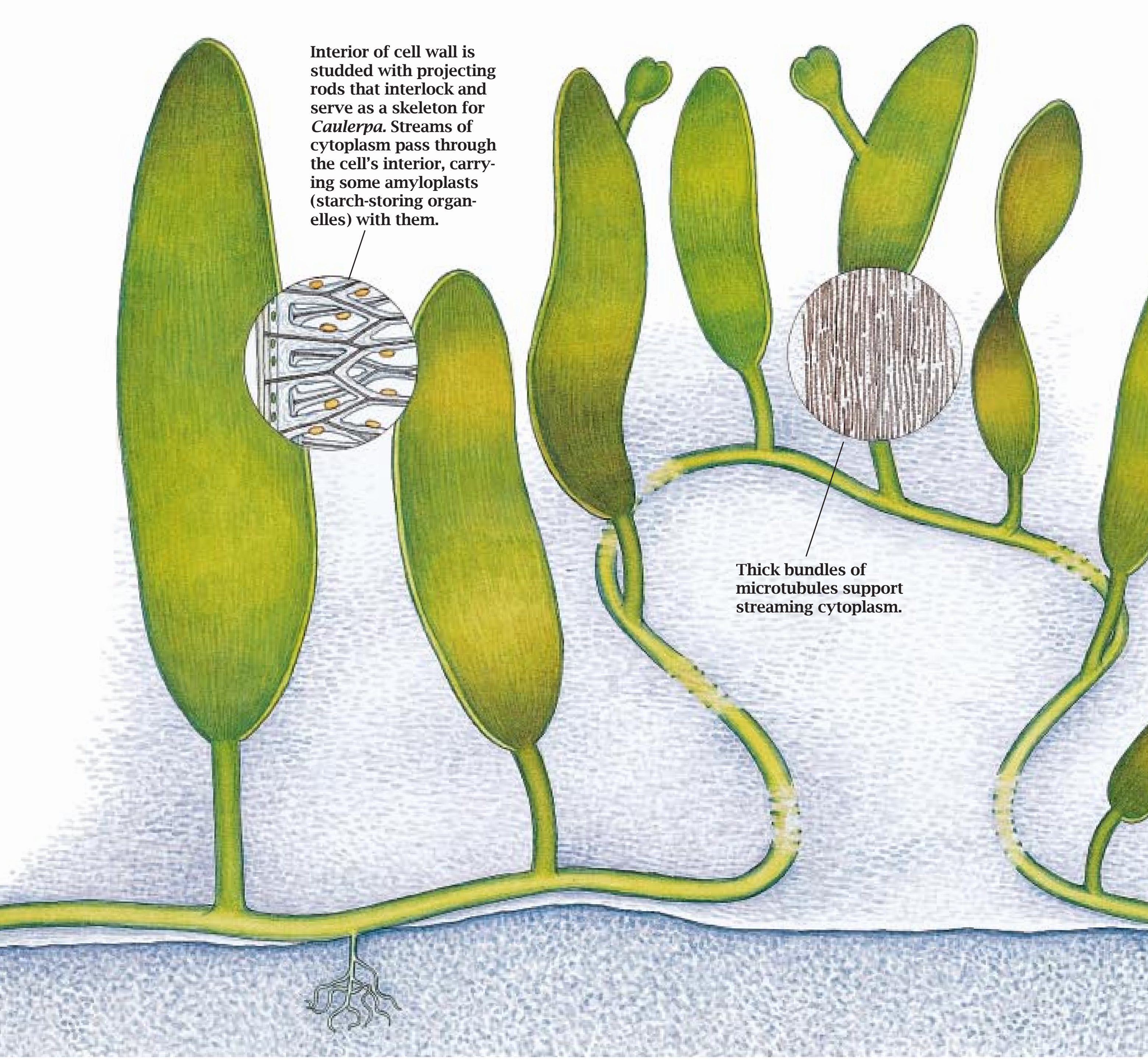
Caulerpa

This tropical alga is the world's largest single-celled organism. Yet it differentiates into a complex structure of leaves, stems and roots

by William P. Jacobs



Interior of cell wall is studded with projecting rods that interlock and serve as a skeleton for *Caulerpa.* Streams of cytoplasm pass through the cell's interior, carrying some amyloplasts (starch-storing organelles) with them.

Thick bundles of microtubules support streaming cytoplasm.

