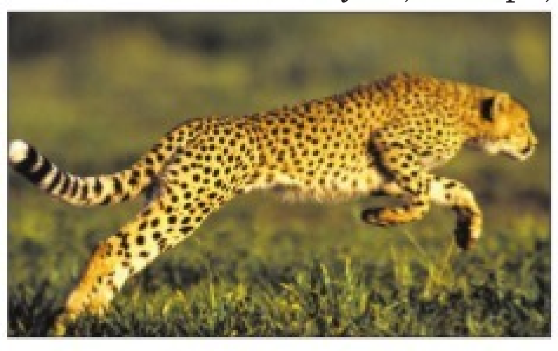
Counting the tiger's stripes

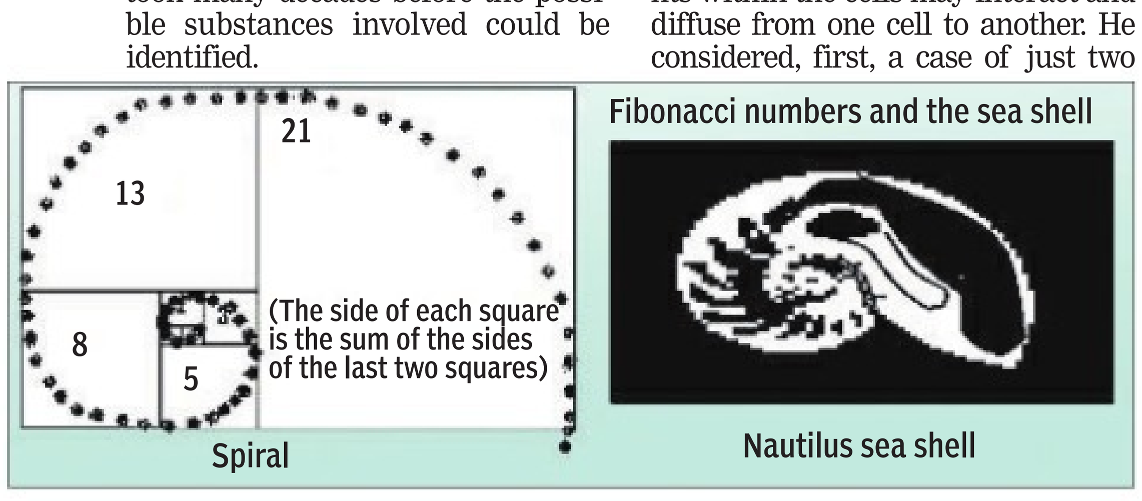
NUMBERS AND MATHEMATICS HELP UNDERSTAND ANIMAL COATS AND THE WAY LEAVES GROW ON PLANTS, WRITES **S ANANTHANARAYANAN**

Evolutionary biologists have explanations for why animals have patterns on their coats or even birds on their wings, but the mechanism by which the patterns actually come about has not been understood. The legendary Alan Turing created a mathematical framework of how chemical interactions could lead to the periodic expression of growth factors, but it took many decades before the possible substances involved could be identified.





The tiger has stripes, the leopard has spots and the cheetah has both spots and stripes.



The patterns on the coats or animals are a familiar sight and clearly help them blend with the surroundings — for concealment, for safety or for greater stealth in the hunt. It is not difficult to understand that the chance appearance of features like the stripes on the tiger or the zebra or the spots on the leopard would have conferred a survival advantage, and genes that made for these features soon became the rule for the species.

How leaves are spaced to catch the most of sunshine and rainfall.

But genes lead to particular proteins and tissue types, so how do colours get distributed? How, in fact, does tissue grow in specific shapes and how do patterns, or even limbs, grow in specific directions?

