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CRITICAL FOCUS

Brian J. Ford

Lineage Without Limit: How Cells Can Resist Ageing

Can the study of the living cell reveal the secret of ageing in our quest to live longer?

It's one of the first sad lessons of life: we die. Sappho wrote about its inevitability in Fragment 58, composed about 600 BC, and around 350 BC Aristotle wrote a lengthy treatise exploring the "vital heat" that drives human metabolism, commenting on its inevitable decay. Our modern microscopical insights explain why it happens. We now recognize that, within each cell nucleus, the telomeres progressively shorten. The genome becomes unstable as mutations accumulate. Cells progressively lose viability and, after about 50 mitotic divisions, they undergo apoptosis and die. It is inevitable … or is it?

Buds are formed by *Hydra vulgaris* as its primary means of reproduction without sex. Curiously, the species is dioecious, existing as either male or female, though the budding vegetative forms are indistinguishable. Sexual reproduction is a relatively rare phenomenon invoked only when environmental conditions are severely compromised.

Sometimes cells do not age, and plenty of living things don't fit the pattern. Many microbes lack any sexual reproductive phase but continue to divide indefinitely without accumulating the supposedly inevitable damage that leads to senescence. Many plants reproduce vegetatively, their cells remaining as brisk, bright and bountiful as they were originally. Animals, too; the common polyp *Hydra vulgaris* reproduces through asexual buds that eventually separate from the parent and live independently. Their cells do not age. They resist senescence, and they remind us that ageing and death are not the inevitable consequence of living that we customarily claim.

Currently, billions of dollars are being invested in the quest to stay alive at all costs. People diet. They cram themselves with superfoods and life-preserving supplements to boost their cells. The recently dead are being deep-frozen, waiting to be revived in the future, to carry on living as if nothing had happened. Cryogenics is now an expanding industry. And all the while, our cells are doing what they are supposed to do; ageing naturally and aiming to shut down when the time comes.

ARE MICROBES TRULY IMMORTAL?

We encounter immortality when young. Ev-

ery child learns that bacteria essentially reproduce through binary fission, so the parent becomes the offspring. There is no senescence, there is no death; bacteria today are the same as those our ancestors knew. Those of us in microscopy qualify that, because we are aware of microbial conjugation, spore formation, zygote formation, and the rest. The first bacterial species reproduced exclusively vegetatively, they lacked even the capacity to produce dormant endospores. Genera that can form spores, such as *Clostridium* and *Bacillus*, are a small minority, about 1% of all bacteria, and they evolved to endure extreme conditions such as desiccation, lack of nutriment, or radiation through a state of acquired dormancy.

The majority of bacteria, including familiar pathogens like *Escherichia coli* and *Staphylococcus aureus*, lack this facility to spore and remain metabolically active under favorable conditions, endlessly reproducing vegetatively without end. Bacterial cells have evolved a range of mechanisms which preserve their genetic integrity. During replication, genetic repair distinguishes newly synthesized strands of DNA and can excise a mismatch. Recombinational repair reinstates double-strand breaks using a sister chromatid as a template and thus avoids accumulating genetic fragmentation. Nuclear repair mechanisms have been identified in *E. coli* allowing the cell to detect dangerous deletions, through complex systems of damage recognition, incision, and resynthesis using uncompromising genetic components as a template.

These bacteria demonstrate remarkable evolutionary stability in reproductive fitness and vigor over extended time-scales, which clearly extend to millions of years. Part of this is due to Darwinian evolution, which favors the survival of individual bacteria with high growth rates, metabolic efficiency, and increased adaptability, while the less able individual cells undergo senescence and disappear from the community. It is particularly interesting to note that *E. coli* cultured over 50,000 generations reveal a progressive increase in relative fitness compared to the ancestral strain. Today's evolved populations excel in growth rate and resource utilization in their controlled environments and can even out-compete with their wild-strain predecessors. Genomic analysis indicates that *E. coli* lineages, dating back some tens of millions of years, retain those core physiological traits that are most conducive to sustained proliferation. Unlike human cells, they don't age with time.

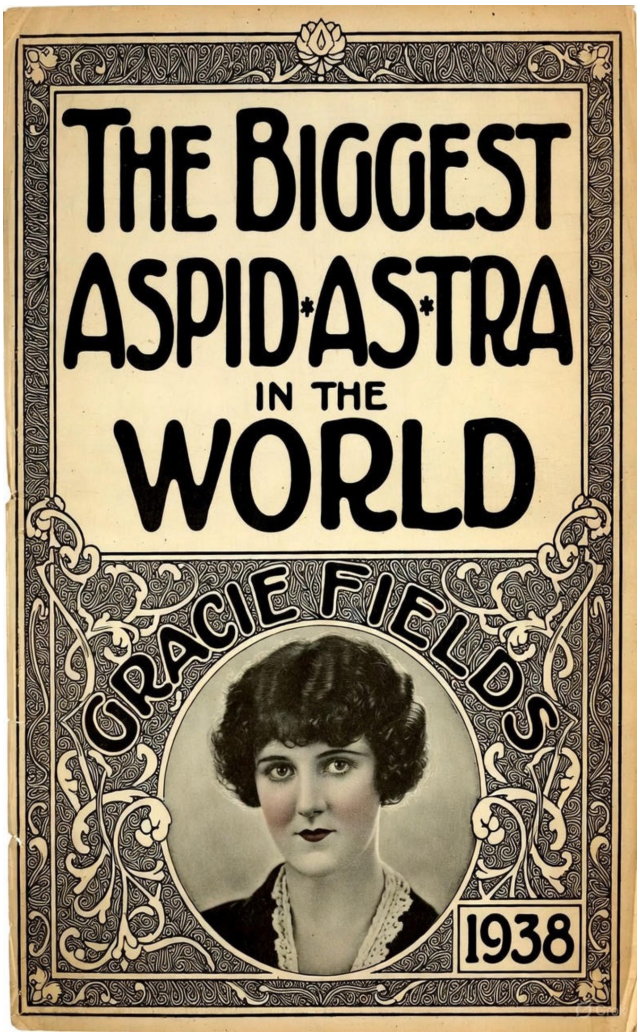
Similarly, *Staph. aureus* exploits fitness for the environment shaped by host-pathogen interactions and the pressures of selection. Successful clones seem to evolve compensatory mechanisms when challenged by antibiotic exposure. Genomic analysis shows that staphylococci have conserved their capacity for virulence and survival, adapting without diminishing their core physiology, over millions of years. Staphylococci don't get old.

