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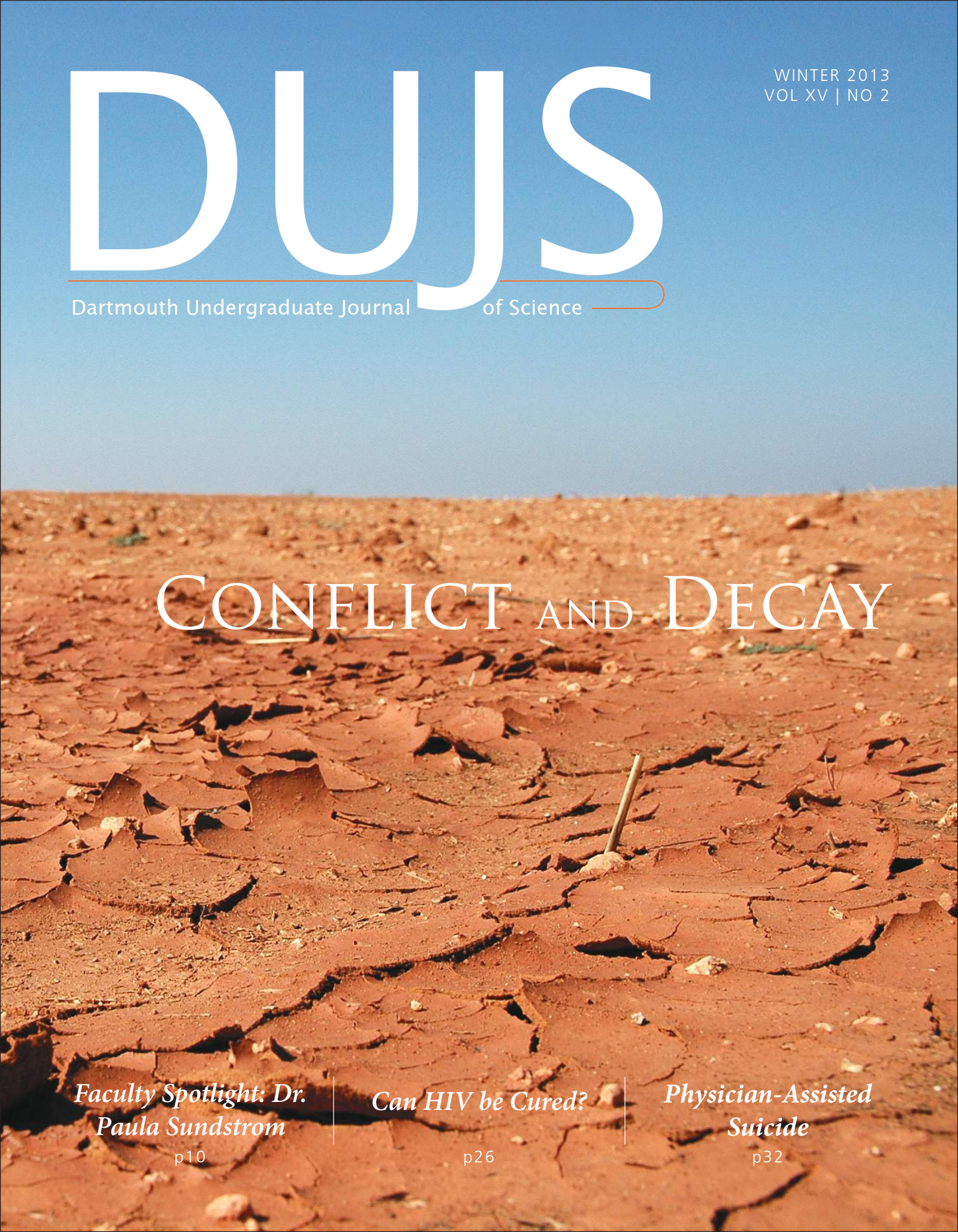

Conflict and Decay

COVER IMAGE

From iStockphoto

Drought Stricken Land

Adjustments by Steven Chen '15.

SUBMISSIONS

The *DUJS* welcomes submissions from all Dartmouth undergraduates. Please see dujs.dartmouth.edu for more information on the submission process. Letters to the Editor and requests for correction may be e-mailed to the *DUJS* or sent to:

DUJS
Hinman Box 6225
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Hanover, NH 03755

ABOUT US

The *DUJS* prints quarterly journals that include science news and review articles, along with research by undergraduates. Weekly Dartmouth Science News articles are also posted to *DUJS Online*.

Note from the Editorial Board

Dear Reader,

Conflict and decay are found throughout the natural world. For instance, organisms must compete with one another for limited environmental resources while decay enables a breakdown of organic materials into their basic components to be reincorporated into living organisms. However, the theme of "Conflict and Decay" can also be applied to human interactions. While the positive implications of scientific and technological progress are clear, the darker side of innovation is also apparent under the service of crime and warfare.

This issue's review articles tackle the issue of "Conflict and Decay" through an array of topics. Kristen Flint talks about the pros and cons of biodegradable plastic. Mackenzie Foley describes potential new dangers with genetically engineered bioweapons. Barry Chen's article studies the utility of computer forensics at the intersection of science and law. Xiongfei Zhang addresses recent advances in DNA analysis for archaic genomic sequencing. A discussion on the effect of climate change on the decline of Mayan civilization can be found in Na Eun Oh's article on climate history. Stephanie Alden has written a review on the remarkable story of the Berlin Patient. Shinri Kamei examines the history of nuclear technology in the military. Finally, Danny Wong investigates whether physician-assisted suicide is legally and ethically justifiable.

Our faculty interview of this issue features Paula Sundstrom, Ph.D., a Professor of Microbiology and Immunology at the Geisel School of Medicine. Dr. Sundstrom's research interests focus on the molecular mechanisms of fungal pathogenesis in candidiasis, the most common fungal infection in humans.

In addition, we are excited to announce that we are now accepting submissions from students at fellow undergraduate institutions. In this issue, we feature two external submissions. In a review article, Brown University student Michael Yanagisawa describes the topic of biomimicry inspired from the self-cleaning mechanism of the lotus leaf. In another review article, Riyad Seervai, also of Brown University, summarizes the progress in unveiling sexual reproduction and gametogenesis in *Saccharomyces cerevisiae*. We look forward to featuring more external articles from undergraduates in our future publications.

Finally, we have recently re-launched our website, "DUJS Online," now updated several times a week. We are also proud to announce the creation of a new comic series, "Interdisciplinary Studies," which draws out "what-if?" scenarios of famous scientists throughout history. Inspired by members of the *DUJS* and drawn by Jenny Liu, a talented Dartmouth freshman, this comic will sure to pique your scientific interests and tickle your funny bone.

Thank you for reading the *DUJS*, and we hope you enjoy this issue.

Sincerely,

The *DUJS* Editorial Board

DUJS
Hinman Box 6225
Dartmouth College
Hanover, NH 03755
(603) 646-8714
http://dujs.dartmouth.edu
dujs@dartmouth.edu

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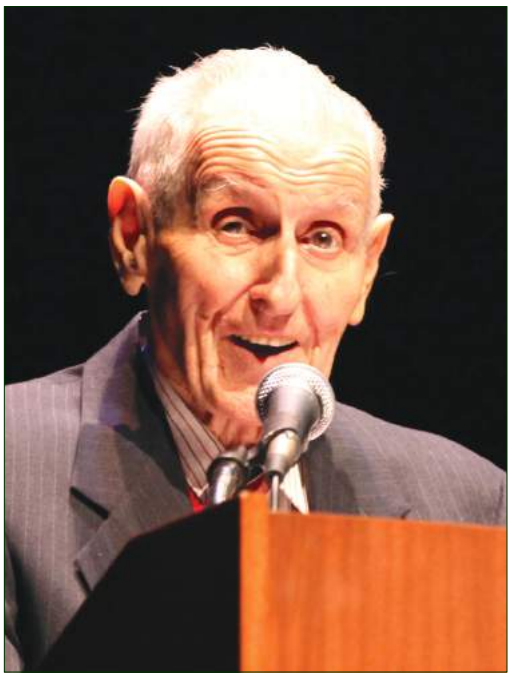

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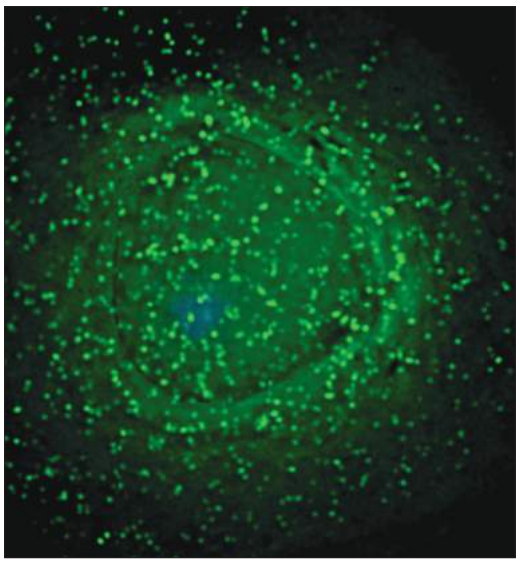

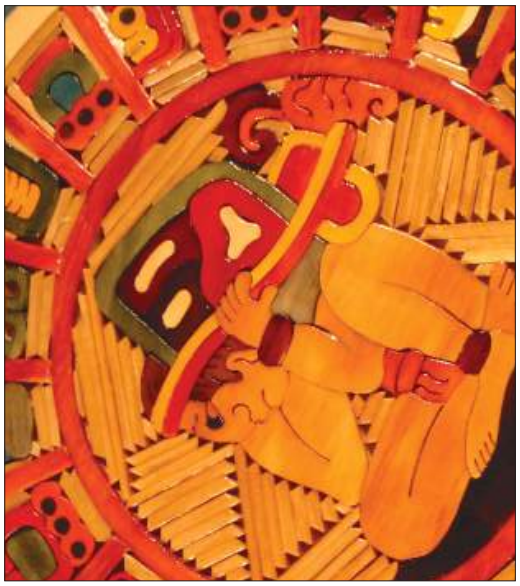

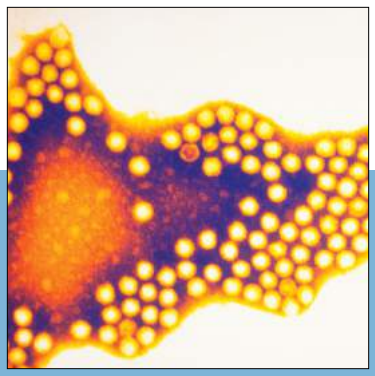

Science News

Recent Discoveries Throughout Science

COMPILED BY SCOTT GLADSTONE

Biology

Nature Chemistry, *Four-strand DNA structure found in cells*

In a paper recently published in *Nature Chemistry*, Shankar Balasubramanian, of the University of Cambridge, has identified a new four-strand DNA structure.

These square-shaped DNA molcules are rich in guanine, one of the four nucleotides of DNA. According to the publication, these structures are easily created in the laboratory and have been examined extensively under such conditions. Scientists had long believed that these G-quadruplex structures occasionally form naturally in the DNA of living cells, and the publication provides strong evidence that these unusual structures not only occur in cells, but also have important biological functions.

Of the various roles that G-quadruplex structures may serve, the most significant may be their role as the protective tips of chromosomal DNA, known as telomeres. Telomeres are known to be rich in guanine, making them likely candidates to fold into the four-stranded structure. As evidence of this structure's protective role, studies in cancer cells have shown that small molecules that bind and stabilize these structures cause DNA damage at the telomeres.

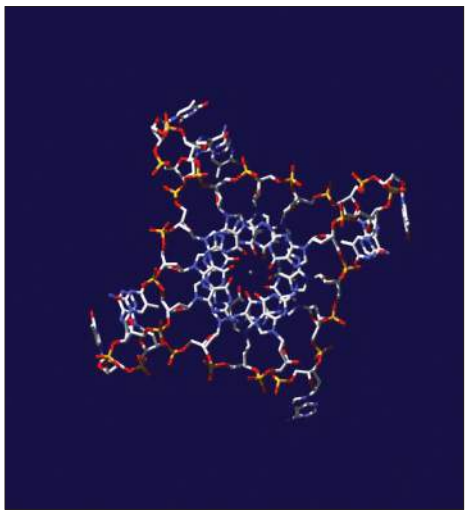

Image courtesy of Alison Abbot. Retrieved from http://www.nature.com/news/four-strand-dna-structure-found-in-cells-1.12253 (accessed 20 February 2013)

G-quadruplex DNA structure model, built using data from x-ray crystallography

Aside from investigating telomeres, Balasubramian and his team investigated the entire human genome in search of other guanine-rich sequences where the quadruplex structure could also be created. If identified, the structure could be implicated in gene regulation. Its involvement in cancer development has already been hypothesized. The presence of G-quadruplex structures in other genes is supported by experiment. Balasubramian's team used an engineered antibody to bind tightly to the quadruplex DNA structure and noticed that, when incubated in culture, the antibody bound only one quarter of the time to telomeres and the remainder of the time to other sites in the chromosomes.

As Balasubramian says, "It's early days, but if we can map exactly where the G-quadruplex structures pop up in the genome, we may learn how better to control genes or other cellular processes that go awry in diseases like cancer. That's the long-term vision."

Engineering

Scientific American, *Novel Solar Photovoltaic cells achieve record efficiency using nanoscale structures*

A novel solar photovoltaic cell composed primarily of indium and phosphorus has achieved record efficiency using nanowire-based structures. To compose such a cell, microscopic flecks of gold are first arranged on a semiconductor background and, using the gold as seeds, 1.5 micrometer tall wires are grown out of chemically modified indium and phosphorus. These nanowires are then etched clean with hydrochloric acid and confined to 180 nanometers in diameter. Exposed to the sun, the nanowire-based cell can convert nearly 14 percent of incoming solar light to electricity, a new record that opens up more possibilities for cheap and effective solar power.

Research recently published by *Science* has shown that solar cells with this construction can deliver electricity comparable to traditional indium-phosphide solar cells. Yet these nanowires only cover 12 percent of the device's surface. Physicist Magnus Borgstrom of Lund University in Sweden argues that, with less required materials, this novel design could prove to be significantly cheaper than what is currently available in the market.

The promise of cheaper, more efficient solar power requires three major design innovations. First, a novel semiconductor should absorb most of the light from the sun; according to Borgstrom, the nanowire structure can absorb "71 percent of the light above the band gap, and we can certainly increase that." Second, improved design will come with the development of techniques to control the nanowires and their chemical constituents more finely, allowing for reduced costs and higher electric yield. Finally, individual cells can be built into multijunction solar cells, which are compound devices that incorporate several types of semiconductor material in order to absorb as much energy in solar radiation as possible. Such cells currently are the most efficient photovoltaic devices in the word, allowing for the conversion of more than 43 percent of sunlight energy into electricity.

However, while the future of solar cell development may seem to favor the development of multijunctional cells, they are also the most expensive type of photovoltaic cell model. Ideally, Borgstrom hopes to leverage the techniques of nanoscale wire development to grow larger cell structures. As he states, "once large-area structures can be grown, concentration will not be necessary anymore."

Environment

The New York Times, *Temperature Rising: How High Could the Tide Go?*

Climate change and global warming have been capturing news headlines in the past decade, citing broken records and unprecedented transformations in the physical makeup of the Earth and its

Image retrieved from http://en.wikipedia.org/wiki/File:PV_solar_roof_mount_and_rack.jpg (accessed 20 February 2013)

Photovoltaic solar panels mounted on the roof of a home in Berkeley, California. A new type of solar cell composed of indium and phosphorous is achieving record efficiencies using novel nanowire-based structures.

atmosphere. Recent research by Maureen Raymo and her team from Columbia University has focused on one significant implication of higher temperatures and climate change: rising sea levels around the world.

Discussing the recent superstorm Hurricane Sandy that devastated northeastern United States, the experts state that "storm tide [like that of Hurricane Sandy] could become routine along American coastlines by late in this century if the ocean rises as fast as [scientists] expect." In previous research, scientists showed that for every couple of degrees Fahrenheit that the Earth warms, enough of the polar ice cap melts to raise the global sea level by 25 to 30 feet. Since the temperature of the Earth is predicted to rise several degrees over the next century due to human emissions of greenhouse gases, sea level increases of dozens of feet above the current sea level can be expected.

These predictions are based on empirical evidence collected by researchers like Dr. Raymo, who have investigated beaches around the world to determine past sea levels. According to Raymo, the Earth has warmed up many times throughout its history, for purely natural reasons, and these transitions have often featured "huge shifts in climate, partial collapse of the polar ice sheets and substantial increases in sea level." Convincing skeptics of these truths is one of the hardest parts of these scientists' jobs, says Raymo, "I wish I could take people that question the significance of sea level rise out in the field with me. Because you just walk them up 30 or 40 feet in elevation above today's sea level and show them a fossil beach, with shells the size of a fist eroding out, and they can look at it with their own eyes and say, 'wow, you didn't just make that up.'"

Scientists who study climate history spend a significant amount of time determining what causes a transition period. They investigate everything from wobbles in the Earth's orbit to the influence of impacting space debris. However, regardless of the specific cause, scientists agree that carbon dioxide appears to have played a crucial role. According to the article, when changes in Earth's orbit caused the Earth to cool, a large amount of carbon dioxide entered the ocean, reducing the heat-trapping properties of the atmosphere and thus amplifying the cooling effect. Conversely, when shifts in insolation led to initial warming, carbon dioxide emerged from the ocean and increased the temperature of the Earth. Based on this result, experts have come to the conclusion that "carbon dioxide [is] the master control knob of the Earth's climate." Thus, with recent increases in human carbon dioxide emissions, it is equivalent to "twisting the Earth's thermostat hard to the right."

In order to determine how future sea levels may change, scientists like Dr. Raymo are looking back to historic carbon dioxide levels. Specifically, Dr. Raymo has focused in on the Pliocene Epoch, a period roughly three million years ago when the carbon dioxide level in the air was 400 parts per million – a level that is predicted to be reached again in the next few years. Raymo and colleagues have begun a world search to find, date, and to measure Pliocene beaches on every continent and then use a computer model to carefully account for the changing sea level. To date, the team has located Pliocene beaches "as low as 38 feet and as high as 111 feet above modern sea level. In North America, the team has found that the Pliocene ocean encroached as far as 90 miles inland from the current shoreline."

A large body of evidence suggests that ice sheets atop Greenland and Western Antarctica are vulnerable to global warming, but together these bodies can only account for a 40-foot rise in sea level. With the average Pliocene beach around 80 feet above the current level, this implies that East Antarctica, previously thought to be stable, may be vulnerable to melting as well. While Dr. Raymo's study has granted us a new, higher threshold on the worst-case scenario for rising seae levels, the study still does not suggest how fast the rise in ocean level could occur. Nevertheless,

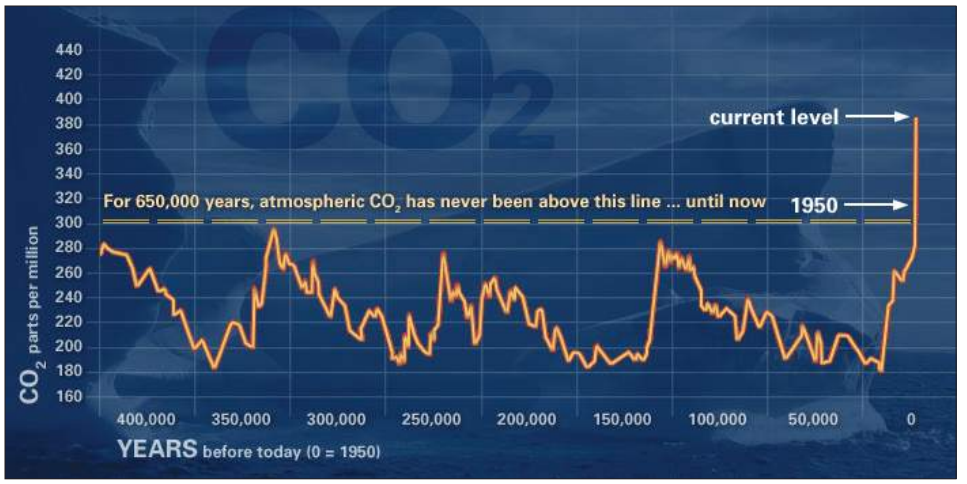

Image courtesy of NASA. Retrieved from http://en.wikipedia.org/wiki/File:Evidence_CO2.jpg (accessed 20 February 2013)

Atmospheric carbon dioxide levels in parts per million. Data consist of ice core proxy data for the past 650,000 years and modern measurements.

calculations by Climate Central, a research group, suggest that "once the ocean has risen five feet, storm tides comparable to those of Hurricane Sandy could occur about every 15 years in New York City," and scientists say that, in the 22nd century, the problem would "probably become far worse, and the rise would then continue for many centuries, perhaps thousands of years."

"At every point, as our knowledge increases," Dr. Raymo said, "we've always discovered that the climate system is more sensitive than we thought it could be, not less."

Evolution

Science, *First Known "Social Chromosome" Found*

A cluster of genes has been identified in the fire ant *Solenopsis invicta* that scientists believe controls for social organization. These fire ants are known for two distinct types of social structures. Researchers previously thought that the different groups' physiological and behavioral distinctions were derived from a variant in a single gene, but a new study provides evidence that the gene in question is actually a "bundle of some 600 other genes, versions of which are all inherited together." This "supergene" is the primary part of what scientists are considering the first discovered social chromosome.

The social stratification in the fire ant community begins at the top with the winged queen, according to lead researcher and evolutionary geneticist Laurent Keller of the University of Lausanne in Switzerland. From this queen, two different social structures can develop. The first social structure features a large and fertile monogyne queen. The monogyne queen is capable of flying long distances to start her colony once she mates, and she must wait until her larvae grow up into workers before her colony is complete. According to the article, "a monogyne colony will accept only the original queen and kill any other that shows up."

In contrast, the second social structure features a smaller polygyne queen who needs mature workers to help set up a colony. Polygyne communities will accept queens from nearby nests as long as they are also polygyne.

In 1998, entomologist and geneticist Kenneth Ross of the University of Georgia in Athens showed that the two groups of fire ants have distinct versions of a gene known as Gp-9. As stated in the publication, "All of the monogynes had two copies of one form; among the polygynes, many had one normal and one mutated copy of the gene. At first glance, the finding made sense. The gene encodes an odor receptor that enables the insects to detect scents, so presumably the polygyne worker ants could smell a kindred queen." But upon further investigation, Keller and colleagues found a chromosome pair containing the Gp-9 gene on about 500 offspring of monogyne and polygyne queens.

The team's main strategy was to determine the integrity of the Gp-9 gene after recombination, a genetic process where two copies of a gene (one from each parent) swap DNA sequences in order to create an offspring with unique genetic material. But, if many genes are going to stick together through generations in order to be hereditary, recombination cannot occur. In the fire ants, a group of "about 600 genes surrounding Gp-9 showed a complete absence of recombination," the researchers reported in *Nature*. This cluster of genes takes up greater than half of the chromosomes occupied by Gp-9 and includes "most of the genes that account for differences between polygyne and monogyne groups."

"This is a spectacular piece of work," says Kenneth Ross, who was not involved in the study. "They've unlocked a whole new mechanism for how a supergene can determine something as complex as behavior."

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Questions: On the Human Body

For more, visit dujs.dartmouth.edu

COMPILED BY ANDREW ZURIECK

A new feature for *DUJS* Online is to ask authors to ponder scientific questions about the world around us. These questions cover topics relevant to modern science or are generally very interesting to a broader audience. In this issue, we have selected a subset of our featured articles pertaining to the human body. Specifically, our authors discuss the mind, bodily functions such as crying, stem cells, and aging. The human body is remarkably complex, and we hope this compilation of articles inspires the reader to critically think about the inner workings of our biological and evolutionary adaptations.