Swimming with faceplate and snorkel over a lagoon where *Caulerpa* grows, you would be unlikely to notice anything unusual about this green plant protruding from the coral sand. It looks much like the sea grasses that also thrive in warm, shallow seas around the world. The horizontal stem and branched, leafy form of *Caulerpa* resemble those of many higher plants, such as the bracken fern or the strawberry plant. But an internal examination reveals the alga's uniqueness. *Caulerpa* is the largest, most differentiated single-celled organism in the world. No cell wall or membrane separates each of the many nuclei and their adjacent cytoplasm from the others.

This unusual, unexpected organism remains unknown to most biologists, although it was first described almost 150 years ago. By its very existence, *Caulerpa* is a gauntlet flung in the face of biological convention. No single cell should be capable of growing to a length of two or three feet, much less differentiating into separate organs, such as a stem, roots and leaves. The questions raised by *Caulerpa*'s peculiar structure have intrigued the few biologists who have investigated its development.

All other organisms of such size and complexity consist of hundreds of thousands of microscopic cells. In each cell, a membrane encloses a limited volume of cytoplasm and a single nucleus. Most plants also secrete a cell wall outside the membrane. So ubiquitous is this organization that between 1838 and 1839 Matthias Schleiden of the University of Jena in Germany and Theodor Schwann of the University of Louvain in Belgium enshrined their observations in the form of the "cell theory." They posited that the cell is the basic unit of biological structure and function in both plants and animals. In the many decades since then, thousands of observations have converted the cell theory into a broadly accepted generalization that forms the basis of current ideas about biological development. Those ideas attribute the microscopic size of the average cell to the limited range of influence of the enclosed nucleus over its surrounding cytoplasm.

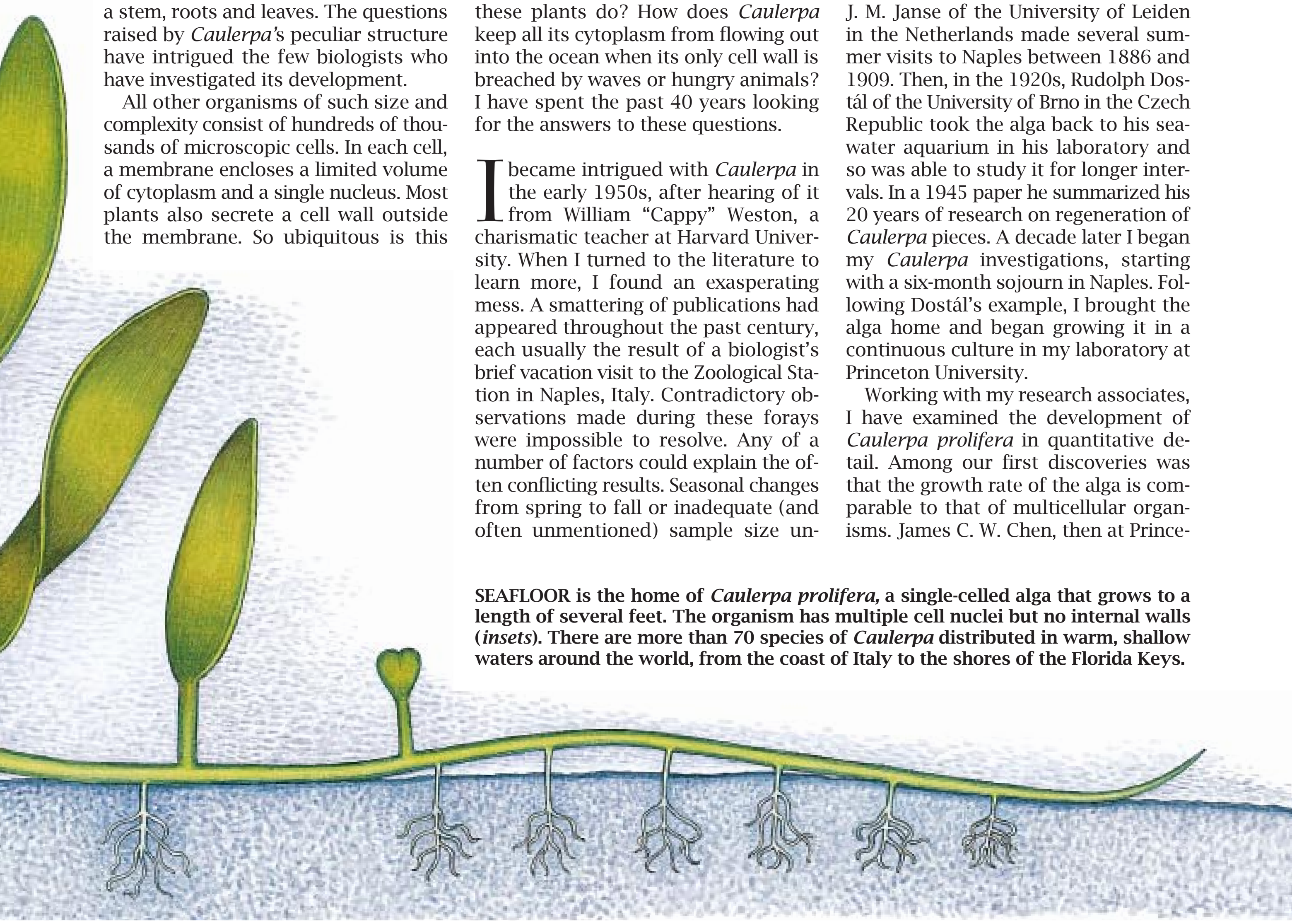
So how can *Caulerpa* grow to its macroscopic size and complexity without the compartmentalization that other organisms seem to need? Is *Caulerpa* handicapped by its single-celled form? Do hormones coordinate its development and regeneration, as they do in higher plants? If so, does *Caulerpa* employ the same chemical messengers as these plants do? How does *Caulerpa* keep all its cytoplasm from flowing out into the ocean when its only cell wall is breached by waves or hungry animals? I have spent the past 40 years looking for the answers to these questions.

