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DIVERSITY

Of beans and genes

Several human genes involved in digestion have diverged along cultural lines. Research suggests these adaptations influence the range of foods tolerated and even certain diseases.

BY MICHAEL EISENSTEIN

Nathaniel Dominy was surprised to find that diet-related genes, like people, sometimes simply repeat themselves to get a point across rather than change the message.

In 2007 while most evolutionary biologists were looking for evidence of selection in the form of genetic mutations, Dominy and colleagues learned that people with high-starch diets had additional copies of the gene coding salivary amylase and that these repeats increased production of the carbohydrate-processing enzyme. "Few would have expected at the time that [these repeats] could have any effect at all — and they had a big effect," recalls Dominy, an anthropologist at Dartmouth College in New Hampshire.

This discovery also offered proof to the growing number of evolutionary geneticists who believe that culture-specific factors, such as diet, have had as powerful an effect on human evolution as more obvious externalities like climate and habitat — with some even suggesting that these factors could have accelerated the overall evolutionary pace. "I don't think we have the data yet to make those claims," cautions Mark Stoneking, a population geneticist at the Max Planck Institute in Leipzig, Germany. "[But] there certainly has been recent evolution in modern humans because of responses to natural selection, and culture may very well be playing a role in a lot of those."

THE GENETICS OF LUNCH

Even if you can enjoy a cold glass of milk without then feeling sick to your stomach, chances are you know somebody who can't. In fact, adult lactose intolerance is the biologically 'normal' state of affairs. "The general pattern in mammals is to lose lactase expression after weaning," explains Dallas Swallow, a geneticist at University College London.

Nevertheless, adults with 'lactase persistence' are widespread in many parts of the world. For example, lactase persistence is characteristic of 89%–96% of Scandinavian and British people, is widespread among pastoralist cultures in Africa and the Middle East, but appears in only 1% of Chinese individuals.

Although one single nucleotide polymorphism (SNP) affecting lactase gene expression accounts for the vast majority of European instances, this trait seems to have arisen independently in different regions of Africa as a result of several distinct yet tightly-clustered variations within a regulatory segment of the lactase gene. "That's convergent or parallel evolution," says Swallow. "The same phenotype is being selected, with different mutations causing that phenotype."

Each of these variants is thought to have emerged within the last 10,000 years, roughly coinciding with the emergence of agriculture and dairy farming, and conferring obvious advantages on those cultures. "Milk is nutritionally good, and if you don't have lactase you can't digest the main carbohydrates in it: you might get diarrhoea or flatulence, and you've lost a source of food, water and calcium," says Swallow. "In the context of African tribes, the most plausible thing is that it was a source of clean, nutritious liquid."

Most geneticists cite lactase persistence as a leading example of recent human evolution driven by shifts in culture and diet. "This happens to be a 'low-hanging fruit'," says Sarah Tishkoff, a geneticist at the University of Pennsylvania. "It's a Mendelian trait and it left a really strong selection signature." Identifying other, equally clear examples has proven challenging, although the subsequent amylase breakthrough by Dominy and colleagues suggests that other ▶

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such traces are there to be found.

Today, many people enjoy starch-rich diets as a matter of choice, but for early humans, tubers and other starchy plants might have been an essential staple in lean times (see The first supper, page S8). "Amylase is the only enzyme that can hydrolyze starch," says Dominy. "If you can produce a lot of amylase, you have a big advantage in the sense that you can extract and assimilate carbohydrates almost instantaneously at the level of the mouth."

The number of copies of the salivary amylase gene, *AMY1*, was already known to vary among individuals. In partnership with Anne Stone and then graduate student George Perry at Arizona State University, Dominy demonstrated that not only does this copy number directly correlate with enzyme levels, but the average copy number within a population also correlates with the starch content of their traditional diet. For example, the Japanese routinely consume large amounts of rice and other starch, whereas the Yakut, a Siberian hunting and fishing culture, have a diet based on fish and meat; these differences are reflected at the level of the *AMY1* gene in Japanese and Yakut populations. "Even though they are closely related genetically, and geographically not separated by a great distance, there's a difference in the number of copies in these two populations on average," says Dominy.

SUFFERING FOR HEALTH

The shift from an active foraging lifestyle to a more sedentary agricultural existence also appears to have introduced selective pressures, as populations struggled to survive nutritional deficiencies. Some intriguing but enigmatic signs of lifestyle-specific adaptation have been detected in the gene encoding N-acetyltransferase 2 (NAT2), an enzyme that is best known for its role in drug metabolism, but which also contributes to the processing of toxins ingested from plants and well-cooked meat. In a series of recent studies, geneticist Lluis Quintana-Murci of the Institut Pasteur and colleagues investigated the extent by which different populations express NAT2 variants that acetylate — and thereby help break down — target molecules quickly or slowly. "We showed that most hunter-gatherer populations present fast-acetylation alleles," he says, "whereas the slower acetylators are very common in farmer-descended populations, like with most Europeans and particularly in the Middle East." NAT2 is also associated with the metabolism of folate, the natural form of folic acid, normally obtained from leafy greens or animal liver. Quintana-Murci's team proposed a model in which the sharp drop in folate intake associated with a shift to a grain- and cereal-rich diet favoured the emergence of alleles that suppress use of folate reserves, although he emphasizes that this is purely speculative until further data are available.