Is Our Collective IQ Increasing?

Na Eun Oh '16

The Flynn Effect is the observation that intelligence quotient (IQ) test scores have, on average, increased significantly from the 1930s to the present day.

Psychologists revise IQ tests every couple of years to maintain an average score of 100. Almost invariably, new samples of test takers record average scores of well over 100 when administered an older version of the IQ test (1).

Richard Zynn was actually the first to note this effect when he observed the pattern in Japanese people in 1982 (2). However, the trend bears the name of James Flynn, as he documented and publicized the effect on a larger scale.

The U.S. population has shown an increase of about 3 points in the average IQ score every decade. The Flynn Effect seems to be more pronounced in populations that are typically considered to be from "more developed" areas, such as in Scandinavia. Recent studies show that the Flynn effect may soon fail to hold for a few developed nations. If this trend continues, and nations with lower average IQ scores continue to show improvement in scores according to the Flynn effect, then discrepancies between average IQ scores among different nations could eventually disappear (3).

Researchers debate whether increases in IQ test scores really correlate to an increase in intelligence, or if these increases simply reflect an improvement of test taking abilities. The learned content hypothesis states, "Massive IQ gains do not represent the sort of skill gains that might be identified with increase intelligence" (1). If the learned content hypothesis is true, then increases in IQ score would not imply gains in general problem solving ability.

The trend of increasing IQ scores over time is generally attributed to both an increase in intelligence and to an improvement of test-specific skills. The greatest increases in scores over time tend to be associated with a reduction of culturally oriented content in IQ tests. These culturally reduced tests place a greater focus on the importance of problem solving and minimize the need for skills specific to a certain culture or familiarity with certain words or images.

The implications of the Flynn Effect are debated amongst psychologists. Those who argue that intelligence is most strongly correlated with IQ tests that place a large focus on problem-solving ability would likely argue that intelligence increases with each cultural generation, (1,3). However, Flynn has reported that the French, who have displayed large increases in average IQ scores, in the range of 15 to 20 points from 1952 to 1982, have not reported of a dramatic increase in genius or mathematical and scientific discovery during their generation. During the 1980s, the number of patents issued in France actually decreased. In 1987, Flynn argued that IQ tests measure abstract problem solving ability, which is different from the real world problem solving ability that most strongly corresponds to functional intelligence. However, Flynn stated in his 2007 paper that improvements in abstract problem solving and formal education could mean a greater appreciation of science – a field that deals largely with the abstract (3).

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Why Do We Cry?

Shawn (Yifei) Xie '16

We cry when we are in despair. We tear up when we yawn. Our eyes swell

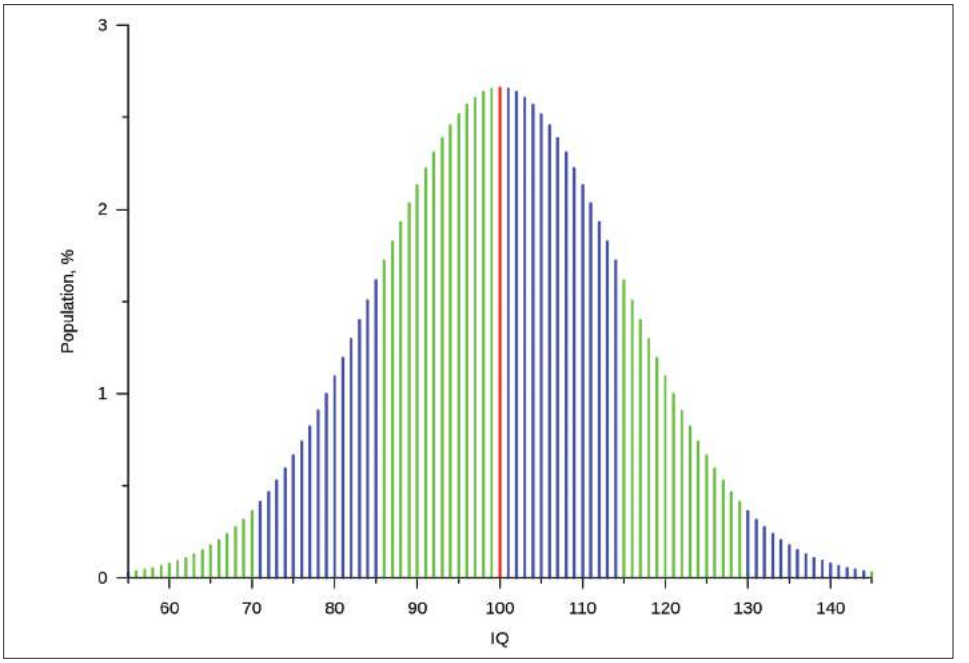

Image retrieved from http://upload.wikimedia.org/wikipedia/commons/thumb/f/f7/IQ_curve.svg/2000px-IQ_curve.svg.png (accessed 22 February 2013).

Psychologists revise the IQ test every couple years in order to maintain an average score of 100. Scores have been increasing gradually since the 1930s.

up during moments of immense joy. But why do we cry in the first place? What biological functions does crying serve? While the scientific community struggles to provide concrete explanations for this teary condition, researchers have offered several plausible theories based on the chemical composition of tears and the apparent evolutionary advantages of shedding these salty droplets.

In a sense, we actually cry every second of our lives. The lacrimal gland under the upper eyelid constantly secretes protein-rich, antibacterial liquid at a rate up to two microliters per minute. The fluid flows down from the outer edge of the eyeball toward the cornea, and lubricates the entire eye surface every time we blink. Excess liquid then drains into the nasal canal via the tear duct on the bottom eyelid.

Of course, we do not normally call this process "crying." Rather, we are more concerned with the times when the secretion of lacrimal fluid overwhelms the draining capacity of the tear duct—when tears overflow and roll down our cheeks.

One stimulant of crying is irritation. If sand gets into our eyes, the lacrimal gland secrets excess fluid to help us wash out the irritant. Similarly, we cry when an onion releases sulfuric fumes that acidify the eye surface. Secretion of more tears lowers the eye's acidity. Even yawning is a form of irritation—the manipulation of facial muscles near the eye squeezes the lacrimal gland and forces out excess tears.

The type of crying that we are most familiar with is the shedding of tears as an emotional response. Why we release tears when we feel grief, hurt or joy still confounds the scientific community, but several observations have been made.

Emotionally charged tears contain several protein-based hormones produced at times of stress—specifically prolactin, adrenocorticotropic hormone and leucine encephalin. The altered chemical composition of these tears have led scientists to speculate that crying is the body's method of ridding excess stress hormones. Otherwise, stress hormones would reach harmful levels within the body. Indeed, this explanation fits well with the anecdotal knowledge that crying allows us to feel better.

More recently, researchers observed that emotional tears could also serve an evolutionary purpose. Excess lacrimal fluid disables the fight-or-flight response when it blurs our vision. By revealing our vulnerability, crying could be seen as a signal for defeat or submission, thereby eliciting mercy or sympathy from an attacker. Similarly, such a show of weakness can evoke pity and lead to a gain support from a larger community.

So why do we cry? Observationally, we cry because we have something in our eyes, or because we are part of a species that, through evolution, recognizes certain social cues. But more importantly, crying is a form of human expression.

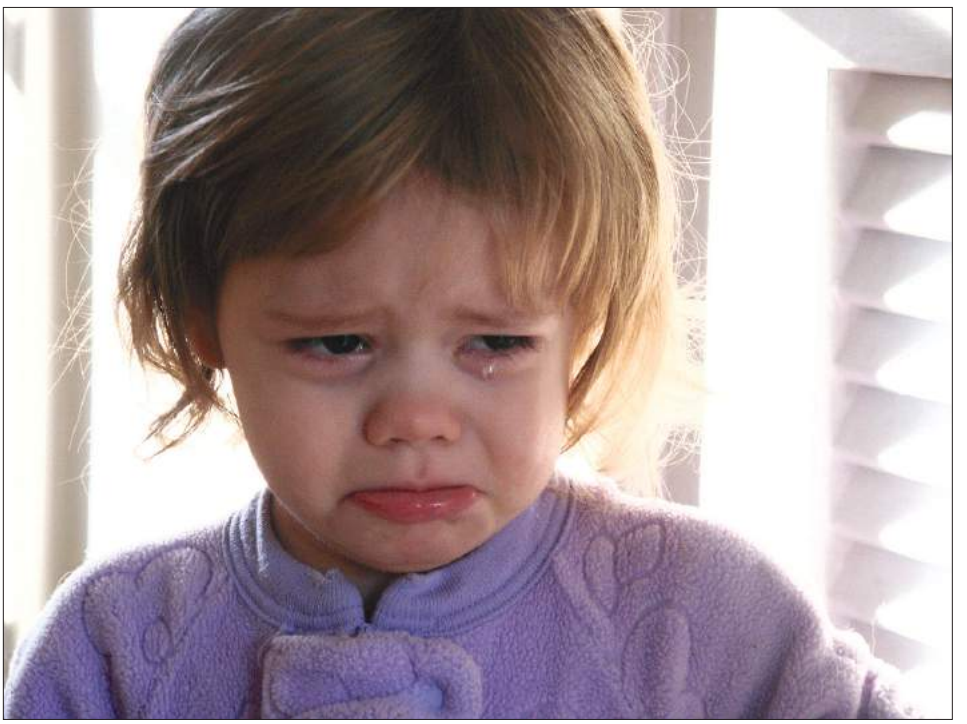

Image retrieved from http://upload.wikimedia.org/wikipedia/commons/thumb/c/c0/Crying-girl.jpg/1280px-Crying-girl.jpg (accessed 22 February 2013).

We cry when we're in despair. We tear up when we yawn. But why do we cry in the first place?

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Stem Cells: The Solution to Living Over 100 Years?

Miguel Peña '16

Many scientists have attempted to find the "Fountain of Youth." Although many genetic and medical treatments have increased the average human life expectancy, these treatments have yet to increase lifespan over a hundred years.

However, this statement may no longer hold true in the future. In a recent issue of *Nature Communications,* Mitra Lavasani, *et al.* (2012) conducted an experiment involving fast-aging, genetically engineered mice. These mice have a usual lifespan of 21 days. A few days before the mice reached their predicted maximum life span, the injections were delivered at the Institute for Regenerative Medicine in Pittsburgh. The results were astounding. The elderly mice lived approximately 70 days – three times their normal lifespan. In human terms, that would be the equivalent of an 80-year-old living to be 200.

Specifically, the investigators studied the effects of injecting muscle-derived stem/progenitor cells (MDSPCs) into a murine progeria model (fast-aging mice). Since age-related degenerative changes are universal in the musculoskeletal system, the impact on the musculoskeletal system by murine muscle-derived stem/progenitor cells (MDSPCs) became the primary focus of the experiments. MDSPCs are multipotent cells isolated from postnatal skeletal muscle. They have the capacity for

long-term proliferation, are resistant to oxidative and inflammatory stress, show multilineage differentiation and self-renew, induce neovascularization, and stimulate regeneration of bone, skeletal, and cardiac muscles. These characteristics raise the possibility that the loss of MDSPCs or related perivascular progenitor cells could contribute to sarcopenia, osteoporosis and other age-associated degenerative diseases (1).

The results of Lavasani's study indicate that MDSPC function is adversely affected by aging. Since the transplantation of functional MDSPCs was sufficient to rescue MDSPC dysfunction and extend the healthspan and lifespan of progeroid mice, investigators suspected that MDSPC dysfunction might have direct contribution to age-related degeneration.

Numerous other studies have provided evidence that the number and/or function of diverse adult stem cell populations decline with aging. However, these correlative studies do not rule out the possibility that the decline in the stem cell population have a causative role in aging.

With these results, it is reasonable to conclude that MDSPCs may have therapeutic value for delaying age-related functional decline in human progeria. It will be of great importance to test other adult stem cell types for a similar therapeutic effect (1).

This is a huge breakthrough for medicine because it will not only extend the life of humans in the future, but will also delay symptoms correlated with aging.

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Why Does Your Skin Age?

Angelica Carrillo Leal '14

Skin aging is a fact of life; everyone will face it sooner or later. In order to understand how this process works, it is important to know the basics of skin. The epidermis, dermis, and subcutaneous tissue are the three layers that compose the human skin. Epidermis is the skin's surface, a layer rich in keratin that provides toughness and water resistance. This layer of skin is where dead cells are shed and where melanin (a dark pigment) is found. The second and thicker layer is the dermis, which is composed of nerves, fats, blood vessels, elastin, and collagen fibers, which provide elasticity. Finally, the subcutaneous layer is composed of fat, keeping us warm and holding our internal organs in place (1).

Intrinsic aging is the aging process that takes place over the years regardless of external influences. After the age of 20, one percent less of collagen is produced in the dermis each year (2). The collagen and elastin fibers become thicker, more clumped, and looser, resulting in inelastic and brittle skin and eventually in wrinkling and sagging (3). In our twenties, the skin's exfoliation process decreases by 28% as well, causing dead skin cells to accumulate and stick together for longer periods of time. In our thirties, the transfer of moisture from the dermis to the epidermis is slowed and fat cells start to shrink. These effects make the skin look dull and thin. In our forties, collagen is no longer produced. The collagen and elastin fibers break, thicken, stiffen, clump together, and lose their elasticity. This results in wrinkles and aging lines. Finally, in our fifties, the skin becomes dry and is easily bruised, damaged, or broken because the sebaceous (oil) glands have decreased in size. In women, menopause causes a decrease in estrogen levels, leaving the skin drier, thinner, more sensitive, and less toned.

A second type of aging is extrinsic aging. Unlike the previous cause of skin aging, it can be controlled because it is a result of environmental damage. Extrinsic aging appears as a thickening of the cornified layer (outermost layer of epidermis), precancerous changes (an example is actinic keratosis), skin cancer, formation of freckles and sunspots, and huge losses of collagen, elastin, and glycosaminoglycans (GAGs). As a result of these processes, the skin becomes rough, uneven in tone, and wrinkled (2).

Free radicals (electron-hungry molecules or atoms) are the cause of these chemical changes. When electrons are pulled from other molecules, chemical structures and biological functions are altered. Environmental influences, such as pollution, smoking, and ultraviolet radiation, generate free radicals. Antioxidant enzymes and molecules, such as vitamin C, vitamin E, and carotenoid pigments, can protect us from free radicals, but their damage occurs anyway. Glucose, a fuel necessary for our bodies, presents another threat. It forms plastic-like molecules known as age-related glycation end-products (AGES) by crosslinking with proteins. These complexes hurt skin

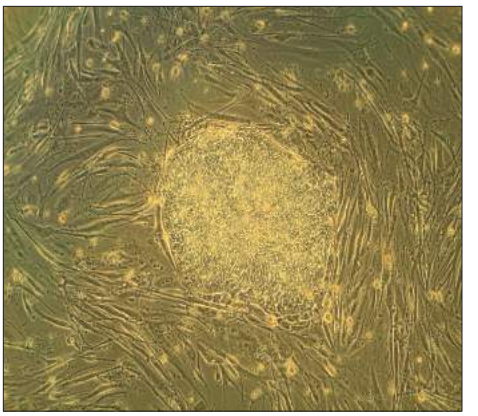

Image retrieved from http://en.wikipedia.org/wiki/File:Humanstemcell.JPG (accessed 22 February 2013).

Stem cells may allow humans to live over 100 years, according to a recent study published in *Nature Communications.*

proteins by causing them to be more brittle and less elastic. Also, because the skin is the barrier between body and environment, ultraviolet radiation causes damage to DNA and molecules and results in the generation of free radicals, making it the crucial factor in the acceleration of wrinkling skin. The skin protects from damage to exposure of sunlight by producing more melanin. [3]

Our body is able to repair damaged proteins, but they will not work as well. Prevention is the key to minimizing wrinkles. An SPF of at least 35 is necessary for sun protection against UVA and UVB. After the age of 25, it is recommended to use Retin-A as an anti-aging cream. It is a vitamin A derivative that can cause redness and peeling during the first two months of use, but overtime it improves fine lines, pores, brown spots, and precancerous changes. Chemical peels and non-invasive lasers can build collagen and improve the skin's appearance too (2).

Aging is under the control of genetic repair mechanics and can be affected by lifestyle influences. Measures to protect our skin will keep it from aging more than it needs to.

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Paula R. Sundstrom, Ph.D.

Professor of Microbiology and Immunology, Geisel School of Medicine

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What has been the focus of your research at Dartmouth?

I study a fungal pathogen called *Candida albicans*. I've been studying how this fungus interacts with host cells, how it attaches, invades, and manipulates the host epithelium to cause disease.

What kinds of investigations do you conduct?

I incubate the fungus with oral epithelial cells, I study how the epithelial cells react to the fungus, and I study the gene expression changes of the oral epithelial cells. My reason for doing this is to develop therapeutic strategies to prevent thrush, which causes white patches on the oral cavity and is often seen in immunosuppressed patient as masses of fungi and keratinocytes. And so in patients that are immunocompromised or immunosuppressed, the fungus is actually able to invade and create a sort of biofilm on the surface. I'm trying to figure out how the keratinocytes respond to the fungus and what is damaging the keratinocytes. I would like to ultimately be able to find a pathway that *Candida* is manipulating to damage the keratinocytes and then try to find pharmaceutical agents that could help protect the keratinocytes. There is a lot of gene expression and analysis using software to try to understand which keratinocyte pathways are being manipulated by *Candida*.

I also do standard molecular biology. I've been working with this fungus for a very long time. I actually had an amazing discovery when I identified a gene that encodes a protein that can attach covalently to our oral epithelial cells. Most microbes attach via electrostatic bonds, and the covalent bond is ten times stronger than any other kinds of bonds. This allows the fungus to attach very tightly to the mucosal surfaces and not be very easily washed away by swallowing and saliva. This is a very exciting discovery, and to make this discovery, I used genetics. I discovered the gene using an immunoscreening approach, and then I knocked out the gene in *Candida* and showed that they didn't pair this way. I used biochemical approaches to prove that the protein was functioning in this covalent cross-linking.

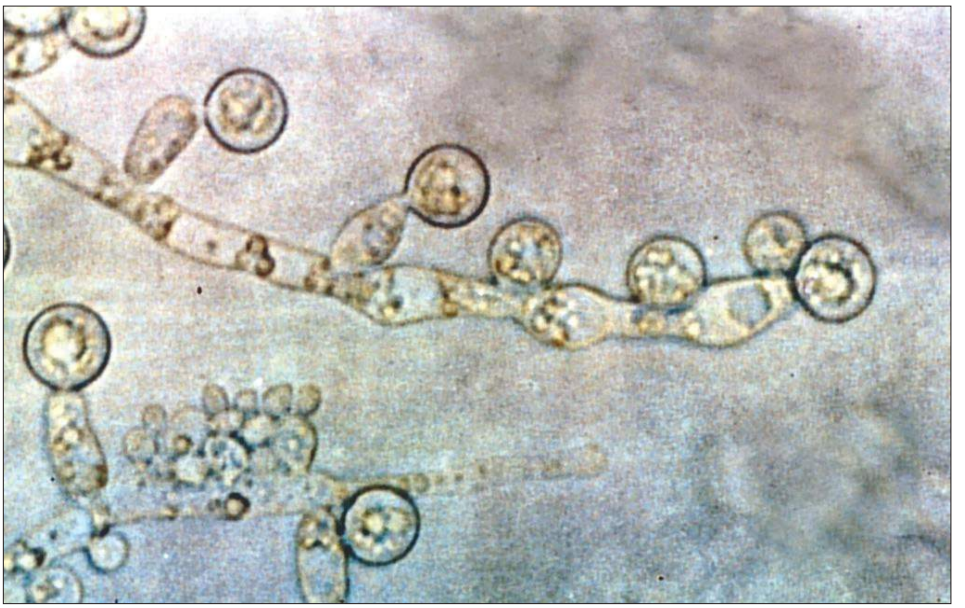

Image retrieved from http://en.wikipedia.org/wiki/File:Candida_albicans_2.jpg (accessed 22 February 2013).

Candida albicans is a fungal pathogen capable of causing disease in immunocompromised patients. It is also found in harmless quantities in the mouth and gastrointestinal tract of 80% of the human population.

Is it very rare that these sorts of proteins attach covalently?

Actually, this protein is very interesting. It's like a hybrid of a fungal protein and a mammalian protein, because covalently attaching proteins are actually found in mammalian cells. The enzyme involved in this category of covalent attachment is called the transglutaminase, and it uses side chains of glutamine residues to cross-link one protein to another, so that a side chain of glutamine residues will attach to a lysine residues on another protein, and so you get an isodipeptide bond between the side chains of these two amino acids. The N-terminus of this protein is very much like mammalian substrates of this transglutaminase, in terms of the amino acid sequence, in terms of being a repeated protein, and in terms of having the glutamine amino acid repeat. It's the type of protein that the host enzyme likes to use as a substrate for cross-linking. The carboxy-terminal end of the protein of the fungal protein is very rich in hydroxy amino acids like serine or threonine and gets heavily glycosylated, and at the C-terminus it is covalently linked to the backbone of the fungal cell wall, so this protein has one end linked into the fungus and the other end is cross-linked to a host. It's a very special type of protein, and in evolutionary time, it's a relatively new protein. It's a protein that has evolved to allow *Candida* to attach very tightly to the places where it causes disease.

How large of a time commitment does your research require? How do you feel about balancing research and undergraduate teaching?

Research takes a lot of time, but the undergraduate teaching I do here is my favorite thing that I do, because I have the opportunity to introduce research to undergraduates. It's what I do in my class [Biology 67: Biology of Fungi and Parasites that Cause Disease]. We do it by reading papers—primary research papers coming

from labs like mine that are making active contributions to biology.

When we read scientific papers, we try to read them fast. It's very complicated, and we don't have time to see whether the abstracts and conclusions are supported by the data. The question we address is, "Can we look at this and really see what's going on?" By doing that, the students get an introduction to research. We talk about the journals that publish the research and what their goals are, the researchers and authors of the paper, the senior author and why they're doing this research. We talk about the data and what the data means, whether they have good controls, whether they're supporting their conclusions in more than one way, and in the end, we evaluate the papers in terms of whether it could lead to even more interesting research that will one day help patients, and we eventually start to see what research is about. We see good papers that bring out new ideas and exhibit different types of experiments done in different ways. We can predict that if certain conclusions are true, we can do one experiment and have it come out one way, and we can do another experiment and have it come out another way. We see how rigorous, how good, and how solid the data is. And I can see the light bulbs turning on, and it's very fun for me. Students are often intimidated at the beginning of the course. I ask questions about the data and the paper, and they struggle to find the answers, but then after a while, they get used to it, and they start to really come up with same questions themselves. It's amazing the insights they manage to find after their minds become attuned to looking at the data and applying what they have learned in their classes to real research.

It's such a big step from textbook learning.

