If not a modern human or a Neanderthal, then to what species does the owner of the Denisova finger belong? Candidates are thin on the ground. *Homo erectus* was the first hominin to move from Africa to Eurasia, some 1.9 million years ago. There is fossil evidence that *H. erectus* survived in Indonesia as recently as 100,000 years ago, but nothing to suggest that the species was present in mainland Asia at anything approaching the time of the Denisova sample. The DNA sequence also argues against this individual being *H. erectus*.

By comparing the sequence of the Denisova mtDNA with those of Neanderthals and modern humans, Krause *et al.*[1] were able to draw a family tree showing the three species' evolutionary relationships (Fig. 2). The tree revealed that the common ancestor of the species dates to about 1 million years ago. If modern humans evolved in Africa, then this ancestor must also have been in Africa, making it impossible for the Denisova hominin to be descended from the *H. erectus* populations that moved to Europe 900,000 years earlier. The Denisova sequence is also distinct from that of the immediate ancestors of Neanderthals, which split away from the lineage leading to modern humans some 450,000 years ago, much later than the branch leading to Denisova (Fig. 2). Left with few alternatives, Krause *et al.* make the logical deduction that the Denisova DNA sequence represents an unknown type of hominin that left Africa in a previously unsuspected migration about 1 million years ago, and that survived in at least some parts of Eurasia until 40,000 years ago or later.

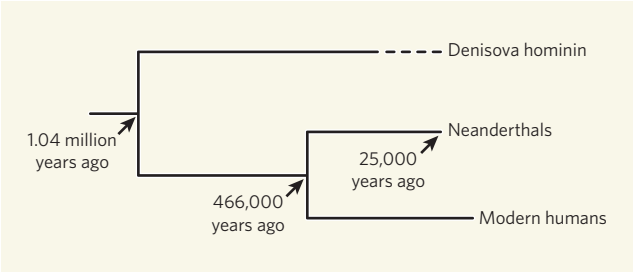

Figure 2 | Evolutionary relationships between modern humans, Neanderthals and the unknown Denisova hominin. Krause and colleagues[1] used the molecular clock to compare the mitochondrial DNA sequences of modern humans, Neanderthals and the Denisova human. The molecular clock uses the rate at which mutations accumulate in a DNA sequence as a way of dating the branch points in an evolutionary tree. Their analysis[1] shows that the lineage leading to the Denisova hominin branched from that leading to modern humans and Neanderthals just over 1 million years ago. Modern humans and Neanderthals shared a common lineage for the next 550,000 years before their two lineages diverged about 466,000 years ago.

What next? The relationship between the Denisova sample and Neanderthals and modern humans will become clearer when nuclear DNA is obtained. And if less-fragmentary remains with the same mtDNA can be identified, their morphological examination might place the Denisova hominin in the broader scheme of human evolution.

A final question is whether there are other big surprises around the corner. Krause *et al.* point out that it is unlikely that ancient DNA will ever be recovered from extinct hominins from warmer parts of the world, because under these conditions DNA does not survive for more than a few thousand years. But there are many other interesting archaeological sites in the cooler latitudes. The demonstration that a bone fragment can provide evidence for an unknown hominin will surely prompt more studies of this kind and, possibly, increase the crowd of ancestors that early modern humans met when they travelled into Eurasia. ■

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MICROFLUIDICS

Exploiting elephants in the room

Robert C. R. Wootton and Andrew J. deMello

Microfluidic devices have many applications in chemistry and biology, but practical hitches associated with their use are often overlooked. One such device that optimizes catalysts tackles these issues head-on.

Reporting in the *Journal of the American Chemical Society*, Kreutz *et al.*[1] describe their use of an 'evolutionary' algorithm, implemented in a microfluidic system, to discover active catalysts. The algorithm mimics genetic processes associated with reproduction, so that variations in selection pressure allow improved formulations of a multicomponent catalyst to be developed with each iteration of the algorithm. This approach leverages the high-throughput nature of microfluidics for testing large numbers of formulations, and so allows the rapid screening of catalysts using reactive systems that were previously considered to be too time-consuming for this purpose.