In a celebrated 1951-52 paper, Turing considered a simplified case of just a few cells and how chemical agents within the cells may interact and diffuse from one cell to another. He considered, first, a case of just two cells and, then, a case of a ring of cells, where agents that he called *morphogens*, or *generators of form*, would interact and then diffuse from cell to cell at different speeds, reinforcing or cancelling their effects at different places. He simplified the case to be studied so that the mathematics of the way mechanical or electrical disturbances spread could be applied to the progress of morphogens and he found that the effects of the chemical agents manifested in waves, with crests and troughs, just like waves in a vibrating string or electromagnetic waves.

The theory, when applied to the animal called the *hydra*, a simple, tubular creature that has a ring of tentacles at one end, led naturally to the regularly spaced concentration of morphogens in the ring that forms an end of the hydra, to give rise to the ring of tentacles. Although without speaking of what the morphogens may be, Turing's theory of "reaction and diffusion" was able to explain the black and white patches on *Friesian cows*, which he was interested in. The theory was carried forward by others and the stripes that appear on the back of the tiger are now understood as a pattern of pigmentation laid down by periodic waves of diffusion of chemicals in the animal's embryo.

More complex patterns appear by considering more sets of waves. Just as one set of waves can lead to a stripe pattern, like the waves at the seaside, two sets of waves that cross each other would lead to a distribution of points or peaks, leading to a spotted pattern, like the coat of the leopard. The wave pattern is seen to be affected by different factors that include the dimensions of the area in which they occur, which determine the "boundary conditions". This, in turn, leads to more conclusions about the spots or stripes on the tails of the great cats. The tiger has stripes on its body and tail and the leopard has spots on the body and the tail.

The cheetah has spots on the body and stripes on the tail, but no animal has spots on the tail and stripes on the body. The reason seems to be the space available for two sets of waves, which lead to spots. If these could exist in the tail, for a spotted one, then they can certainly exist in the body, and the body must be spotted, rather than striped!

Recent studies of the ridges on the roof of the mouth of the common mouse have now identified a hormone called *Fibroblast Growth Factor* and a protein called *Sonic Hedgehog* as the chemicals involved. Other studies of the shapes of the cactus plant and the role of a hormone called *auxin*, by Allen Newel at the University of Arizona, have found that three sets of waves could be the reason for cells to grow in particular shapes. Tom W Hiscock and Sean G Megason at Harvard University note that there can be other drivers, besides the Turing process, of periodic phenomena, like cell-based mechanisms or mechanical properties, like elasticity, of a system. They have just published in the journal *Cell Systems* an integration of the different models in the form of a combined mathematical formulation to find bases for more features. Why the stripes of the tiger are vertical, while those of the zebra can be both ways, for example, or and even why the fingers grow along the length of the arm and not any other way.

The work of Newel, in fact, connects the waves of morphogens with some fascinating numbers that are found to appear in the plant kingdom. The *Fibonacci series* is a list of numbers where each is formed by adding the previous two, like this: 0, 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, 144… The interesting property of this series is that ratio of each pair of successive numbers gets closer and closer to 1.61803398874… the so called *Golden Ratio*, which appears in geometry, aesthetics, music, art and architecture. The ratio of successive numbers in the Fibonacci series, in fact, gets closer and closer to an infinitely long, non-repeating decimal number that actually cannot be expressed exactly as a ratio of two integers (and is hence known as an "irrational number").

~~Series:-0 1-1-2 3-5-8 13-21-34-89 Ratio: 0-1-2 1.5 1.666 1.6-1.625-1.615-1.619 -1.618~~ Fibonacci numbers are also found to appear everywhere in nature. One typical instance is the placement of leaves along the stalk of a plant. The angular separation of the leaves, as one goes up the stalk, is found to form a Fibonacci series. The fact that the ratio of successive numbers in the series tends to be an irrational number has an important consequence — that no leaf in the plant will ever find itself exactly above or below another leaf. This property accurately optimises the function of the leaves to receive the best of sunlight or rainfall, to make for the energy efficiency of natural systems to have evolved to be at the maximum that is possible.

