

such as occur in other organisms in which UAA and UAG specify termination of protein synthesis. However, although UGA in *B. nonstop* is decoded to specify the amino acid tryptophan, no tRNA-bearing tryptophan that had a matching anticodon for UGA was found.

Instead, the tryptophan-bearing tRNA present in *B. nonstop* retains the standard anticodon for 'reading' UGG (the standard codon for tryptophan). However, Kachale and colleagues report that this tRNA also recognizes the codon UGA. This tRNA is different from other tRNAs: the 'arm' that projects the tRNA anticodon is shorter than normal by one base pair (Fig. 1). This 'short arm' feature greatly enhances UGA reading by the tRNA. Interestingly, this feature is also present in an organism called *Condylostoma*, for which it was previously unknown how UGA might specify an amino acid.

Why did such drastic changes evolve in how *B. nonstop* interprets the genetic code? Infectious agents lead to natural selection of successive host variants that are resistant to infection and can thwart an invader's countermeasures. *B. nonstop* is a host for many viruses, and Kachale *et al.* suggest that the adoption of such variant genetic decoding might have helped it in the 'arms race' against infectious invaders.

The authors also point out another possible selective force. The DNA of *B. nonstop* has a notably lower content of G- and C-containing nucleotides (C is cytosine, another nucleotide base) than do other species in the Trypanosomatidae family, suggesting that, during its evolution, *B. nonstop* gained many mutations that increased its content of A- and T-containing nucleotides (T is the base thymine). This probably resulted in amino-acid-specifying codons changing their sequences to become richer in A or T sequences. For example, G-to-A mutations in the UGG codon, which normally encodes tryptophan, would turn the codon into UGA, UAG or UAA. Making each of these three codons specify amino acids rather than prematurely signifying termination was probably advantageous.

Mutations in tRNAs at positions distant from the anticodon are known to affect various aspects of the fidelity of protein synthesis, with one in a tryptophan tRNA characterized in detail⁸. Kachale and colleagues' finding is striking, in that it provides a natural example of how a change in tRNA shape alters codon recognition without altering the anticodon itself.

Although tRNA databases contain sequences and secondary structures of many tRNA molecules, codon specificity is assigned mainly, although not entirely, on the basis of the molecules' anticodons⁹. The curious case of *B. nonstop* tryptophan tRNA reminds us of the outstanding challenges in making an accurate assignment of a tRNA's codon specificity. For example, the human genome encodes

more than 400 tRNA-encoding genes and many more tRNA-like sequences⁹. For most tRNAs, their codon specificity is predicted purely computationally, and it is not known how variations in the sequences of these genes might affect their codon-decoding properties.

Part of the challenge in the correct assignment of a tRNA's codon specificity is due to the presence of numerous nucleotide modifications introduced after tRNA synthesis. Moreover, predicting modification status is not trivial, especially considering that previously unknown types of modification are continually being discovered¹⁰. Perhaps the eventual solution to this challenge can be facilitated by the deep-learning approaches that in the past few years have propelled an enormous leap in scientists' ability to predict protein 3D structures.

However, that deep-learning success was enabled by the availability of vast relevant experimental data. Although work over many decades has provided extensive information about the relationship between tRNA structures and codon recognition, the scope of the available 3D structures of tRNAs interacting with their codons is limited by comparison. Better availability of cryo-electron microscopy, given the increasing power of

this technique to produce high-resolution atomic structural data, might eventually make a major contribution in generating the data sets required for machine learning to tackle this problem.

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Hydrology

Spring waters on Mount Fuji run deep

Lauren Somers

A trio of tracers has debunked a long-held model of the origins of spring water on Mount Fuji, revealing interactions between shallow and deep aquifer layers, and providing a fresh approach for probing mountain groundwater flow.

Mountains have been described as the water towers of the world^{1,2} – they receive large amounts of precipitation, store snow and ice, and their high altitudes mean that water undergoes only low levels of evaporation and transpiration. Mountain rivers are crucial water resources both for mountain inhabitants and for downstream lowland populations. Groundwater stored in mountains is now recognized as an important water resource, but our understanding of groundwater flow paths in mountains is limited by a lack of field data and by the shortcomings of conventional hydrogeological methods³. Writing in *Nature Water*, Schilling *et al.*⁴ report an analytical approach that provides insights into how water can move through the subsurface in rugged mountain environments.

The authors focus on Mount Fuji in Japan,

a World Heritage site of the United Nations cultural organization UNESCO and one of the world's most iconic mountains. The groundwater and cold, groundwater-fed springs that emerge from this volcano provide millions of people with safe drinking water, drive tourism and supply agricultural production. Abundant precipitation on the highest part of the mountain recharges layers of aquifers composed of the rock basalt (Fig. 1), which formed from repeated volcanic eruptions.

It was thought that Mount Fuji's recharged groundwater flows laterally through the more-permeable layers near the surface to feed springs close to the foot of the volcano, and that the spring waters do not pass through the deeper aquifers. This interpretation was derived from studies based on widely used hydrogeological methods, which analysed

shallow groundwater levels, stable isotopes in the water molecules, and the most abundant dissolved ions⁵. However, these methods might not be effective in settings, such as Mount Fuji, that have relatively uniform rock types and in which the isotopic signatures of groundwater at different depths overlap as a result of their shared meteoric origin (that is, from precipitation).

Schilling *et al.* took a different approach. They analysed three unconventional tracers of groundwater: helium, vanadium and microbial environmental DNA (eDNA). Each of these tracers provided evidence of connections to deep groundwater and yielded insights into groundwater flow paths.