Pointing out the 'elephant in the room' is the basis of good peer review and often inspires great scientific developments. Most branches of science and engineering have at least one dark secret — usually something that is understood within a community but, although it does not present a real problem, is generally not discussed in publications. This is normally because it contradicts statements made in early (often overly hopeful) publications in the field: statements that no one now takes seriously, but that might be politically problematic to refute.

Within the field of microfluidics, the elephant tends to involve the materials used to make lab-on-a-chip devices. It is not uncommon to find that the polymers used to make such chips must be extensively modified to make them compatible with the chemistry under investigation[2]. The same is true for glass and silicon microfluidic devices used as reactors for biological processes, in which the channel surfaces are often chemically treated (passivated) before use to prevent proteins or DNA from sticking to them[3]. In truth, the chip material plays as important a part in the performance of a microfluidic device as the design, pumping mode, detection method or any other parameter. Selecting a material that is compatible with your process can be challenging, but must be attempted because modifying one material to behave like another is rarely a complete success. One can strap antlers to a zebra, but it still won't be a reindeer.

Similarly, within droplet-based microfluidic systems, in which microscopic droplets of solutions within immiscible carrier fluids are used as miniature reaction vessels, the elephant in the room is droplet leakage. Microdroplet (or segmented flow) systems are generally assumed to provide perfect compartmentalization of each reaction, with no inter-droplet crosstalk[4]. But although this is essentially valid for reactions involving large biomolecules, droplets can be alarmingly porous to smaller molecules.

Optimization processes based on genetic algorithms have their own problem. A genetic algorithm is a method for turning a population of multivariate individuals into a new population in which each member has a different set of characteristics, normally by mimicking the processes of variation and selection present in evolutionary systems. It is generally accepted that speciation by biological evolutionary processes is, to say the least, multigenerational. The timescales involved

in protein evolution, and therefore macrobiotic speciation, are now well understood, and closely match the timescales documented in the fossil record[5]. The elephant in the room for many genetic-algorithm optimization processes is therefore that the number of generations required for optimization may be high, whereas the possible throughput of experiments is often low.

Kreutz *et al.*[1] have combined solutions to all of the above issues into a system that holds real promise for developing useful answers to synthetic problems involving multivariate optimizations. The authors studied the oxidation of methane (CH_4) to methanol (CH_3OH, an industrially important bulk chemical) using oxygen gas. This process is of increasing interest because of its environmentally friendly credentials; oxygen is readily available from the atmosphere, and produces no unpleasant waste products, unlike many other oxidants. But catalyst turnover numbers for the reaction (the number of moles of substrate converted by each mole of the catalyst before it is inactivated) have been low, as have the yields in many cases.

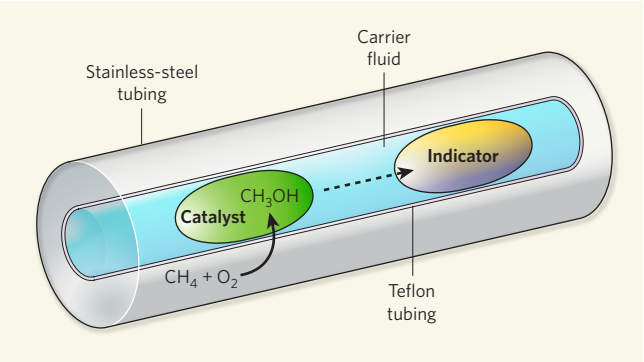

Figure 1 | Screening catalysts in a microfluidic device. Kreutz *et al.*[1] have designed a microfluidic system that screens the activity of catalysts for the oxidation of methane (CH_4) to methanol (CH_3OH), using oxygen as the oxidant. Microdroplets of catalyst solutions are passed through Teflon tubes by a carrier fluid. The Teflon tubes are enclosed by stainless-steel tubes, into which pressurized CH_4 and O_2 are introduced. The gas mixture diffuses through the Teflon tubing and, in the presence of active catalysts, reacts to produce methanol (CH_3OH). At high temperatures, the methanol diffuses through the carrier fluid to a neighbouring droplet, which contains a colorimetric indicator. The indicator changes from yellow to purple in the presence of methanol, with the intensity of the purple colour corresponding to the activity of the catalyst. (Figure adapted from ref. 1.)