I became intrigued with *Caulerpa* in the early 1950s, after hearing of it from William "Cappy" Weston, a charismatic teacher at Harvard University. When I turned to the literature to learn more, I found an exasperating mess. A smattering of publications had appeared throughout the past century, each usually the result of a biologist's brief vacation visit to the Zoological Station in Naples, Italy. Contradictory observations made during these forays were impossible to resolve. Any of a number of factors could explain the often conflicting results. Seasonal changes from spring to fall or inadequate (and often unmentioned) sample size undoubtedly affected the findings. The visitors rarely had time to repeat any experiment. And, of course, many worked before the days of statistical analyses, leaving the reader to guess at the reality of differences reported.

Only after two European biologists decided to investigate *Caulerpa* over longer periods could one have some confidence in the published reports. J. M. Janse of the University of Leiden in the Netherlands made several summer visits to Naples between 1886 and 1909. Then, in the 1920s, Rudolph Dostál of the University of Brno in the Czech Republic took the alga back to his seawater aquarium in his laboratory and so was able to study it for longer intervals. In a 1945 paper he summarized his 20 years of research on regeneration of *Caulerpa* pieces. A decade later I began my *Caulerpa* investigations, starting with a six-month sojourn in Naples. Following Dostál's example, I brought the alga home and began growing it in a continuous culture in my laboratory at Princeton University.

Working with my research associates, I have examined the development of *Caulerpa prolifera* in quantitative detail. Among our first discoveries was that the growth rate of the alga is comparable to that of multicellular organisms. James C. W. Chen, then at Prince-

WILLIAM P. JACOBS has written a dozen or so papers on giant single-celled organisms. He has also studied the hormones of land plants, their transport in shoots and roots, and their role in controlling differentiation, regeneration, growth and longevity. He received both his A.B. and Ph.D. from Harvard University. In 1948 he started as an assistant professor of biology at Princeton University, and he is now professor emeritus there. A Guggenheim Fellow, Jacobs has served on numerous committees, including advisory panels for the National Aeronautics and Space Administration.



SEAFLOOR is the home of *Caulerpa prolifera,* a single-celled alga that grows to a length of several feet. The organism has multiple cell nuclei but no internal walls (*insets*). There are more than 70 species of *Caulerpa* distributed in warm, shallow waters around the world, from the coast of Italy to the shores of the Florida Keys.

ton, and I took daily photographs to measure its growth. We found that the rhizome (the cylindrical stem of the plant) grew roughly 4.6 millimeters a day, a rate similar to that observed for stems of several multicellular plants.