While natural selection can explain how plants, and even snail shells, seashells, and a host of natural shapes have selected the most efficient and economical dimensions, the question always arises of how do species ensure that they grow according to these dimensions. The work of Newel and colleagues provides an illuminating lead — they find that the waves of the three morphogens involved in the cactus plant are connected by the Golden Ratio; the number of wave crests of the third wave is actually the sum of the numbers in the first two waves!

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DEFT METHODS OF REPLICATION

TAPAN KUMAR MAITRA EXPLAINS HOW DNA CLONING TECHNIQUES PERMIT INDIVIDUAL GENE SEQUENCES TO BE PRODUCED IN LARGE QUANTITIES

The power of restriction enzymes is the ease with which they allow a desired segment of DNA, usually one containing a specific gene, to be inserted into a cloning vector and replicate when introduced into bacterial cells. This process of generating copies of specific DNA fragments is called DNA cloning. Although the specific details of cloning procedures vary, the following five steps are typically involved

1. Insertion of DNA into a cloning vector: Most vectors used for DNA cloning are themselves recombinant DNA molecules, designed specifically for this purpose. For example, when bacteriophage l DNA is used as a cloning vector, the phage DNA has had some of its non-essential genes removed to make room in the phage head for spliced-in DNA. Plasmids used as cloning vectors usually have a variety of restriction sites and often carry genes that confer antibiotic resistance on their host cells. The antibiotic-resistance genes facilitate the selection stage (4), while the presence of multiple kinds of restriction sites allows the plasmid to incorporate DNA fragments prepared with a variety of different restriction enzymes.

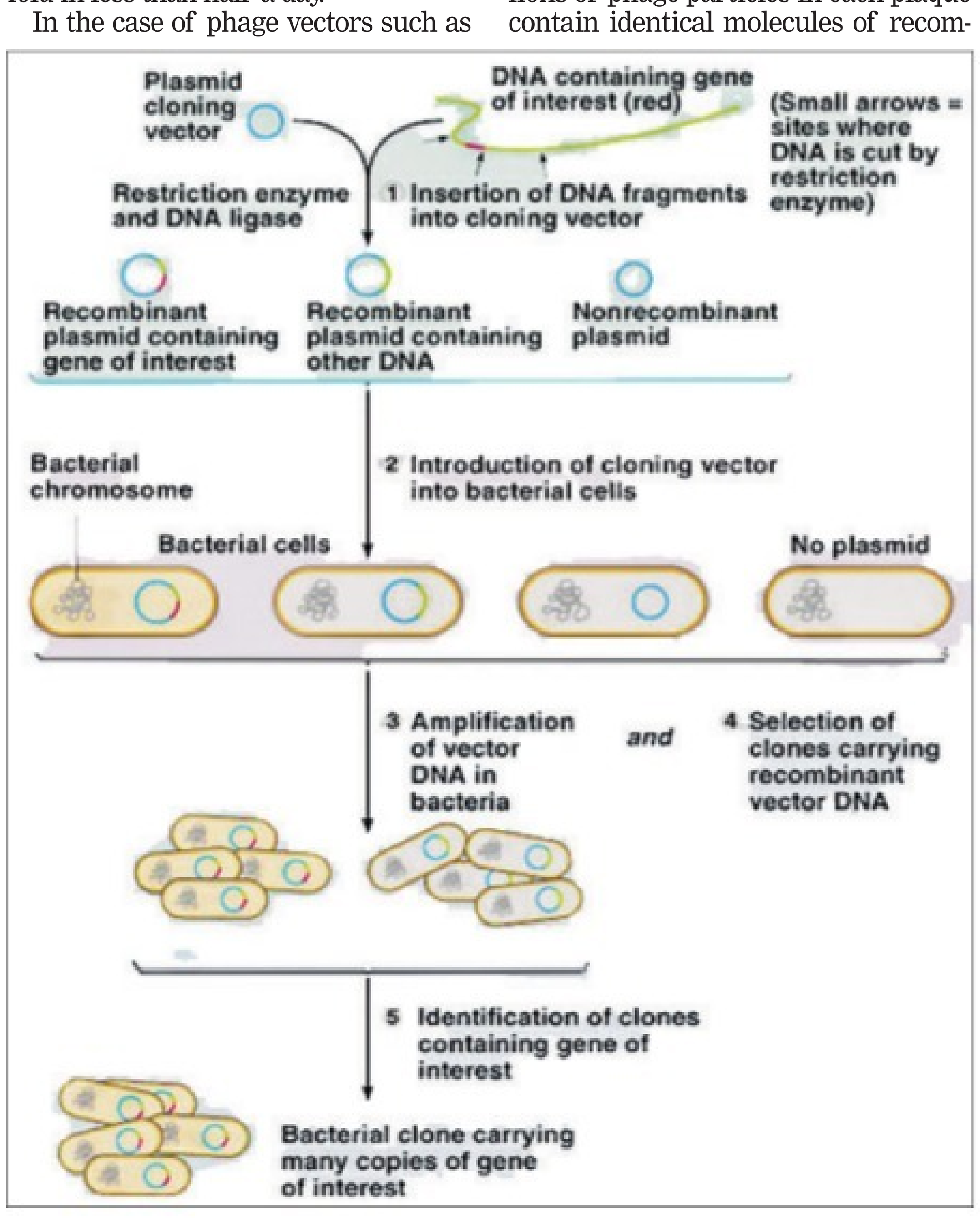
An example of a commonly used plasmid vector is *pUC19* ("puck-19"). Because it carries a gene that confers resistance to the antibiotic ampicillin *(ampR)*, bacteria containing the plasmid can be easily identified by their ability to grow in the presence of ampicillin. The pUC19 plasmid also has 11 different restriction sites clustered in a region containing the *lacZ* gene, which codes for the enzyme ?-galactosidase. Integration of foreign DNA at any of these restriction sites will disrupt the *lacZ* gene, thereby blocking the production of ?-galactosidase. As we will see shortly, this disruption in ?-galactosidase production can be used later in the cloning process to detect the presence of plasmids containing foreign DNA.