It is a very big step; I always get questions at the beginning of class, "What are the textbooks, I want to start reading ahead," but I rarely use the same papers from one year to the next. It's very nice for me because I read some papers very carefully that I may otherwise gloss over, but the closest thing we use to a textbook are review articles written by people who are actually generating the primary research. These review articles introduce the background and show how the new data fits into the accepted canon of research. They are very exciting to read,

Photo Courtesy of Paula Sundstrom

Dr. Paula Sundstrom, Geisel School of Medicine at Dartmouth College. Sundstrom recieved her Ph.D. in 1986 from the University of Washingston and joined the Dartmouth Faculty in the Fall of 2003.

since these are the people who are doing the research, and they have the most interesting questions, and they write about it really well, more than what you would see in a textbook. For each major research paper we read, we usually read a review by the same person, and we discuss the review and have questions, and by the time we read the research paper, the students are ready to go. They have a story already and can see how this new paper adds to the story. It's quite a bit different, and the students aren't used to that. And they come into class and see the assignments involving paper X and "explain this" and "what does that show"—very specific questions. And we split up the labor too; Student A talks about this paper, Student B talks about another. The students work in teams to talk about the questions. They have quite a bit of preparation to do for each class; it's not just me sitting there telling them what to write down in their notes for a few hours. And the sooner that the students start really covering the class material themselves, the sooner they start learning really well. In the last part of the class, the students start giving presentations. They choose their own papers based on the criteria detailed in class, and they have to do an oral presentation and a written paper about their oral presentations. They give a background, they really go into the data, and they really evaluate their papers and see whether it holds up to what the authors are actually saying. They determine whether the paper is actually a good paper. And we have consultants for each presentation that come with prepared questions and generate class discussion. It's pretty intense for the students; they work very hard to get these presentations together.

What prompted you to teach this class this way?

I started teaching this sort of material when I was at Ohio State University (OSU). I was at OSU for about ten years, and I started co-teaching a class. When we went over papers, it didn't sit very well with me about the teaching. Later, I followed my own instincts in terms of creating a class that really goes into the primary literature and involves the students. And so I did, and as the years go by, I do change the class in format and material, but I continue to follow my own instincts about teaching. Oftentimes you see graduate students in Ph.D. exams who don't really know what they're talking about, they're in a lab and someone has told them what they're doing, and they can kind of fill in the blanks but they're not thinking about it themselves. In this class, I'm trying to develop these skills for thinking critically about data. We talk about the next experiments that can stem from the new data, then they start to think about putting themselves in the driver's seats for doing these experiments and taking responsibility for what happens in the lab. That's a big part of why I teach my class this way; I want my students to take ownership of the science.

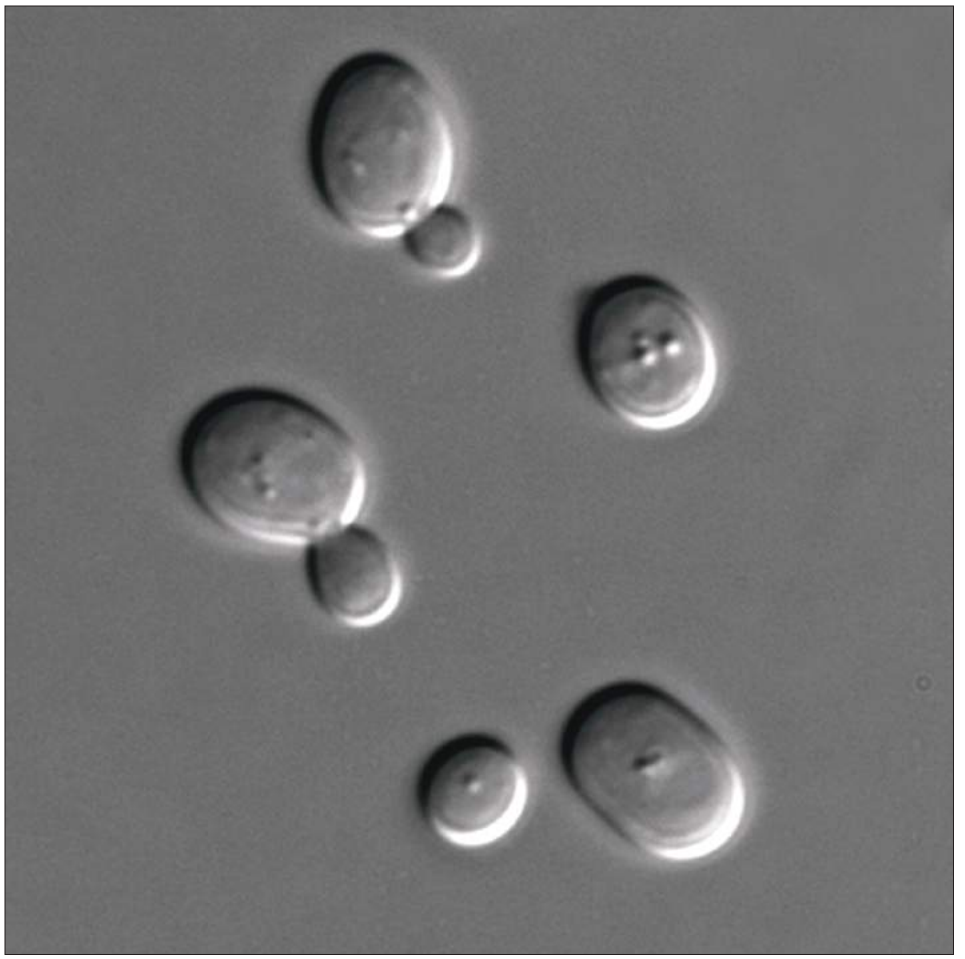

Image retrieved from http://en.wikipedia.org/wiki/File:Candida_albicans_2.jpg (accessed 22 February 2013).

Saccharomyces serevisiae is a common yeast used in baking and brewing. Though fungus are often associated with decay, they can also serve many useful roles.

Do you think more classes should be taught this way?

I don't like to presume; there are so many other outstanding ways to teach. I've heard other professors say that they simply don't have time to do this. I take the papers and come up with questions to get the students to think, and it does take time to do that. It takes time to get the paper ready. I wouldn't like to impose that one anyone. I do think that it really works well, and there are many benefits to doing classes this way for the students and for me. We get to really know each other well over the course of the class—I'm able to write pretty good letters for medical schools and other professional pursuits following graduation. When I grade, I usually write the better part of a page to each student covering each part of the grade—class participation, the exams, the presentations—and so after doing that for each student, it's not difficult to write a letter of each student. That's a very good benefit of my class. It's usually a very small class—nine now—but I think we have a system that works very well.

What advice would you have for those who want to get started in scientific research?

It takes a certain sort of mindset; research is thinking logically, and you have to find out whether that's you. Certain people may have never considered doing research, and they take my class and suddenly know what they want to do, but sometimes it's hard to know. Researchers are in the lab a lot. The best thing to do is just to go into a lab and work with a grad student or a postdoc and just do something simple at first. It may be just doing an assay for someone, and it may be kind of repetitive, but it's something you can take a small amount of time to learn and how it fits into the big research picture. And if you like what's going on in the lab, you can get more involved with an independent project. I know a lot of people do it this way; it's harder not to have lab savvy before starting your own lab project. You can do this in the right lab with the right support, but this is an easier way in. Also, if you ease into things, you might find out that you like what's happening in another lab more than swhat is in your current lab; you can make more intelligent choices for what's right for you.

Switching gears, what do you have to say about public perception of fungi?

Most people don't know much about fungi; it's taught as a pretty small part of high school biology. The public perception, I'm not quite sure about, but when something comes out in the news about meningitis, say, people will become very fearful. Fungi are interesting and important on so many levels, though. In terms of death and decay, they're huge players in the decomposition of organic matter. They have specialized enzymes for doing so, and they can then adsorb these for growth. They help recycle waste and dead stuff all around. They have special ways of growing that allow them to do that; they have threadlike structures called hyphae that allow them to spread and degrade in different areas. Without decay, we'd just be living in piles of stuff! There are lots of positive interactions between fungi and plant roots; they intertwine. Also, fungi are used in industry to make drugs like cyclosporine and penicillin; they're also used in the food industry to make wine, beer, and cheese. People make a lot of money off of fungi. However, fungi can also be pathogens and pests in taking down crops; their spores can spread through the air. Public perception can depend on what you do; farmers probably have a different perception of fungi than most people. They play a large part in life, but we're not always aware of what they do.

Biodegradable Plastic

The Promises and Consequences of Biodegradable Plastic

KRISTEN FLINT

Image courtesy of USDA. Retrieved from http://commons.wikimedia.org/wiki/File:BiodegradablePlasticUtensils2.jpg (accessed 20 February 2013)

Figure 1: Knives, forks, and spoons made from a biodegradable starch-polyester material. Biodegradable plastics offer a promising alternative to petroleum-based plastic, but the high cost and low yield associated with their production pose significant obstacles.

Living "green" has become a popular trend in the last twenty years, and reducing oil consumption remains an important goal for the sustainably-minded today. One of the top uses of crude oil is the production of plastics. In 2006, 331 million barrels of petroleum were used for plastics, compared to 2 million barrels used for fuel (1). These plastics later end up in landfills, where they take years to break down. Petroleum-based plastics are becoming more expensive as oil prices continue to increase, and plastic accumulation in landfills takes up valuable space and threatens the environment. The economic and ecological drawbacks of petroleum-based plastics have pushed researchers to develop and investigate biodegradable plastics as an environmentally-friendly alternative.

During the 1920s, Maurice Lemoigne, a French researcher, discovered the first biodegradable plastic from his work with the bacterium *Bacillus megaterium* (2). Lemoigne isolated poly-3-hydroxybuturate (PHB), but his work went unnoticed for many years. Decades later, microbiologists in the United States and Great Britain independently discovered PHB in 1957 and 1958, respectively (2). However, research into biodegradable plastics slowed until the oil crisis of the 1970s (3). As nations faced the reality of rising oil prices, they encouraged research for synthesizing alternatives to petroleum-based products. However, when the oil crisis died down, interest in biodegradable plastics research decreased once more. In the 1990s, the research again became popular, this time in the biomedical industry. Between medical applications and fluctuating oil prices, research in biodegradable plastics has become steady and profitable. So far, researchers have invented several different types of plastics and a variety of manufacturing methods.

Polyhydroxyalkanoates (PHA)

PHB is a type of polyhydroxyalkanoate, or PHA (2). PHAs are biodegradable thermoplastics that are synthesized by many different types of bacteria. When bacteria develop in nutrient-deficient environments, bacteria create PHAs as food and energy reserves, which are then stored as insoluble granules in the cytoplasm (2,3). Depending on their molecular composition, PHAs have varying physical properties, but all PHAs biodegrade in carbon dioxide and water (3). PHAs can degrade in either aerobic or anaerobic environments through thermal degradation or enzymatic hydrolysis (3). Bacteria, algae, and fungi use extracellular enzymes to depolymerize, or break down, PHAs and, through a process known as mineralization, absorb the remaining fragments for use as minerals (4). Manufacturers can alter the properties of PHAs by changing their structure or composition. PHAs have a variety of applications in water-resistant surfaces, binders, synthetic paper, medical devices, electronic parts, food packaging, and agriculture (3). However, PHAs currently have economic drawbacks that limit their use. Until recently, PHAs have had high production costs, low yields, and limited availability (3). Consequently, they have not been able to displace the petroleum-based plastics that manufacturers can cheaply create in bulk.

One method of reducing the cost of production is to create a polymer blend

of PHAs with renewable materials, such as starch or cellulose, a technique that requires a smaller amount of PHA per unit (4). The blends still have properties that are easy to modify, providing a viable, less expensive alternative to pure PHAs (4). Blends also degrade better than PHAs without renewable materials. When PHAs are blended with hydrophilic polymers, more water can penetrate the plastic and increase the efficiency of degradation (5). The sole caveat to using blends is that when creating the blends, manufacturers must mix the biodegradable substance with the renewable sources thoroughly to avoid having small starch or cellulose particles that interrupt the plastic's biodegradation and harm the environment (4).

Synthesis of PHA with Plants

Production costs are major impediments to the marketability of biodegradable plastics. Researchers have experimented with various methods of production, but one of the most promising techniques is cultivating PHAs in transgenic plants. Synthesizing PHAs through bacterial fermentation costs five times as much as the production of petroleum-based plastics because of low yields per bacterium (6). By using transgenic plants to produce PHAs, net yields increase at a lower cost. The plants can grow PHAs by redirecting cytoplasmic acetyl-CoA present in the plant to produce PHB (6). However, redirecting cytoplasmic acetyl-CoA stunts plant growth and negatively affects yield of both PHB and the plant itself (7). To avoid this side effect, researchers have instead targeted PHB production to specific areas of the plant with existing high levels of acetyl-CoA, such as chloroplasts (7). When synthesized in this manner, PHB yields make up 15% of the plant's dry weight, dramatically lowering the cost of production (6).

Polylactide (PLA)

Another popular biodegradable plastic is polylactide, or PLA. PLA is a synthetic polyester that biodegrades within a year, decaying much faster than conventional petroleum-based plastics (8). The creation of PLA involves bacterial fermentation, similar to the fermentation in the synthesis of PHAs. This fermentation creates lactic acid, which is then polymerized (5,8). Manufacturers use PLA because its method of synthesis is more economical than the synthesis of other biodegradable plastics. Scientists can already produce lactic acid inexpensively, so the cost of producing PLA is less than the cost of producing PHAs (8). Furthermore, PLA is biocompatible and can be utilized in biomedical applications, such as in medical plates and screws that can be degraded and absorbed by the body (8). However, PLA exhibits several physical and mechanical properties that make it more difficult to use than PHAs or other plastic options for applications outside of biomedicine. PLA is brittle, thermally unstable, and hydrophobic (4). PLA degrades by hydrolysis with no need for external enzymes, but creates a large build-up of lactic acid during degradation, which can cause problems in biomedical applications (4,8). Like PHAs, PLA has a variety of physical and mechanical properties that can be changed by altering its chemical structure and molecular weight (8). Manufacturers can also blend PLA with renewable polymers to alter its properties and lower production costs (4).

Environmental Effects

Researchers have worked on developing biodegradable plastics with the hopes of bettering the environment, but the production methods and applications of biodegradable plastics could still be detrimental to environmental and human health. The waste management infrastructure currently recycles regular plastic waste, incinerates it, or places it in a landfill (9). Mixing biodegradable plastics into the regular waste infrastructure poses some dangers to the environment. Biodegradable plastics behave differently when recycled, and have the potential to negatively influence to human health. To be effective in food packaging, plastics must exhibit gas permeability, chemical resistance, and tensile strength (10). If the food packaging materials are recycled, their physical properties could change, allowing degraded chemical compounds and external contaminants to enter the food (10). On top of that, plastics contaminated with food are difficult to recycle, and blended plastics sometimes leave behind starch residues that can further contaminate the recycling process (9,10). Another option for biodegradable plastic waste is incineration with energy capture, so that the energy that goes into producing the plastic could be reclaimed during decomposition. However, incineration of biodegradable plastics does not create any more energy than petroleum-based plastics, so the environmental effects of the two are roughly equivalent (9). The third option is landfilling biodegradable plastics. However, when biodegradable plastics decompose, they produce methane gas, a major contributor to global warming (9). While methane gas could be collected and used as an energy source, capturing that energy would be another expense, and some of the gas would still escape. Thus, the biodegradable nature of these plastics poses economic and ecological problems in the current waste management infrastructure.

To assess the environmental costs and benefits of biodegradable waste, James Levis and Morton Barlaz, researchers at the Department of Civil, Construction, and Environmental Engineering at North Carolina State University, developed an equation for the "global warming potential" of waste. Their equation considers landfill construction, operations, cover placement, gas management, maintenance, and monitoring (11). With this equation, researchers can estimate the emissions released during the production and disposal of a biodegradable substance and compare the figures to the estimated emissions for producing and disposing petroleum-based substances. The substance with lower overall estimated emissions is considered to be "better" for the environment. In their study, Levis and Barlaz used a hypothetical biodegradable polymer and found that petroleum-based polymers may have a less negative impact on the environment than biodegradable plastics (11).

Conclusion

Biodegradable plastics offer a promising alternative to petroleum-based plastics. While petroleum-based products use oil in their manufacturing and take up space in landfills, biodegradable

Image courtesy of Trevor MacInnis. Retrieved from http://commons.wikimedia.org/wiki/File:Fuel_Barrels.JPG (accessed 20 February 2013)

Figure 2: In 2006, 331 million barrels of petroleum were used for making plastic.

plastics can be synthesized in bacteria or plants and have the potential to be disposed of in a way that is less damaging to the environment. Biodegradable plastics have a variety of applications, from agriculture and food packaging to biomedical devices and tableware. The major obstacles to replacing petroleum-based plastics with biodegradable plastics are high costs and low yields associated with existing methods of biodegradable plastic production. With more research into plant-based manufacturing systems, these obstacles are being overcome. Finally, the last obstacle to surmount is the proper disposal of biodegradable plastics. In order for biodegradable plastics to be effectively disposed of, the current waste management infrastructure must change, or methods with less economic and environmental costs must be developed.

CONTACT KRISTEN FLINT AT KRISTEN.L.FLINT.14@DARTMOUTH.EDU

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Genetically Engineered Bioweapons

A New Breed of Weapons for Modern Warfare

MACKENZIE FOLEY

Genome sequencing has given rise to a new generation of genetically engineered bioweapons carrying the potential to change the nature of modern warfare and defense.

Introduction

Biological weapons are designed to spread disease among people, plants, and animals through the introduction of toxins and microorganisms such as viruses and bacteria. The method through which a biological weapon is deployed depends on the agent itself, its preparation, its durability, and the route of infection. Attackers may disperse these agents through aerosols or food and water supplies (1).

Although bioweapons have been used in war for many centuries, a recent surge in genetic understanding, as well as a rapid growth in computational power, has allowed genetic engineering to play a larger role in the development of new bioweapons. In the bioweapon industry, genetic engineering can be used to manipulate genes to create new pathogenic characteristics aimed at enhancing the efficacy of the weapon through increased survivability, infectivity, virulence, and drug resistance (2). While the positive societal implications of improved biotechnology are apparent, the "black biology" of bioweapon development may be "one of the gravest threats we will face" (2).

Limits of Past Bioweapons

Prior to recent advances in genetic engineering, bioweapons were exclusively natural pathogens. Agents must fulfill numerous prerequisites to be considered effective military bioweapons, and most naturally occurring pathogens are ill suited for this purpose (3). First, bioweapons must be produced in large quantities. A pathogen can be obtained from the natural environment if enough can be collected to allow purification and testing of its properties. Otherwise, pathogens could be produced in a microbiology laboratory or bank, a process which is limited by pathogen accessibility and the safety with which the pathogens can be handled in facilities. To replicate viruses and some bacteria, living cells are required. The growth of large quantities of an agent can be limited by equipment, space, and the health risks associated with the handling of hazardous germs (1). In addition to large-scale production, effective bioweapons must act quickly, be environmentally robust, and their effects must be treatable for those who are implementing the bioweapon (3).

Recent Advances

As researchers continue to transition from the era of DNA sequencing into the era of DNA synthesis, it may soon become feasible to synthesize any virus whose DNA sequence is known (4). This was first demonstrated in 2001 when Dr. Eckard Wimmer re-created the poliovirus and again in 2005 when Dr. Jeffrey Taubenberger and Terrence Tumpey re-created the 1918 influenza virus (1). The progress of DNA synthesis technology will also allow for the creation of novel pathogens. According to biological warfare expert Dr. Steven Block, genetically engineered pathogens "could be made safer to handle, easier to distribute, capable of ethnic specificity, or be made to cause higher mortality rates" (2).

The growing accessibility of DNA synthesis capabilities, computational power, and information means that a growing number of people will have the capacity to produce bioweapons. Scientists have been able to transform the four letters of DNA—A (adenine), C (cytosine), G (guanine), and T (thymine)—into the ones and zeroes of binary code. This transformation makes genetic engineering a matter of electronic manipulation, which decreases the cost of the technique (4). According to former Secretary of State Hillary Clinton, "the emerging gene synthesis industry is making genetic material more widely available […] A crude but effective terrorist weapon can be made using a small sample of any number of widely available pathogens, inexpensive equipment, and college-level chemistry and biology." (5)

Techniques to Enhance Efficacy of Bioweapons

Scientists and genetic engineers are considering several techniques to increase the efficacy of pathogens in warfare.

1. Binary Biological Weapons

This technique involves inserting plasmids, small bacterial DNA fragments, into the DNA of other bacteria in order to increase virulence or other pathogenic properties within the host bacteria (2).

2. Designer Genes

According to the European Bioinformatics Institute, as of December 2012, scientists had sequenced the genomes of 3139 viruses, 1016 plasmids, and 2167 bacteria, some of which are published on the internet and are therefore accessible to the public (6). With complete genomes available and the aforementioned advances in gene synthesis, scientists will soon be able to design pathogens by creating synthetic genes, synthetic viruses, and possibly entirely new organisms (2).

3. Gene Therapy

Gene therapy involves repairing or replacing a gene of an organism, permanently changing its genetic composition. By replacing existing genes with harmful genes, this technique can be used to manufacture bioweapons (2).

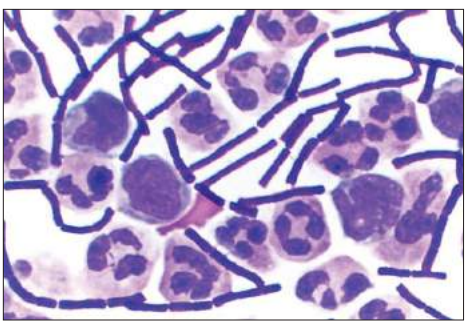

Image retrieved from http://commons.wikimedia.org/wiki/File:Gram_Stain_Anthrax.jpg (accessed 20 February 2013)

Figure 1: Gram stained cerebrospinal fluid showing gram-positive anthrax baccilli.

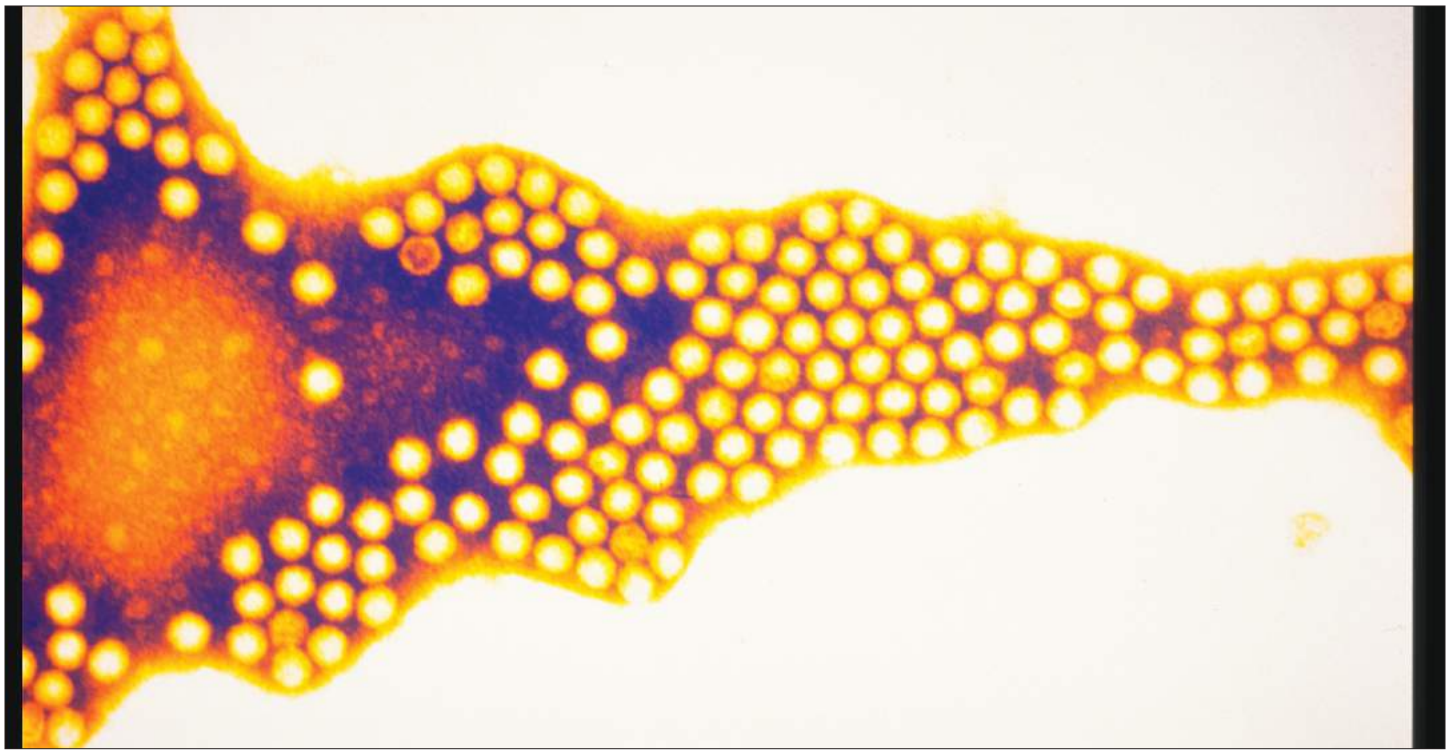

Image courtesy of Sanofi Pasteur. Retrieved from http://www.flickr.com/photos/sanofi-pasteur/5280384448/ (accessed 20 February 2013)

Figure 2: Virus responsible for poliomyelitus (picornavirus). Through improved vaccination efforts, polio will soon be eradicated from the globe.

