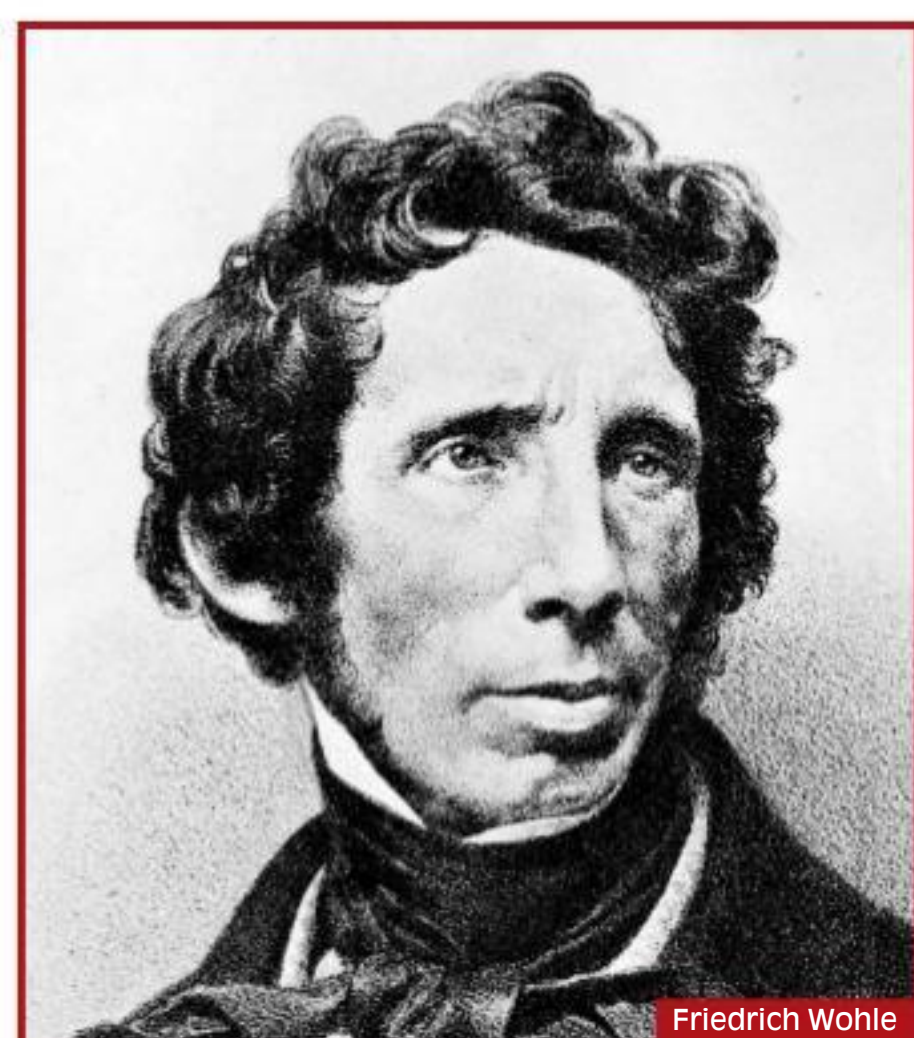
"Reflection matrix microscope is the next-generation technology that goes beyond the limitations of conventional optical microscopes," professor Choi said. "This will allow us to widen our understanding of the light propagation through scattering media and expand the scope of applications that an optical microscope can explore."

This microscope also has the advantage of being able to be used in conjunction with conventional two-photon microscopes that are being used in life sciences, removing aberrations in the image. The team demonstrated this by taking fluorescent images of a dendritic spine of a neuron behind a mouse's skull -- something that would not normally be possible without removing brain tissue entirely.