Yet there is another mechanism at work. Our accepted concept of binary fission is that each bacterial cell produces two daughter cells which are identical in all respects. Fission in bacteria can be asymmetric. In some species, notably *E. coli*, replicative ageing has been characterized by the emergence of a progressive decline in cellular function, reduced growth rates, diminished reproductive efficiency, and a progressive loss of physiological efficiency. When the bacterial cell divides, one of the offspring bears the accumulated damage, whereas the other perpetuates the intact genome. This replicative asymmetry would enhance population-level fitness by favoring the survival of rejuvenated offspring, so that the apparent ability of bacteria to overcome ageing is due to the self-sacrifice of genetically damaged or physiologically compromised cells. It is Darwinism: the unfit cells undergo apoptosis, while the repaired, revived and rejuvenated bacteria go on to populate tomorrow.

A noteworthy example is *Bacillus fastidiosus* which has been isolated after surviving for 3.5-million-years in soil samples from Siberia. Most of that time (though not all) saw the bacteria dormant in permafrost, though on revival in the laboratory, the active cells proved to exhibit robust repair mechanisms. These organisms are being intensively studied in the hope of learning more of their reproductive strategies and their facility to compensate for accumulated genetic deterioration. Those studying senescence are hopeful of harnessing the survival strategies evolved by such bacteria in the ceaseless quest for human longevity. As we will discover, it isn't so simple.

THE EVERLASTING HOUSE PLANT

If you want to see true immortality, then it is the angiosperms that hold the key. Plants exhibit a form of biological immortality that transcends anything we observe in the animal world. I first pondered this as a child. It was a conspicuously obvious example: my grandparents in Brighton, on the English south coast, lived in Edwardian style with a magnificent *Aspidistra* plant taking pride of place in the window of their drawing-room. This graceful plant had become popular in late Victorian times; *A. elatior* had been brought in from Japan and most homes had a specimen of this



The Victorian houseplant *Aspidistra sp*. was common in British homes, and some 40,000 tons were vegetatively produced at its height of popularity. Its name was mis-spelt on the sheet music (and on the record label) for many years. Gracie Fields, in 1937 the highest paid film actress in the world, never pronounced it correctly in thousands of her performances.

or related species. They were graceful, elegant monocotyledons and were always reproduced by splitting the rhizomes and growing on from a small plant to one of potentially monstrous size.

An aspidistra was so tolerant of poor light levels, low soil moisture, and chronic neglect, that it became popularly known as the Cast-Iron Plant. There was even a vaudeville song about it, "The Biggest Aspidistra in the World" recorded in November 1938 by British vaudeville star Gracie Fields for her film "Keep Smiling." It was composed by lyricist Tommie Connor with musical composer Jimmy Harper (under the pseudonym James S. Hancock) and the song became an instant hit. During the Second World War, Britain's most powerful shortwave radio transmitter, used for propaganda broadcasts into Nazi-occupied Europe, was code-named "Aspidistra." It was a name everyone knew.

What so intrigued me was that the plant was always propagated by vegetative reproduction – yet it never aged. Its cells remained as vigorous, as verdant, and as vital as when it was first brought to Britain from Japan. Nobody planted any seeds; the plant was perpetually split and passed on, so each British plant was, in essence, part of the original. There were tons of it across the country. And not one showed signs of senescence – this was a plant that could live forever.

In my early teens, my grandparents gave me a cutting of their *Tradescantia zebrina*, that rambling houseplant with striped purplish leaves. My grandparents had obtained theirs from a cutting given to them by my great-grandparents in Cornwall and was an old plant which they knew as Wandering Jew. Cuttings had also been passed to my aunt and uncle, and I grew mine in my childhood bedroom. At school we were regaled by the way plants germinated, flourished, and died; yet here was one certainly over a century old. The total amount of plant in the family would fill a small room yet it was all vegetatively reproduced – it was all, biologically, the same plant. Most intriguing of all, to my young mind, is that it didn't age. My plant was a vigorous, sturdy, fresh and delightful phenotype. It had avoided senescence; this plant was immortal. I subsequently passed on cuttings to my friends and relatives … many are growing still.

Other genera were popular in other countries. Visiting cousins in Sweden, I noticed how many homes had loops of *Hoya kerrii* dangling across windows, hanging from bookcases, flourishing on household shelves, and popularly known "hjärtporslinsblomma." A native of Southeast Asia, particularly Thailand, it has thick, glossy, succulent cordate leaves, and its appeal in Sweden stems from its low-maintenance nature, its tolerance for indirect light and cooler temperatures, perfect for Scandinavia. Its heart-shaped foliage makes it popular as a romantic gift (often presented around Valentine's Day).

Like *Tradescantia*, it is propagated by cuttings. It's estimated that three-quarters of Swedish homes have indoor plants, each one weighing about a pound (roughly 0.5 kg); so – since there are about 5 million households in Sweden – the total amount of house plants could weigh some 2,500 tons, the great majority being *H. kerrii* descended from a few little plants. The tonnage of *Tradescantia* plants in Britain is dwindling, whatever it was; fashions change in domestic horticul-

ture as they do in every walk of life, and they're not fashionable any longer.

PLANTS CAN PERPETUALLY REJUVENATE

House plants are often said to be long-lived because they grow slowly, they have low rates of photosynthetic activity (and thus endure minimal levels of oxidative stress), and are rarely affected by the traumas that a leaf might experience in the wild. Yet plenty of vascular plants grow naturally and retain the proclivity for perpetual youth.

