

Ultrasensitive radiocarbon detection

Radiocarbon is rare, forming no more than one part per trillion of the total carbon content of the atmosphere. An optical method allows radiocarbon to be detected at roughly 25-fold lower levels than this, opening up fresh avenues of research.

RICHARD N. ZARE

Radiocarbon dating is an invaluable technique for determining the age of carbon-containing samples up to about 50,000 years old. Until now, the only method available for measuring levels of radiocarbon (carbon-14) in a sample has been high-energy accelerator mass spectrometry, but the apparatus involved is bulky, expensive and complex. Reporting in *Physical Review Letters*, Galli *et al.*¹ describe an optical technique that might overcome these problems. Their approach promises to greatly extend the use of radiocarbon measurements for dating, and as a tracer technique for following the fate of organic compounds in the body. It forms part of a growing revolution that is replacing mass spectrometry with optical methods for isotope analysis^{2–4}.

On Earth, there are three naturally occurring isotopes of carbon. The most abundant of these (99%) is carbon-12, with most of the rest being carbon-13. But carbon-14 also occurs in trace amounts, forming only as much as 1 part per trillion (0.000000001%) of the carbon in Earth's atmosphere. Unlike ¹²C and ¹³C, ¹⁴C is radioactive, decaying with a half-life of about 5,730 years — a fact that makes it potentially useful as a radiolabel for several applications.

Carbon-14 is mainly produced in Earth's atmosphere from the bombardment of nitrogen molecules by cosmic rays. Plants fix atmospheric carbon dioxide during photosynthesis, and so the level of ¹⁴C in plants (and in animals that eat plants) when they die approximately equals the level of the isotope in the atmosphere at that time. Because the amount of ¹⁴C in dead organisms subsequently decreases as a result of radioactive decay, the date of carbon fixation (or death) can be determined by measuring the amount of the isotope in the remains. This is the basis of radiocarbon dating, the technique that has been a workhorse for estimating the age of organic remains from archaeological sites. In practice, the ratio of the number of ¹⁴C atoms to the total number of other carbon atoms in a sample is measured.

Because of the paucity of ¹⁴C, radiocarbon dating presents a huge technical challenge.

Most isotope-ratio measurements are carried out using mass spectrometry, in which ions are weighed by measuring their trajectories in electric and/or magnetic fields in a vacuum. Standard mass spectrometers, however, do not have sufficient resolution to distinguish the small mass difference between ¹⁴C and ¹⁴N, the most common isotope of nitrogen. The abundant presence of ¹⁴N therefore tends to mask any signal from radiocarbon.

High-energy accelerator mass spectrometry can overcome this problem. For this technique, the sample must first be turned into solid carbon (graphite) using a series of chemical transformations. It is then bombarded with caesium ions to produce negatively charged carbon ions, which are accelerated by a positive voltage of millions of volts. The negative ions, by now travelling at a few per cent of the speed of light, are subsequently converted into positive ions by an electron stripper (which consists of a gas or a thin foil), before the ions' masses are determined. Because nitrogen atoms do not form stable negative ions, the resulting data are free from nitrogen interference. But although accelerator mass spectrometers are powerful tools, they are also costly. Establishing and maintaining such an instrument costs millions of dollars, and so they tend to be found only at national facilities.

A much simpler approach is to completely oxidize a sample so that every carbon atom is turned into carbon dioxide³. The various isotopic forms of carbon dioxide can then be distinguished from each other because each has a slightly different infrared spectrum (they absorb slightly different frequencies of infrared light). All that is required to determine the ratios of carbon isotopes in a sample of carbon dioxide is to precisely measure the intensities of the spectral lines that correspond to infrared absorption for each isotopic form of the gas. Other compounds, such as water vapour and nitrogen, do not interfere in the infrared spectrum, either because they have different infrared spectra or because they do not have 'allowed' infrared transitions — that is, quantum mechanics prevents the molecules from undergoing energy transitions that would be detected in the infrared.

This optical approach has already been used³ to determine the carbon-isotope ratio

of carbon dioxide containing ¹²C and ¹³C, but the low concentration of ¹⁴C has made its measurement in carbon dioxide extremely difficult. Using an ultrasensitive technique called saturated-absorption cavity ring-down spectroscopy⁵, Galli *et al.*¹ have now succeeded in measuring the ratio of ¹⁴C to total carbon at values well below radiocarbon's natural abundance in carbon dioxide.