4. Stealth Viruses

Stealth viruses are viral infections that enter cells and remain dormant for an extended amount of time until triggered externally to cause disease. In the context of warfare, these viruses could be spread to a large population, and activation could either be delayed or used as a threat for blackmail (2).

5. Host-Swapping Diseases

Much like the naturally occurring West Nile and Ebola viruses, animal viruses could potentially be genetically modified and developed to infect humans as a potent biowarfare tactic (2).

6. Designer Diseases

Biotechnology may be used to manipulate cellular mechanisms to cause disease. For example, an agent could be designed to induce cells to multiply uncontrollably, as in cancer, or to initiate apoptosis, programmed cell death (2).

7. Personalized Bioweapons

In coming years it may be conceivable to design a pathogen that targets a specific person's genome. This agent may spread through populations showing minimal or no symptoms, yet it would be fatal to the intended target (4).

Biodefense

In addition to creating bioweapons, the emerging tools of genetic knowledge and biological technology may be used as a means of defense against these weapons.

1. Human Genome Literacy

As scientific research continues to reveal the functions of specific genes and how genetic components affect disease in humans, vaccines and drugs can be designed to combat particular pathogens based on analysis of their particular molecular effect on the human cell (2).

2. Immune System Enhancement

In addition to enabling more effective drug development, human genome literacy allows for a better understanding of the immune system. Thus, genetic engineering can be used to enhance human immune response to pathogens. As an example, Dr. Ken Alibek is conducting cellular research in pursuit of protection against the bioweapon anthrax (2).

3. Viral and Bacterial Genome Literacy

Decoding the genomes of viruses and bacteria will lead to molecular explanations behind virulence and drug resistance. With this information, bacteria can be engineered to produce bioregulators against pathogens. For example, Xoma Corporation has patented a bactericidal/permeability-increasing (BPI) protein, made from genes inserted into bacterial DNA, which reverses the resistance characteristic of particular bacteria against some popular antibiotics (2).

4. Efficient Bio-Agent Detection and Identification Equipment

Because the capability of comparing genomes using DNA assays has already been acquired, such technology may be developed to identify pathogens using information from bacterial and viral genomes. Such a detector could be used to identify the composition of bioweapons based on their genomes, reducing present-day delays in resultant treatment and/or preventive measures (2).

5. New Vaccines

Current scientific research projects involve genetic manipulation of viruses to create vaccines that provide immunity against multiple diseases with a single treatment (2).

6. New Antibiotics and Antiviral Drugs

Currently, antibiotic drugs target DNA synthesis, protein synthesis, and cell-wall synthesis processes in bacterial cells. With an increased understanding of microbial genomes, other proteins essential to bacterial viability can be targeted to create new classes of antibiotics. Eventually, broad-spectrum, rather than protein-specific, anti-microbial drugs may be developed (2).

Future of Warfare

"The revolution in molecular biology and biotechnology can be considered as a potential Revolution of Military Affairs (RMA)," states Colonel Michael Ainscough, MD, MPH (2). According to Andrew Krepinevich, who originally coined the term RMA, "technological advancement, incorporation of this new technology into military systems, military operational advancement, and organizational adaptation in a way that fundamentally alters the character and conduct of conflict" are the four components that make up an RMA. For instance, the Gulf War has been classified as the beginning of the space information warfare RMA. "From the technological advances in biotechnology, biowarfare with genetically engineered pathogens may constitute a future such RMA," says Ainscough (2).

In addition, the exponential increase in computational power combined with the accessibility of genetic information and biological tools to the general public and lack of governmental regulation raise concerns about the threat of biowarfare arising from outside the military (7). The US government has cited the efforts of terrorist networks, such as al Qaida, to recruit scientists capable of creating bioweapons as a national security concern and "has urged countries to be more open about their efforts to clamp down on the threat of bioweapons" (5).

Despite these efforts, biological research that can potentially lead to bioweapon development is "far more international, far more spread out, and far more diverse than nuclear science […] researchers communicate much more rapidly with one another by means that no government can control […] this was not true in the nuclear era," according to David Kay, former chief U.S. weapons inspector in Iraq (7). Kay is "extraordinarily pessimistic that we [the United States] will take any of the necessary steps to avoid the threat of bioweapons absent their first actual use" (7).

"There are those who say: 'the First World War was chemical; the Second World War was nuclear; and that the Third World War – God forbid – will be biological'" (2).

CONTACT MACKENZIE FOLEY AT MACKENZIE.A.FOLEY.16@DARTMOUTH.EDU

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Computer Forensics in Criminal Investigations

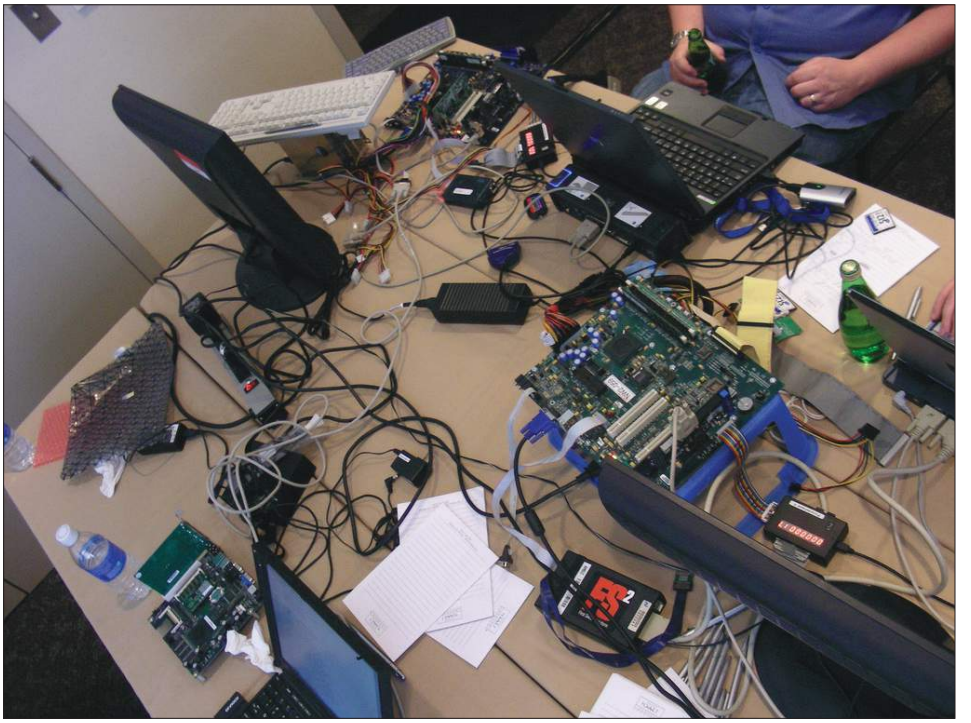
At the Intersection of Science and Law

BARRY CHEN

Computer forensics integrates the fields of computer science and law to investigate crime. For digital evidence to be legally admissible in court, investigators must follow proper legal procedures when recovering and analyzing data from computer systems. Unfortunately, laws written before the era of computer forensics are often outdated and cannot adequately assess the techniques used in a computer system search. The inability of the law to keep pace with technological advancements may ultimately limit the use of computer forensics evidence in court. Privacy advocates are growing especially concerned that computer searches may be a breach of a suspect's human rights. Furthermore, as methods for encryption and anonymity grow more advanced, technology may be abused by helping criminals hide their actions. Ultimately, the role of technology in computer forensics may not reach its full potential due to legal boundaries and potential malicious intentions.

Computer forensics has been indispensable in the conviction of many well-known criminals, including terrorists, sexual predators, and murderers. Terrorist organizations may use the Internet to recruit members, and sexual predators may use social networking sites to stalk potential victims. However, most criminals fail to cover their tracks when using technology to implement their crimes. They fail to realize that computer files and data remain on their hard drive even when deleted, allowing investigators to track their criminal activity. Even if criminals delete their incriminating files, the data remains in a binary format due to "data remanence" or the residual representation of data (1). File deletion merely renames the file and hides it from the user; the original file can still be recovered (2).

Eventually, data may be overwritten and lost due to the volatile nature of memory, a storage area for used data. A random access memory chip (RAM) retrieves data from memory to help programs to run more efficiently. However, each time a computer is switched on, the RAM loses some of its stored data. Therefore, RAM is referred to as volatile memory, while data preserved in a hard drive is known as persistent memory. The RAM is constantly swapping seldom used data to the hard drive to open up space in memory for newer data. Over time, though, the contents in the swap file may also be overwritten. Thus, investigators may lose more evidence the longer they wait since computer data does not persist indefinitely. Fortunately, computer scientists have engineered equipment that can copy the computer's contents without turning on the machine. The contents can then be safely used by lawyers and detectives for analysis (2).



Retrieved from http://en.wikipedia.org/wiki/File:Original-facebook.jpg (accessed 03 October 2012)

Figure 1: Computer system hacking. Computer forensics has been essential in convicting many well known criminals, including terrorists, sexual predators, and murderers.

Global Position System (GPS) software embedded in smartphones and satellite navigation (satnav) systems can also aid prosecutors by tracking the whereabouts of a suspect. Since companies that develop software for computer forensics also develop products for satellite navigators, they are well-equipped with the tools and technology necessary for acquiring GPS evidence.

However, the evidence that can be recovered from GPS software is limited to only a list of addresses. Current GPS software does not record the time when the address was archived, whether the address was inputted by a person or automatically recorded, or whether the owner's intent for entering the address was associated with the crime. Despite these limitations, GPS evidence has still been crucial to the success of many prosecutions. In one famous example, four armed suspects accused of robbing a bank in the United Kingdom were convicted because each suspect owned a vehicle whose satnav held incriminating evidence, including the bank's address and the addresses of the other three suspects. The Scottish National High-Tech Crime Unit searched a suspect's TomTom, a GPS device, to obtain thousands of addresses that the vehicle passed by. Many of the addresses turned out to be the scenes of criminal offenses (3). In 2011, U.S. forces successfully found the Pakistani compound where Osama bin Laden was killed by

tracking satellite phone calls made by his bodyguard (4).

While GPS evidence on its own may not be enough to establish a motive, GPS evidence can still provide invaluable leads or confirm a hunch. For example, contact lists, language preferences, and settings all may be used to establish a suspect's identity or identify accomplices. Evidence from GPS software and mobile devices can be a valuable supplement to other forms of evidence (3).

Some criminals have grown more cautious by hiding incriminating data through encryption techniques. However, according to Andy Spruill, senior director of risk management for Guidance Software, most criminals "don't have the knowledge or patience to implement [encryption software] on a continued-use basis." The minority of criminals who do encrypt their files may only use partial encryption. If only a few files on a hard drive are encrypted, investigators can analyze unencrypted copies found elsewhere on the device to find the information they are seeking. Furthermore, since most computer users tend to reuse passwords, investigators can locate passwords in more easily decipherable formats to gain access to protected files. Computer data are also oftentimes redundant - Microsoft Word makes copies each time a document is modified so that deleting the document may not permanently remove it from the hard drive. With so many forms of back-up, it is difficult for criminals to completely delete incriminating computer evidence (5).

While investigators can exploit computer system glitches to obtain evidence, technological limitations can often compromise a computer search. A common protocol for handling a mobile device found at a crime scene is to turn the power off. Investigators want to preserve the battery and prevent an outside source from using the remote wipe feature on the phone's contents. When the phone is turned off, the phone cannot receive text messages and other data that may overwrite the evidence currently stored in the device. However, turning off the device has its own consequences, potentially causing data to be lost and downloaded files to be corrupted (1).

To solve such problems, computer engineers have developed technology for shielding a device from connecting to a cellular carrier's network. Computer forensic scientists no longer need to turn off the device to isolate it. For example, radio frequency (RF) shielded test enclosure boxes help keep signals from entering or leaving the device. A Faraday bag, used in conjunction with conductive mesh, can also isolate a mobile device. Using these techniques, investigators can safely transport mobile devices to the lab while the device is turned on (1).

However, GPS software and Faraday bags are not foolproof. A cell phone isolated in a Faraday bag may adamantly search for a signal, depleting the phone's battery power. When searching for a network, cell phones are also losing data (1).

According to Professor David Last of University of Bangor, Wales, errors in locating signals may range up to 300 meters when obstructions are present. While "95 percent of [GPS] measurements fall within 5 metres of the true position" in clear and open areas, large geographical barriers and skyscrapers may severely block and reflect satellite signals. Interference from solar weather may also disrupt signals. Criminals even purposely use jammers to disrupt tracking systems. Investigators must carefully audit communications channels and monitoring systems used in tracking systems. In doing so, they can better avoid skepticism from the jury by being able to give a clearer and more precise estimate of the amount of error afflicting GPS measurements. Otherwise, the defense can suppress the GPS evidence if the measurements are significantly faulty and unreliable (3).

While the Fourth and Fifth Amendments were written long before the era of computers, both concepts still apply to the practice of computer forensics. The amendments serve to protect basic human rights by preventing unreasonable search and seizure and self-incrimination. In the case of *United States v. Finley*, the defendant claimed that "a cell phone was analogous to a closed container," suggesting that investigators should exercise the same restraint and caution in searching cell phones as they would in a bag or a private home. Generally, investigators must first obtain a search warrant, which is typically given by the court in order to obtain and preserve evidence that can be easily destroyed (1). However, exceptions to the rule have been observed in *United States v. Ortiz*; investigators legally retrieved telephone numbers of "finite memory" from a suspect's pager without a warrant because the contents of the pager can be easily altered when incoming messages overwrite currently stored data. Searches without a warrant "incident to arrest" are permissible because they help to prevent fragile data of evidentiary value from being lost (6). They consist mostly of scanning the device's contents using the keyboard and menu options. More advanced searches incident to arrest may include the use of a mobile lab, which allows for the immediate download of cellular phone data (7). However, according to *United States v. Curry*, searches "incident to arrest" can only be conducted "substantially contemporaneous with the arrest" (1). If investigators want to conduct further post-arrest forensic analysis, proper legal authorization must first be obtained (7).

Proper legal procedures are often vague and burdensome for investigators, especially since laws may vary from state to state. Some states may have a stricter policy regarding warrantless searches. In *United States v. Park*, the court ruled that since cell phones can hold a greater quantity of data than pagers, its contents are less likely to be lost; a warrantless cell phone search is thus unnecessary and unjustified. Similarly, in *United States v. Wall*, the court decided that "searching through information stored on a cell phone is analogous to a search of a sealed letter" (6). Even if investigators manage to obtain a search warrant, the evidence they find may still be suppressed if their forensic procedures fail to follow legal procedures. For example, looking through unopened mail and unread texts or not carefully documenting the chain of custody may constitute an improper search (1). With so many boundaries and inconsistencies in the legal system, it is often difficult for investigators to successfully perform their jobs.

Different state and national legal systems plague computer forensics as well. When an Estonian was charged with computer crimes in 2007, Russia refused to provide legal cooperation because it had not criminalized computer crimes yet. Russia received severe Distributed Denial of Service attacks for its lack of cooperation (8).

In addition to a faulty legal system, the accessibility of advanced technology may be afflicting computer forensics. The North Atlantic Treaty Organization (NATO) defines cyber terrorism as "a cyber attack using or exploiting computer or communication networks to cause

sufficient destruction to generate fear or to intimidate a society into an ideological goal" (8) As computer systems grow more powerful, criminals may also abuse computer systems to commit crimes such as software theft, terrorism, and sexual harassment (9). For example, stalkers can abuse the Tor Project, an anonymizing tool for victims of cybercrimes to safely report abuses, to instead hide their identities when they commit crimes of harassment. The technology is too advanced for the digital trail of cybercrimes to be tracked. As encryption programs grow stronger and more popular, forensic investigators may no longer be able to decode the hidden digital evidence.

Conclusion

For computer forensics to progress, the law must keep pace with technological advancements. Clear and consistent legal procedures regarding computer system searches must be developed so that police and investigators can be properly trained. An International Code of Ethics for Cyber Crime and Cyber Terrorism should also be established to develop protocols for "obtaining and preserving evidence, maintaining the chain of custody of that evidence across borders," and "clear[ing] up any difference in language issues." Following these measures may be the first steps to resolving the technological and legal limitations afflicting computer forensics. Interpol, the International Criminal Police Organization, has developed a Computer Crime Manual with "training courses" and "a rapid information exchange system" that serves as a foundation for international cooperation (8). Lastly, the criminal abuse of technology can be limited by equipping the police department with state-of-the-art training and equipment for forensic analysis. Only then is the world safely prepared to face the future of technology. As one author predicts, "the next world war will be fought with bits and bytes, not bullets and bombs" (8).

CONTACT BARRY CHEN AT BARRY.Y.CHEN.16@DARTMOUTH.EDU

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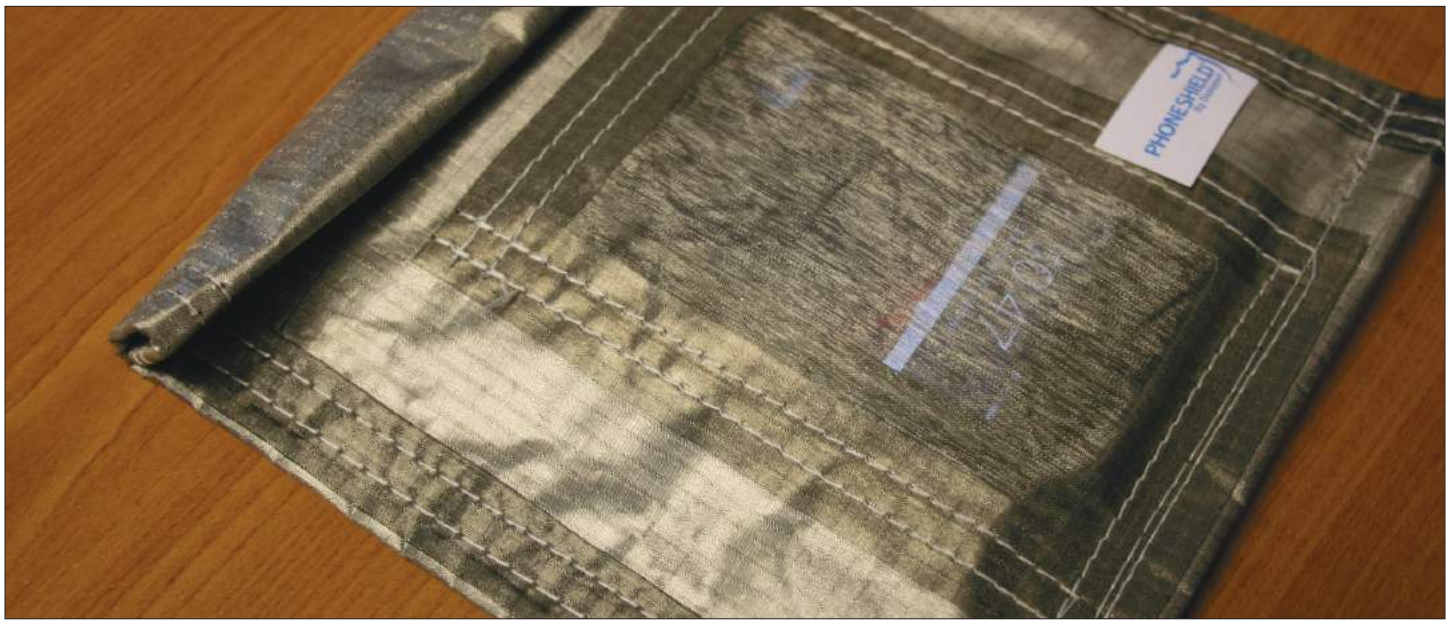

Image retrieved from http://commons.wikimedia.org/wiki/File:RF_bag_with_iPhone.jpg (accessed 20 February 2013)

Figure 2: Radio frequency bag with iPhone inside for reducing data loss. These bags keep radio signals from entering or leaving the device.

High-Quality Archaic Human Genome Sequencing

Advances in DNA Analysis

XIONGFEI ZHANG

Image retrieved from http://en.wikipedia.org/wiki/File:Turist_den-peschera.jpg (accessed 20 February 2013)

Figure 1: Tourists in front of the Denisova Cave, where the Denosivan bone fragment was found.

Recent advances in DNA preparation and sequencing have enabled high-quality analysis of archaic human genomes. Previously sequenced genomes of archaic humans, including the Neanderthal genome in 2010, were relatively low in quality due to the degraded state of the DNA samples. Information that could be inferred from these low-quality genomes was limited. A new method, invented in 2012, enabled a higher-quality rendition of the previously sequenced Denisovan genome. Analysis of the high-quality Denisovan genomic sequence suggests inbreeding between ancestors of Denisovans and ancestors of modern humans. The high-quality sequencing also gave insights to Denisovan population characteristics.

Initial Sequencing

In March 2010, scientists found a humanoid finger bone belonging to a juvenile female who lived 50,000 years ago in Denisova cave in the Altai Mountains, Siberia (1). An international group led by Svante Pääbo, a paleogeneticist at the Max Planck Institute, set out to sequence the DNA fragments left in the finger bone. From the initial analysis published in May 2010, the group found that the finger bone was neither Neanderthal nor modern human but rather an undiscovered archaic human. The archaic human was named Denisovan after the location where the bone fragment was found. Due to the age and small amount of DNA remaining in the finger bone, the group was only able to achieve 1.9x coverage (every nucleotide in the genome was sequenced 1.9 times on average) (1). This means the genome was too low in quality to be used to accurately document the finer genetic differences between Denisovans and modern humans.

New Method

In August 2012, the group published a new, high-quality version of the Denisovan genome. A new preparation method for DNA libraries, a collection of DNA fragments stored for sequencing, developed by Matthias Meyer allowed the group to increase the Denisovan genome coverage (2). DNA for sequencing is usually prepared in the double-stranded state. However, sequencing single-stranded DNA might produce more data from archaic DNA sources. The denaturing of double-stranded DNA into single-stranded DNA effectively doubles representation in the library (2). The protocol did not use DNA purification methods to prevent a possible loss in yield.

The libraries were sequenced with the Illumina Genome Analyzer platform. This produced reads, or short sequences of DNA, that were about 35 base pairs. The short reads were aligned to the modern human genome. This resulted in around 31x coverage of the approximately 1.86 billion base pairs of the human autosomal genome to which the reads were mapped. 99 percent of the nucleotides have been sequenced 10 or more times (2).