The primary cellular mechanism in plants that prevents ageing involves the indefinite capacity of stem cells to correct themselves as they proliferate in the shoot and root. These totipotent cells are found in the meristem, and they avoid senescence through robust DNA repair mechanisms that work ceaselessly to maintain genetic integrity. You will find it said that plant meristems exhibit a stratified organization, with a central quiescent zone of slowly dividing stem cells that minimizes the chances of mutation, while peripheral zones utilize rapid division for growth. That is clearly nonsense. The apical cell of any meristem must divide as rapidly as any other; it endlessly churns out new cells that replicate and subsequently specialize to create the tissue layers that we see in the adult plant – medulla, xylem, phloem, and the rest. Such stem cells can retain their genetic integrity indefinitely through post-replication surveillance that can correct a base-pair mismatch or deletion, and methodically excises and replaces any erroneous DNA. The telomeres of these stem cells do not suffer ageing; plant meristems express high levels of the enzyme telomerase, a ribonucleoprotein complex that adds repeats to the telomeres, thereby counteracting for loss. Their telomeres do not shorten – and here lies a clue to potential immortality.

STONE AGE TREES ALIVE TODAY

Individual plants can live for thousands of years. Yes, I know you'll immediately think of the Bristle-Cone Pine tree *Pinus longaeva*, which everybody has heard about. These remarkable trees are found in the Ancient Bristlecone Pine Forest within the White Mountains of California. One of those trees (they won't show visitors which one) has a verified age of 5,067 years, determined through dendrochronological analysis of its wood-core samples that reveal the annual growth rings. This means it germinated around 3,051 BC – that is the early Bronze Age, the dawn of civilization. The pyramids of Egypt hadn't been thought of, and Stonehenge lay in the future. America was populated by early hunter-gatherer communities, and the first earth ovens were being developed. Yet this small specimen of *P. longaeva* was already growing contentedly in the Californian sun, and it has been there ever since.

Always in the Top Ten of popular American houseplants, *Tradescantia zebrina* was introduced from Mexico some 200 years ago. Although it produces small flowers, it rarely sets seeds and they are of low viability, for the plant is virtually always reproduced from cuttings. Telomerase remains active, and its genetic complement resets at every mitotic division.

It is, however, a youngster. There is a Norway Spruce *Picea abies* named Old Tjikko in the Fulufjället National Park in Sweden, a clonal tree with an estimated age of approximately 9,500 years. It will have known the last Ice Age yet lives today and is almost twice as ancient as the oldest Bristle-Cone Pine. Its longevity arises from a persistent subterranean root system that regenerates new trunks every few centuries; the current visible trunk is only about 600 years old, but carbon-14 dating of the massive subterranean rootstock confirms that it's all the same plant, and germinated around 7,480 BC.

Then there is the Creosote Bush *Larrea tridentata* known as King Clone. It is a clonal plant thriving in the Mojave Desert and is said to be 11,700 years old. The plant expands outward from a central root crown, forming a ring some 65 feet (20 meters) in diameter. *L. tridentata* has remarkable drought tolerance and secretes chemical defenses against herbivores, allowing this genetic individual to endure the arid, nutrient-depleted environment where a standalone shrub would rarely exceed 150 years. This astonishing plant germinated when early humans were using stone tools,

This stand of Quaking Aspen, *Populus tremuloides*, named Pando, may have been growing for as long as 80,000 years. It is a feature of Fishlake National Forest, Utah, and new aerial trees are regularly produced from a subterranean rootstock. They are all clones; the genetics of the entire plant is continually replenished, so it does not age.

mammoths roamed the tundra, saber-toothed cats were on the prowl, and the fabled four-ton Giant Sloth *Megatherium americanum*, standing 20 feet tall (6 meters), still held sway in the forests. That was then – and the same plant which was germinating in those far-off prehistoric times is still alive today.

In Perry County, Pennsylvania, is a clonal plant that dates back over 13,000 years; the Box Huckleberry *Gaylussacia brachycera* which grew to extend over 6,500 feet (some 2,000 meters). However, the demand for free movement of the automobile – which pays little heed to American heritage – led to the construction of Route 22/322 in the 1970s and almost all of that vast plant was ripped up and destroyed. Just 20 acres (8 hectares) remains. It is an astonishing survivor since the time of the Clovis culture of prehistoric America and a era when woolly mammoths *Mammuthus primigenius* and the giant American mastodon *Mammut americanum* roamed the steppes. The same rootstock is still sending up aerial branches, genetically intact and as vibrant now as it was then, no matter what the highway installers might do. It will be growing on when we are likely to have caused our own extinction.

We must go back even further to nominate the germination of the Huon Pine *Lagarostrobos franklinii* in the rainforests of Mount Read, Tasmania. This tree propagates through layering, where branches form roots upon contacting the moist soil and grow to form interconnected stands that occupy many acres (several hectares). This vegetative mode of reproduction sustains the colony amid the cool, wet mountainous climate. Individual stems are known to be over 3,000 years old. It is their slow and compact rate of growth and dense, resinous wood that further contribute to its resilience against decay and insect attack. The rootstock from which this cloned colony still springs has survived for 43,000 years. Ancestral species of humanoid were abundant; Denisovans populated Siberia and much of SE Asia, while Europe and much of West Asia were inhabited by Neanderthals. The new arrival *Homo sapiens* was invading from the south, coexisting with these other hominids. The Huon Pine is recognized as the oldest living thing.

A couple of decades ago, I was the editorial consultant for that prodigious book, and were I there now, I'd suggest a rival candidate. It's one we all know: the Quaking Aspen, *Populus tremuloides*, the most widespread tree in the whole of North America, ranging from Alaska down to the middle of Mexico, from New Jersey in the East across to California in the West. The best studied is Pando, located in Fishlake National Forest, Utah, which extends over 106 acres (43 hectares) and comprises some 47,000 stems, all arising from the same slowly expanding rootstock – they're all the same plant. It weighs 6,000 tons, making it the largest living organism in the world. And its age? The individual stems tend to be replaced after 150 years, and the subterranean rootstock from which they arise had been variously dated to around 14,000 years. In the last two years, Rozenn M. Pineau and her colleagues in Atlanta and at the University of Chicago have analyzed over 500 tissue samples using high-throughput DNA sequencing with phylogenetic modeling to detect mutation rates, in comparison with data from known and well-studied aspen examples (see Pineau, Rozenn M., et al., "Mosaic of Somatic Mutations in the Ancient and Still-Living Aspen Clone, Pando," *bioRxiv*, 2024; https://10.1101/2024.10.19.619233.