The pattern of *Caulerpa*'s development, however, differs from the more complex growth seen in higher plants. Most multicellular plant organs mature at rates that vary with time, but *Caulerpa* elongates at a constant speed. In multicellular plants, some individual cells that contain multiple nuclei (as *Caulerpa*'s large cell does) also demonstrate this pattern. Thus, extended periods of constant growth may be typical of multinucleated cell structure, common both to *Caulerpa* and to certain cells in higher plants.

We were surprised to find that the elongation rates of all three organs of *Caulerpa*—stem, root and leaf—do not differ significantly from one another. In higher plants, the speed of growth varies from organ to organ. Our results from *Caulerpa* suggest that whatever factor limits development of the stem must pervade the entire plant so that it also limits the growth of the other two types of organs.

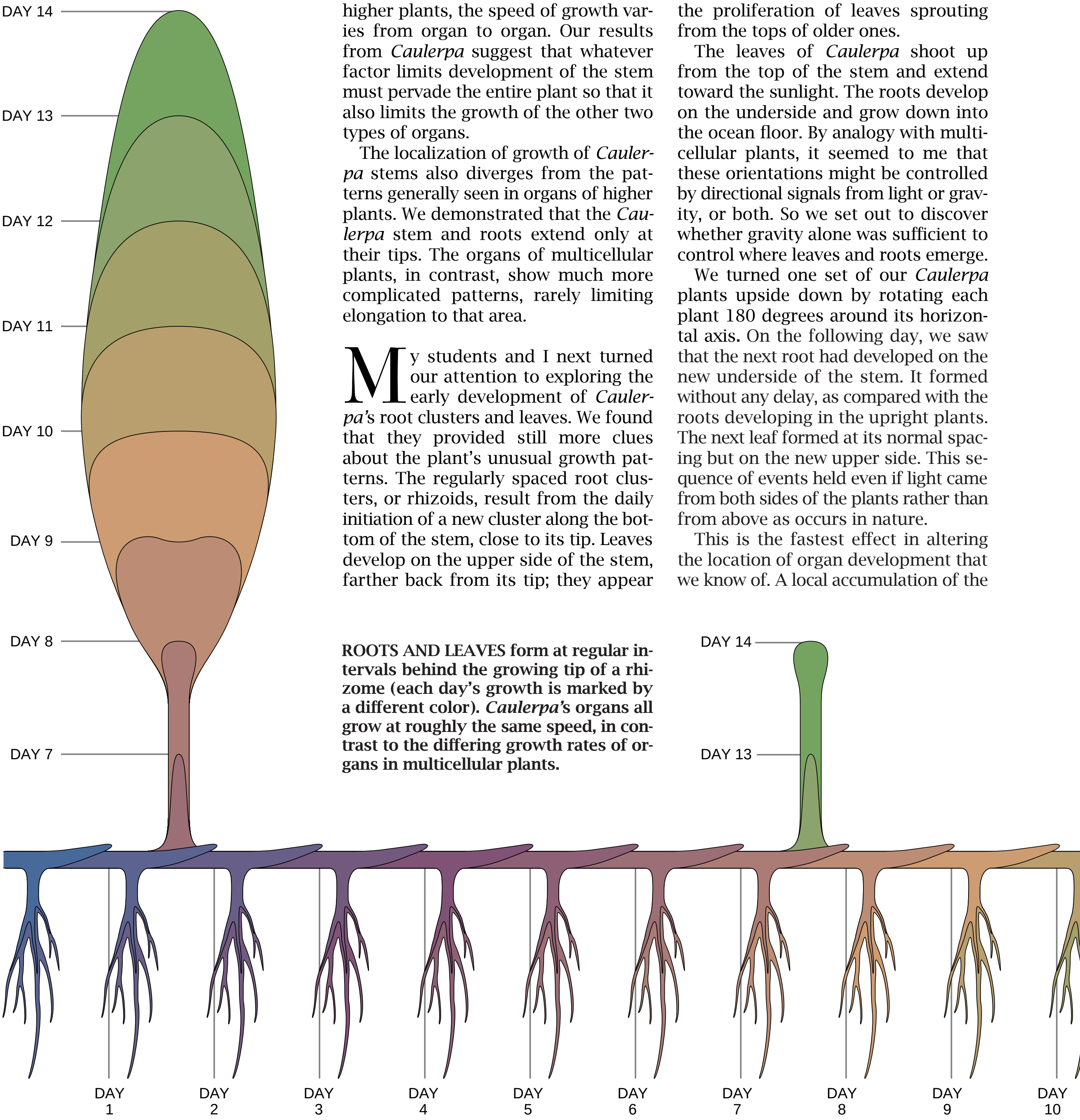
The localization of growth of *Caulerpa* stems also diverges from the patterns generally seen in organs of higher plants. We demonstrated that the *Caulerpa* stem and roots extend only at their tips. The organs of multicellular plants, in contrast, show much more complicated patterns, rarely limiting elongation to that area.