The group calculated the average error rate to be 0.13 percent of nucleotides sequences. This was done by comparing the constructed sequence with highly conserved regions within primates, with the assumption that deviances from highly conserved base pairs were errors. The group inferred 0.07 percent male modern human DNA contamination (2). Since the Denisovan DNA sample came from a female individual, any alignments to the human Y chromosome were considered contaminants.

Findings

For comparison, the group also sequenced the genomes of 11 modern day humans from various ethnic groups: a San, Mbuti, Mandenka, Yoruba, and Dinka from Africa; a French and Sardinian from Europe; a Han, Dai, and Papuan from Asia; and a Karitiana from South America (2). The group identified sites that are variable in a modern West African individual, who is not thought to be influenced by Neanderthal or Denisovan genetic flow (3), and counted the occurrence of derived alleles in Neanderthal or Denisovan but not in Chimpanzee. The group estimates that the Denisovan and modern human populations diverged around 170,000 to 700,000 years ago (large span due to uncertainty of human mutation rates) (2).

Image courtesy of Max Planck Institute for Evolutionary Anthropology. Retrieved from http://i.livescience.com/images/i/000/030/560/i02/denisovan-bone-120830.jpeg?1346348451 (accessed 20 February 2012)

Figure 2: The Denosivan fossil finger (a replica shown here with a U.S. penny).

From the initial analysis in 2010, the group found Denisovan DNA in Southeast Asian populations and concluded that ancestors of modern humans probably interbred with the Denisovans (1). Out of the 11 modern human populations sequenced in the new study, Papuans derive the most genetic material (6 percent) from Denisovans (2). Papuans also share more alleles with Denisvan genomes on the autosome chromosomes than on the X chromosome. A possible explanation is that genetic flow introduced to Papuan populations originated from Denisovan males.

The high quality of the new Denisovan genome allowed accurate measurement of its heterozygosity, or the percent of nucleotides that are different between the maternal and paternal genomes. Heterozygosity can also be used to measure genetic variation in the population. The group estimated that Denisovan heterozygosity to be 0.022 percent (2). This is a low percentage compared to the heterogyzosity of modern humans. Unusually long stretches of homozygosity were not detected, excluding immediate inbreeding as a possible explanation. The conclusion was that Denisovans have very low genetic diversity compared to that of modern humans. This implied that the Denisovans had a dwindling population at the same time when modern humans had an expanding population.

Although no phenotype information exists about Denisovans, the group could infer that the Denisovan girl had brown hair, skin, and eyes. The Denisovan female sequence carried alleles that are associated with dark pigmentation in modern humans.

The group also concluded that Denisovans contained 23 chromosomes, not 24 as in chimpanzees. A fusion of two chromosomes created human chromosome 2. The fusion event is characterized by a human-specific DNA sequence repeat. There are 12 occurrences in the Denisovan genome where sequences contained the unique repeat. The same test performed with the chimpanzee genome failed to produce the unique repeat. The group concludes that Denisovans shared the same two chromosome fusion event as shown in modern humans (2).

The sequencing of an archaic human allows comparison with modern humans for the identification of traits unique to modern humans. The group identified 111,812 single nucleotide changes (SNCs), including 269 changes that cause amino acid changes, and 9,499 nucleotide insertions and deletions distinguishing modern human genomes from that of Denisovans and chimpanzees. These changes were found in strongly conserved genes, including genes associated with brain function. One of the genes, CNTNAP3, is associated with speech formation. There is speculation that critical components of speech and synaptic transmission may have only arisen in modern humans. There were also 34 differences in genes associated with skin and eye diseases in modern humans. It seems possible that physiology of the skin and eye have diverged since the last common ancestor between Denisovans and modern humans (2).

Future

There are many unresolved questions about the Denisovan humans. The observation that the Denisovans have low genetic variability conflicts with the diversity of the Denisovan's habitats, which ranged from Siberia to south eastern Asia and Austrialia. While it is possible that the Denisovans had a quick population growth phase, more research is needed to determine their true geographic range and to see whether populations in Siberia and populations further south were genetically related.

The new method employed in the 2012 study will provide a path for other ancient humans to be sequenced. Two Denisovan teeth were also discovered in the Denisova cave. More Denisovans could be sequenced to determine the population characteristics of the ancient humans. The new method for sequencing archaic DNA could be used to improve the Neanderthal genome. A new high-quality sequence of Neanderthal genome could be used to determine the timeline of Neanderthal population expansion. If the Neanderthal expansion timeline is consistent with the Denisovan timeline, the population model could be used to inform the diversity of the population in the Out-of-Africa theory.

Conclusion

Although Denisovans are thus far only represented by one finger bone and two teeth, they are currently the most well-known archaic human genetically – including Neanderthals of which there are hundreds of fossil records (4).

The differences between modern and archaic humans are important to catalog in order to further understanding of why archaic humans eventually became extinct and how modern humans expanded their population.

CONTACT XIONGFEI ZHANG AT XIONGFEI.ZHANG.16@DARTMOUTH.EDU

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Climate Change and the Decline of Mayan Civilization

A Look into Climate History

NA EUN OH

Introduction

New evidence shows that droughts were a significant factor in the fall of the Mayan civilization. Paleoclimatologist Douglas Kennett of Pennsylvania State University led an international team of researchers that analyzed a 2,000 year–old stalagmite from a cave in South Belize (1). Kennett obtained semiannual climate records through his analysis, and compared this record to historical events. The team proposes that high rainfall favored Mayan population growth between 440 and 660 C.E. Then, between 660 and 1000 C.E., a drying trend led to increased warfare and the division of political states (2). A drought lasting between 1020 and 1100 C.E. occurred in the midst of the population collapse, which marked the end of the Classic Mayan civilization (2,3).

The Mayan civilization experienced its peak during the Classic period (250–950 C.E.). The Mayans lived in the lowlands of the Yucatán Peninsula, which includes parts of southern Mexico, Guatemala, and Belize. Between 750-950 C.E., the Mayan civilization deteriorated as the Mayans abandoned many of their major cities. Archaeologists call this period the Terminal Classic collapse, and the cause of this demise remains unclear (3).

Early Theories for Mayan Decline

Prior to the development of the drought theory on Mayan civilization's demise, researchers had suggested soil erosion as the cause of the civilization's downfall. The Mayans chopped down forests to create greater farmland, resulting in soil erosion that would have made farming more difficult. However, recent studies of Guatemalan Lake Salpetén's sediments show little erosion during the Terminal Classic period. In fact, Flavio Anselmetti of the Swiss Federal Institute of Aquatic Science and Technology found that clay soil deposits mostly accumulated during the early Mayan era (4). Though a variety of other reasons have been suggested, drought currently remains the most accepted.

The Yucatán Peninsula, where the Mayans resided, is a seasonal desert. The region depends on heavy summer rains that provide as much as 90 percent of the annual precipitation. Annual precipitation also varies drastically across the peninsula. Surface water often dissolves the limestone bedrock of the Yucatán, and also creates caves and underground rivers. Because of these underground formations, surface water is scarce. Therefore, the Mayans used artificial reservoirs as their source of water during their four- to five-month-long winter dry spells. Tikal, a Maya city, had enough reservoirs to supply 10,000 people for 18 months. However, the reservoirs still depended on seasonal rain to replenish their supply (3).

While Douglas Kennet's data provides greater support for the drought theory, it is not a new concept. David A. Hodell had proposed the idea in 1995 after analyzing sediment records in Lake Chichancanab. The lake is located in Yucatán, Mexico and possesses gastropod and ostracod shells with varying levels of the isotope ^{18}O. A small percent of $H_2{}^{18}O$ naturally resides in the lake water, but when temperatures rise, the proportion of $H_2{}^{18}O$ becomes greater. This is due to unequal levels of evaporation between $H_2{}^{18}O$ and $H_2{}^{16}O$. Since $H_2{}^{16}O$ is lighter than $H_2{}^{18}O$, $H_2{}^{16}O$ water evaporates more readily. Higher levels of $H_2{}^{18}O$ occur with greater evaporative loss, or in dry climates. David Hodell studied gastropod and ostracod shells because the carbonate in the shells reflects the higher ^{18}O levels. The shells also incorporated gypsum and calcite. Lake Chichancanab is saturated in both calcite and gypsum, but in drier climates there is a higher gypsum/calcite ratio. The shells also demonstrate this pattern.

Hodell's analysis of the shells suggested the existence of a drought during the latter portion of the Mayan Classical period, but it was too broad. The dating for the shells has an error margin of 35 to 65 years. Hodell's analysis was too broad a time frame to link with historical events (5).

Other scientists have studied lake and marine sediments to infer the climate as well. Studies conducted in Lake Punta Laguna in Mexico and the Macal Chasm in Belize also provided time frames that were too broad to compare with the complex political changes. A study performed in the Cariaco Basin in Venezuela had greater precision, but the location was too far from the Mayans. The different regions may have experienced varying climate.

Current Climate Research

In comparison to previous climate studies, Douglas Kennett's dating of the samples has a smaller margin of error. Kennett's team uses high resolution uranium-thorium dating. The steady radioactive decay of uranium-234 to thorium-230 has a half-life of 245,500 years and a small error range of 17 years. With a higher precision provided by the uranium-thorium dating, the research team can project dates within a couple of decades rather than centuries (6,7). Additionally, York Balum Cave, the original location of the Chaac stalagmite, is located within 200 km of major Mayan centers such as Tikal and Copan. The close proximity supports the validity of Kennett's data, as the two sites should experience the same climate system. The precise nature of the stalagmite's dating allows the research team to compare their climate data with complex historical records.

To obtain climate data, Kennett measured the concentrations of $^{18}O_2$ and $^{16}O_2$ in 0.1 mm increments of the stalagmite.

Image courtesy of Sémhur. Retrieved from http://en.wikipedia.org/wiki/File:Maya_civilization_location_map-blank.svg (accessed 20 February 2013)

Figure 1: A map of the Classical Mayan Civilization.

Image courtesy of Truthanado. Retrieved from http://commons.wikimedia.org/wiki/File:MayanCalendar.png (accessed 20 February 2013)

Figure 2: A Mayan calendar created by a modern crafsman.

Each 0.1 mm increment represented half of a year. Higher relative levels of ^{18}O suggest a drier climate while lower relative levels a suggest wetter climate (2). The validity of the stalagmite's climate information was confirmed using a Hendy test. The Hendy test assesses isotopic equilibrium based on two conditions: a lack of correlation between the presence of isotopes $^{18}O_2$ and ^{13}C, and a consistent level of $^{18}O_2$ for each growth layer of the stalagmite (8).

Kennett's climate data is skewed due to evaporation within the caves. Because there was another small level of evaporation, the $^{18}O_2$ levels are higher and suggest slightly drier climates (2). The conversion of the $^{18}O_2$ levels to quantitative rainfall measurements is currently unobtainable, so Kennett uses the data to describe periods as either wet or dry (6).

Correlations Between Data and History

Douglas Kennett's research confirms the long "drying trend" predicted by earlier studies. He maps the drying trend to have started in 640 C.E. and peaked in 1020 C.E. Kennett's data also show particularly long droughts between 200-300 C.E., 820-870 C.E., 1020-1100 C.E., and 1530-580 C.E. Short but very severe droughts also occurred in 420, 930, and 1800 C.E.

The drying trend shown in the data from 200-300 C.E. matches the demise of El Mirador, a pre-Mayan settlement located in northern Guatemala. However, this correlation is contrasted with the persistence and expansion of other societies such as the

Evidence points to high levels of rainfall between 400 and 500 C.E., the same time period as the Early Classic expansion. The heavy rainfall continually recharged the urban water storage systems and would explain the growing influences of Tikal and other Mayan centers. The period of 440-500 C.E. also had the best recorded ruling Maya lineages. The 820-870 C.E. drought predicted by the research matches the Terminal Classic period. Kennett's research also supports a previous prediction of a 40 percent decrease in summer rainfall during the Terminal Classic period (2). Kennet's research team also found that war related events increased during the early stages of a drying trend that spans from 640-1020 C.E. From 750-775, C.E. rulers commissioned monuments at unprecedented rates. These monuments possessed text that pointed to status rivalry, war, and strategic alliances. A sudden drop in the number of texts at key Maya centers that followed provides evidence for the failure of Mayan political systems.

The Classical Mayan civilization ended with gradual depopulation and a shift of political power to northern parts of the Yucatán Peninsula.

Kennett's research team suggests a two stage collapse of the Classical Maya civilization. The first stage starts with the 660 C.E. drying trend that increased warfare and political destabilization. This primary stage worsens with the dry interval between 800-900 C.E., which causes a reduction of agricultural activity and more political disintegration. The second stage is a more gradual population decline punctuated by spurts of more drastic population reductions during the driest interval between 1020-1100 C.E (2).

While the drought that occurred from 1530-1580 C.E. was beyond the Mayan Classical period, Kennett's team compared their climate data with Yucatán records. Records matched the data to a drought that occurred between 1535-1575 C.E. Historical accounts also linked this drought to famine, disease, death, and population relocation. This example shows the impact of dry conditions to populations.

Criticisms and Conclusions

Kennett's research accomplishes a more accurate estimate of the region's paleoclimate. However, Arlen Chase of the University of Central Florida believes that more climatic records are needed, because of microclimatic variations within the Mayan civilization. The Yucatán Peninsula does experience drastically different annual precipitation among its regions. The Maya migration to northern Yucatán, which is much drier, also conflicts with the drought theory. Andrew Scherer, an archaeologist of Brown University, also questions the link between less rainfall and meager harvests. Corn, a major crop for the Maya, requires only 400 to 600 mm of rain, and the Yucatán peninsula gets an average of 2000 to 3000 mm of annual rainfall. If precipitation dropped only 40 percent during the Terminal Classic period, then the decrease in rainfall might not have affected food supplies as drastically as the drought theory assumes.

Kennett's interdisciplinary study shows climate change in a social context (6).

CONTACT NA EUN OH AT
NA.EUN.OH.16@DARTMOUTH.EDU

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Can HIV Be Cured?

The Remarkable Story of the Berlin Patient

STEPHANIE ALDEN

The human immunodeficiency virus (HIV) infection and its more advanced counterpart, acquired immunodeficiency syndrome (AIDS), affect approximately 34 million people worldwide, with the number of infected patients growing by over two million every year (1). Since first being detected in 1981, AIDS has claimed the lives of almost 30 million people around the world (1). Scientists have searched for a cure for years without success, but the treatment of Timothy Brown, also known as the "Berlin Patient," may change that forever.

Overview

HIV harms its hosts by targeting immune cells, which leaves infected individuals prone to opportunistic infections. HIV initially attaches to proteins called CD4 receptors. These receptors, found on the surface of white blood cells, aid in the recognition of foreign substances (2). The main targets are T-helper cells, which fight infection by activating other immune cells (2). HIV must also interact with a chemokine receptor on the cell that binds to cell-signaling molecules (2). Once the virus binds to the target cell, it enters and releases its genetic contents into the cytoplasm (2).

HIV is a retrovirus, meaning that its genetic information is stored in the form of single-stranded RNA. Before the virus can overtake the cell, an enzyme called reverse transcriptase must convert this RNA to double-stranded DNA. This viral DNA integrates itself into the DNA of the cell, creating infected DNA called a provirus. The provirus subsequently replicates to create more HIV viruses using the host's own biological machinery (2). The exact mechanism that the virus uses to kill immune cells is unknown, but its effects have been identified.

Depending on the person and the severity of infection, the time between infection and the initial manifestation of symptoms, ranging from rashes to fatigue, varies from months to years (3). An individual is diagnosed with AIDS when he or she is HIV positive, has a CD4+ T cell count below 200 cells/mm^3, and/or has an AIDS-related infection, which can take years to develop (3). These AIDS-related infections include several types of cancer and a range of parasitic, viral, fungal, and bacterial infections such as tuberculosis and meningitis. These infections, not HIV, are the direct cause of death in most patients (3).

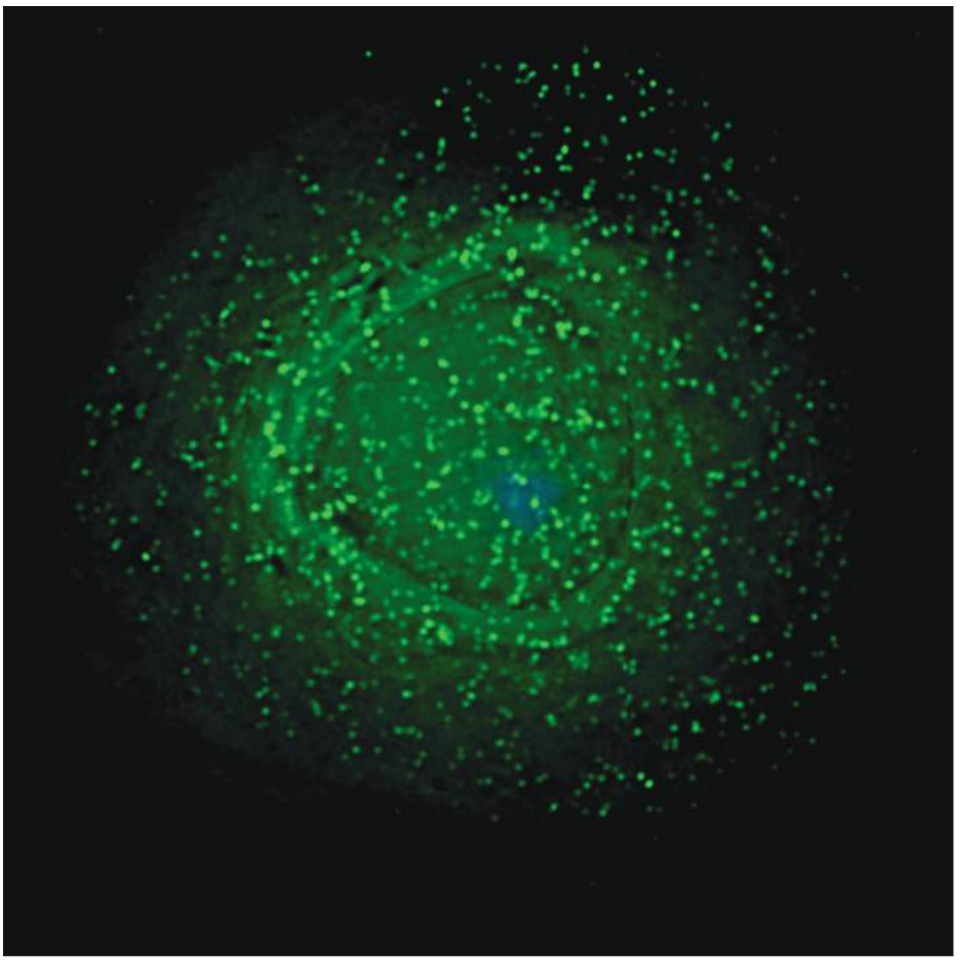

Image courtesy of L Gross. Retrieved http://commons.wikimedia.org/wiki/File:HIV_on_macrophage.png (accessed 21 February 2013)

Figure 1: HIV-1 particles assembling at the surface of an infected macrophage. Since first being detected in 1981, AIDS has claimed the lives of approximately 34 million people.

History of Treatment

Early treatment of HIV/AIDS in the 1980s used antiretroviral drugs that blocked the enzyme necessary to convert RNA to DNA (4). In the early stages of treatment, doctors used one drug at a time to block HIV. However, after years of this method, they realized that the virus could quickly develop resistance, rendering a one-drug approach ineffective (4).

With the development of different types of antiretroviral drugs targeting different enzymes in the HIV life cycle, doctors now use highly active antiretroviral therapy (HAART), a "cocktail" of HIV drugs (4). By using this method, when the virus develops a resistance to one drug, two or three other drugs may still suppress the virus by blocking different stages in viral development. This treatment has only been able to slow the progression from HIV to AIDS, but new stem cell treatments may give hope to HIV/AIDS patients around the world.

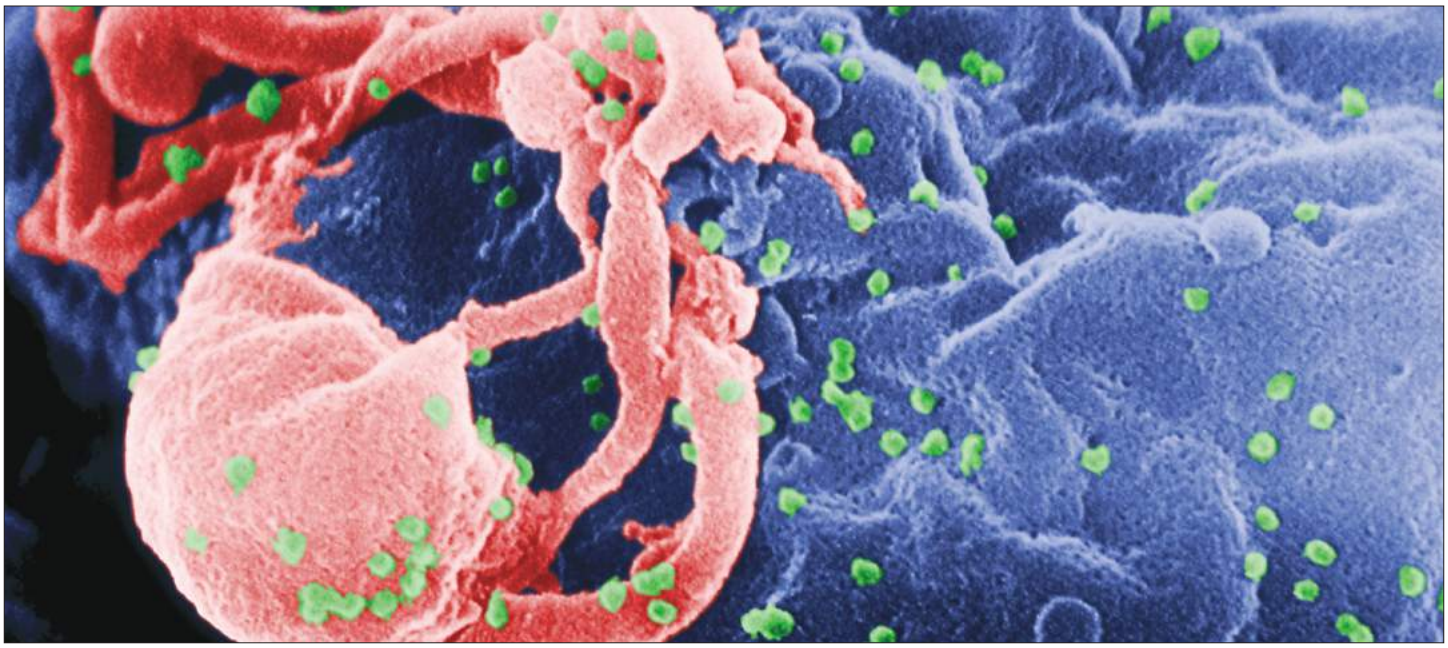

Image courtesy of C. Goldsmith (CDC). Retrieved http://commons.wikimedia.org/wiki/File:HIV-budding-Color.jpg (accessed 21 February 2013)

Figure 2: Scanning electron micrograph of HIV-1 budding (in green) from cultured lymphocyte. AIDS-related infections include several types of cancer and a range of parasitic, viral, fungal and bacterial infections such as tuberculosis and meningitis.

