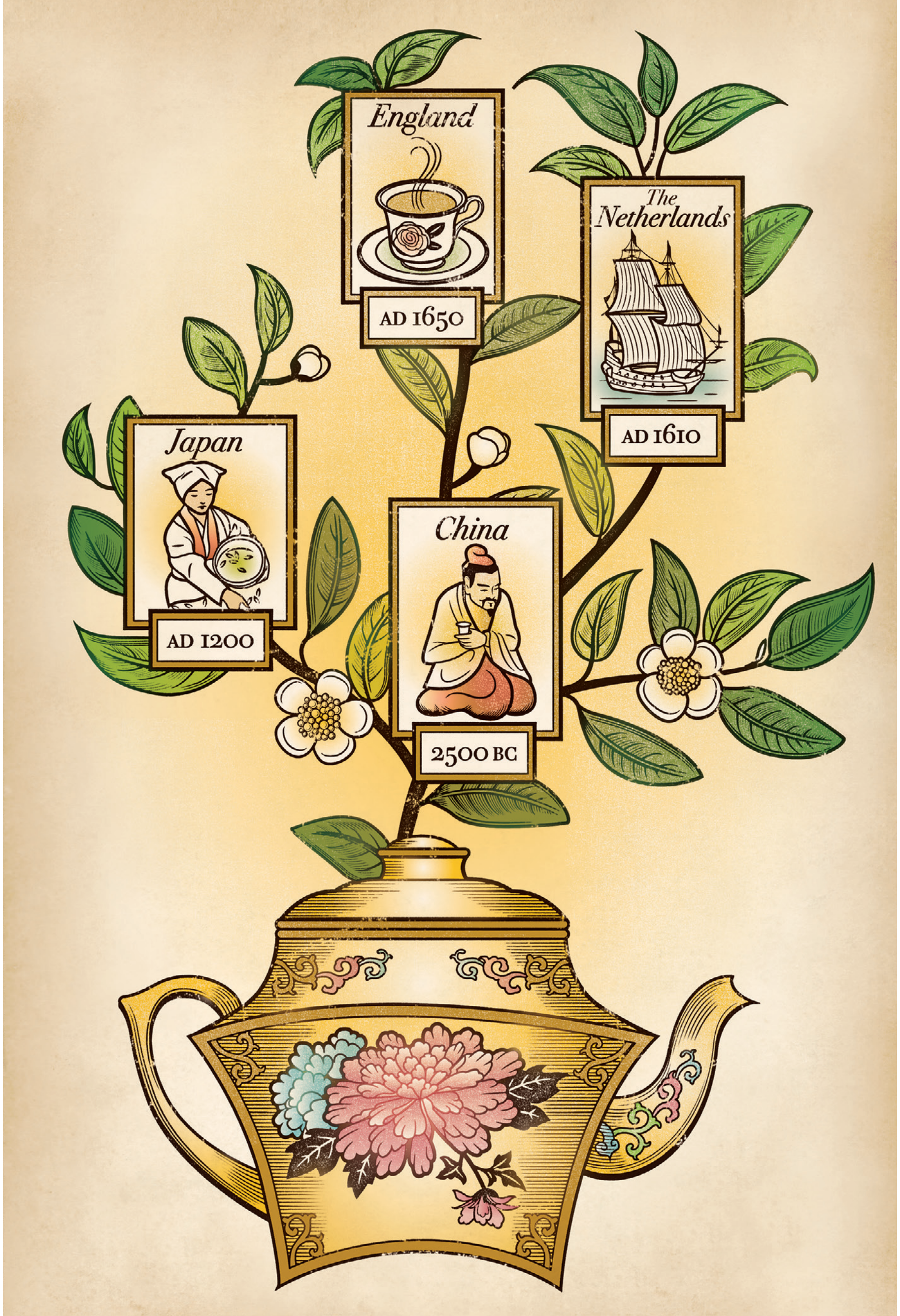

SUSAN BURGHART

AGRICULTURE

Making tea

Genetic studies of today's tea trees are providing clues to how the plant was first domesticated.

BY LIAM DREW

In around AD 500, according to legend, the Buddhist monk Bodhidharma spent nine years facing the wall of a cave, silently meditating yet remaining awake and focused. Eventually, though, he dozed off, and when he awoke he was so angry with himself that he ripped off his eyelids and threw them to the ground in disgust. From this discarded flesh grew a plant from which Bodhidharma's followers could make a beverage that both stimulated their minds and calmed their nerves. It was the first tea plant, and the drink was perfect for meditating monks.

