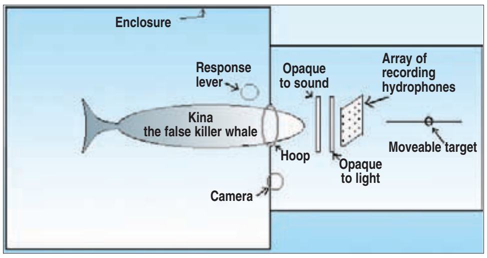
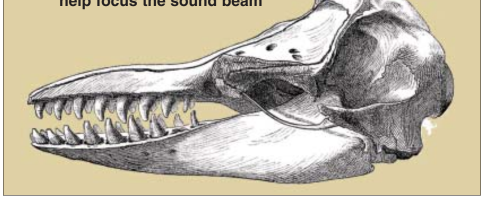
Whales and the acoustic squint

How these mammals use sonar to locate prey in total darkness has fallen to research, says
s ananthanarayanan




Sketch of a false killer whale skull showing the concave rear, which may help focus the sound beam

TOOTHED whales, which include dolphins, are able to precisely locate and snap up very small fish or other food that they come across, even in deep water where there is hardly any visibility. They use sonar, or echolocation, of course, but how they achieve the uncanny accuracy has not been understood. Laura Kloepper from the University of Hawaii, USA, and her PhD adviser, Paul Nachtigall, now report in the *Journal of Experimental Biology* their finding that a dolphin species called the *false killer whale* (because of its resemblance) is able to focus and adjust its beam of sound waves to get the most crisp echoes and the best fix on its target.

The dolphin creates high pitched, ie, ultrasonic *clicks* for echolocation from nasal structures in a region called the *melon* in its forehead. The sound is emitted by structures of fatty tissue, called *monkey lips*, which are made to slap together by forcing air from the lungs — to create the echolocation pulses of sound. The sound waves pass through fatty material in the melon, which minimises reflection at the contact with water so that the sound waves pass into the water with the least loss of energy. The rear portion of the melon is hard bone and is concave, to focus or direct the sound waves in a beam, like a searchlight.

It has been known that toothed whales (unlike *baleen* whales, which filter seawater through their teeth to capture prey) are able to adapt the frequency and the intensity of the sound clicks, according to changes in the environment. Beams of sound waves, or even radar beams, become narrower when the radiation is of higher frequency. It was thought that variations in the beam width of toothed whale sounds may be related to the frequency alone. But the bony rear surface of the melon is covered with air spaces, whose pressure and size are variable and do change during emission of clicks, and these could act to focus the beam independent of frequency. The fatty material that fills the melon could also behave like a lens to widen or narrow the beam. The array of muscles and tendons that surround the melon strongly suggest that this is the case, although actual changes in the echolocation beam, according to need, had not been demonstrated.

Experiment

Kloepper and Nachtigal decided to find out with the help of Kina, a trained and well-adapted false killer whale that has lives within the Marine Mammal Research Programme at the University of Hawaii. The experimental set-up consisted of two pens, separated by an opening like a hoop. Kina was trained to take position in one pen, with her head inside the hoop up to her first fin, and try to locate a specific object placed in the other pen. The line of sight to the object was blocked by a screen that allowed no light and another screen that was opaque to sound enabled the experiment, with sound waves, to start and stop. Kina was trained to touch a response lever when she spotted the standard target object, or to stay still if she spotted a different object. The standard object was an aluminium cylinder, about five inches long and the other objects were similar cylinders of lesser and even lesser thickness.

Kina was rewarded with a fish every time she identified the object correctly, as the standard or different cylinder. An array of underwater microphones also picked up the sounds emitted by Kina and recorded the strength of the sound beam at different points to estimate how narrow or wide the beam was during each echolocation event.


Laura Kloepper and Paul Nachtigall.

The standard object was one that Kina was able to identify comfortably, during trials, while the cylinders of lesser thickness were "difficult" and "more difficult" targets, as revealed in trials. The experiment was then to get Kina to identify the three objects, randomly presented at different distances, 2.5, four and seven metres, over a period of some weeks. And during the attempts, to record the frequency and spread of the sound beam that Kina used in the effort.

Discovery

The first look at the results showed that beams of higher frequency were narrower in all cases, which is hardly significant because such narrowing or widening is the property of acoustic beams. But what was significant was that the frequencies were not evenly distributed over different distances and levels of difficulty. Analysis of the beam width at the same frequency and different difficulty levels or at different distances revealed that the changes in beam area did not correlate in all cases to the frequency alone. It was seen that beam areas decreased for difficult targets and increased for greater distances.