The Berlin Patient

Timothy Brown had been infected with HIV for almost eleven years when he was diagnosed with acute myeloid leukemia. This cancer affects both bone marrow--a tissue that helps form blood cells--and white blood cells (5). To treat both his acute myeloid leukemia and HIV at the same time, doctors had to come up with a new treatment protocol beyond HAART and chemotherapy to keep Brown alive.

Dr. Gero Hutter, an oncologist from Berlin, decided to give Brown a stem cell transplant with peripheral blood stem cells from a matched donor (5). These stem cells can differentiate into healthy CD4+ T cells and replace formerly infected, cancerous cells (6).

This donor's cells differed from Brown's in that they were homozygous for a 32 base pair deletion in the part of DNA that codes for chemokine receptor 5 (CCR5) (5). This receptor is one of the aforementioned co-receptors that HIV uses to infect host cells. This 32 base pair deletion prevents the production of a normal, active CCR5 receptor (5). Without this receptor, HIV cannot enter and infect host cells, and the virus cannot replicate (5).

Before the first transplant, Brown underwent an intense round of chemotherapy and total body irradiation to kill diseased white blood cells. Following the transplant, both chemotherapy and HAART were halted until Brown had a relapse of acute myeloid leukemia (6). He then underwent another round of chemotherapy and a second transplant, which then left him free of cancer and with an undetectable load of HIV (6).

After the transplants, healthy donor-derived CD4+ T-cells reconstituted Brown's immune system (6). Further testing showed that T-cells throughout his body had the same rare mutation found in the donor that prevented the HIV virus from infecting cells. This meant that CD4+ T-cells derived from Brown's body could not be found in the immune system (6). Replacement of host cells with donor cells also reduced the amount of HIV virus in Brown's body, thus further reducing the chance that Brown's new cells could be infected (6).

Controversy

Doctors had not expected Brown's HIV to become completely undetectable (5). Instead, they believed the virus would attempt to infect T-cells during immune reconstitution via a different co-receptor called CXCR4 (6). However, there is still a possibility that HIV may lie latent somewhere in Brown's body.

Due to the significance of Brown's case, researchers around the world are interested in studying him. Recently, a team at the University of California, San Francisco found traces of viral nucleic acid in blood cells and tissue samples that did not match the virus found in Brown at the outset of infection (7). However, researchers at the University of California, San Diego conducted similar tests and found no traces of HIV in Brown's system (7).

Nonetheless, the traces of viral nucleic acid have caused many to question whether Brown has really been cured, but researchers at both institutions assert that these segments of viral DNA are either contaminants or are not actively replicating (7). Either way, Timothy Brown no longer has actively replicating HIV in his system, and most experts deem him cured.

Further Treatments and Future Directions

While Brown is the first patient to be cured of HIV, he may not be the only one. Two men in Boston, both of who suffered from HIV infection and lymphoma, a type of blood cancer, are also HIV-free following stem cell transplants. Unlike Brown, these patients received bone marrow transplants from donors who were not homozygous for the 32 base pair deletion (8). Instead, doctors simply kept these patients on HAART while they underwent stem cell transplantation (8).

Donor cells helped rid the patients' bodies of infected cells by replacing them while the HAART protected the donor cells from infection (8). Both patients saw reduced levels of HIV the first few months after transplantation, and over three years later, HIV was undetectable in both patients (8). However, both patients are still on HAART, so researchers are hesitant to

call this method a "cure" (8).

Other researchers are trying to find treatments to rid the body of HIV without costly and dangerous stem cell transplants and surgeries. Researchers at the University of North Carolina at Chapel Hill recently discovered that vorinostat, a drug normally used to treat certain lymphomas, could awaken hidden HIV viruses in affected patients (9). Vorinostat inhibits histone deacetylase, an enzyme that condenses and silences DNA, thus enabling HIV to hide in cells. In this case, inhibition of the enzyme enables the production of the HIV virus and allows for the identification of infected cells that may serve as viral reservoirs (9). With the proper treatment, methods such as this could potentially lead to elimination of the virus from the body.

In France, scientists have tried to recreate elite controllers, patients who are HIV positive but whose bodies are able to prevent the virus from replicating and destroying their immune systems. Researchers identified and treated individuals with HAART for three years within ten weeks of infection (10). After years without treatment, these patients still have detectable levels of HIV, but, like the bodies of elite controllers, their bodies can control the virus (10). Charline Bacchus, the leader of this study, explained that HIV lies mainly in short-lived T cells in these patients and believes that this difference helps the infection from spreading (10).

Additional areas of exploration include gene therapy to alter patients' DNA, the creation of more targeted drug treatments, and other ways to bring the virus out of its latent stage and identify it. The two viable paths to a cure identified by scientists are total elimination of the virus from the host--a sterilizing cure--and the strengthening of the immune system, which enables it to fight off HIV--also known as a functional cure.

Conclusion

While researchers have made great strides in the fight against HIV/AIDS, there is still much work to be done. Other options discussed throughout this article are still in early stages of development and may only work in a small percentage of patients. The most successful treatment to date, stem cell transplants, are highly expensive and have a high mortality rate, ranging from 20 to 35 percent depending on the facility and condition of the patient (11). Furthermore, early treatment of HIV may only strengthen the immune system in five to 15 percent of patients (10).

There is still much to learn about HIV and the way it affects cells before an appropriate cure accessible to all infected patients can be found.

CONTACT STEPHANIE ALDEN AT STEPHANIE.L.ALDEN.16@DARTMOUTH.EDU

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Nuclear Marine Propulsion

The History of Marine Nuclear Technology

SHINRI KAMEI

Nuclear marine propulsion, like microwaves, was a brainchild of war. While research into atomic fission had started in the late 19th century, World War II provided a new sense of urgency for scientists. Nuclear technology made leaps in the six years leading to the conclusion of WWII, culminating in the creation of the atomic bomb.

In the wake of WWII, the world entered its "atoms for peace" days. People envisioned revolutionizing the world with nuclear powered cars, fridges, and ships (1). By the end of the decade, The Cold War continued to push the progress of nuclear research, although by the end of the decade, even the sea had adopted nuclear energy.

On the seas, leagues away from a port, the scarcity of resources limited warship capabilities. The longer the time spent at sea, the more equipment and food was needed. However, these obstacles were small in relation to the thousands of pounds in bunker fuel that ships demanded.

Nuclear power was perfect for the largest of ships spending months away from home. While merchant and commercial ships proved too small for a nuclear reactor to power efficiently, hulking icebreakers and submarines were suitable for nuclear energy. Unlike conventional fuel sources, reactor cores do not require refueling for over 10 years (2). It took the world's superpowers years and millions of dollars of research to arrive at these conclusions, but the introduction of nuclear reactors to ships began a new era of seafaring. The limits on how long vessels could stay away from port would soon shift to depend on their human occupants.

Image courtesy of U.S. Navy. Retrieved from http://en.wikipedia.org/wiki/File:Hyman_Rickover_inspecting_USS_Nautilus.jpg (accessed 21 February 2013)

Figure 1: Captain Hyman G. Rickover, the "Father of the Nuclear Submarine," inspects the USS *Nautilus* in 1954.

The History of Nuclear Marine Propulsion

First Steps

Rising tensions between the United States and the Soviet Union caused the United States to bulk up its military resources. The nation needed bigger and better planes and ships, and in the wake of the destruction caused by "Little Boy" and "Fat Man" over Hiroshima and Nagasaki, respectively, the focus was on the atom. The United States managed to show that nuclear fission could indeed power a jet engine, but the project was cancelled in 1955 and an operational aircraft was never developed (3). Research into nuclear fission for the seas was more fruitful, and models today continue to use a system used by many nuclear land reactors.

The United States Navy beat the Soviet Union to the nuclear powered ship. The development of a nuclear propulsion plant was authorized by Congress in July 1951. Captain Hyman G. Rickover led the Naval Reactors Branch of the Atomic Energy Commission and would go on to be known as the father of the nuclear submarine (see Fig. 1).

By 1955, the navy had placed a nuclear propulsion reactor in the USS *Nautilius*. The first lady, Mamie Eisenhower, christened the ship with champagne. After the ship set sail, its operators signaled back "UNDERWAY ON NUCLEAR POWER" in Morse code. The next month, *Nautilus* departed for southern waters, travelling from New London to San Juan, Puerto Rico.

It travelled 1381 miles, a distance ten times longer than any other previous submarine, in less than 90 hours, setting a record for the longest period of submergence for a submarine. And for the first time, the submarine maintained an average speed of 16 knots for more than an hour, two times faster than the speed of most submarines during WWII (4).

Expansion

By 1962, the U.S. had 26 nuclear submarines with 30 more on the way. The U.S. shared its technology with the United Kingdom while Russian, French, and Chinese developments progressed independently. By this time, the nation was eager to see its work profit. The work to apply the new technology to commercial ships had begun. That same year, a retired naval captain aboard the NS *Savannah* (see Fig. 2), the world's first nuclear merchant ship, observed the following:

"I stood in the *Savannah's* swept-back superstructure as she moved out into the Atlantic. She slipped along at an effortless 17 knots with only 60 percent power. I heard no noise save the swish of water at her sides. She was clean as a sailing ship; the nuclear plant gave off no smut or smoke or exhaust" (5).

Germany's *Otto Hahn* and Japan's *Mutsu* soon followed, but these ships actually proved that using fossil fuels for commercial ships was more economically efficient (6). Issues of liability, which could span across numerous nations in the case of an accident, made commissioning nuclear ships tedious. Nuclear energy systems aboard ships would also need to be extremely small, thus the uranium fuel had to be highly enriched. In the wrong hands, the fuel could be used to power atomic bombs (1).

As a research team in the United Kingdom in the 1960s reported, processing nuclear waste was difficult, and storing it for long lengths aboard was impossible. The ships would need access to a port where the waste could be dropped off and handled. At the time, they concluded that nuclear reactors could only be used on large ships that were run intensively on a few long routes with established terminal facilities (7).

The Brussels Convention on the Liability of Operators of Nuclear Ships was a multinational agreement specific to nuclear ships and would have made operation more feasible for commercial ships. After its initial stages of development, disagreements regarding the operation of military vessels blocked the convention, and the agreement was never ratified (8). Nuclear merchant ships ended up as a luxury that could not be diplomatically afforded, and only one more was ever produced.

How Nuclear Propulsion Works

Adaptations for the Seas

Nuclear reactors aboard ships are not immune to the perils of sea. Although the ship can move with degrees of freedom that land reactors cannot endure, space and weight aboard is limited, and the ship is isolated at sea. The reactor must be able to withstand the rolling and pitching seas, be able to generate higher power per unit of space, and fulfill stability requirements. While land reactors produce thousands of megawatts of power, a marine reactor only produces a few hundred megawatts.

The reactor also needs to be extremely sturdy and reliable. However skilled the personnel, maintenance away from port is difficult, and a breakdown can leave a ship stranded at sea with its contaminants seeping across international waters.

The reactor is made to minimize risk. In U.S. Naval submarines, the reactor is typically housed in a cylindrical section in the belly of the submarine, sandwiched between shielded bulkheads. One hundred tons of lead shielding surrounds each reactor. Only the inside of the reactor, roughly the size of a garbage can, is inaccessible for inspection and replacement (1). It relies on its long core life and the ship's relatively low energy demand to continue operation. By contrast, a land reactor is inspected and refueled every eighteen months (10).

Pressurized Water Reactor

In a typical nuclear reactor, a pressurized water reactor generates steam that rushes through turbines to produce energy. Pellets of uranium oxide arranged in tubes form the reactor core, and fission in these rods release neutrons from the nucleus of uranium. This release generates energy, and a moderator, typically water, slows down these neurons in order to further fission in other atoms. Neutron-absorbing material such as cadmium and hafmium are inserted or withdrawn from the core to control the reaction rate (9).

The massive amounts of energy released by the core heat up the water in the primary coolant loop of the reactor. The loop holds the water at high pressures and prevents it from evaporating until the water reaches the steam generator. Once the water evaporates, the steam travels through the main turbine. The turbine's spin generates electricity.

The steam occupies almost 2,000 times the volume that an equal amount of water would. It moves to the condenser, where electricity-powered pumps circulate water through the cooling system. The water then cools the steam enough so that the steam condenses, later to be reheated and sent through the reactor again.

The primary danger of nuclear energy lies in this condensation process. In the case that the system is cut off from the outside power grid, as in the recent Fukushima explosion, heated steam does not return to water. The build-up of this high-volume steam ultimately leads to a pressure explosion of steam—not, contrary to a common misconception, of the core

Image courtesy of NARA. Retrieved from http://en.wikipedia.org/wiki/File:NSsavannah-1962.jpg (accessed 21 February 2013)

Figure 2: The NS Savannah, the first nuclear-powered merchant ship, in 1962. The failure of the Brussels Convention severely limited the number of nuclear merchant ships.

itself. Every nuclear energy system is equipped with backup upon backup in the case of failure, and the failure of all of these systems is extremely rare (Fig. 3).

Nuclear Marine Propulsion Today

At the end of the Cold War in 1989, 400 nuclear submarines were operational or under construction. Since then, at least 300 have been scrapped due to weapons reduction programs, and there are currently about 120 vessels in operation, including newly constructed ones.

Since the commercial nuclear ship industry closed in the 1970's, lack of competition and incentive have driven naval suppliers to a lull in development. Unlike in markets of other military weapons systems, such as aerospace and electronics, prices have crept up. Producers of civilian reactors could not and did not have the financial incentive to match the complexity of maritime reactors (10).

However, recent changes have returned interest to this market. Shipping currently accounts for 5% of greenhouse gas emissions, and the world is focused on climate change. Gen4 Energy, an outgrowth of Los Alamos National Laboratory, has developed a small modular reactor that produces 25 MW using low enriched uranium, while a typical reactor generates 1500 MW (1). The power and fuel choice of the reactor makes what this company calls "nuclear batteries" a breakthrough for the nuclear world.

Nuclear power creates close to zero emissions and is cost-effective. While the per-unit cost of fuel is higher than that of traditional fuels such as coal or gas, the overall cost per unit of energy is much lower in nuclear energy. Regardless, concerns about safety continue to impede the development of nuclear power. Gen4's nuclear battery is also under scrutiny because its small size makes it vulnerable to theft and abuse.

Nuclear technology has made massive strides since its conception in the early 20th century. Its applications have been limited due to safety concerns, but the new war against global warming is poised to drive its progress. In a span of a few years, safety risks may be minimized and nuclear power—aboard ships and even in cars—may be the norm. Perhaps a reprisal of the Atoms for Peace days will come at last.

CONTACT SHINRI KAMEI AT SHINRI.KAMEI.16@DARTMOUTH.EDU

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Physician-Assisted Suicide

The Arguments and Criticisms Surrounding PAS

DANNY WONG

Physician-assisted suicide (PAS) is the provision of medication for the purpose of bringing about death. Throughout history, physicians have secretly practiced PAS and euthanasia, in which doctors directly administer lethal drugs. In the United States, ethical and legal debates over PAS sprung into prominence in 1990 when Jack Kevorkian brought the topic to the public's attention. Kevorkian, nicknamed Dr. Death, advocated its legalization and, until his death, had conducted over 130 assisted suicides (1). In June 1997, the U.S. Supreme Court ruled that individuals do not have a constitutional right to PAS. But the court did not prohibit the practice either. Rather, it allowed the states to decide for themselves. As of early 2013, Oregon, Washington, and Montana are the only three states that have legalized PAS, having done so in 1997, 2008, and 2009, respectively (2). Whether the option of PAS is a constitutional right, an act of compassion, or a violation of the traditional principles underlying medicine, the concerns surrounding PAS continue to carry implications for patients near the end of life.

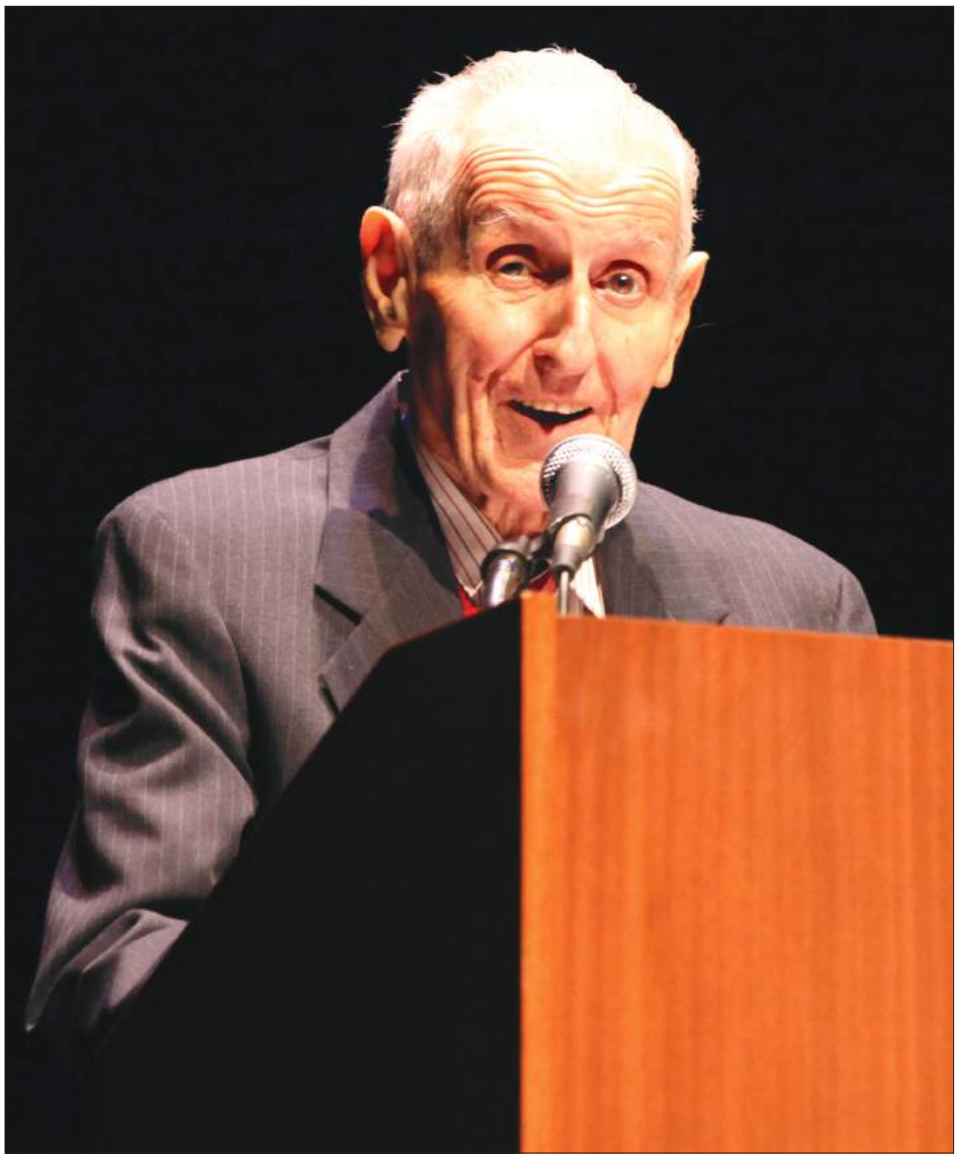

Image courtesy of Greg Asatrian. Retrieved from http://commons.wikimedia.org/wiki/File:KevorkianUCLARoyce.jpg (accessed 21 February 2013)

Figure 1: Jack Kevorkian, nicknamed Dr. Death, participated in over 130 assisted suicides. He died in June, 2011.

Reasons for Requesting PAS

Investigators have conducted studies on patient rationale for requesting assisted suicide. Results indicate that uncontrollable pain is not the most important factor among patients interested in PAS. Instead, patients more frequently cite depression, hopelessness, and psychological distress as reasons for PAS (3). Other motivations include loss of autonomy, dignity, and fatigue at the end of life, which may result from an inability to participate in once-enjoyable activities (4,5). A 2005 investigation of the Oregon health care system demonstrated that patients requesting assisted suicide are more likely to be younger, unmarried, and have a higher degree of education. Patients with a college education are almost eight times more likely to use PAS than those without a high school education. The most frequent conditions suffered by PAS-seeking individuals include amyotrophic lateral sclerosis, HIV/AIDS, and malignant cancer (4). In particular, cancer patients made 31 of the first 43 requests for assisted suicide (3).

Arguments Supporting PAS Legalization

Supporters of PAS argue that physicians have a responsibility to relieve suffering and to respect patient autonomy. Forms of suffering not only include physical and psychological issues but also interpersonal and existential matters; for example, a patient may consider himself a burden to his family and incapable of enjoying life. Despite available counseling and technology, medical interventions may not alleviate patient suffering. In such cases, supporters indicate that having the option of assisted suicide is an act of compassion that respects patient autonomy (5). Proponents argue that it is wrong to leave patients in a state of unbearable pain, and that uncertainty over whether the physician will honor the patient's desires may cause a

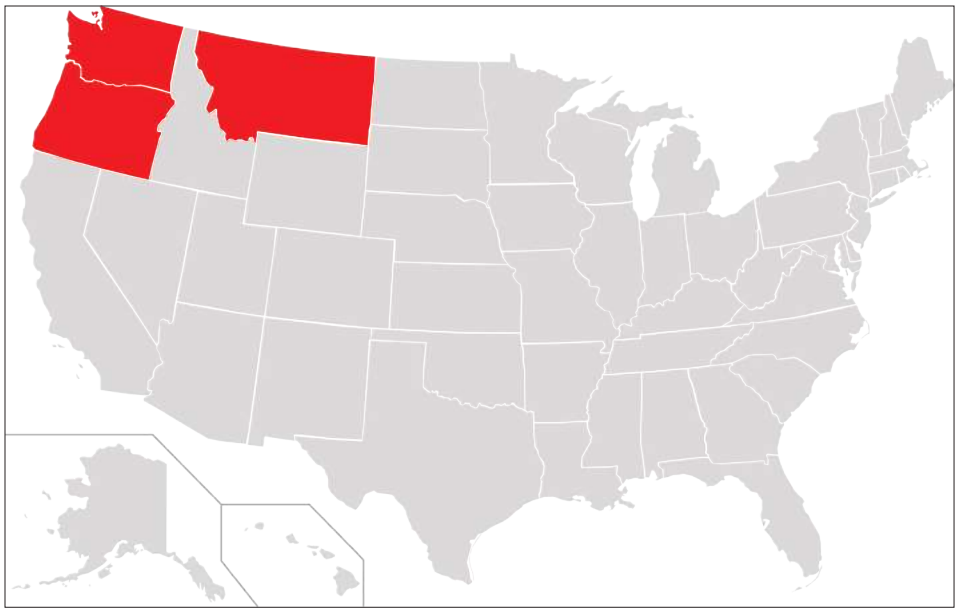

Image courtesy of Lokal_Profil. Retrieved from http://commons.wikimedia.org/wiki/File:Map_of_USA_highlighting_euthanasia.svg (accessed 21 February 2013)

Figure 2: Map of the United States highlighting states that have legalized physician-assisted suicide.

rift in the patient-physician relationship (6). Pointing out the fact that patients have the legal right to refuse treatment, which may hasten death, proponents also claim that individuals should have the right to request medical interventions that can directly result in death (7). Some patients nearing the end of life may simply prefer assisted suicide to heavy sedation or withdrawal from life support. Furthermore, the decision to undergo PAS, contrary to the decision to end life-sustaining treatment, is always an active one made by the patient him or herself (6). Lastly, supporters point out that the choice between life and death is a personal matter that should not be regulated by the government (5).