The authors therefore designed a droplet-based microfluidic system that used a genetic algorithm to optimize the properties of a methane-oxidation catalyst. The catalyst consisted of three components, each of which could be varied. The team began by screening 48 different combinations of components for their collective catalytic activity, with every combination tested in its own droplet. Each combination consisted of three 'genes', sets of compounds that might act as one of the three components of the catalyst. The authors identified the best genes (those that produced the most active catalysts) and either 'mutated' them by changing a few of their components at random, or crossed them with other genes. This generated a new set of 48 genes for a subsequent round of screening.

Over eight generations of screening, the team generated an up to sevenfold increase in the fitness score — a combination of yield and turnover number — of their catalytic system, and also identified the relevant catalytic components that enhanced fitness. This is an amazing achievement given the relatively small number of component combinations (384) explored. For comparison, there are hundreds of millions of possible combinations.

The method is also notable because of the consummate use of the properties of both the techniques and the materials. The chip material chosen by Kreutz *et al.* was polytetrafluoroethylene (Teflon), a fluorous material in which all the hydrogens of the hydrocarbon chains have been replaced with fluorine atoms. Similarly, the droplets were carried through the microfluidic device by a fluorous oil. Such fluorous materials are extremely permeable to gases, particularly oxygen, so the authors simply perfused the gaseous starting materials for the reactions through the walls of the chip (Fig. 1).

Kreutz and colleagues monitored the production of methanol in their system using droplet crosstalk. In this approach, catalyst-bearing droplets were flanked by droplets containing colorimetric indicators. These indicators responded to methanol diffusing from adjacent catalyst-bearing droplets by changing colour, thus allowing a rapid, on-line assessment of reaction progress. What's more, the authors proved that their genetic algorithm was practically useful in a high-throughput microfluidic device. Kreutz *et al.* have thus directly addressed — and, in the case of droplet crosstalk, harnessed — the three elephants in the room associated with microfluidic devices and genetic algorithms.

This combination of techniques and materials[1] opens up the possibility of the evolutionary development of other, more complex catalysts and even of artificial enzymes. The applications are therefore widespread within biomolecular and chemical sciences. However, challenges do remain. Notably, although gases readily permeate Teflon, liquids do not. Similarly, the rate of mass transfer through the walls of the droplet-carrying tubes is limited by the wall's permeability, thickness and the pressure differential across it. These effects might sometimes combine to cause a paucity of starting materials, artificially slowing fast reactions. Regardless, it will be fascinating to see what discoveries will be made as a result of Kreutz and colleagues' approach. Rarely has a herd of elephants seemed so productive. ■

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EVOLUTIONARY BIOLOGY

A flourishing of fish forms

Michael Alfaro and Francesco Santini

According to an innovative exercise in 'morphospace analysis', modern fish owe their stunning diversity in part to an ecological cleaning of the slate by the mass extinction at the end of the Cretaceous.

Fish come in a bewildering diversity of shapes: trumpetfish, sea horses, pufferfish, cowfish and anglerfish are just a few examples. Size, too, varies tremendously in living species. Some gobies reach less than a centimetre as adults, whereas the oarfish stretches well over 12 metres and the ocean sunfish can weigh more than a car. These groups, along with almost two-thirds of all other fish, belong to a particularly conspicuous section of the fish tree of life, the spiny-finned fishes (Acanthomorpha). With 18,000 species, spiny-fins comprise almost a third of all vertebrates.

Despite, or perhaps because of, this incredible richness, biologists understand surprisingly little about the tempo of spiny-fin diversification. As he reports in *Proceedings of the Royal Society*, Friedman[1] has searched for the origins and underlying causes of shape diversity in spiny-fins across nearly