The plant's recently sequenced genome tells a different story, however, meaning that scientists will have to construct a more plausible account of tea's transformation from a plant growing wild in China to a crop that is the basis of the world's second-most popular drink, after water. Every day the world's population consumes more than 2 billion cups of tea. Tea is grown commercially in more than 60 countries and yields an annual harvest of more than 5 million tonnes of leaves, which are plucked or cut from the plants' freshest growth.

The tea plant's journey is reflected in its name, *Camellia sinensis*. *Camellia* indicates that tea is a woody plant, closely related to the ornamental bushes that have earned a place in innumerable gardens owing to their flowers, and *sinensis* signifies its Chinese origins.

The spread of tea production and consumption from China to the rest of the world is well documented. Tea was taken to Japan by another Buddhist priest in around the year 1200. The Dutch brought tea to Europe in 1610, and the English developed a taste for it around 50 years later. Until the mid-nineteenth century, China supplied the West with tea, but after decades of tension, resulting in the Opium Wars, Britain sought to cultivate tea for itself in India. From there, tea farming spread through the British Empire and beyond.

But it is harder to determine when, where and why tea was first domesticated, as that occurred before reliable written records began to be kept. It is thought to have been first used in China as a medicinal herb, probably favoured for its mild stimulatory properties, before becoming a beverage revered for its delicate flavours. Present estimates place this first use at 3,500–4,000 years ago. But, "The first unambiguous mention of tea in a text came from an employment contract from about 2,000 years ago," says Lawrence Zhang, a historian at the Hong Kong University of Science and Technology. "One of the things the servant was supposed to do was go to market and buy this plant for his master."

The earliest archaeological evidence of tea drinking falls into a similar timeframe. In 2016, the distinctive molecular components of tea were found in plant matter collected in northeastern China and Tibet, and then carbon-dated to about 2,100 years old[1]. But to go further back, to the earliest domestic history of tea, biologists are looking for clues in the DNA of today's tea plants.

CHOOSING TRAITS

It is overly simplistic to imagine that there is a moment at which a wild plant transforms into an agricultural product. "Usually there's an initial domestication followed by a long period of improvement," says Jonathan Wendel, a plant evolutionary genomicist at Iowa State University in Ames. "And that improvement is still going on in many of our plants and animals."

For every plant currently grown by humans, that initial domestication involved humans taking an interest in wild-growing plants — at first gathering fruit or leaves, for example — and then starting to cultivate them for their own use. Consciously or not, growers preferentially propagating the plants that best provide the qualities they want exposes that species to artificial selection.

Over time, this usually results in big changes to the species. For example, teosinte, the wild ancestor of maize (corn), is a highly

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branched wild grass bearing many tiny ears of corn — strikingly different to the robust single stems of cultivated maize that produce just a few large ears. By contrast, however, farmed brazil nuts are almost indistinguishable from their wild forebears.

The origins of tea are clouded by the fact that wild *C. sinensis* plants have never been identified unequivocally. Close cousins of *C. sinensis* grow wild in China and neighbouring countries today, but they clearly belong to different species. And where wild-growing *C. sinensis* has been found, most scientists think that such plants are feral ones descended from crops.

This situation is not particularly unusual. "It's become a truism that the wild forms of most of our domesticated crops don't exist — they can't be found," says Wendel. There are many reasons for this, he explains. The plant might have been rare and driven to extinction, for example. But why ever it was, this means that researchers do not know the point from which tea domestication proceeded. They have not seen the plant that was first exploited by humans, so they do not know which of the modern plant's traits were introduced by people. Rather, they must try to infer this information from hints in the plant's DNA and its biology.

Breeding tea probably selected for traits such as higher yield, perhaps by choosing plants with seasonal uniformity in growth and resistance to cold and disease. But, almost certainly, there would also have been selection for the production of compounds that make drinking tea a pleasurable experience. "Tea's quality is mainly due to its secondary metabolites," says Colin Orians, an ecologist at Tufts University in Medford, Massachusetts. But these chemicals "are not there to make tea taste good for humans", he says. Instead, they are the products of biochemical pathways that aid the tea plant's survival.

"It's become a truism that the wild forms of most of our domesticated crops don't exist."