My students and I next turned our attention to exploring the early development of *Caulerpa*'s root clusters and leaves. We found that they provided still more clues about the plant's unusual growth patterns. The regularly spaced root clusters, or rhizoids, result from the daily initiation of a new cluster along the bottom of the stem, close to its tip. Leaves develop on the upper side of the stem, farther back from its tip; they appear less often and with less regularity than do root clusters. In my cultured *C. prolifera* sample, collected from the Florida Keys, a new leaf formed every five or six days. The leaf initially resembled the pointed cylinder of a new root or stem, but the tip soon grew a flattened, heart-shaped blade and unfolded into a roughly rectangular form four to five inches long. Often another new leaf started growing near the top of the primary one once it had matured. In fact, the species name *prolifera* derives from the proliferation of leaves sprouting from the tops of older ones.

The leaves of *Caulerpa* shoot up from the top of the stem and extend toward the sunlight. The roots develop on the underside and grow down into the ocean floor. By analogy with multicellular plants, it seemed to me that these orientations might be controlled by directional signals from light or gravity, or both. So we set out to discover whether gravity alone was sufficient to control where leaves and roots emerge.

We turned one set of our *Caulerpa* plants upside down by rotating each plant 180 degrees around its horizontal axis. On the following day, we saw that the next root had developed on the new underside of the stem. It formed without any delay, as compared with the roots developing in the upright plants. The next leaf formed at its normal spacing but on the new upper side. This sequence of events held even if light came from both sides of the plants rather than from above as occurs in nature.

This is the fastest effect in altering the location of organ development that we know of. A local accumulation of the



ROOTS AND LEAVES form at regular intervals behind the growing tip of a rhizome (each day's growth is marked by a different color). *Caulerpa*'s organs all grow at roughly the same speed, in contrast to the differing growth rates of organs in multicellular plants.

starch-storing structures inside cells known as amyloplasts apparently triggered the change. Michael B. Matilsky, then at Princeton, and I found that within six hours of inverting the plants there were 54 percent more amyloplasts on the new underside of the stem tip than before. We also noticed that the increased number of these organelles accumulated where the new root cluster would later develop. A corresponding decrease in the number of amyloplasts occurred on the upper side of the stem tip. Apparently the amyloplasts drifted down through the cytoplasm and settled to the bottom of the stem. Farther back from the tip, in the areas where roots do not develop, there was no redistribution of the organelles after inversion. Our results indicate that *Caulerpa* uses amyloplasts to respond to gravity just as higher plants do. Yet its reaction to gravity is somewhat different. Instead of changing only the direction of growth, gravity initiates the development of organs in novel locations on *Caulerpa.*

GROWING SHOOT (shown here in a thin section illuminated by polarized light) orients itself by means of amyloplasts. These starch-storing structures (visible as bright spots) settle onto the bottom side of the shoot as a result of gravity, and root clusters form in response to their accumulation.

Any laboratory study of *Caulerpa* originally required the removal of some alga from the ocean. How does the single-celled plant withstand being torn open by grazing fish or sadistic biologists such as myself? As soon as a leaf or stem is cut, some cytoplasm does stream out into the seawater as one would expect. But a wound plug forms, and a new wall is laid down behind it, sealing off the remainder of the cell. Such self-sealing allows *Caulerpa* to survive substantial loss of leaf area and permits the process of regeneration to begin. Indeed, regeneration of an entire *Caulerpa* plant from a piece of leaf or stem is not uncommon.

Regenerating pieces of *Caulerpa* can often be found in the sea. For many years, the lack of any evidence for other forms of reproduction led biologists to consider regeneration as perhaps the only way the plant reproduced. But in the late 1920s Dostál observed that old leaves formed tiny projections from which cytoplasm oozed. Along with the cytoplasm, the plant released flagellated cells capable of movement. By the end of the 1930s several people had observed sexual reproduction—the fusion of pairs of such cells—in different *Caulerpa* species. Nevertheless, tearing and subsequent regeneration still appear to be the most probable way that *Caulerpa* reproduces in nature.