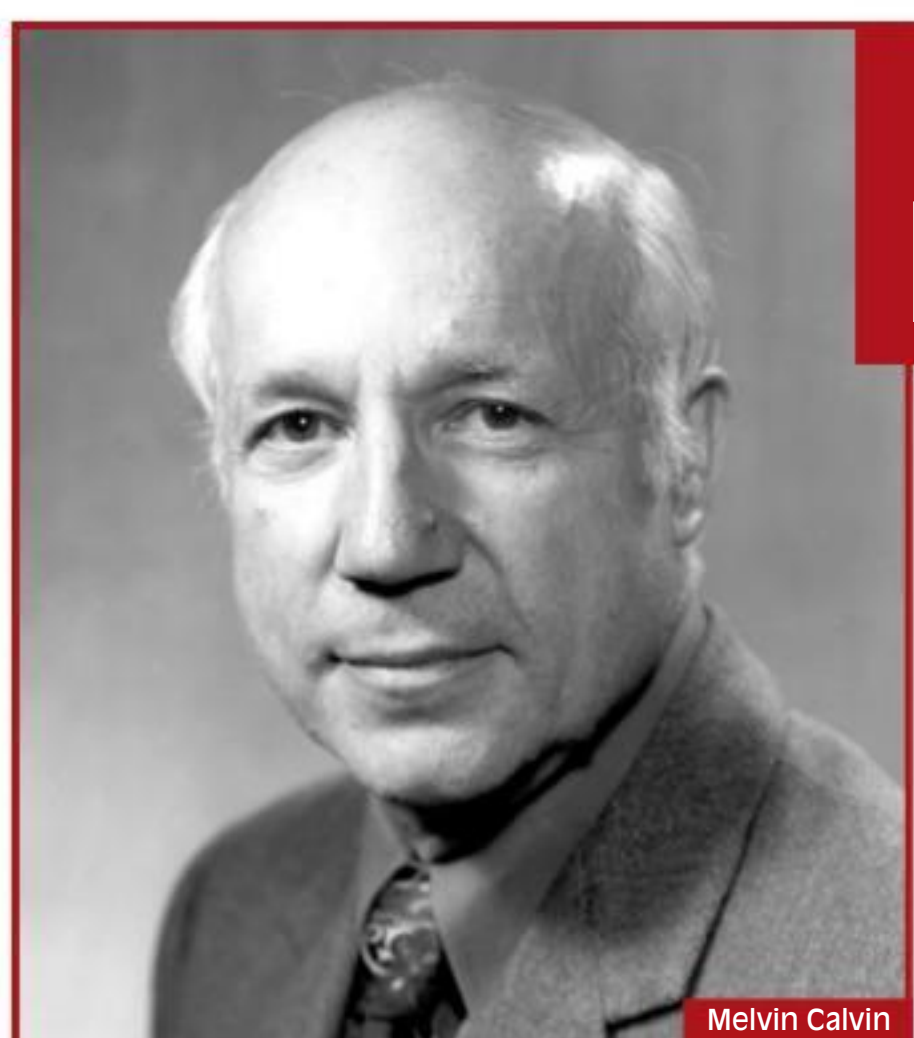
This new development means it is now possible to investigate the brain in its most native states, the scientists claim. "By correcting the wavefront distortion, we can focus light energy on the desired location inside the living tissue", said professor Yoon Seokchan and graduate student Lee Hojun, who conducted the study, in a statement. "Our microscope allows us to investigate fine internal structures deep within living tissues that cannot be resolved by any other means. This will greatly aid us in early disease diagnosis and expedite neuroscience research."

The research, entitled "Laser scanning reflection-matrix microscopy for aberration-free imaging through intact mouse skull", was published in *Nature Communications*.

—THE INDEPENDENT



Friedrich Wohler



Melvin Calvin

BIOCHEMISTRY DOWN THE YEARS

In the early 19th century, principles of chemistry were first applied to the hitherto unchangeable world of biology

ISOTOPE VS RADIOISOTOPE

Different atoms of a given chemical element may have the same atomic number and nearly identical properties but differ in the number of neutrons and hence in atomic weight; an isotope refers to the atoms with a specific number of neutrons and thus a particular atomic weight.

A radioactive isotope, or radioisotope, is an isotope that is unstable, emitting subatomic particles – either alpha or beta particles – and, in some cases, gamma rays as it undergoes spontaneous conversion to a stable form.

TAPAN KUMAR MAITRA

Around the time when cytologists were starting to explore cellular structure with their microscopes, other scientists were making observations that began to explain and clarify cellular function.

Much of what is now called biochemistry dates from a discovery reported by German chemist Friedrich Wohler in 1828. Wohler was a contemporary (as well as fellow countryman) of Matthias Jakob Schleiden and Theodor Schwann. He revolutionised our thinking about biology and chemistry by demonstrating that urea, an organic compound of biological origin, could be synthesised in the laboratory from an inorganic starting material, ammonium cyanate.