Arguments Against PAS Legalization

Opponents of PAS argue that the practice undermines the Hippocratic principles that have governed medicine for thousands of years. Dating back to the fifth century B.C., the Hippocratic Oath states that one "will neither give a deadly drug to anybody who asked for it, nor […] make a suggestion to this effect" (8). Not granting a patient's choice to undergo assisted suicide does not directly imply that the physician has acted without compassion and abandoned the individual. The term "compassion," which means to suffer with, indicates that the physician is obligated to remain with the patient throughout the struggle. Opponents argue that in cases of physical or psychological suffering, the physician should take whatever measures possible to end the pain. However, when dealing with interpersonal or spiritual problems that extend beyond medicinal treatment, health professionals should recruit the necessary individuals to provide the patient comfort and peace; these may include the clergy, family, or friends. Such practices, critics claim, outline the quintessential role of the physician in a patient-physician relationship. Legalizing PAS would effectively compromise the patient-physician relationship and demoralize the physician's role as a healer (5). Furthermore, the legalization of PAS may hinder progress that has been made in palliative care (7).

Opponents also claim that legalizing PAS will widen possibilities for misuse and abuse. For instance, if PAS becomes socially and legally acceptable, the practice could be further extended to include patients with non-terminal illnesses and to those without the capacity to make autonomous decisions. In addition, society may begin viewing the disabled, the elderly, and the sick, among others, as appropriate candidates for PAS. As a result, these vulnerable subgroups may be discriminated against and be coerced into opting for assisted suicide (5).

Criticism of the Current Regulations on PAS

There are a number of guidelines surrounding PAS. For example, Oregon law requires the patient to make two oral requests and one written request. In particular, there must be a 15-day waiting period between oral requests and a two-day lapse between making the written request and receiving medication (2). Oregon law also requires physicians to offer palliative care as an alternative option (9). Aside from these procedural steps, there are several other regulations that are subject to personal opinion. For example, patients must be diagnosed with less than six months to live and be cleared of impaired judgment (2). Whether or not an individual meets these criteria depends upon the health professionals making the assessments. It is difficult to accurately determine how long a patient has left to live and to understand an individual's motivations and state of mind concerning assisted suicide (5).

Despite the legal requirements listed above, critics claim that these safeguards can be easily circumvented. For example, though physicians must present palliative care as another option, they may be uninformed about methods of relieving patient suffering, thereby hindering their ability to offer credible and practical solutions (9). Meanwhile, the law does not require physicians to direct patients to palliative care specialists. In Oregon, physicians referred only 13 percent of the first 142 patients requesting PAS to palliative care consultants. The current situation effectively encourages physicians to offer the option of palliative care for the purpose of meeting legal requirements, rather than for the purpose of relieving patient distress (9).

Critics also pointed out issues with the current legal statutes surrounding psychiatric problems. In particular, Oregon law requires physicians to refer patients to a psychiatrist or psychologist only when the physician believes that an individual suffers from impaired judgment. However, previous studies suggest that physicians are not adequately equipped to diagnose patients with depression or impaired judgment (9). Such a diagnosis would require health professionals to examine the patient's previous experiences with death and to be aware of suicide-associated risk factors, including alcoholism and past episodes of depression. Though the Oregon University Center for Ethics recommends that all PAS cases undergo psychiatric evaluation, physicians have referred patients to psychiatrists at a low and decreasing rate. By 2006, only four percent of PAS cases in Oregon underwent proper evaluation. In addition to determining whether a patient

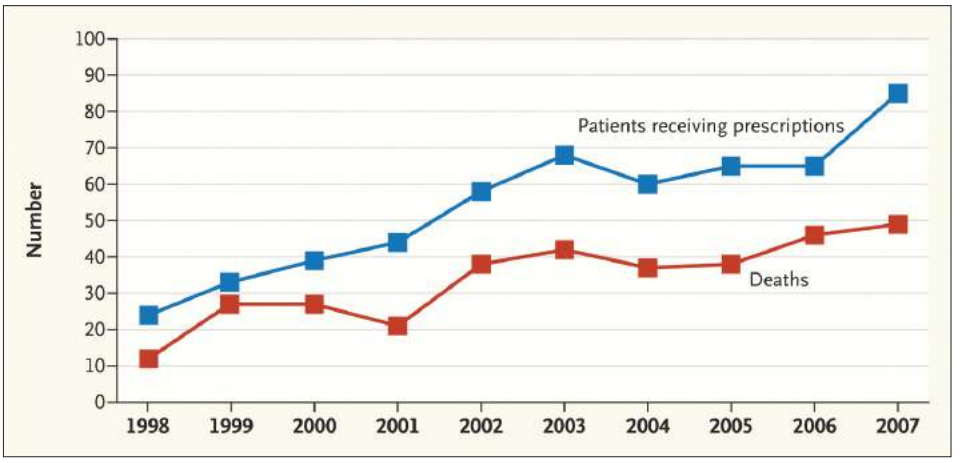

Image courtesy of Ho Yin Au (MIT). Retrieved from http://mit.edu/angles/2010_Hoyin_Au.html (accessed 21 February 2013)

Figure 3: Number of patients receiving prescriptions for drugs for use in assisted death and number of assisted deaths under the Oregon Death with Dignitiy Act, 1998 to 2007. Data from the Oregon Department of Human Services

is capable of making an informed decision, such evaluation may provide mental relief and take away the individual's desire to die. Critics point out that having a professional capable of understanding and relieving a patient's desperation is necessary for the individual to make an informed decision about PAS (9).

Another criticism of the legal system is the lack of safeguards against coercion. Though Oregon law requires patients to have the capacity to voluntarily request assisted suicide, the possibility of coercion still remains, especially among older individuals who are more dependent on their family members. Consider the case of Kate Cheney, an 85-year-old widow diagnosed with terminal stomach cancer. Due to fear of experiencing unbearable pain and of losing bodily control, she looked into the option of PAS and proceeded with the necessary evaluations with the assistance of her daughter Erika. She ultimately received the lethal drugs upon being deemed competent, despite the fact that one psychologist had noted memory defects and possible coercion by her daughter. Cheney decided to take the medication shortly after being placed into a nursing home by her family. Cases like this highlight the coercion that may result from caretaker and financial burdens, among other considerations (9).

Conclusion

Debates concerning assisted suicide continue today. Is PAS ethically and legally justified? Should the principles underlying hospice care be altered to accommodate PAS? Whatever position one takes, it is safe to say that it is critical to improve palliative care and the regulations surrounding health care. To protect patient rights and enable individuals to make informed decisions, the health care system must provide better access to specialists equipped with the necessary tools and understanding and to prevent safeguards surrounding PAS from being circumvented.

CONTACT DANNY WONG AT DANNY.WONG.14@DARTMOUTH.EDU

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Self-Cleaning Surfaces: Inspired by the Lotus

MICHAEL YANAGISAWA, BROWN UNIVERSITY '13

All too often the solutions we desire are closer than we think, simply hiding in nature as secrets waiting to be uncovered. After all, nature has been subject to millions of years of evolutionary pressures, molding each species to fill a specific niche. Only through evolution have birds learned to fly, but only through years of tinkering have we managed to mimick their flight. The steel beams we forge possess only a fraction of the strength of the humble spider's silk. Likewise, every plant can turn carbon dioxide back into oxygen, a process that, if copied, could aid our ailing climate. Seemingly anywhere we look, nature gives new insight into the seemingly insurmountable problems in society (1). Of particular interest in this paper is the lotus leaf. Its remarkable but simple structure gives it superhydrophobic properties, keeping it clean from both debris and water in even the dirtiest habitats. The applications of this modest discovery have been tremendous; never before has science believed materials could clean themselves. Indeed, the invention continues to find new applications. The knowledge gained from the plant demonstrates the power of biomimicry and the sheer wisdom of nature.

The Lotus Leaf

The lotus flower sits above the water, untouched by dirt and filth. Even in muddy waters, its petals remain clean. For its magical purity, the flower has fascinated many people throughout the ages. Many Asian religions admire it for its cleanliness even amidst contamination, an island of beauty floating above unclean waters. Several Hindu deities are associated with the flower, a symbol of purity and divine birth in Buddhism. The unsullied flower remains a symbol of purity even to this day.

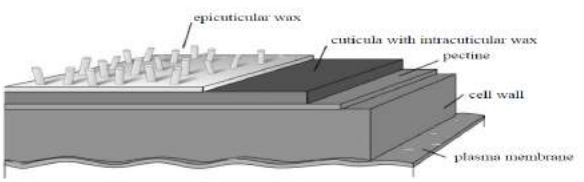

Figure 1: A cartoon of the surface of the lotus leaf. The surface of the leaf is hydrophobic, but the presence of epicuticular wax sculptures makes the surface superhydrophobic. Reproduced from (14).

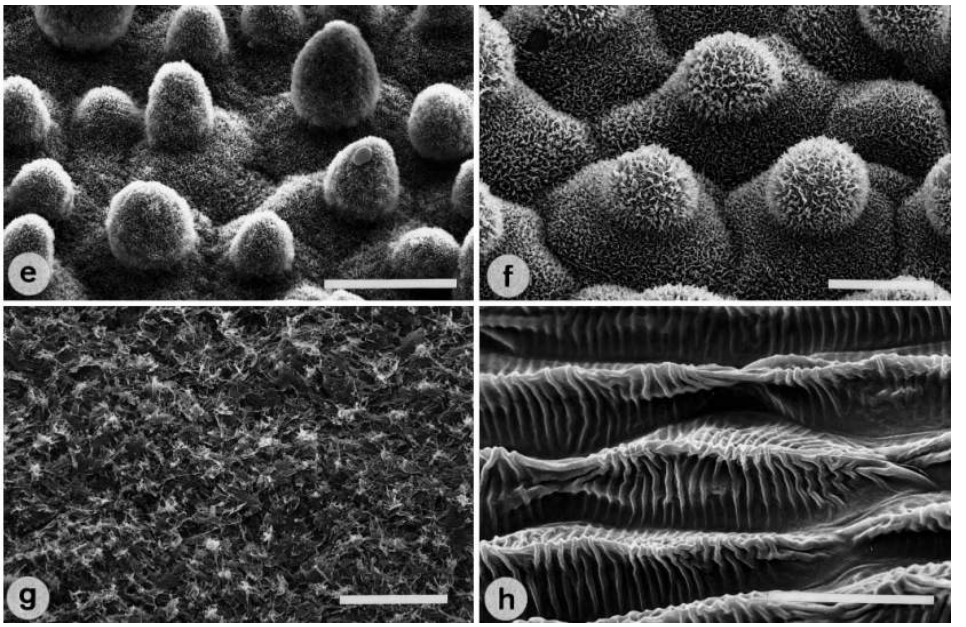

Figure 2: SEM images of the lotus leaf Nelumbo nucifera (e-g) and a composite leaf Mutisia decurrens (h). Note the rough, sculptured surface (e-g) and ribbed surface (h). Bars = 20 μm. Reproduced from (2).

The properties of the lotus petal astounded scientists. Researchers understood regular hydrophobic surfaces, like the waxy surface of many other leaves, but the lotus leaf possessed some unique characteristic. While the other leaves could get dirty, the lotus leaf seemed to be perpetually clean. Water droplets rolling down the leaf seemed to pick up debris like a snowball down a hill. This self-cleaning mechanism allows the flower to retain its cleanliness, a phenomenon that greatly interested scientists.

With the advent of scanning electron microscopy (SEM), scientists began to unravel the mysteries of the lotus leaf. An SEM by Barthlott and Neinhuis revealed that the lotus leaf of Nelumbo nucifera had a number of hydrophobic surface sculptures, creating a rough, textured outer layer of epicuticular wax crystals and epidermal cells (Fig. 1) (2). A close-up of the leaf reveals its complex surface packed with very intricate structures (Fig. 2). These micro-sculptured surfaces were called "non-wettable"—water would bead off the leaves and slide right off. In contrast, the wettable leaves the group observed had a smooth hydrophobic surface, one that allowed water to bead up but still stick to the leaf. Surprisingly, even ultra-smooth surfaces did not exhibit the same properties as the lotus leaf; such surfaces, while expected to be extremely hydrophobic, still let water droplets stick.

Barthlott and Neinhuis called the surface of the lotus leaf superhydrophobic, or more water-repellent than simple hydrophobic surfaces (2). The interaction's contact angle, a measurement of the angle between the water droplet and the surface, was consistently above 150°; an angle this great meant that the water droplet nearly formed a ball on top of the surface (see Fig. 3). In contrast to water, which typically forms a rounded dome on the surface of a molecule, the superhydrophobic surface allows for the water to have very limited contact with the surface itself. The surface tension of water, combined with the micro-textured surface of the lotus leaf, keeps the water droplet spherical. The reduced water-leaf contact area also reduces the amount of water that could "stick" to the leaf, causing the droplet to roll of the leaf even at the slightest inclination (less than 5° of incline for the lotus leaf, and more than 40° for the smooth leaves) (2).

Perhaps even more intriguing is

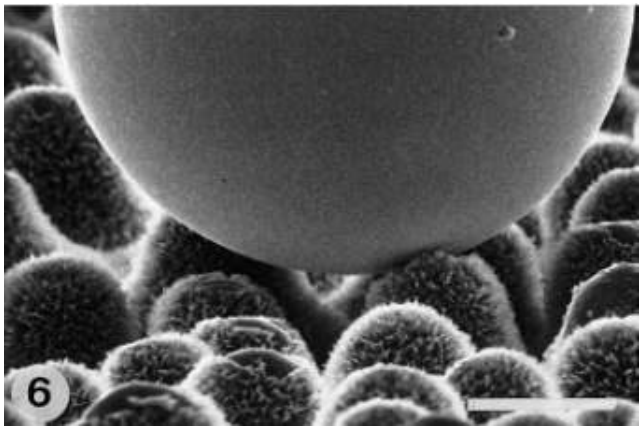

Figure 3: The water droplet forms almost a perfect sphere on the surface of the leaf. Reproduced from (2).

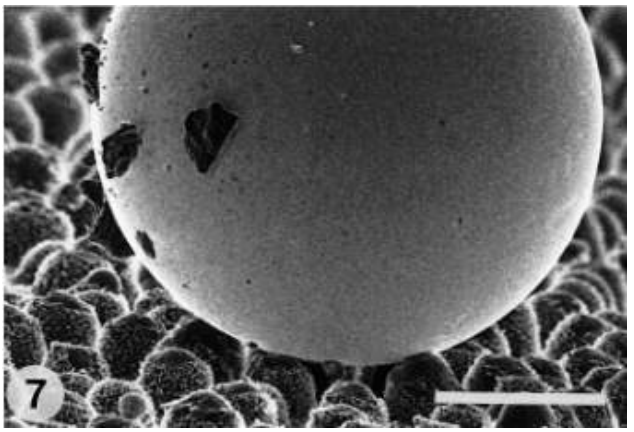

Figure 4: The water droplet capturing particles on the surface of the leaf. Reproduced from (2).

the self-cleaning properties the group observed. The duo conducted a variety of experiments, exposing the surface of the lotus leaf to contaminants varying in size and chemical properties, particles like barium sulfate, spores, soot, and siliconcarbide dust. The lotus flower was then left to wash off in heavy natural rain, resulting in nearly 100% particle removal. The results were similar when the flower was exposed to heavy fog: nearly all of the particles on the lotus leaf were washed away (2). Notably, the water rinse was equally effective on all the particles tested; even hydrophobic contaminants, expected to repel the water droplets, washed off easily with water (2).

The self-cleaning property of textured, superhydrophobic surfaces has been termed the "Lotus-effect," after the enduring symbol of purity. Despite the name, the "Lotus-effect" is not unique to the lotus; other biological surfaces, like plant leaves and even insect bodies, display this same textured layer creating a superhydrophobic surface (2-3).

A Brief Foray into the Physics and Chemistry

The behavior of the micro-studded surface is unexpected. After all, we would initially expect an extremely smooth surface to be the most effective; the water droplet should glide across the surface. The lotus leaf, however, shows us that its texture is the key to superhydrophobicity. Chemists and physicists alike have worked to understand this phenomenon, hypothesizing two processes. The first is the Wenzel model: the texture increases the surface area, thus increasing the hydrophobicity. The second is the Cassie model: air is trapped within the structures' depressions, enhancing the hydrophobic behavior (4). Through observation, the Cassie model has been shown to be more likely. Water droplets rolling off superhydrophobic surfaces have significantly less friction than those of ordinary solids; they seem to move over a thin film of air. The individual sculptures thus allow the droplet to float along the surface. As an analogy, the droplet on a textured surface is like a fakir sitting atop a bed of nails. The fakir can sit happily atop the dangerous spikes because his weight is distributed over the whole bed of nails; no single nail can break through the surface of his skin. Likewise, no single sculpture on the superhydrophobic surface can pierce or indent the water droplet. The addition of micro-sculptures on each individual sculpture only serves to magnify this effect. As a result, the water sitting atop a superhydrophobic surface have also been termed "fakir droplets" (4).

The self-cleaning property is likewise intriguing. This phenomenon is likely due to the interactions among the water droplet, contaminating particles, and the leaf surface. A water droplet will adsorb a particle if its adhesion force is greater than that of the particle and that of the superhydrophobic surface. In other words, if the water-particle attraction is greater than the particle-leaf one, the rolling droplet will collect the particle. Because the leaf's surface is highly textured, the contact area (and thus adhesion force) between the particle and the surface is very low, allowing the particle to be "captured" by the water droplet and removed from the leaf's surface (see Fig. 4). As the droplet rolls along–as it is apt to do because of the low inclination required for movement–it continues to pick up particles on the surface of the leaf, as shown in Fig. 5. In contrast, water on a smooth surface simply trickles down the leaf, pushing the particles without collecting them. The droplets do not collect debris because of the large interfacial area between the particles and surface.

From Leaf to Lab

The convenience of self-cleaning surfaces opens up technology never before imagined. Any surface that regular comes into contact with water can be cleaned using this phenomenon, covering a wide range of uses. Imagine skyscrapers that never need window washers, bathroom tiles that remain scum-free, and park benches that keep clean; self-cleaning materials are a solution to a problem we did not even know existed.

Thus the biological solution became an engineering problem: how can exceedingly small structures be fashioned onto a surface, and more importantly, what structural features are important? Foremost, the peaks of the surface should be hydrophobic and small, prerequisites for a superhydrophobic surface. The peaks of the lotus leaf are characterized by fine, nanoscale details on the microscale structures; each peak has hundreds of even smaller peaks on it. Ideally, the surface should also be durable to increase its longevity. For example,

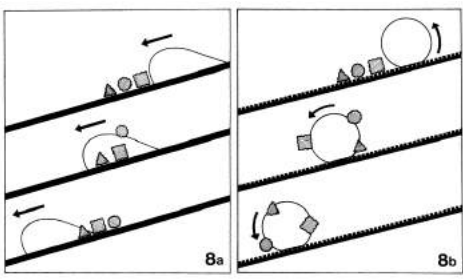

Figure 5: Image on left (8a) shows a particle with a low contact angle that displaces particles; it is not superhydrophilic or self-cleaning. The image on the right (8b) depicts a nearly-spherical water droplet that adheres to particles, effectively cleaning the surface of the leaf. Reproduced from (2).

short peaks are preferred to long ones, as nanoscale materials are susceptible to mechanical stress.

Several techniques have been devised to create microscale sculptures, creating effective superhydrophobic surfaces. Barthlott has proposed stamping, etching, or molding a hydrophobic polymer, or gluing hydrophobic powder to the surface to create the right texture (5). For example, a smooth hydrophobic polymer such as polytetrafluoroethylene (PTFE) can be imprinted with a mesh or covered in a Teflon powder to produce a superhydrophobic surface. Coulson *et al.* have shown that plasma-roughened polymers produce sufficiently textured surfaces; likewise, Chen *et al.* showed two other processes, ion etching and compressing a polymer, which are equally effective (6-7). In short, researchers continue to find new ways to create superhydrophobic surfaces. Such techniques, however, have their drawbacks. While the processes produce effective self-cleaning surfaces, they are also costly and time consuming. The surfaces produced are also hazy, a drawback for applications on windows or lenses. To improve the material, the focus has moved from microscale to nanoscale structures.

Indeed, the new wave of superhydrophobic surfaces has taken advantage of nanoscale materials. The detailed texture on the individual surface sculptures is important to their superhydrophobicity; Cui *et al.* have dip-coated microstructured surfaces with nano-sized silica particles followed by a particular polymer to create an effective and cheap surface (Fig. 6) (8). The fakir's bed of nails has also been applied to the nanoscale level; "nanopin" films, essentially a bed of nanoscale needles or cones, are great superhydrophobic surfaces, producing contact angles up to 178° (Fig. 7) (9). For example, Dorrer and Rühe

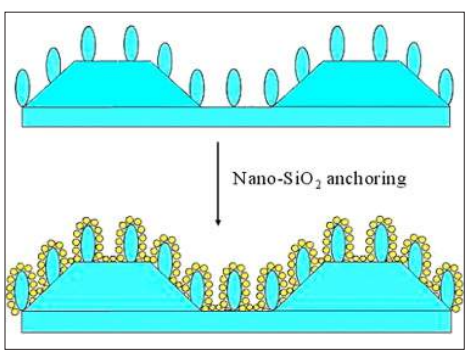

Figure 6: Microstructures on a surface are dip-coated in SiO2 and polymer to create superhydrophobic surfaces. Reproduced from (8).

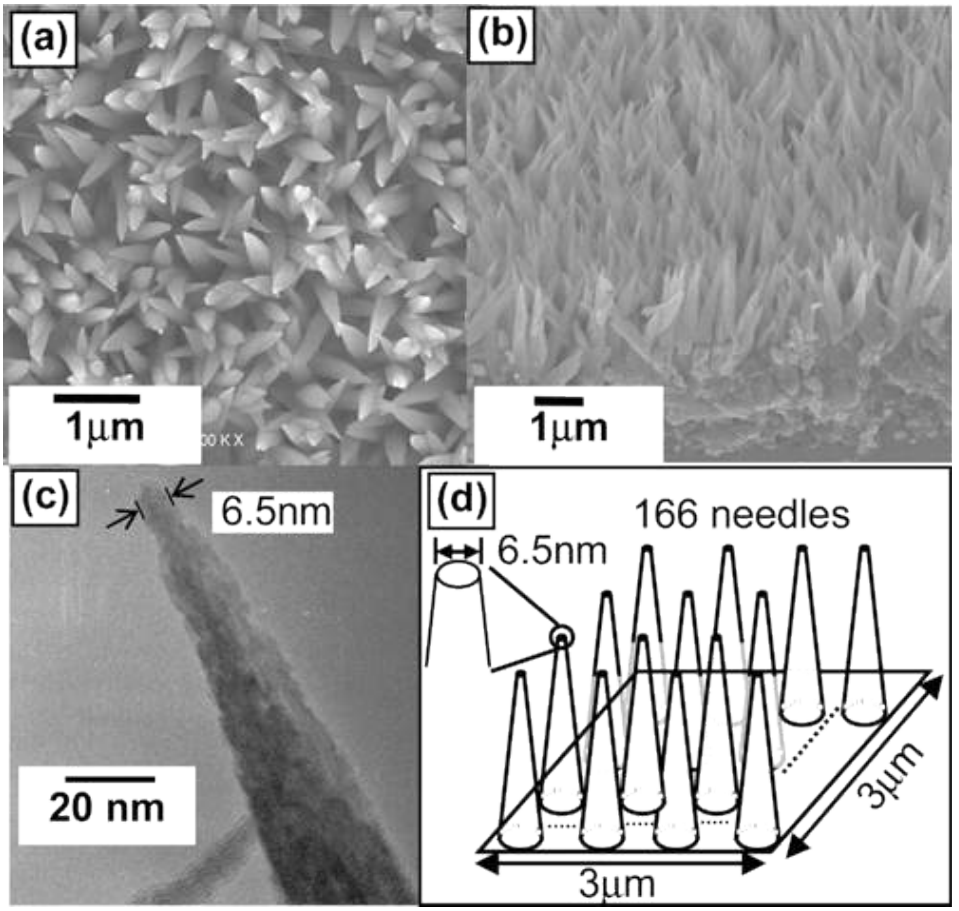

Figure 7: The nanopin structure that produces very good superhydrophobic surfaces. Reproduced from (9).

produced silicon nanoglass that displayed extremely hydrophobic properties with a contact angle of 179°±1° (10). Another research group has created effective transparent superhydrophobic surfaces with the required nanoscale structures (11). Yet another created a "nanotube forest" composed of carbon nanotube standing on end, a structure that looks similar to a bed of pins (12). The spheres that sit atop these surfaces are essentially spherical, showing the efficacy of the nanostructures on superhydrophobicity. The properties also show the success of using lotus leaf's microstructure to inspire useful man-made materials in the lab.