These suggest a far older origin, and their findings are supported by that essential microscopical tool of paleobotanists; palynology. Aspen pollen is found in soil samples dating back tens of thousands of years, and combining all the data gives a date of germination that could be 80,000 years ago. There were fewer than 100,000 *Homo sapiens* in the entire world, and their stone-age culture was slowly emerging (Ford, Brian J. "The Microscope and the Caveman," *The Microscope*, 60:4 pp. 157–165, 2012). Megafauna, from mammoths and giant sloths, to the saber-toothed cat and mastodons – were abundant, the 3-ton woolly rhinoceros *Coelodonta antiquitatis* roamed the northern latitudes (it is featured in the earliest cave paintings), while *Diprotodon optatum* – the biggest-ever marsupial measuring 12 ft (almost 4 meters) from snout to tail and weighing 3 tons – dominated Australasia. This is a prehistoric world, and one where today's surviving Pando

plant was likely becoming established. We should treat it with respect. If you can survive without genetic deterioration for 80,000 years, you can live as long as the environment persists. That's immortality.

VEGETATIVE REPRODUCTION AND VIRGIN BIRTH

Those ancient plants eliminated the sex cells from reproduction and replaced it with complex methods of genetic surveillance and self-repair. They retain their telomeres. And this is what humans would need to do, if we are aiming to extend our lifespan. It is surprising to learn that many animals have already contrived to do this. The result is that they can clone themselves, producing large numbers (billions, in theory) of identical copies of themselves.

Extending this to humanity was the theme of Ira Levin's prescient novel, *The Boys from Brazil* (1976), in which arch Nazi Josef Mengele was found lurking in São Paulo, producing scores of clones of Adolf Hitler, and farming them out to foster-families across Brazil. Mengele's technique in the novel was to extract a nucleus from one of Hitler's cells and inject it into a denucleated donor human ovum. It was greeted as far-fetched and horrifying at the time, though this technique of somatic-cell nuclear transfer was used by the British cell biologist Keith Campbell who discovered that the donor cell had to be put into a quiescent state before it would work. It's astonishing to think that Levin's book was written fifty years ago, and Campbell's cloning of Dolly the sheep became reality 20 years later. It was not a simple task; the team tried 277 times before Dolly was successfully born.

Of those, only 29 embryos developed past 6 days; just three survived to pregnancy, and Dolly was the only one to proceed to full term. When she was born on July 5, 1996 she appeared to be a normal, healthy lamb; but her telomeres were already ageing, and were the same as those from a sheep already 6 years old. She developed severe arthritis aged 5 (when it occurs in sheep it's normally after the age of 10) and she showed signs of cancer within a year – pulmonary adenomatosis was diagnosed, and many believe that she was disposed to it because of imperfect epigenetic programming within her somatic cells. Dolly was not a perfect copy of her mother.

Cattle were first cloned two years after Dolly, and pigs two years after that. Techniques have improved and cloning farm animals is now big business. It's widely used in China, Brazil, Argentina, Italy, South Korea, and the USA, where meat and milk from cloned animals is considered safe. In Britain and the EU, cloning is banned for food production so no cloned creatures or their products are allowed in the human food chain. In China, cloning is not only accepted and supported by government, but it is rapidly scaling up.

Dolly the sheep is seen here with Sir Ian Wilmut the director of the Roslin Institute in Scotland, where the research was carried out. The cells used in the cloning experiment were extracted from ovine mammary tissue samples and the sheep that was born was named from the expansive thoracic physique of Dolly Parton, the singer.

Extending cloning technology to humans was first reported in 2004, when the journal *Science* published an astonishing paper by a South Korean veterinarian named Hwang Woo-suk who reported creating the first cloned human embryos. He followed it a year later (publishing in the same journal) by announcing the creation of 11 patient-specific stem-cell lines using the same somatic-cell nuclear transfer technique the British had developed. He was proclaimed as S. Korea's national hero. There were huge television specials about the man, and commemorative postage stamps were produced to mark his remarkable breakthrough. Huge government grants were offered and Hwang was honored as "the Pride of Korea."

But doubts arose. The TV researchers spoke to junior staff, who revealed that the ova had all been obtained by coercion from juniors; some had been illegally paid for. The project suddenly began to seem dubious. The investigative reporters delved deeper, only to find that the cell lines were fake. Photographs had been doctored, records had been forged, and DNA results had been invented. The results were false.

The following year, *Science* retracted both papers and apologized. Seoul National University fired Hwang, and he was arrested by the police and put on trial. He was convicted of bioethics violations and embezzlement of grants totaling $700,000, and in 2009 he was given a 2 years in jail, but suspended. So he was

In 2004, South Korean veterinarian Hwang Woo-suk claimed to have cloned a human in a Seoul laboratory. He had not. In spite of his dishonest claims, and the grants he fraudulently obtained, he was neither fined nor jailed. However, he was the first person to successfully clone a dog, and in 2006 students held a candle-lit vigil in support.

never fined, nor did he spend a day in prison. He suffered no legal penalty. And there was some serious science behind it all. Although the fact is often ignored by scholars and serious scientists, Hwang did succeed in successfully cloning a dog for the first time. After a large number of unsuccessful trials, he produced a healthy clone named Snuppy from an Afghan Hound on April 24, 2005. It was no foolproof, repeatable technique, for he used 1,095 eggs that were fertilized and transferred into a total of 123 surrogates. Only 2 pups were born, and one died soon after from pneumonia. Snuppy alone survived, and its DNA was independently sequenced by others to substantiate that he was indeed an identical clone. The achievement was announced in *Nature*.