We can't be sure why each of tea's components evolved, Orians says, but some general principles provide clues. The caffeine that gives tea its stimulatory effects is a neurotoxin to insects and other invertebrates, and might have antimicrobial benefits. Catechins — compounds that contribute to tea's bitterness and are credited with mediating the potential health benefits of tea drinking — are flavonoids, which are a range of antioxidant molecules that help plants to deal with oxidative stress. Some also offer the plant protection from herbivores or shield it from ultraviolet radiation. And theanine — the chemical linked to tea's potential calming effects — is an amino acid that is likely to contribute to nitrogen biochemistry and the synthesis of plant material.

Some combination of these compounds first drew people to wild tea plants, but since then, their relative abundances have probably been shifted by artificial selection. "I have no doubt that we started liking tea because of the caffeine," says Orians, "but we like our stimulants to taste good too." Early texts about tea, dating from the eighth century, show that it was often prepared with extra flavourings such as onion, ginger, salt or orange, suggesting that the tea alone was unpalatable. The taste was improved by innovations in processing the leaves — these methods enabled the production of green, white, black and oolong teas from the same plant — but tea was also likely to have been bred for better taste. Certainly, there is much experimentation in growing tea cultivars — varieties created through selective breeding — with new flavour profiles, even today. But it is not clear when flavour began to drive selection.

Tea harvesting in Assam, India.

A, C, G AND TEA

In the past two decades, genetic analyses have transformed understanding of the origins of many crops, including maize, olives and rice. Now, tea is joining them.

As plants are domesticated, they become ever more genetically distinct from their wild ancestors. They accumulate mutations that underlie the traits for which growers select, and variants found on regions of chromosomes close to those mutations can spread alongside them. As time passes, random genetic differences are also amassed. Therefore, the species change genetically, and each plant strain that is kept apart from other strains by growers will also develop its own genetic profile. Without a wild ancestor to characterize, these changes can't be observed directly, but cataloguing the genotypes of current strains enables geneticists to infer some of this history.

Analysis of the genetic differences between cultivated strains reveals most reliably how closely related the strains are. The more related that two strains turn out to be, the more recently they shared a common ancestor. Geneticists can therefore analyse today's cultivars to draw family trees that depict their relationships. Deriving such evolutionary histories for cultivated plants is complicated by crosses between cultivars, but the hybrids that result typically have genotypes that are clearly a mixture of two distinct sets of parental genes.

Geneticists can also infer which genome regions have been selected by tea growers. When a favourable genetic trait spreads quickly through a population — owing to farmers choosing to breed only tea plants that have it — an entire chromosomal region hitches a ride. This means that other versions of the genomic region are banished, and that the stretch of the genome will not vary much between strains and individual plants — a sure sign to geneticists that the region contains one or more genes related to a valuable trait.

Researchers have been using genetics to try to determine relationships between tea strains for 20 years, and have applied increasingly sophisticated genetic tools. There are now approximately 1,500 cultivars, which have conventionally been grouped in particular ways. The most obvious divide is between Chinese tea (*C. sinensis* var. *sinensis*) and Assam tea (*C. sinensis* var. *assamica*), which is named after the Assam region in India where it was first grown. Chinese tea has smaller leaves than does Assam tea and is more tolerant of colder climates. Assam tea accounts for only a small fraction of tea grown in China but is widely grown in India and other hot countries. The relationship between these two varieties has long been uncertain, however, and it has also been unclear how other major subtypes, including Khmer tea, are related to them.

Work led by Lian-Ming Gao, a plant evolutionary geneticist at the Chinese Academy of Sciences' Kunming Institute of Botany, suggests that there are three distinct genetic lineages of tea plants. And, provocatively, Gao's team proposes that this finding indicates that tea was domesticated on three separate occasions. The first sort is Chinese tea, which the authors say probably comes from southern China. But they find two distinct types of Assam tea: a Chinese tea from the southwestern province of Yunnan, and an Indian one from the Assam region. Their analyses also shows that Khmer tea is not a separate lineage in its own right, but rather a hybrid of the *assamica* and *sinensis* cultivars

The initial findings were based on genomic fragments from 300 samples of tea from China and 92 from India. Two more studies by Gao's team, using chloroplast DNA and more sophisticated sequencing techniques, have subsequently supported these groupings. It has long been suggested that Chinese and Assam tea might have distinct origins, but the idea that Assam tea consists of two distinct lineages that were domesticated separately is more controversial.

Gao's team then used its genetic data to estimate when the three lineages diverged. Taking the genetic differences between strains,

Colin Orians (with camera) and colleagues hunt for tea green leafhoppers, an insect pest that damages tea leaves (inset), in Shaxian, China.