Commercial Applications

Barthlott and Neinhuis, the duo who published the paper on the Lotus-effect, patented the "Lotus-effect" design in 1998. As the patent states, "the capability of self-cleaning of the plant surfaces is not so much dependent on the kind of wax, but on the surface structure of such waxes," reiterating the elevations and depressions that characterize the superhydrophobic surface (5). Barthlott realized the potential of the technology and quickly began to develop and market Lotus-Effect materials; the Lotus-Effect® company bears claim to over one hundred patents. The company produces a huge spread of products, ranging from sanitary ceramics to eyeglasses, to roof tiles and car windshields. Lotus-Effect® paint specimens left outdoors for six years degraded significantly less than those coated with biocide-laden paint (13). This application shows the eco-friendly potential for the superhydrophobic products, an accidental but beneficial side effect.

Sto Corp. has likewise applied the lotus' technology to painted surfaces. The StoCoat Lotusan® paint is unique in that it has very high levels of water and dirt-repellence; a coat of paint in essence creates a superhydrophobic layer. No longer do walls need to be cleaned by hand; rain water alone suffices. Nanostructured surfaces on glass would be useful on rainy days; clear side and back windows would make driving safer. For this purpose, NeverWetTM has created a spray coating that creates a superhydrophobic surface. The magic behind the spray is nothing more than the lotus effect bundled into a neat package.

Conclusion

The story of the lotus leaf shows the potential of biomimetic materials in everyday life. From simple admiration in ancient Asian cultures, the lotus leaf has grown into a surface notable for its self-cleaning properties. The hydrophobic coating in combination with the micro-sculptured surface creates a superhydrophobic surface, one that has been copied and patented. Through the simple plant we may live with the luxury of self-cleaning materials; people all over feel a sense of relief in anticipation the technology's potential. The story of the lotus leaf is but one application of one biological phenomenon, though. It makes us wonder what other discoveries nature keeps hidden.

CONTACT MICHAEL YANAGISAWA AT MICHAEL_YANAGISAWA@BROWN.EDU

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Sexual Reproduction and Gametogenesis in *Saccharomyces cerevisiae*

Implications for Replicative Aging

RIYAD SEERVAI, BROWN UNIVERSITY '13

While aging makes the somatic body mortal, reproduction maintains the germ-line as immortal. In this paper, Riyad Seervai reviews findings showing that sexual reproduction in Saccharomyces cerevisiae *resets the replicative aging clock through a number of morphological and biochemical processes. I also discuss the connection between mating and aging in other model organisms.*

Introduction

In 1885, Samuel Butler famously stated, "A hen is an egg's way of making a new egg." This revolutionary concept has had important ramifications on the way that scientists understand reproduction and longevity. The only way for a germ cell to pass on its DNA is through the intermediate of a free-living, independent organism that is capable of mating. Sexual selection drives the evolution of organisms in order to satisfy the needs of the germ line. Germ cells are thus immortal, able to defy time because they live on through the somatic cells of the body. However, studies in Saccharomyces cerevisiae from Dr. Angelica Amon's Lab at MIT have shown that this is not always the case, and that somatic cells are able to defy time through germ line functions as well.

Saccharomyces cerevisiae, commonly referred to as budding yeast or bakers' yeast, has stood out as a model organism for understanding a variety of biological processes. The small size of its genome, the ease with which it can be manipulated, and its short life-cycle have made it a strong candidate for studies in reproduction, both sexual and vegetative. In recent years, yeast has also stood out as a prime candidate for studies of cellular and organismal aging (1), due to their cells' finite replicative capacity (2). This paper shows how these two biological processes – reproduction and gamete formation, and aging – are connected in ways that were previously unknown. The paper describes the processes of sexual reproduction and aging in *S. cerevisiae*. Then, it proceeds to analyze Amon's studies in order to provide an understanding of how the two processes defy Chronos and Thanatos, thus maintaining the unsolved mystery of the chicken and the egg.

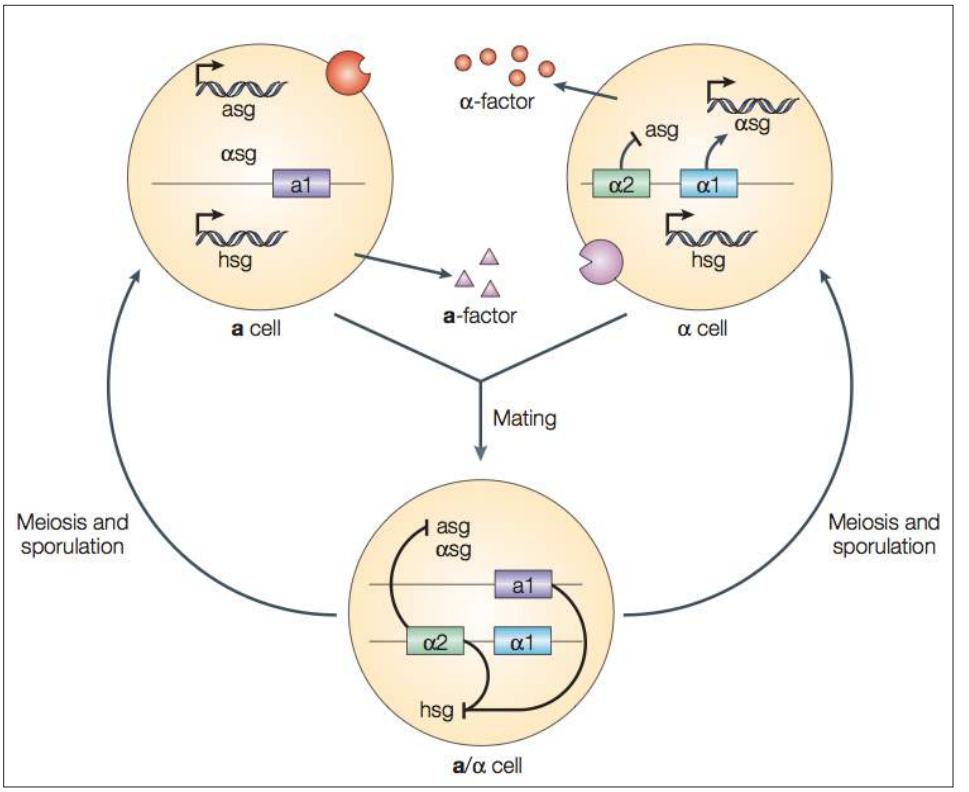

Figure 1: Genetic characterization of the mating cycle in *S. cerevisiae*. a and α cells express different genes that are involved in attracting the other mating type during sexual reproduction. Reproduced from (4).

Yeast Mating 101

The history of mating in *S. cerevisiae* dates back to over half a century ago. It was determined that when conditions are favorable, two haploid yeast spores fuse together in order to form a diploid hybrid cell (3). This required that the cells be viable, remain in a haploid state, and have opposite mating types. It was surprising to note that budding yeast was not a simple, asexual organism. On the contrary, yeast have two mating type (MAT) loci, MATa and MATα, which confer a and α mating types to the haploid cells. The loci have been genetically determined (4), and it has been shown that the MATa1 locus activates a-specific genes (asg) responsible for the production of a-pheromone and a receptor for the α–factor. The α locus contains two loci, MATα1 and MATα2, which are responsible for suppressing the a-specific genes (α1) and activating the α-specific genes (α2). Both a and α cells express haploid-specific genes (hsg), which are involved in the mating process.

In order for two heterothallic yeast cells to mate and form a diploid, they must be attracted to each other. The a cell releases a-factor (or pheromone) that lands on the α cell's receptor for a-pheromone. The α cell attracts the a cell in a similar fashion (5). Upon sensing the opposite mating pheromone, the cells get arrested in an unbudded stage of the cell cycle in which DNA replication has not yet taken place – that is, the cells are still haploid (6). This is the optimal stage during which nuclear fusion, DNA replication, and meiosis can occur without lethal mistakes (7). The arrested cells now undergo an alteration in morphology to form elongated,

asymmetrical projections that have affectionately been dubbed "shmoos" after Al Capp's mythical comic strip character (7). After two cells have undergone the process of shmooing, the cell membranes fuse, allowing the two haploid nuclei to form a diploid cell. Fig. 1 shows the life cycle of *S. cerevisiae* from the haploid to diploid states. It is important to note that in the diploid state, the MATa1 and MATα2 are responsible for downregulating the haploid-specific genes so that the cell cannot mate. The MATα2 locus also blocks expression of the asg and αsg. During the process of sporulation, the diploid cells exit the cell cycle at the G1 stage (8). Under specific conditions, cells undergo meiosis and form a tetrad of spores/gametes and a spore wall. The mother cell now collapses to form an ascus, and the haploid spores are often referred to as ascospores (for a detailed review of sporulation, see (9)). This completes the mating cycle in *S. cerevisiae*.

Aging in *S. cerevisiae*

Yeast cells, like all other organisms, undergo a process of senescence/aging. There are two assays that define the life span of a yeast cell (Fig. 2). The first, Chronological Life Span (CLS), is the amount of time that a cell can exist in the G0 stage (10). In order to test this, cells can be suspended in an undivided state, and a subset of cells is allowed to restart replication at different time points. The CLS is the time at which the cells can no longer reenter the cell cycle (11). Mortimer and Johnson determined the second, Replicative Life Span (RLS), by counting the number of daughter cells that a single mother cell could produce before its death (2). For the rest of this paper, and the articles it discusses, aging implies replicative aging and life span implies RLS.

Aging in yeast is a result of various cellular and biochemical processes. Sinclaire and Guarente showed that Extrachromosomal rDNA Circles (ERCs) are a major cause of aging in yeast (12). ERCs are formed through homologous recombination between repeats within the rDNA array on chromosome XII. These accumulate exponentially in the mother cell and lead to nucleolar fragmentation. Yeast cells have only one nucleolus, which is separated from the rest of the nucleoplasm (11). Recent work suggests that changes in the nucleolus, and not the mere presence of ERCs, trigger aging through

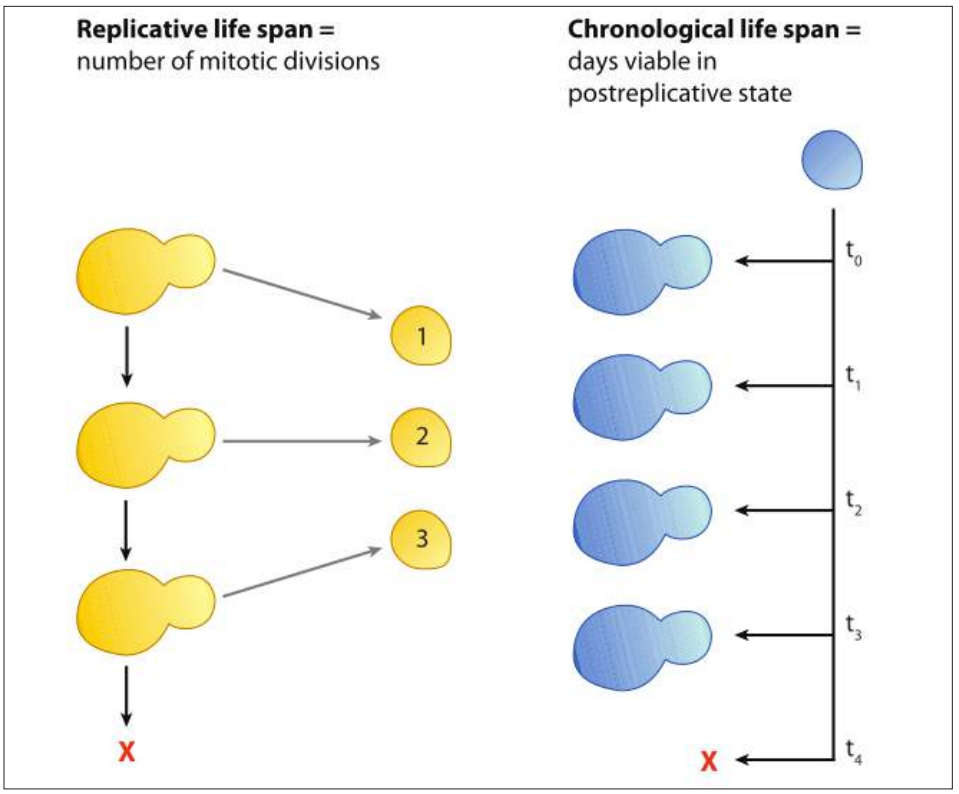

Figure 2: Replicative versus Chronological Life Span. The life span in yeast is measured using two different parameters: the number of times a cell can undergo mitosis, and the number of days a cell remains viable in the post-replicative state. Reproduced from (11).

illegitimate recombination, transcriptional and translational defects, and trafficking errors (10). Trafficking defects between the nucleus and cytoplasm are also a result of leaks in deteriorating nuclear pores (13).

Heavily carbonylated proteins form aggregates and are retained in aging cells (14). These are associated with heat shock protein 104 (hsp104), a chaperone protein that acts in tandem with Hsp40 and Hsp70, to dissociate, resolubilize, and refold damaged proteins (15). Oxidation is another important form of damage, and mitochondrial dysfunction is a major source of reactive oxygen species (ROS) in aging cells. With aggregates and ERCs, oxidatively damaged proteins are an important source of aging in mother cells. Unlike in mammals, the length of telomeres remains relatively constant throughout the RLS of yeast cells, and does not feature as a part of their senescence phenotype (16).

An Old Hen Yields Sprouts into a Young Chick

In 2011, Unal and colleagues from the Amon Laboratory published a paper in *Science* showing that the resetting of RLS in yeast occurs during the process of gametogenesis (17). They began by asking the main question itself – does sporulation of old yeast cells reset the RLS in spores? It was noted that old cells pre-sporulation died out much faster than young ones (Fig. 3A). However, old cells post-sporulation behaved the same as young ones – they produced as many as 30 new buds before their death (Fig. 3B). There was no difference in the RLS within the four spores of a tetrad. Thus, it was quite clear that sporulation resets RLS. The group set out to discover how this process works. In order to test for protein aggregates in cells pre- and post-sporulation, they labeled Hsp104 with enhanced Green Fluorescent Protein (eGFP) and analyzed the signal in both groups of cells. The number of cells with Hsp104 foci decreased significantly during formation of the tetrad. It was shown at a later stage that the level of carbonylated proteins (mentioned previously) is the same between aged spores and young cells (10). Gametogenesis also eliminates age-related ERCs and nucleolar aberrations. Fob1, a nucleolar protein, was seen to localize in the cells after sporulation. Both young and aged spores have a single Fob1-eGFP locus, which is representative of a single nucleolus. Gametogenesis resets the RLS by eliminating the morphological and biochemical symptoms of senescence.

The group sought to uncover which aspects of gametogenesis were critical to

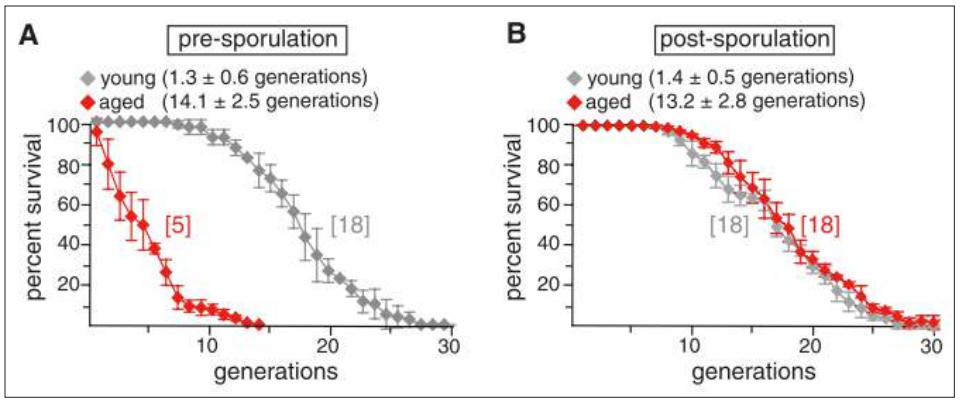

Figure 3: Gametogenesis resets RLS in *S. cerevisiae*. (A) RLS of young and aged yeast cells before sporulation. (B) RLS of cells post-sporulation. Reproduced from (17).

resetting the RLS. They found that Ndt80, a meiosis-specific transcription factor required for exit from pachytene (18), was indispensible to the successful completion of the process. Ndt80 activates genes that play a role in the meiosis and sporulation pathways (19, 20). The fact that some of these genes also function in mitotic cells is significant, as Amon's group found that transient expression of Ndt80 in vegetative cells could significantly extend the RLS by clearing protein aggregates and restoring the nuclear morphology. Although it is unclear whether sporulation- and Ndt80-induced RLS resetting use the exact same mechanisms, it is believed that the two must share some common processes. Unal and Amon speculate that Ndt80 could act by targeting Heh1 and Nur1, LEM (LAP2, Emerin, MAN1) domain proteins induced during sporulation (10, 20). Ndt80 might also act through remodeling leaky nuclear pores in aged cells during gametogenesis.

Conclusion

The fact that expression of a sporulation-specific transcription factor can extend the RLS of vegetatively growing cells has important ramifications for the way we think about sex and aging. First, it provides us with an opportunity to dissect the molecular mechanisms of cellular aging using Ndt80 expression as a switch to trigger a reversal of the aging phenotype. More importantly, the finding indicates that at least a subset of the factors required for rejuvenation during gametogenesis can function outside the developmental program (10). *Saccharomyces cerevisiae* has been used as a model to study various age-related diseases such as the neurodegenerative Huntington's and Parkinson's diseases, and it would be interesting to see the effect of Ndt80 expression in these disease models (11).

Yeast is not the only organism that shows reversal of aging through activation of gene expression programs normally limited to the germ line. Curran *et al.* showed that acquisition of germline characteristics by somatic cells have contributed to the increased health and survival of long-lived Caenorhabditis elegans mutants (20). *C. elegans* has served as a model for understandings Alzheimer's Disease and cancer, in which gametogenesis clears the age-related buildup of oxidatively damaged proteins (22). Interestingly, Ndt80 is closely related to the p53 family of transcription factors (10), and Cep-1, the *C. elegans* p53 homologue, is responsible for regulating meiotic chromosomal segregation during gametogenesis (23). It has been suggested that p53 was originally intended for repair of double-stranded breaks, and then evolved to accommodate general stress response and tumor suppression (10).

In mammals, senescence is primarily due to shortening of the telomeres (24), and the resetting of telomere length takes place during embryogenesis rather than gametogenesis (10). Telomere homeostasis is crucial for longevity in mammals; regulation of other age-related cellular changes are still unknown. In the meantime, the finding that sexual reproduction and gametogenesis resets the aging clock in non-mammalian model organisms leaves us with much to ponder over in terms of the dichotomy between the hen and the egg. Butler (1885) was wise indeed, but he was not omniscient. One wonders what his egg would have thought on the issue!

CONTACT RIYAD SEERVAI AT RIYAD_SEERVAI@BROWN.EDU

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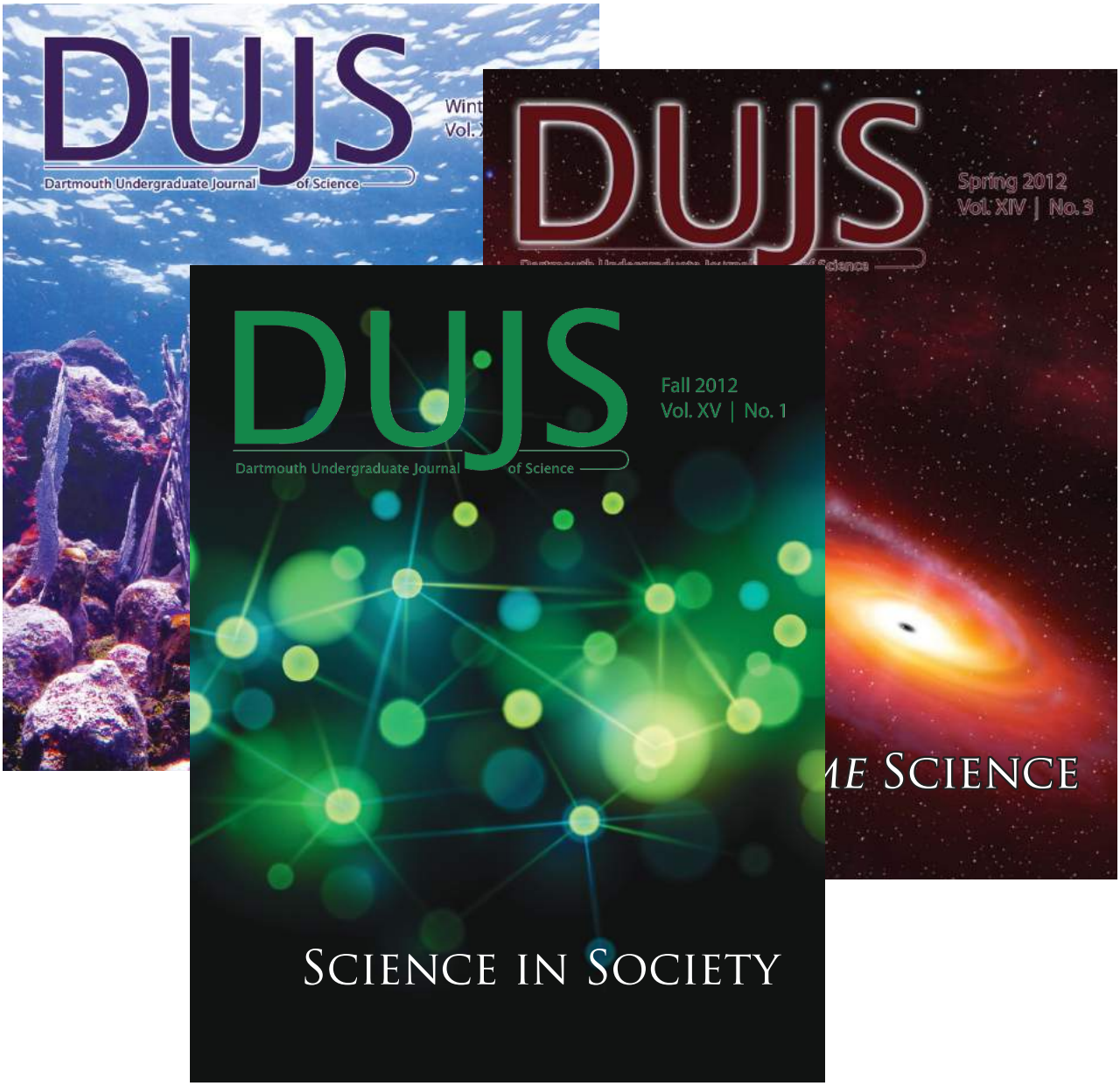

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