This was a major scientific success; dogs are much harder to clone than many other mammals because their eggs are ovulated at an immature stage. Under the microscope they are dark and fatty and very hard to work with. The timing of dog ovulation and their heat cycles is unreliable and many laboratories had tried for years and had failed. Hwang's team were the first to conquer these obstacles.

And now? Hwang Woo-suk runs his own institute, Sooam Biotech, on the outskirts of Seoul, where he clones dogs commercially. He can clone yours. It costs $100,000 plus mailing of samples to S. Korea (Sooam Biotech provides a kit) and they guarantee success. There are no refunds, but – if the first attempt fails – they keep trying. In the past 20 years they have cloned over 1,000 dogs for anxious owners, and are the most desirable cloning center for pets. It's much cheaper elsewhere (the US company ViaGen can clone your pet for $50,000) but Hwang is the celebrities' choice.

HUMAN CLONING AND THE LAW

The House of Representatives first voted to ban human cloning in a bill sponsored by Republican Dave Weldon (after 6 hours of debate) on July 31, 2001, with a penalty of 10 years imprisonment and million-dollar fines, and on March 8, 2005, just a couple of weeks before Snuppy emerged, the United Nations passed their Declaration on Human Cloning. It called on all member states to prohibit all forms of human cloning inasmuch as they are incompatible with human dignity and the protection of human life. The wording was deliberately ambiguous. It bans reproductive cloning, which is seen as violating human dignity, though it does leave room for experiments on therapeutic cloning. It is, moreover, a non-binding resolution, and no enforcement penalties exist. It was passed with 84 votes in favor, 30 voted against, with 37 abstentions.

Experiments with human cells have been announced. In 2001, Robert Lanza and Michael West of Advanced Cell Technology created early human embryos which developed only to the 6-cell stage. No stem cells were produced. In the following year in France, Brigitte Boisselier of the Clonaid company, announced they had produced a cloned human baby named Eve, but it turned out to be a hoax. Samuel Wood led a team at the American company Stemagen, who successfully produced 5 cloned blastocysts, representing the very earliest stages of embryology. They were not permitted to grow further. The first viable human stem cells were cloned by a research team at Oregon Health and Science University headed by Soukhrat Mitalipov.

Currently there are experiments under way in China, Japan, S. Korea, and the USA, all designed to harness early embryonic tissues in modeling disease. It is now known how to reset telomere length during reprogramming, and at last human viable stem cells can be routinely produced.

JURASSIC PARK IN THE REAL WORLD

The plot of *Jurassic Park* is so ingenious. Its author, Michael Crichton, was a Harvard-educated physician, and the notion of a droplet of blood trapped in a mos-

The Auroch was an impressive wild ox weighing some 1,500 pounds (750 kg) and standing 6 feet (1.8 meters) tall at the shoulder. This image is about six feet (roughly) and was painted in ochre and charcoal 17,000 years ago in the caves of Lascaux, France. Aurochs survived in European forests until the mid 17th century.

Selective back-breeding by German zoo-keeper brothers Lutz and Heinz Heck gave rise to oxen looking very like Aurochs, though their genetics (and behavior) are closer to domesticated cattle. The indomitability of the breed was said to exemplify fascism, and the research was funded by the Nazis through Reichsmarschall Hermann Göring.

quito perfectly preserved in amber is almost feasible. Crichton originally drafted the book as the story of a graduate student struggling to clone a dinosaur, only later changing it to a billionaire's theme park. The novel appeared in 1990 and Stephen Spielberg's movie was released just 3 years later. For the first time, we saw vivid and realistic dinosaurs on the cinema screen. The fact that the ground cannot normally support the weight of such enormous beasts was to emerge later (Ford, Brian J. *Too Big to Walk, the New Science of Dinosaurs*, New York and London: HarperCollins, 2018) but the movie became a classic, and has grossed over a billion dollars. The entire series of movies generated $6 billion – an astonishing return for a movie that cost $63 million to make.

In fact, any blood in amber will now be in the form of a degraded residue rich in haem, but without usable DNA. A beautifully preserved mosquito discovered in amber from Montana and dating back 46 million years, revealed traces of haem in analytical spectra, but only a few base-pairs from DNA. An older specimen from Myanmar dates back 99 million years and also contained slight traces of haem, but no hemoglobin, and no genomic DNA. Crichton's book remains fiction but it's close enough to reality to endure as a fascinating story.

RECREATING THE DEAD

The first attempt to revive an extinct species was the case of the Auroch, *Bos primigenius*, a huge ancestral species of cattle that became extinct in the remote forests of Poland in the 17th century. Aurochs stood 6 feet (1.8 meters) tall at the shoulder and weighed 1,500 pounds (700 kg). They had elongated, lyre-shaped horns and were known for their aggression. Aurochs were hunted by stone-age communities – there are graphic pictures of them on cave paintings dating back 35,000 years, and images continued for the following 25,000 years. Around 10,000 years ago, farmers in Turkey and Syria began to select for docility and a more manageable physique, and modern cattle emerged as a newly created species, *Bos taurus*. Some 2,000 years later in Pakistan, a different approach to breeding gave rise to cattle with a hump: the Zebu, *B. indicus*. From these, every one of today's 1.5 billion domestic cattle are descended. Aurochs were untamable, and remained a wild animal whose populations diminished as their habitat was lost, and through hunting by humans. The final survivor was a cow in the Jaktorów Forest of Poland, who died in 1627. Her skull was sent to Sweden, where it is on display to this day at the Livrustkammaren Museum in Stockholm.

But wait, this massive ox was powerful, intimidating, dominant, and untamable; it seemed to embody the hallmark of fascism and, in the 1930s, attempts were made to recreate the mighty Auroch as a symbol of Hitler's new Germany. Two brothers, Lutz and Heinz Heck, both directors of German zoos, launched a selective breeding program to recreate the Auroch. They chose Hungarian Grey cattle, for size and the horns, Scottish Highland cattle for hardiness and the characteristic shaggy coat, and Spanish fighting bulls for their aggression and physical strength, and (with characteristic modesty) the brothers decided to name the result Heck cattle. This was the first program in his-

tory to attempt to revive an extinct species.