The authors found concentrations and isotopic signatures of helium in spring water that were indicative of helium derived from Earth's mantle, particularly in springs near the Fujikawa-kako Fault Zone (Japan's most tectonically active fault zone). This finding suggests that the fault zone provides a pathway for vertical water flow between the different layers of aquifers, rather than just lateral flow through aquifers near the surface.

Vanadium concentrations in spring water are known to increase with time spent in basalt aquifers⁶. Schilling *et al.* found that vanadium levels in spring water on Mount Fuji were variable and generally lower than in groundwater taken from wells on the mountain. Furthermore, vanadium concentrations in the groundwater and spring samples analysed show a strong negative correlation with $\delta^{18}\text{O}$ (a measure of the abundance of the stable oxygen-18 isotope in the water). This indicates that the springs have variable origins, and that those with longer flow paths are recharged at higher elevations (where precipitation has low $\delta^{18}\text{O}$).

Finally, the authors analysed environmental DNA dissolved in water samples, and found DNA characteristic of two orders of archaea microorganisms that have adapted to living in deep groundwater. The presence of this DNA in the spring water also suggests that deep groundwater wells up to the surface.

The three tracers in this study have all been used previously (see, for example, refs 6 and 7), but combining them, as Schilling and colleagues have done, is new. Each tracer comes with caveats (as described by the authors) that would limit the interpretation of their results if used individually. But the helium, vanadium and eDNA results correlate well with each other across the sites investigated at Mount Fuji, providing confidence in the authors' interpretation that some meteoric groundwater wells up from the depths, crossing low-permeability layers between aquifers to contribute to spring flow.

The findings also demonstrate the shortcomings of conventional hydrogeological methods in certain settings. The deep

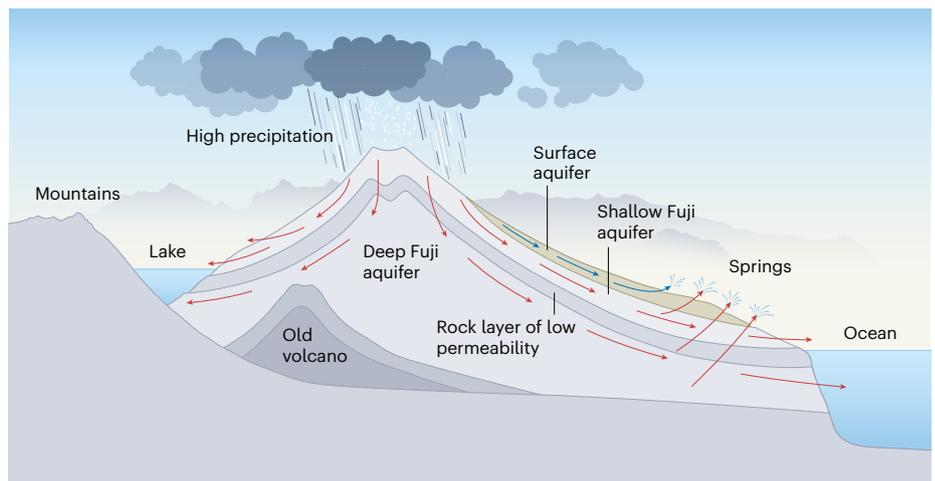


Figure 1 | Groundwater flow in Mount Fuji. Mount Fuji contains layers of aquifers produced from successive volcanic eruptions; the remains of an old volcano that preceded Mount Fuji are also buried beneath the aquifers. Precipitation at the top of the mountain was thought to pass only through the aquifer near the surface before emerging at springs lower down (blue arrows). Using a combination of unconventional groundwater tracers (helium, vanadium and environmental DNA), Schilling *et al.*⁴ show that some of the springs produce water that has passed through the shallow and deep Fuji aquifers (red arrows) – something that had been overlooked by conventional analytical methods in previous studies.

hydrological connections identified by the authors had not been detected by conventional methods, even though the groundwater system at Mount Fuji has been studied extensively⁵.

The authors point out that Mount Fuji is highly unusual in that it is a volcano built up from many layers of permeable basalt. It also hosts many springs and deep wells, which provide access to groundwater that is often not possible in remote mountain regions. Vanadium and helium are viable groundwater tracers in this setting, and could also be useful at other volcanic islands. However,

“The authors found concentrations and isotopic signatures of helium in spring water that were indicative of helium derived from Earth’s mantle.”

these tracers, and the reported findings about groundwater flow, might not be directly translatable to non-volcanic mountains. By contrast, the use of eDNA is not limited to volcanic environments. Originally used in microbiology research⁷, eDNA has been used as a hydrological tracer only in the past few years, and is just beginning to be used in mountain hydrology⁸.

Although Schilling *et al.* find evidence of deep groundwater input to some springs, they find that others are fed mainly by shallow groundwater, in line with the previous understanding of the local hydrogeology. The next steps should be both to quantify the

proportion of deep groundwater that contributes to different spring and stream flows and to the water extracted for human activities, and to determine the uncertainties in these numbers. This is crucial, partly because the quality and quantity of water stored at Mount Fuji is declining, but also because of climate change – knowledge of groundwater flow is needed to work out how the hydrogeological system will respond to future change.

Millions of people around the world depend on mountain hydrological systems that are being altered by climate change, but the inner workings of mountain groundwater systems remain unclear in many regions, which precludes effective groundwater management³. Schilling and colleagues' study provides insights and tools that begin to address this challenge.

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