How a specific gene of interest residing in a foreign DNA source is inserted into a plasmid cloning vector, using *pUC19* as the vector and a restriction enzyme that cleaves pUC19 at a single site within the *lacZ* gene can be seen in the illustration. Incubation with the restriction enzyme cuts the plasmid at that site (1), making the DNA linear (opening the circle). The same restriction enzyme is used to cleave the molecule containing the gene to be cloned (2). The sticky-ended fragments of foreign DNA are then incubated with the linearised vector molecules under conditions that favour base pairing (3), followed by treatment with DNA ligase to link the molecules covalently (4). Only the recombinant plasmid contains the desired fragment of foreign DNA. In practice, however, a variety of DNA products will be present, including non-recombinant plasmids and recombinant plasmids containing other fragments generated by the action of the restriction enzyme.

2. Introduction of the recombinant vector into bacterial cells: Once foreign DNA has been inserted into a cloning vector, the resulting recombinant vector is replicated by introducing it into an appropriate host cell, usually the bacterium *E coli*. Cloning vectors are introduced into bacteria in one of two ways. If the cloning vector is phage DNA, it is incorporated into phage particles that are then used to infect an appropriate cell population. Plasmids, on the other hand, are simply introduced into the medium surrounding the target cells. Both prokaryotic and eukaryotic cells will take up plasmid DNA from the external medium, although special treatments are usually necessary to enhance the efficiency of the process. The addition of calcium ions, for example, markedly increases the rate at which cells take up DNA from the external environment.