In their technique, the authors placed a gas sample between two or more highly reflecting mirrors that form an optical cavity. Infrared light that is incident on the cavity continually circulates within it, so that it takes many round trips. This effectively increases the optical path length of the light, allowing infrared absorption by the gas to be detected with a sensitivity that vastly exceeds what can be achieved in traditional absorption experiments.

Another feature of the cavity is that, when the infrared light source is interrupted, the radiant energy stored in the cavity 'rings down' — it decreases over time. Using a powerful infrared laser to 'saturate' the vibrational-rotational transitions in carbon dioxide that correspond to infrared absorption, Galli *et al.* used the rate of ring down as an excellent absolute measure of the concentration of absorbers inside the cavity (an approach that has previously been reported for infrared spectroscopy⁵). The authors obtained a linear concentration response down to a detection limit of about 43 parts per quadrillion, which makes their technique quite well suited for radiodating carbonaceous samples. It may also have applications in positron emission tomography (an imaging technique used in medicine for body scans), which often requires⁶ monitoring of carbon dioxide labelled with carbon-11, an artificial radioactive isotope of carbon.

Galli and colleagues say that the size of their experimental set-up is roughly two square metres in area, about 100 times smaller than the footprint of typical accelerator mass spectrometers. Furthermore, the equipment costs only about US\$400,000 — many times less than an accelerator mass spectrometer. For widespread adoption of the infrared technology, however, it will be necessary to reduce the cost even further, say by a factor of five or ten. Even so, an infrared method for measuring isotope ratios represents a real breakthrough because of the many possible uses of the technique. And there are other advantages. For example, in mass spectrometry, an ion from a sample is counted only once because its measurement neutralizes it. But infrared-absorption measurements do not destroy the sample, allowing it to be repeatedly analysed.

With further improvements, the infrared technique may well become the method of choice for measuring the isotope ratios of many common elements. Moreover, if the anticipated cost reductions are realized, the measurement of isotope ratios might become

a widely used tool in determining the origins of materials used for a broad range of purposes, from environmental monitoring to medical research. ■

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Species choked and blended

The appearance of new ecological niches propels the evolution of species, but the converse can also occur. A study shows that changing lake habitats have caused extinctions and reduced the genetic differences between species. [SEE ARTICLE P.357](#)

JEFFREY S. MCKINNON & ERIC B. TAYLOR

Conventional wisdom long held that even if individuals of two different species could mate with each other, their offspring were doomed to early death or sterility. But a different view is taking hold: that it is often adaptations to different environments that cause species to separate, such that hybrid offspring fail because of their poor fit to resources, rather than through intrinsic shortcomings¹. As a consequence, changes to particular environmental conditions that previously kept species distinct could increase genetic mixing, and thereby reduce species number. On page 357 of this issue, Vonlanthen *et al.*² provide evidence that human alterations

1. Galli, I. *et al. Phys. Rev. Lett.* **107**, 270802 (2011).
2. Crosson, E. R. *et al. Anal. Chem.* **74**, 2003–2007 (2002).
3. Zare, R. N. *et al. Proc. Natl Acad. Sci. USA* **106**, 10928–10932 (2009).
4. Kasyutich, V. L. & Martin, P. A. *Infrared Phys. Technol.* **55**, 60–66 (2012).
5. Giusfredi, G. *et al. Phys. Rev. Lett.* **104**, 110801 (2010).
6. Kasyutich, V., McMahon, L. A., Barnhart, T. & Martin, P. A. *Appl. Phys. B* **93**, 701–711 (2008).

to lake habitats have eroded barriers between species and contributed to extinctions.

The authors' study of 17 Swiss lakes shows that glacial melting in the past 12,000 years provided ecological opportunities, in the form of new environmental niches, that led to diversification of whitefish species, as has been reported for other freshwater fishes³. Whitefish species divergence is characterized by, for example, differences in body size and the number of 'gill rakers' — cartilaginous structures that protrude from fish gills and are involved in feeding (Fig. 1). Large-bodied whitefish, which have fewer gill rakers, typically feed from the bottom of lakes and spawn in shallow water in winter, whereas smaller species, which have more gill rakers, tend to

feed in open water and spawn much deeper.