Both versions of the NAT2 enzyme carry certain disadvantages, as acetylation can actually enhance rather than reduce the toxicity of certain compounds: fast acetylation is linked with colon and lung cancers, whereas slow acetylation is associated with prostate and bladder cancers. Therefore, any nutritional benefits are likely to be closely balanced against the potentially harmful outcomes of NAT2 variation.

There are a number of other instances where selective pressures appear to have resulted in a trade-off. For example, although the twenty-odd T2R proteins involved in bitter taste perception represent a potent early warning system for harmful compounds, the genes encoding these factors also exhibit a striking level of variability (see More than meets the mouth, page S18). Unusual patterns of distribution have been observed for several variants that could alter the sensitivity of the mouth to bitter chemicals. "It's clear that there are ethnic differences in the composition of T2R haplotypes," says Wolfgang Meyerhof, a geneticist at the German Institute of Human Nutrition in Nuthetal.

Most traits are complicated and multifactorial in nature.

In a study of taste variation in central African populations by Meyerhof and colleagues, one low-sensitivity variant of the T2R16 bitter receptor, which normally responds to cyanogenic glycosides found in the starchy tuber cassava, was found to be unexpectedly common. These glycosides are metabolized in the gut to release toxic cyanide. The researchers speculated that the health costs of consuming potentially toxic compounds must be balanced by some sort of positive selection, perhaps arising from enhanced resistance against the malarial parasites that are widespread in this region, to sustain this allele in the population.

In fact, there are several instances where the benefits of lowering pathogen susceptibility are apparently sufficient to select for otherwise deleterious alleles. "Infectious disease is probably one of the strongest selective forces in the past or ever," says Tishkoff. She points to the example of glucose-6-phosphate dehydrogenase (G6PD) deficiency, a widespread enzymopathy associated with blood cell defects and potentially severe toxic reaction to foods including the fava bean (shown in main image, page S13). The gene variants associated with G6PD deficiency, also known as 'favism', are widespread in several ethnic groups that routinely eat these beans. Their prevalence, in spite of the near-term dietary and health costs, could result from the protection these variants confer against malaria.

As with lactase persistence, the strong adaptive advantages of this phenotype are demonstrated by its independent appearance in diverse populations; distinct G6PD-deficient alleles have emerged in Africa, the Mediterranean and the Middle East. More recently, Quintana-Murci and colleagues determined that an allele of this gene is prevalent in Southeast Asia, which results in only moderate enzyme deficiency, appears to protect against *Plasmodium vivax* — an unexpected finding, as *P. vivax* is seldom lethal and was presumed to represent a much less potent force for short-term human evolution than its highly dangerous relative *P. falciparum*. "It's about the consequences," says Quintana-Murci. "*P. vivax* could be important in childhood, or in women who are pregnant and infected — maybe they won't die, but their babies are born with low birth weight, which eventually weakens them and raises their chances of dying."

However, not all phenotypes can be directly linked to genetic variation. As years of genome-wide association studies have demonstrated, most traits are considerably more complicated and multifactorial in nature, and tracking

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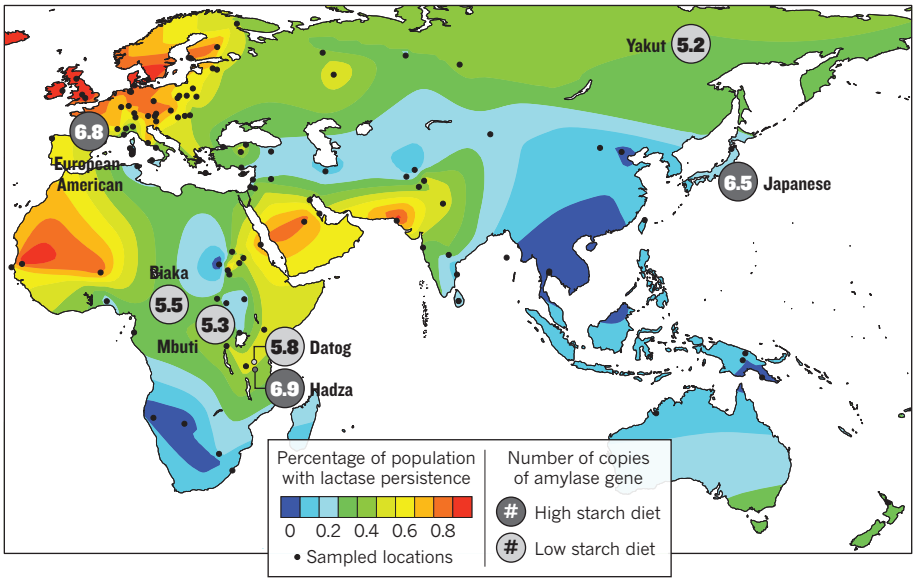
Milk provides many nutrients to those who can tolerate it (see map on opposite page).