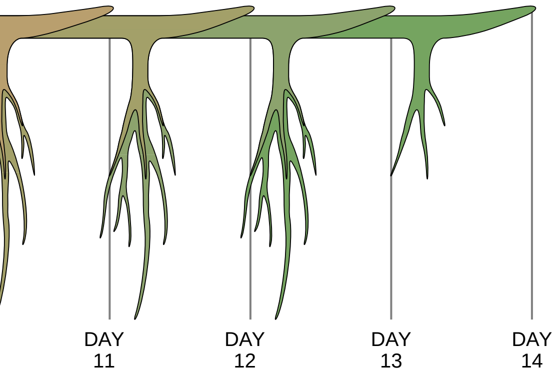
An experimental version of a wound plug permits closer study of *Caulerpa's* regeneration mechanisms. In 1904 Janse discovered that pressing together opposite walls of the cell and clamping them would lead in a few days to the development of a new cell wall known as a pressure wall. More recently I have found that this protective response happens much faster, on the order of minutes. We can then cut the alga apart at pressure walls with little loss of cytoplasm and observe the regeneration of small pieces of *Caulerpa.* Such pieces cannot regenerate otherwise, because there is not enough cytoplasm remaining to reorganize the cellular material and trigger growth.

Regeneration of the whole plant from large pieces occurs naturally; laboratory studies indicate an unexpected directionality to the process. For example, when we cut a 50-millimeter-long piece from a *Caulerpa* leaf, an entire plant regenerated in a particular polar sequence [*see illustration on next page*]. First, roots developed at the cut mad- closer to the stem. Later, a new stem grew only a few millimeters away from this cut. On the outer half of the leaf section, a new leaf began to regenerate. Most organisms exhibit such polarity in normal development as well as in regeneration (if they are capable of that). But in multicellular plants, such regeneration is usually attributed to the polar movement of growth substances through thousands of cells. Demonstration of a similar trait in single-celled *Caulerpa* surprised many.

We also used pressure walls to alter developmental pathways in regenerating pieces of *Caulerpa.* A transverse pressure wall made just below a growing leaf places the bud physiologically above the base of the section. Because the sprout sits near the bottom of its stem, the little nubbin that would have grown out as a leaf will adjust to become a root. Similarly, a bud starting as a root can often be induced to change to a leaf instead.

In view of the striking effect of gravity on the development of the stem tip, we also wondered how gravity might affect regeneration. Because leaves also contain the starch-storing amyloplasts, perhaps their gravity-induced resettling could alter regeneration patterns as well. But the pattern and number of regenerated organs were unchanged by inverting the leaf pieces. For instance, roots regenerated exclusively on the original stem end of the leaf piece, whether that end was up or down relative to gravity.

It is still unclear what determines the



directionality of regeneration of pieces of *Caulerpa.* The most likely candidate is the striking cytoplasmic streaming. Strands of moving cellular material are easily visible in the leaves, even under the low magnification of a dissecting microscope. The broad channels run roughly parallel to the long axis of the leaf, and the direction of the flow can be opposite in adjacent strands. The streams may move organ-forming substances in a particular direction, thus substituting for the myriad direction-specific transporting cells of higher plants.

If one ignored the fact that these regenerating fragments are simply multinucleated pieces of a single cell, *Caulerpa's* polar regeneration seems much like the well-known regeneration of pieces of other plants. Do *Caulerpa* species—and by extension do other algae—use hormones to coordinate development and regeneration? If so, are the hormones chemically related to those used by presumably more highly evolved plants?