However, increased human activity around the lakes dramatically altered the lakes' ecology during the twentieth century. Higher nutrient levels in the water caused eutrophication, in which algal populations increase, water quality is reduced and oxygen levels at the lake bottom decrease. Vonlanthen *et al.*¹ propose that these conditions compressed the depth range in which whitefish could spawn, bringing previously separated species together to breed, forming hybrids. Whitefish feeding patterns were probably also affected, through reductions in zooplankton diversity and possibly in the density of bottom-dwelling prey (Fig. 1), which would also have reduced opportunities for exploiting ecological variation.

Vonlanthen and colleagues' data show that the extent of species loss for each lake correlates with the severity of that lake's eutrophication. But did these extinctions result exclusively from demographic decline — the extinction process we usually think of, in which deaths outnumber births? Or was reverse speciation at play, in which characteristics that once defined distinct species are merged into a single hybrid species?

The authors report² several lines of evidence suggesting a role for reverse speciation in the lakes. First, the severity of eutrophication is the best predictor of genetic differentiation of modern whitefish — lakes that suffered the greatest eutrophication contain species that are the least genetically different from each other. Historical DNA samples also allowed Vonlanthen and colleagues to document a progressive reduction in whitefish genetic differentiation in one of the lakes (Lake Constance) between 1926 and 2004. Furthermore, they find strong genetic traces of the extinct whitefish species

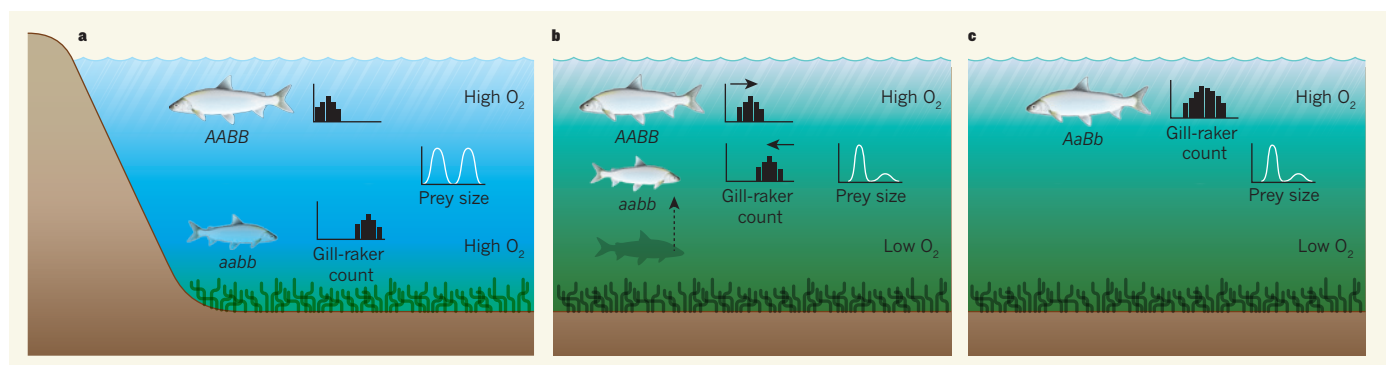


Figure 1 | Loss of fish biodiversity through eutrophication. **a**, Before human activity raised nutrient levels in lake waters, the Swiss lakes studied by Vonlanthen *et al.*² were well oxygenated at all depths, and there were diverse invertebrate prey communities in both the open water (suggested by other studies to be generally smaller prey, represented by the left side of the prey-size distribution) and at the bottom (generally larger prey, right side of distribution). These resources supported genetically distinct species of whitefish (represented as AAbb and aabb) with different characteristics, including their body size and number of gill rakers — cartilaginous protrusions from the gills. Large-bodied whitefish with fewer gill rakers generally fed from the bottom and spawned in

shallow water, whereas small-bodied species with more gill rakers typically fed in open water and spawned much deeper. **b**, Lake eutrophication led to lower oxygen levels, especially at depth, driving deep-spawning species into shallower water, where they spawned with other species to form hybrids. Simultaneously, the fishes' prey became less diverse, thereby reducing divergent selection — the process by which different ecological niches provide a selective pressure for species to have distinct characteristics. **c**, Increased hybridization and reduced divergent selection, as well as demographic decline, resulted in extinction of the deeper-spawning species, with the remaining species being a genetic hybrid (AaBb) and possessing an intermediate number of gill rakers.