A similar project was the attempt in South Africa to recreate the Quagga, *Equus quagga*, a kind of Zebra that had been extinct since 1883 when the last Quagga died at the Amsterdam Zoo. In 1987 Eric Harley, a geneticist at the University of Cape Town, embarked on a program of back-breeding just as the Heck brothers had done. Teams of helpers screened thousands of Plains Zebras in Namibia and KwaZulu-Natal for individuals that looked most like the Quagga, which had a brownish background color between stripes, rather than white, and few stripes at all on the rear of the body or on the hind legs. Harley's team bred from the most Quagga-like animals, and in 2023, detailed DNA comparison at the University of Cape Town confirmed that the latest of these Quaggas are genetically very close to the skin cells in samples taken from extinct animals.

There are now 150 of these animals in game reserves across South Africa, many living in semi-wild conditions. They are not 100% genetically identical to the 1883 Quagga, but externally they are identical, and genetically they have recovered most of the original Quagga variation through selection alone. This project is widely regarded as the only successful de-extinction by breeding ever to succeed, and the Quagga is the only extinct animal that walks the earth again in viable, breeding herds, even though obtained by selective breeding rather than cloning.

This was the first successful recreation of an extinct mammal, but it was not the last. The Pyrenean Ibex, *Capra pyrenaica*, closely related to the Iberian Ibex, was the first mammal to be declared extinct in the present century. The sole surviving Pyrenean Ibex, a female named Celia, was killed by a falling tree in January, 2000, and the species was declared extinct.

A team of Spanish cell biologists led by Jose Folk at the Centro de Investigación y Tecnologia Agroalimentaria in Zaragoza had already taken skin cells from Celia's ear in 1999, and had frozen them in liquid nitrogen. They resolved to revive the species by using somatic cell nuclear transfer, the same technique that had created Dolly the sheep. They took nuclei from carefully thawed skin cells, and inserted them into 154 previously enucleated goat eggs. The resulting embryos were implanted into surrogate domestic goats. Only 7 pregnancies resulted, and only 1 resulted in a live birth. A single female kid was born by Cesarean section, and proved to be genetically 100% pure Pyrenean Ibex. This was the first animal ever brought back from extinction with a full complement of original DNA. It is a triumph for genetics and for cell biology.

The excitement did not last for long. The little kid died just 7 minutes later, due to severe lung defects. The newborn Ibex could not breathe because the lungs were partially fused, and had abnormal lobes, a defect later linked to incomplete epigenetic reprogramming. The same team tried again with improved protocols, in 2008 and 2010, using better cryoprotectants and epigenetic drugs, but produced no live births at all. However the frozen cell line from Celia is still stored in Zaragoza and will be used in subsequent research.

PROSPECTS FOR THE FUTURE

Even though the Spanish experiments were not a success, they did prove that reviving extinct species is certainly possible, providing we have a source of viable DNA from the preserved remains. Several experiments are currently underway. One of the most famous mass extinctions was that of the Passenger Pigeon, *Ectopistes migratorius*, which was once the commonest bird across North America but was hunted to extinction by 1914. A team in North Carolina has already extracted DNA from museum specimens.

The Dire Wolf, *Aenocyon dirus*, a large predatory animal that became extinct some 13,000 years ago, is currently under study at the Colossal Biosciences company in Texas. Recreated specimens will be genetically modified Gray Wolf proxies, but it is believed that they will provide animals that look extremely similar to their extinct predecessors. The same company have just reassembled the genome of the Thylacine or Tasmanian tiger, *Thylacinus cynocephalus*, which became extinct in 1936, and they now plan to see the first viable thylacine pups around 2028. The most impressive experiment that Colossal Biosciences have in mind is to revive the Woolly Mammoth, *Mammuthus primigenius*, which became extinct about 4,000 years ago. They have good DNA samples, and they also have in mind to recreate the Moa, *Anomalopteryx spp.*, which were wiped out around 1500 AD, and even the legendary Dodo, *Raphus cucullatus*, which became extinct in 1662. Meanwhile, a company named Revive and Restore in California are thinking to re-create the Great Auk, *Pinguinus impennis*, and the Heath Hen, *Tympanuchus cupido*, within the next decade.

Many of these will be hybrids, with their signature DNA added to the genome of existing species. The Dodo, for example, will be a genetically modified Nicobar Pigeon *Caloenas nicobarica* which will provide most of the DNA. Specific morphological traits like body size, leg thickness, bill shape and repression of wing development will be added using CRISPR, the expansively-named Clustered Regularly Interspaced

Short Palindromic Repeats technology, to insert some 5-8% Dodo DNA into the genome – about 125,000 base-pair edits in a genome of 1.3 billion genes. It will look like a Dodo, but it won't behave like one; it's still a Nicobar Pigeon with pretensions.

HUMAN CELLS THAT LIVE FOREVER

We have seen how varied are the methods that living things have evolved to perpetuate their cells intact over countless generations. Surely, humans ought to be able to do the same? If a grove of aspen trees or a water-flea can exist for millions of years without any deterioration in genetic architecture (or shortening of their chromosomes' telomeres) then – why not us? Our idea that cells must age, and are doomed inevitably to deteriorate, is not true. Human cells can live for an extended period – HeLa cells have been grown in culture since 1951 and are known around the world.