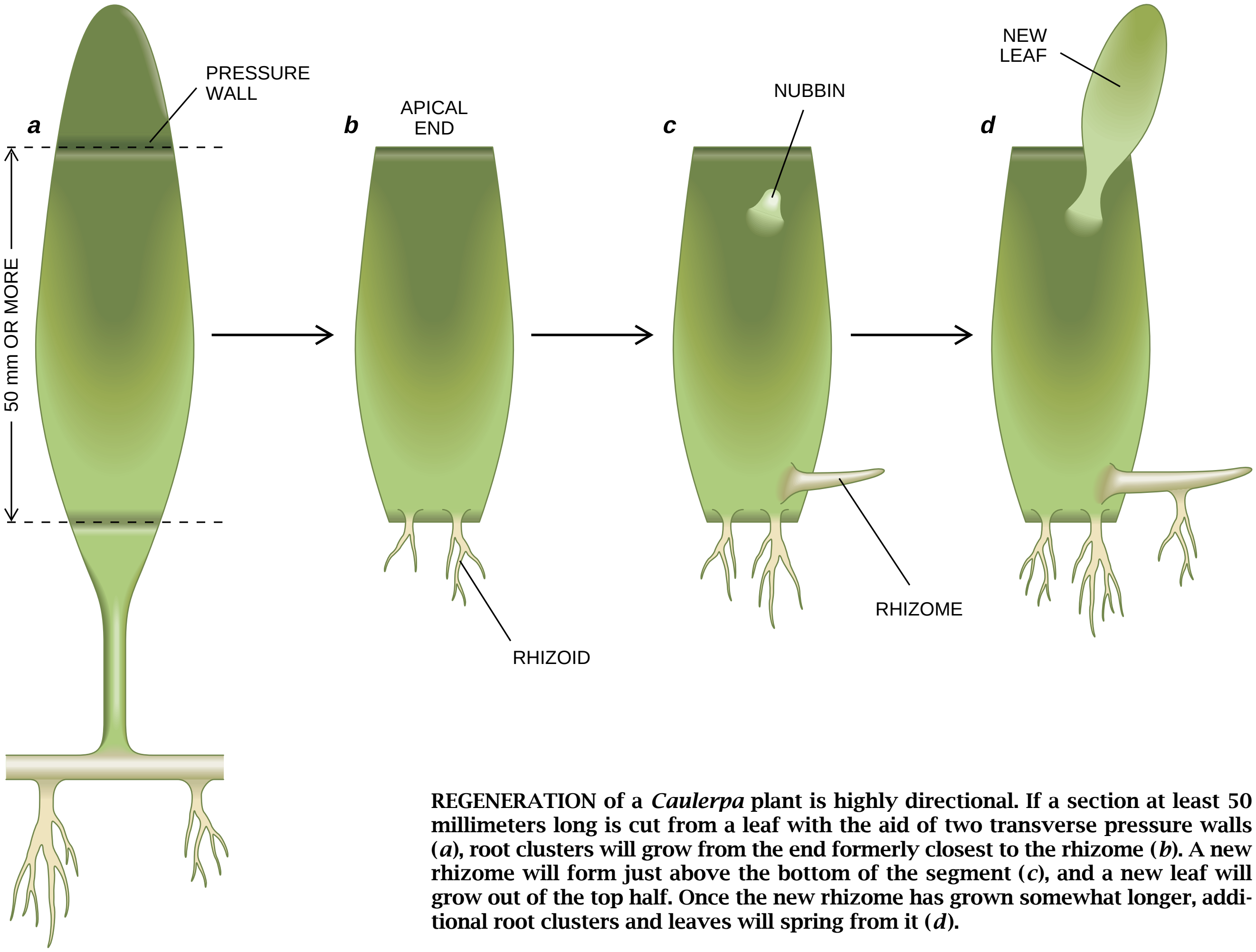
The hormone indole-3-acetic acid (IAA) helps to control regeneration in multicellular plants. We have recently documented conclusively, using gas chromatography and mass spectrometry, that IAA is present in *Caulerpa.* Work done earlier by Ned Kefford and Arun Mishra of the University of Hawaii, Clinton J. Dawes of the University of South Florida and Henry Augier of the University of Aix-Marseille II in France showed that addition of IAA to the seawater in which *Caulerpa* thrives stimulates its growth patterns. But even with evidence that IAA is present and active in *Caulerpa,* we still wondered if the action of IAA depended on selective distribution of the hormone, as it does in multicellular plants. No evidence exists for the formation of pockets of IAA within *Caulerpa.* To the contrary, if IAA labeled with radioactive carbon is added to the tip of a *Caulerpa* leaf, the radioactivity—and presumably IAA—spreads uniformly along the stem.