MILK AND STARCH CONSUMPTION

Global distribution of genes related to ability to digest starchy foods and lactose in milk.

SOURCE: REF. 3, 4.



down these complex changes will require alternative allele-hunting tactics.

A TANGLED WEB

University of Chicago geneticist Anna Di Rienzo recently tried to identify allelic variants that differ in frequency among populations residing in similar geographic regions or ecosystems but who have distinct diets or modes of subsistence, such as farming or foraging. Through this approach, her team uncovered various hints of genetic adaptation in carbohydrate metabolism and folate production, associated with the adoption of diets based on roots and tubers. Conversely, cultures with a cereal-rich diet were more likely to produce a truncated, hyperactive version of PLPR2 — an enzyme responsible for breaking down plant glycolipids — than their non-cereal-consuming counterparts. "There is a consistent frequency shift between populations," says Di Rienzo. "The stop codon [in *PLPR2*] occurs always at higher frequencies in populations that have a cereal-rich diet compared to populations that don't and yet live in the same geographic region."

Yet it remains a challenge to piece together such minor genetic variations scattered throughout the genome. "These aren't mutations that will knock you dead," says Di Rienzo. "They make subtle changes to gene function or expression, and detecting those subtle changes can really be quite hard."

For more recent adaptations, the mutations can also be very rare, making it difficult to detect clear patterns. Even when the data seem to suggest the presence of selective pressure behind a given variant, it is essential to have a solid understanding of the cultural history of the region to eliminate demographic biases. "If a lot of the individuals you've tested have the same great-grandparents, it's quite a different story from if they were relatively unrelated," explains Swallow.

Most importantly, studies need to demonstrate clear functional contributions from a particular variant or subset of variants, and arrive at plausible reasons for why these changes are adaptive in some cases but not in others. "We want to know what the biology is that's being affected by these unusual patterns," says Stoneking. "How many of them are real, and how many are false positives, and what are the underlying stories? That's sort of where the field is a bit stuck at the moment."

IT TAKES A COMMUNITY

Clearly, scouring for signals of recent evolution amid the tens of thousands of interconnected human genes and regulatory regions can be compared to finding the proverbial needle in the haystack — but what if that haystack is far bigger than most people think?

Jeremy Nicholson, a biological chemist at Imperial College London, points out the tremendous diversity of the intestinal microbial flora, citing a report in 2010 which showed that Europeans each carry a complement of at least 160 bacterial species, with more than 536,000 bacterial genes between them — well over 20-times the human gene count[1]. "It actually should be thought of as a multicellular organism with a very large genome," he adds.

Even with our limited understanding of the microbial communities that thrive in our digestive tract and elsewhere in the body, it's increasingly clear that their net genomic output is inextricably linked with our own metabolic function, and the composition and activity of these communities is a direct by-product of our environment, culture and diet. "The gut microbial community can be viewed as a metabolic organ — an organ within an organ; they sense, adjust to, and process components of our diet, and their metabolic products profoundly influence our physiology," says Jeffrey Gordon, a microbiologist at Washington University in St Louis, Missouri. "It's like bringing a set of utensils to a dinner party that the host does not have."

Nicholson has already found some compelling evidence that genes expressed by the gut flora have effects that reach far beyond the digestive tract. "We've found deep compartmental connections between microbial status and bile acid metabolism," he says, "[And] there are some staggering connectivities between blood pressure and gut microbial metabolites."

Research from Gordon's lab has shown there are differences in the sets of bacterial species that reside in the guts of individuals, even identical twins. "Certainly less than 10% — and it might even be less than 2%—of the bugs that are in you are also in me," says Nicholson. Gordon and others are confident that the impact of cultural variation is at least as strong.

Our understanding of the genetic basis of even relatively well-characterized phenomena pertaining to dietary variation, like lactase persistence, could be confounded by the impact of these commensals. "There are Chinese students who come to the West who can drink quite a lot of milk even though they come from a genetic background where they're not lactase persistent," says Swallow. "We think that's due to adaptation of the intestinal flora." It also appears possible that the considerably smaller, but potentially equally diverse, microbial communities in our mouths may play an important role in the early stages of meal digestion, as indicated by a recent study that suggests oral bacteria may facilitate the processing of wheat gluten.

One of the most striking findings comes from a recent study by a team at France's Centre National de la Recherche Scientifique, presenting strong evidence that Japanese individuals can digest seaweed carbohydrates more efficiently[2]. This was made possible by an ancestral gene transfer event from kelp-borne bacteria that endowed their gut flora with the capacity to produce porphyranase and agarase enzymes. This adaptation is seemingly absent in North Americans who have not historically consumed raw kelp. Microbe-watchers like Nicholson suggest that this study could be a strong indicator of the future, as the research community begins to come to terms with the extent to which human genetic effects on diet might be overwhelmed by the bacteria we carry. "It's a piece of genius," he says. "It's something I use in my slide presentations now to worry geneticists." ■

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