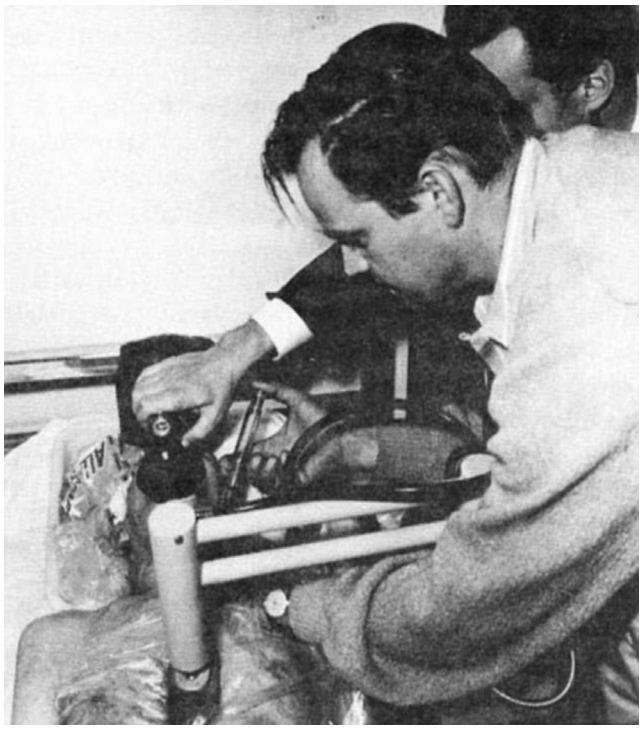
They originated in 1951, in a woman patient named Henrietta Lacks's at the Johns Hopkins Hospital in Baltimore. She was an impecunious Black tobacco farm worker, and Johns Hopkins was the only hospital around that would consider treating Black patients (bear in mind this is only 74 years ago, within living memory of many readers). They took a routine biopsy which revealed the presence of an aggressive cervical carcinoma. George Gey, head of the tissue culture unit at Johns Hopkins, received 2 cell samples: one from the tumor, and one from the healthy cervical tissue. The tumor cells grew aggressively in culture – the first human cells ever to do so indefinitely. Within six months Ms. Lacks' cancer had spread and she was given radiation therapy. Sadly it failed to eliminate the tumor and she died of uremic poisoning after the cancer blocked her ureters. Henrietta Lacks was buried in an unmarked grave in Clover, VA. George Gey meanwhile distributed the HeLa cell cultures to laboratories worldwide and by 1954 they had become the standard human cell line. Within 20 years, HeLa cells were acting like independent microbes, contaminating hundreds of other cell lines, which remains a problem today.

Although Henrietta Lacks herself died in 1951, there are approximately 60 million tons of her cells alive in laboratories today. It is the largest living entity that humans have ever propagated. Ms. Lacks may have died in 1951, but her cells live on and they have given rise to important developments in the decades since her sad demise.

Although people like to celebrate them as examples of human immortality, the cells themselves have undergone catastrophic genetic change. Starting with a healthy complement of 46 chromosomes, they have given rise to hyper-triploid strains; indeed strains of HeLa cells with up to 80 chromosomes are known. There are more than 20 copies of some deleterious genes in many of these genomes, along with hundreds of translocations, deletions, and misreadings that have occurred over the years. The nuclei of HeLa cells have accumulated thousands of point mutations, and there are now so many different HeLa sub-strains that we lose count. The karyotype is continually evolving.

The HeLa cells in which we culture viruses originated in 1951 in cancer tissue taken from Henrietta Lacks, an impecunious tobacco farm worker. Research into vaccine production worth many billions of dollars has been the result, and in 2013 her family were offered a settlement that recognized the importance of the HeLa cell line.

The main reason for their apparent immortality is the very high level of telomerase activity, a phenomenon found only in embryonic stem cells or the most aggressive malignancies. The telomeres of the chromosomes in HeLa cells cultures can be 20 KB long, and this has remained remarkably stable for over 70 years of continuous culture. This active expression of telomerase was present in Henrietta Lacks's original cervical tumor in 1951, and it was locked in before the first cell culture was established. Every time a HeLa cell divides the telomerase adds back-up to 100 base pairs lost from the ends, so the telomere retains its length and the cells never reach the Hayflick limit. They just keep dividing. HeLa telomeres are long, stable, and kept that way by very high telomerase activity, and this is one of the best examples of the classic cancer telomere maintenance mechanism. Genetically, the rest of the cell is a catastrophic genetic confusion, but the telomere system continues to work perfectly – and perpetually.



Robert Nelson is seen infusing dimethyl sulfoxide into the freshly deceased body of James Bedford in 1967. Bedford was the first person to be cryonically preserved in liquid nitrogen. Whoever might take it upon themselves to attempt reviving someone from the heady, hedonistic days of the 1960s remains undecided.

HOW LONG SHOULD ANIMALS LIVE?

The oldest vertebrate in the world is a Greenland Shark, *Somniosus microcephalus*, which could be 512 years old. Radiocarbon dating confirms this extraordinary lifespan. Ming, an Ocean Quahog Clam *Arctica islandica*, was shown to have lived for 507 years. Had it not been opened and sampled in 2006, it would be alive today. On the volcanic island of Saint Helena in the South Atlantic ocean you will find Jonathan, the world's oldest living land animal. He is a giant tortoise, *Aldabrachelys gigantea*, and was brought to the island as an adult in 1832, aged about 50. He is believed to be at least 250 years old. Charles Darwin collected a Galápagos Giant Tortoise, *Chelonoidis nigra*, on his celebrated travels aboard HMS Beagle. I saw the magnificent creature, named Harriet, in Fleay's Fauna Reserve, a zoo in Queensland, Australia. Harriet died in 2006 – though genetic analysis later suggested she was a subspecies found only on the island of Santa Cruz, and Charles Darwin did not visit that island. In any event, she lived to be almost 180 years old, far longer than most vertebrate species.

Mammals can experience longevity too, and I am not thinking of elephants which do not live as long as we can. The longest-living mammal known is a Bowhead Whale, *Balaena mysticetus*, aged 211 years, with harpoon tips from the early 1800s still embedded in its blubber. The Fin Whale, *Balaenoptera physalus*, has been known to live to some 115 years, though humans are the only other mammals that come close. Currently, the oldest person is Ethel Caterham who lives in Surrey, England. She was born on August 21, 1909, and is currently 116 years old. In the mammalian world, only the Bow Whale lives longer.