Gibberellins are another class of growth hormones active in many multicellular plants. Early studies reported that extracts of *Caulerpa,* when added to higher plants, triggered growth patterns just as pure gibberellins do. But my recent, more extensive analysis using mass spectrometry revealed no known gibberellin or gibberellin metabolite in such *Caulerpa* extracts. Was the gibberellinlike activity seen in *Caulerpa* caused by a chemical that did not have the basic structure of the compound but that happened to show similar activity? Or was the activity from yet another, as yet unknown, gibberellin that could be added to the already long list of more than 70 of these compounds?

We surmise that hormones produce their effects in *Caulerpa* by interaction with substances or organelles whose distribution does vary. For example, the amyloplasts that accumulate at the bottom of the growing stem tip may promote root formation by working in concert with either IAA or the gibberellinlike substance. The interaction maintains gravity-directed growth and initiates root formation at specific sites.

Although the details of many of *Caulerpa's* hormonal interactions are unclear, most of its organelles are known and resemble those of higher plants. In the peripheral cytoplasm, chloroplasts used in photosynthesis and many small nuclei stream along with the starch-storing amyloplasts. A large cytoplasmic sac, or vacuole, of convoluted shape sits in the center of the cell.

The only visibly unusual features are



REGENERATION of a *Caulerpa* plant is highly directional. If a section at least 50 millimeters long is cut from a leaf with the aid of two transverse pressure walls (*a*), root clusters will grow from the end formerly closest to the rhizome (*b*). A new rhizome will form just above the bottom of the segment (*c*), and a new leaf will grow out of the top half. Once the new rhizome has grown somewhat longer, additional root clusters and leaves will spring from it (*d*).

***CAULERPA MEXICANA* is one of several species of the alga whose leaves are deeply fringed. The roots attach to coral fragments or rocks on the bottom of lagoons. Global distribution of *Caulerpa* species (each recognizable by its distinctive form) makes it clear that a single-celled structure is no bar to successful competition with "higher" plants.**

numerous rods that project in from the cell wall that encloses the whole plant. The rods run both perpendicularly and parallel to the long axes of the organs, forming a dense, interconnecting network. Janse counted 850 rods per square millimeter in the older part of the leaf and five times as many in the tip. The rods are sheathed in cytoplasm, and their density throughout the organism partially compensates for the lack of cell membrane surface. They appear to serve as a supporting skeleton for the huge cell. A few investigators have suggested that they also serve as conduits to and from the surrounding sea.

Dinkar D. Sabnis, working with me at Princeton, looked at *Caulerpa* with an electron microscope and found a similarly unusual distribution of tiny fibers known as microtubules. We determined that sheets or thick bundles of microtubules, evenly spaced and uniformly oriented, were arrayed in the internal layer of cytoplasm where streaming occurs. Our suggestion that microtubules were related in some way to streaming was later confirmed by other researchers. Inoculating the plant with the alkaloid compound colchicine disassembled the microtubules (as expected from research on various multicellular organisms) and stopped the streaming.

Many of the riddles posed by *Caulerpa's* existence are now understood. Despite its uniquely large, single-celled organization, *Caulerpa* competes successfully with multicellular organisms that inhabit the world's warmer seas. It grows as fast as many of its land-based, multicellular, distant relatives. *Caulerpa* has proved so hardy that one species is raised commercially in seawater pens in the Philippines for use in fresh green salads. It readily regenerates entire plants from pieces of stem or leaf and does so in a temporal and spatial pattern resembling that seen in more highly evolved, multicellular plants. The fact that 73 species of *Caulerpa* exist around the world, making them far from rare in the tropical algal flora, suggests that their single-celled construction is not a great handicap.

Musing over the current knowledge we have about *Caulerpa* and the many questions still remaining, I find that evolution has provided more possibilities than we have tended to expect. If *Caulerpa* is this prominent as a large, highly differentiated, multinucleated single cell, what are the ultimate lengths to which this structure could be carried? I can see nothing that would preclude an even larger unicellular organism so long as it lives in the sea. There the buoyant water substitutes for the internal support provided to land plants by their cell walls. Might we one day discover a huge algal equivalent of "Audrey II," the outrageously large Venus flytrap of *The Little Shop of Horrors*?

FURTHER READING

ARE ANGIOSPERM HORMONES PRESENT IN, AND USED AS HORMONES BY, ALGAE? William P. Jacobs in *Plant Growth Substances 1985.* Edited by Martin Bopp. Springer-Verlag, 1986.

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