3. Amplification of the recombinant vector in bacteria: After they have taken up the recombinant cloning vector, the host bacteria are plated out on a nutrient medium so that the recombinant DNA vector can be replicated, or amplified. In the case of a plasmid vector, the bacteria proliferate and form colonies, each derived from a single cell. Under favourable conditions, *E coli* will divide every 22 minutes, giving rise to a billion cells in less than 11 hours. As the bacteria multiply, the recombinant plasmids also replicate, producing an enormous number of vector molecules containing foreign DNA fragments. Under such conditions, a single recombinant plasmid introduced into one cell will be amplified several hundred or even billion fold in less than half a day.

In the case of phage vectors such as phage l, a slightly different procedure is used. Phage particles containing recombinant DNA are mixed with bacterial cells and the mixture is then placed on a culture medium under conditions that produce a continuous "lawn" of bacteria across the plate. Each time a phage particle infects a cell, it is replicated and eventually causes the cell to rupture and die. The released phage particles can then infect neighbouring cells, repeating the process again. This cycle eventually produces a clear zone of dead bacteria called a plaque, which contains large numbers of replicated phage particles derived by replication from a single type of recombinant phage. The millions of phage particles in each plaque contain identical molecules of recombinant phage DNA.



An overview of DNA cloning in bacteria using a plasmid vector. The plasmids most widely used as cloning vectors are typically about 1/1000 the size of the bacterial chromosome.

4. Selection of cells containing recombinant DNA: During amplification of the cloning vector, procedures are introduced that preferentially select for the growth of those cells that have successfully incorporated the vector. For plasmid vectors such as *pUC19*, the selection method is based on the plasmid's antibiotic-resistance genes. For example, all bacteria carrying the recombinant plasmids generated resistant to the antibiotic ampicillin, since all plasmids have an intact ampicillin-resistance gene. The amp^R gene is a selectable marker, which allows only the cells carrying plasmids to grow on culture medium containing ampicillin (the medium "selects for" the growth of the ampicillin-resistant cells).

A different approach is used with phage cloning vectors, which are usually derived from phage A DNA molecules that are only about 70 per cent as long as normal phage DNA. As a result, these molecules are too small to be packaged into functional phage particles. But if an additional fragment of DNA is inserted into the middle of such a cloning vector, it creates a recombinant molecule that is larger and thus capable of being assembled into a functional phage. Hence when phage cloning vectors are employed, the only particles that can successfully infect bacterial cells are those, which contain an inserted foreign DNA sequence.

5. Identification of clones containing the DNA of interest: The bacterial colonies present on the Petri dishes at the end of step (4) are likely to contain at least as many different kinds of fragments as there are restriction sites in the DNA used in step (1). The final stage in any recombinant DNA procedure is therefore screening the bacterial colonies (or phage plaques) to identify those that contain the specific DNA fragment of interest. This is frequently the most difficult step in DNA cloning.

A number of techniques for screening colonies of bacteria exist. The particular technique used depends on what the researcher knows about the gene being cloned. If something is known about the base sequence of the gene of interest, the researcher can employ a nucleic acid probe, a single stranded molecule of DNA or RNA that can identify a desired DNA sequence by base-pairing with it. Nucleic acid probes are labeled either with radioactivity or with some other chemical group that allows the probe to the easily visualised. The researcher prepares a labelled DNA or RNA probe containing all or part of the nucleotide sequence of interest and uses it to tag the colonies that contain complementary DNA.

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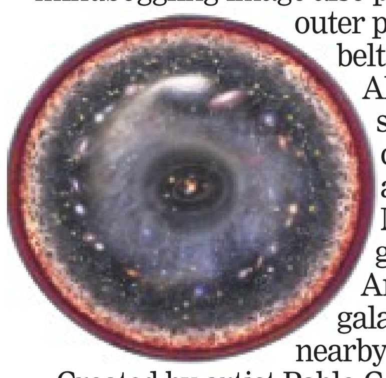
PLUS POINTS

Entire universe

This stunning spherical image manages to cram in the entire universe, including our Solar System at the centre. The mindboggling image also packs in the outer planets, Kuiper belt, Oort cloud, Alpha Cetauri star and the cosmic web along with the Milky Way galaxy, Andromeda galaxy and other nearby galaxies.