The results are consistent with a tendency to concentrate the beam width, so that there is more reflected information in the case of a difficult target. Such concentration may be by using a higher frequency, which also leads to better resolution of images. But statistical analysis was able to distinguish the widening that was because of the change of frequency and to identify a positive widening which was not related to frequency, but came about with increasing distance. Although the beam appeared to widen at the array which was placed close to Kina, plotting the path of sound waves revealed that the beams that appeared widest at the array were not spreading out but were focused on farthest targets, while narrow beams aught then to be focused at shorter distances.

The results thus showed that Kina was able to adjust both the frequency and the focus of the sound beam so that the maximum energy could be reflected back by the target object, for the best identification. The results reported are a first confirmation that there is actual and constructive focusing of the sound beam by the dolphin. The anatomy of the animal strongly suggests that the action is by varying reflectivity of the concave cavity behind the source of sound, by the action of muscles and the pressure of the air sacs. The young false killer whale then learns to use the ability to resolve morsels that drift across its acoustic viewfinder, much like human babies learn to focus and coordinate their eyes to touch and grasp objects.

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A cosmetic leap of faith

jayanthi a pushkaran **reports on how the perils of using nanotechnology in beauty products are being ignored**

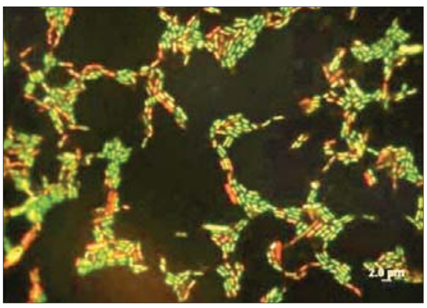
THE latest Sunsilk advert doing the rounds on TV showcases its new range of hair care products. The breakthrough formulations claim to provide root-to-tip nourishment to each strand. The patented formula entails nanoparticles with a highly concentrated protein compound that coats every strand and supposedly provides even deposition across the strand. These products are based on nanotechnology, the new kid on the block of mega-technologies that has become a huge hit in the cosmetic sector.

A report by Thomson Reuters suggests that cosmetic firms have doubled their investment in nanotechnology R&D over the past seven years. Products trademarked with the term "nano" in the brand name have also risen. Cosmetic giants like L'Oréal, Amorepacific, Avon and Proctor & Gamble are using nanotechnology not only to enhance the efficacy of their products but also to raise the bar in the market by achieving technology-based product differentiation. L'Oréal is one of the largest nanotechnology patent holders in the USA and has devoted $600 million of its annual $17 billion revenues to nanotechnology research.

India's cosmetic market is being targeted by premium global cosmetic brands due to its favourable demographics and tremendous annual growth rate (15 to 20 per cent). Market experts have observed a parallel shift in the demand from functional to specialised cosmetic products. Earlier this year, the International Trade Administration of the US Department of Commerce identified India as a vibrant spot for American firms. It is time to reflect upon the potential implications of this technology.

Why is cosmetic industry so fond of nanotechnology? The answer lies in the unique properties that these particles hold. At the physical range of 1-100 nm, nanoparticles display improved properties (physical, chemical or biological) that enable application of new phenomenon based on their size.

Nanotechnology is used in lotions to make wrinkles disappear, in serums to enhance breasts and in sunscreens. The mineral-based pigments of titanium and zinc oxide, commonly used in sunscreens, are greasy and leave a white residue on application. But when reduced to nanosized bits, the pigments become transparent and more penetrating. Likewise, nanosized emulsifiers used in shampoos, hair conditioners and make-up removers yield a less oily mixture when they are broken down. In anti-ageing products, nanoparticles are used for their potential to topically deliver antioxidants, vitamins and minerals for skin rejuvenation.



Using aquatic microbes as their "canary-in-a-cage", US scientists say that nanoparticles now being added to cosmetics, sunscreens and hundreds of other personal care products may be harmful to the environment.

However, the long term health impacts of nanoparticles remain poorly understood. We assume that if a cosmetic product is available in the market, it has been checked for safety. But experiences from different parts of the world suggest otherwise. L'Oréal lists several products under its anti-ageing Revitalift product line containing nano-sized ingredients. However, neither the word "nano" appears on the label, nor are they tested for their nano-properties in India.