Until then, it had been widely held that living organisms were a world unto themselves, not governed by the laws of chemistry and physics that apply to the non-living world. By showing that a compound made by living organisms – a "bio-chemical" – could be synthesised in a laboratory just like any other chemical, Wohler helped to break down the conceptual distinction between the living and non-living worlds and to dispel the notion that biochemical processes were somehow exempt from the laws of chemistry and physics.

Another major advance came about 40 years later, when Louis Pasteur linked the activity of living organisms to specific processes by showing that living yeast cells were needed to carry out the fermentation of sugar into alcohol. This

observation was followed in 1897 by the finding of Eduard and Hans Buchner that fermentation could also take place with extracts from yeast cells – that is, the intact cells themselves were not required. Initially, such extracts were called "ferments," but gradually it became clear that the active agents in the extracts were specific biological catalysts that have since come to be called enzymes.

Significant progress in understanding of cellular function came in the 1920s and 1930s as the biochemical pathways for fermentation and the related cellular processes were elucidated. It was a period dominated by German biochemists such as Gustav Embden, Otto Meyerhof, Otto Warburg, and Hans Krebs.

Several of those men have long since been immortalised by the pathways that bear their names. For example, the Embden-Meyerhof pathway for glycolysis was a major research triumph of the early 1930s. It was followed shortly by the Krebs cycle (also known as the TCA cycle). Both pathways are important because of their role in the process by which cells extract energy from foodstuffs. At about the same time, Fritz Lipmann, an American biochemist, showed that the high-energy compound adenosine triphosphate (ATP) is the principal energy storage compound in most cells.

An important advance in the study of biochemical reactions and pathways came as radioactive isotopes such as ³H, ¹⁴C, and ³²P began to be used to trace the metabolic fate of specific atoms and molecules. Melvin Calvin and

his colleagues at the University of California, Berkeley, were pioneers in this field as they traced the fate of ¹⁴C-labelled carbon dioxide, ¹⁴CO₂, in illuminated algal cells that were actively photosynthesising. Their work, carried out in the late 1940s and early 1950s, led to the elucidation of the Calvin cycle, as the most common pathway for photosynthetic carbon metabolism is called. The Calvin cycle was the first metabolic pathway to be elucidated using a radioisotope.

Biochemistry took another major step forward with the development of centrifugation as a means of separating and isolating subcellular structures and macromolecules on the basis of size, shape, and/or density, a process called subcellular fractionation. Centrifugation techniques used for this purpose include differential centrifugation and density gradient centrifugation, which separate organelles and other subcellular structures based on size and/or density differences, and equilibrium density centrifugation, a powerful technique for resolving organelles and macromolecules based on density differences.

Especially useful for the resolution of small organelles and macromolecules is the ultracentrifuge, which was developed in Sweden by Theodor Svedberg in the late 1920s. An ultracentrifuge is capable of very high speeds – over 100,000 rpm – and can thereby subject samples to forces exceeding 500,000 times the force of gravity. In many ways, the ultracentrifuge is as significant to biochemistry as the electron microscope is to cytology. In fact, both instruments were developed at about the same time,

so the ability to see organelles and other subcellular structures came almost simultaneously with the capability to isolate and purify them.

Other biochemical techniques that have proven very useful for the isolation and purification of subcellular components include chromatography and electrophoresis. Chromatography is a general term that includes a variety of techniques in which a mixture of molecules in solution is progressively fractionated as the solution flows over a nonmobile absorbing phase, usually contained in a column. Chromatographic techniques separate molecules based on size, charge, or affinity for specific molecules or functional groups.

Electrophoresis, on the other hand, refers to several related techniques that utilise an electrical field to separate molecules based on their mobility. The rate at which any given molecule moves during electrophoresis depends upon its charge and size. The most common medium for electrophoretic separation of proteins and nucleic acids is a gel of either polyacrylamide or agarose.

With an enhanced ability to see subcellular structures, fractionate, and isolate them, cytologists and biochemists began to realise the extent to which their respective observations on cellular structure and function could complement each other, thereby laying the foundations for modern cell biology.

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