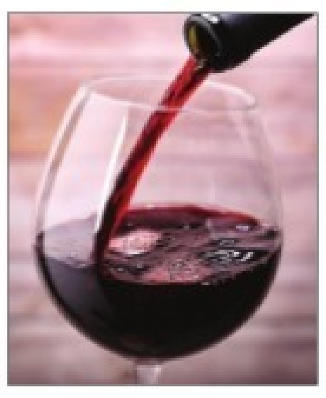


Created by artist Pablo Carlos Budassi, the jaw-dropping image is based on logarithmic maps of the universe compiled by researchers from Princeton University along with images from Nasa's telescopes and space probes. Logarithmic maps are used for visualising inconceivably huge areas such as the universe because each step on the axes increases by a factor of 10. However, they're not very user-friendly to look at. Budassi got the idea of fitting the universe maps into one tiny image while making hexaflexagons — paper polygons with many sides — for his son's birthday. "When I was drawing hexaflexagons for my son's birthday souvenirs, I started drawing central views of the cosmos and the Solar System. That day the idea of a logarithmic view came, and in the next days I was able to assemble it with Photoshop using images from Nasa and some textures created on my own," he told *Tech Insider.*

Wine lovers beware

If you've ever woken up surprised by how terrible you feel because of the glasses of wine you so innocently sipped the night before, scientists may have at least a partial explanation. In a report in the *Journal of Wine Economics*, University of California researchers say that among the nearly 100,000 bottles of wine they sampled from around the world, almost 60 per cent under-reported how much alcohol was in each bottle.

While the discrepancy was, on average, quite small — at just 0.42 per cent more alcohol than labelled — it didn't appear to be accidental. The study suggested winemakers were falsely reporting in part to meet buyer expectations for particular wines. Among the biggest offenders were Chilean and Spanish reds and Chilean and American whites.



"Wineries may have incentives to deliberately distort the information because they perceive a market preference for a particular range of alcohol content for a given style of wine or for other reasons, such as tax avoidance," the researchers write. One UK consumer group is calling for evidence-based labelling that includes a calorie count, which *Wine Spectator* says is now required by the FDA at chain restaurants in the USA. Red wine has also been found to contain arsenic.

Customising ceramics

There's a lot to be said for ceramic parts – they're strong, lightweight, handle heat better than many metals and ideal for crafting parts for airplanes, rockets and even the space shuttle. Now researchers have used a 3-D printer to make customised ceramic parts that have also overcome the Achilles' heel – the tendency to crack. The finding could open the door to a new class of ceramic-body or ceramic-engine jets, perhaps even a hypersonic craft that can fly from New York to Tokyo in a few hours.

The retired space shuttle fleet used ceramic tiles on their undersides to dissipate the atmospheric heating on re-entry, but the tiles were fragile, prone to cracking and often needed to be replaced.

"If you go very fast, about 10 times the speed of sound within the atmosphere, then any vehicle will heat up tremendously because of air friction," said Tobias Schaedler, senior scientist at HRL Laboratories in Malibu, California. "People want to build hypersonic vehicles and you need ceramics for the whole shell of the vehicle." He and colleagues at HRL invented a resin formulation that can be 3-D printed into parts of virtually any shape and size. The printed resin can then be fired, converting it into a high-strength, fully dense ceramic. The resulting material can withstand ultrahigh temperatures in excess of 1,700 degrees Celsius and is 10 times stronger than similar materials.

In fact, Schaedler's team has figured out how to trick ceramics into behaving like plastic. "We have a pre-ceramic resin that you can print like a polymer, then you fire the polymer and it converts to a ceramic," he said. "There is some shrinkage involved, but it's very uniform, so you can predict it."