DS Sankholkar, former head of Technical Regulatory Affairs, Unilever India, argues that the pace of regulation for cosmetic labelling and standard development in India is not in line with technological progress. In fact, Indian norms for labelling and safety tests of cosmetic products, as dictated by the Bureau of Indian Standards and the Drug and Cosmetics Act, have no specific provision to address nanoparticles. Therefore, if titanium dioxide is permitted in cosmetic preparations then nanosized titanium oxide, too, would be treated as harmless and seen within the framework designed for its bulk counterpart.

In the past few years, countries like the USA, UK, Switzerland, Germany, Netherlands, Australia and Japan have started addressing concerns related to the use of nanotechnology in cosmetics. The European Union has passed laws which require all cosmetics having nano-ingredients to be tested for safety before being marketed.

It has been a decade since the Nano Science and Technology Initiative was launched in India, yet there is no policy that regulates nanotechnology. "Though the BIS has developed four standards in nanotechnology, none of them deal with its use in cosmetics," says Madhulika Bhati of the Council of Scientific and Industrial Research.

CSE/Down To Earth Feature Service

Unexpected insights

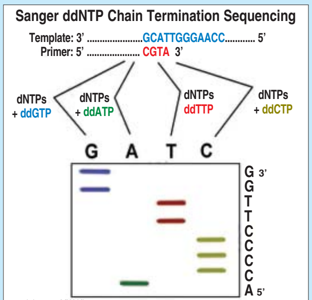
tapan kumar maitra **explains how confirmation of the genetic code is achieved by DNA sequencing**

THE genetic code can also be deciphered by comparing the amino acid sequences of proteins with the nucleotide sequences of the genes that code for them. However, the methods for sequencing nucleic acids had not yet been developed in the 1990s and, as we have seen, the code was solved using cell-free systems with artificial mRNAs.

Methods that allow very rapid sequencing of DNA have recently been developed, and the complete nucleotide sequences of living organisms such as *bacteriophage ØX174* (a virus that infects E.coli) and *SV40* (a virus that infects monkey cells and can cause tumors) have been determined. The nucleotide sequences obtained by the new methods confirmed the genetic code and established that all the possible *codons* are used *in vivo*. They also provided completely unexpected insights into the way genes are organised.

There are several sequencing methods, all of them based on the generation of DNA fragments of different lengths that start at a fixed point and terminate at specific nucleotides. The DNA fragments are separated by size on *polyacrylamide* gels, and the nucleotide sequence is read directly from the gel. The DNA fragments can be produced by chemical cleavage or by enzymatic copying of single-stranded DNA.

Sanger ddNTP Chain Termination Sequencing



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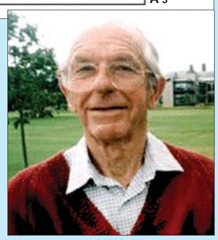
Frederick Sanger.

According to the different steps involved in Frederick Sanger's chain-termination method, single-stranded DNA is copied using DNA *polymerase I* from E. coli. This enzyme will not work on single-stranded DNA unless a short primer is annealed to it, producing a stretch of double-stranded DNA.

The primer has on its *deoxyribose* a free 3'-OH group to which the next nucleotide can be attached. The short primers are generated using restriction *endonucleases*, which are enzymes that cut DNA at specific sites. The primer is then extended using DNA polymerase and radioactive nucleotides. The chains are terminated at specific bases by adding a 2', 3'-dideoxy-nucleotide. Once this has been incorporated, DNA elongation stops. The nucleotide lacks a 3'-OH group in the *sugar moiety*, thus preventing the attachment of the next nucleotide. Four parallel reactions are performed, each containing a small amount of one chain-terminating agent (either ddATP, ddGTP, ddCTP, or ddTTP). These produce DNA chains of various lengths that start at a unique site and end at specific bases.

To read the sequence, the four reactions are *electrophoresed* on polyacrylamide gel that separates the fragments according to size. The fragments appear as a series of bands, each differing in length by one nucleotide. Each of the four tracks in the gel indicates chains terminating at one of the four nucleotides. The DNA sequence is read directly from the gel. For example, the band in position 30 is under the track labelled C and therefore represents a cytosine in the DNA sequence. This figure is a good example of a sequencing gel, and the student is advised to read the sequence unassisted and to compare the results with the sequence indicated in the figure legend. The section between positions 30 and 70 can be read rather easily. The number of nucleotides that can be sequenced with these rapid methods is limited only by the resolution of the polyacryamide gels.

In a good experiment, 200-300 nucleotides can be sequenced in only a day's work. These methods, together with new genetic engineering techniques that allow the preparation of large amounts of purified genes, have led to important advances in our understanding of how genes are organised.

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