THE FINAL THRESHOLD – WAITING FOR ETERNITY

It is 1962. Robert Ettinger, an academic from Michigan, is putting together a lengthy report on a revolutionary new idea – cryopreservation. He foresees an era when dying humans can be perfectly preserved for an eternity in liquid nitrogen, ready to be thawed out and revived when a cure for whatever killed them has been found. His manuscript runs to some 50,000 words, the length of a short book. Ettinger sends it off to major publishers including Doubleday, Simon & Schuster, and Harper & Row, and eventually receives some 30 letters of rejection. One said: "If this is a joke, it isn't funny; if it isn't a joke, it's obscene." He approached literary agents, too; not one would support his ideas.

Ettinger was certain he had a crucial new concept, and decided to publish the book at his own expense. He had Edwards Brothers of Ann Arbor print 1,000 copies for $2,800 (some $30,000 today) and mailed out hundreds to leading authorities including scientists, journalists, and celebrities. Isaac Asimov received one, but did nothing with it. But some of the journalists wrote about his ideas, and they created a buzz – so much so that Doubleday approached him and offered formally to publish the book. It has been in print ever since.

One of the first readers of the new book was James Bedford, a Californian psychologist, in 1967. Bedford was a devotee of sci-fi and avidly digested Ettinger's book. That same year, the Cryonics Society of California was founded, Bedford was quick to join. He was dying from kidney cancer at the age of 43, and he decided to have his body preserved in suspended animation rather than buried. He died on January 12, 1967.

It is a curious paradox on which few trouble to reflect, that every cell in the body is fully alive after a person is declared dead (see: Ford, Brian J. "There is always Life after Death," *The Microscope*, 62:1, pp. 15–24, 2014). Bedford's warm body was rushed over to the Cryonics Society of California in Pasadena where physician Robert Nelson injected him with heparin and dimethyl sulfoxide as cryoprotectants, chilled him to

near freezing, and then immersed him in a flask of liquid nitrogen at -196° C. Since 1982, he has been stored at the Alcor Life Extension Foundation in Scottsdale, AZ, making him the first, and longest, cryopreserved human.

Today, over 500 people have been cryopreserved, and there are 3,000 on waiting lists. Among those who have said they wish to be cryonically preserved after death are Seth MacFarlane of Family Guy, Paris Hilton the heiress, television's Larry King, and music mogul Simon Cowell. None of them seem to accept death as our ultimate earthly destination. American culture tends to avoid dwelling on death. It is popular to say that someone has "passed." That would make no sense in Britain. If you said there, that someone had passed, they'd ask: "Passed what? Wind? Gallstones? Driving test?"

In winter, the Alaskan Wood Frog, *Lithobates sylvaticus*, seems to become a solid block of ice, only to revive and hop away unconcernedly when the warmth of springtime supervenes. Its cells, however, do not freeze; high levels of glucose (up to 550 mmol/liter) and excess urea accumulate in the blood, ensuring the survival of the tissues.

BANISHING TOMORROW

How long should you want to live? Nobody has lived longer than Jeanne Louise Calment, born in France on February 21, 1875, who lived to 122 years and 164 days. It is believed that the prophet Moses suggested that 70 was our natural lifespan. Psalm 90:10 states: "The days of our years are threescore years and ten," though it added that fourscore years might be the result "by reason of strength." Not much has changed; the average lifespan in today's America is 79 years. Moses would recognize that. We can maximize it by nurturing our cells with varied and nourishing foodstuffs, though in modest amounts, and by living an active and constructive life. But who would want to extend it indefinitely? When the time comes, it's entirely appropriate to bow out and free up the space for generations still to come.

Who would undertake to attempt to bring James Bedford back to life? The practical perplexity is imponderable, the moral dilemma overwhelming. We know that life can be restored after freezing; insects and tardigrades, like human sperm and ova, have all been frozen solid in liquid nitrogen, and have been routinely revived. Can vertebrates freeze? The Wood Frog, *Lithobates sylvaticus*, is well known to become a solid block of ice for months in winter and is widely studied as a unique survivor of extreme weather. Two things: first, it's far from unique; several other amphibians do it.* Secondly, no matter how it appears; their cells adapt. As the temperature sinks lower, huge amounts of glucose and urea are produced in the bloodstream. The Wood Frog has been documented to have levels of glucose 100× higher than is ever found in daily life. Osmotic pressure draws water out from within the cytoplasm of each cell, reducing them to semi-solid particles of cytoplasm. Normally, cells rupture when frozen but these cells cannot freeze. There is too little water remaining. About 60% of the body freezes solid, but the cells, in their condensed state, survive. So recovery is possible, though nobody seriously imagines that you could restore a cryopreserved human to normal, healthy life.

Our cells replicate up to the Hayflick limit of about 50 divisions. Our hearts beat about 3.5 billion times, our kidneys process 14 million gallons of fluid (sufficient to fill 21 Olympic sized swimming pools), our livers process over 600,000 tons of blood – the weight of 5 fully laden Nimitz class aircraft carriers – our nails grow over 400 feet (120 meters) and then? It's time to go.

Our cells have contrived to make immortality unattainable. It once seemed likely that there would be a simple genetic switch; turn it off and ageing was ended. Telomeres would be sustained by telomerase, as they are in cancer cells, and we'd simply never age. But evolution has equipped us with interrelated layers of genetic complexity to prevent our achieving that end. Our cells have conspired to ensure we have to live a predetermined life that – even if it inconveniences us – ensures the continuity of the community. Our cells are more ingenious than we think. And the only reason we are intelligent – is because our cells are. ■

*Other amphibians that freeze include the Gray Treefrog, *Hyla versicolor*, of eastern and central USA, which you can often hear singing in the yard when it thaws in spring. The tiny Spring Peeper, *Pseudacris crucifer*, of the eastern and northern States, is similarly capable of being frozen solid, and so is the Siberian Salamander, *Salamandrella keyserlingii*, which spends the winter as a lump of ice.