MAIN: XIN LI; INSET: ERIC R. SCOTT

and then estimating the rate at which genetic changes accumulate in such plants, researchers can calculate when lineages probably last shared a common ancestor. Such calculations suggested that the *sinensis* and *assamica* varieties diverged 22,000 years ago — long before any suggested date for the domestication of tea, and consistent with two wild populations having been domesticated independently.

The date for the Chinese and Indian *assamica* lines splitting is much more recent at 2,770 years ago — after tea was first domesticated. It is therefore open to debate whether these lineages were domesticated independently. Possibly, the *assamica* variety was domesticated only once, and was transported by people from one region to the other, allowing it to evolve separately in the two locations. "There have been three different gene pools demonstrated," Wendel says, "but that's a far cry from three different domestications."

Xiao-Chun Wan, a biochemist at the State Key Laboratory of Tea Plant Biology and Utilization at Anhui Agricultural University in China, is also sceptical about this conclusion. In 2016, Wan's group published a study[2] of tea's evolutionary relationships, also using genomic fragments, that demonstrated a clear separation between domesticated *C. sinensis* and wild tea species, and showed that the *sinensis* variety forms a genetic cluster apart from the *assamica* variety, although he did not compare Indian and Chinese forms of *assamica*.

In the same study, Wan's group also attempted to identify genetic footprints that would reveal the selection process that domesticated tea has undergone. They found preliminary evidence of selection for several enzymes involved in the generation of secondary metabolites, including caffeine. Their work shows the sorts of analysis that should become even more powerful now that a complete genome is available, says Wendel.

The *C. sinensis* var. *assamica* genome[3] was published in 2017, and Wan's group published a draft sequence[4] of the *C. sinensis* var *sinensis* genome in 2018. These data provided insight into the evolution of caffeine biosynthesis in tea. Wan says that the genome, which took a decade for his group to assemble, "provides a solid foundation for the investigation of domestication in tea plants", making it possible to do more detailed surveys of the differences between strains. For a start, comparison of these full genomes indicated that the *assamica* and *sinensis* varieties diverged much earlier than suggested by Gao's team, with the first estimate being 380,000–1,500,000 years ago[4].

The suggestion that the *sinensis* and *assamica* varieties were domesticated independently draws attention to events in the nineteenth century, when Britain first sought to cultivate tea in India. A crucial advance came when, in the 1840s, Robert Fortune, a botanist from Scotland, stole tea plants from China to start plantations in India — and brought Chinese tea farmers with him to do so. Fortune's heist is consistent with the idea that *C. sinensis* was domesticated just once — in China.

At the time of the theft, Britain was already growing some tea in India — but it was the *assamica* variety. In 1823, Robert Bruce, also from Scotland, had travelled along the Assam Valley. There, he learned of a wild tea that was harvested and consumed — sometimes as a vegetable, other times as a fermented drink — by the indigenous Singpho people. Because the plant had larger leaves than the Chinese tea with which he was familiar, Bruce was unsure whether it was a genuine tea. After his death, his brother, Charles Bruce, started to cultivate Assam tea in India — more than a decade before Fortune's exploits.

The Singpho people might therefore have been responsible for a second, independent domestication of tea, although the possibility remains that migrating tribes such as the Shan people of southeast Asia brought this tea to Assam from elsewhere. It might also be the case that Assam tea was domesticated independently in China. But Yunnan, the main province in China in which this tea is grown, is less than 1,000 kilometres from Assam. Agricultural exchange therefore seems possible.

Genetic analyses will elucidate the relationships between *assamica* cultivars. Yet such methods are best deployed in tandem with historical and archaeological evidence.

NEW BREWS

The other problem when defining domestication is that tea varieties are still being refined. Eric Scott, a PhD student at Tufts University who works with Orians on plant defence mechanisms, spent June and July 2017 at Shanfu Tea Company in Shaxian, China, studying how tea growers are using different varieties to make the best version of a newly popular type of tea.

The tea green leafhopper (*Empoasca onukii*) is an insect that eats tea plants, and the conventional response to an attack was to discard the affected leaves. But in the 1930s, farmers in Taiwan found that the surviving leaves yielded an excellent tea. When attacked by leafhoppers, tea plants respond by producing a chemical alarm signal that attracts jumping spiders, a natural predator of leafhoppers. "Those alarm signals just happen to be delicious," says Scott. "They have a really nice honey, fruity aroma that ends up in the processed tea and really increases the quality." This Eastern Beauty tea is fashionable at the moment, so farmers are exploring which varieties are most favourably transformed by their defence mechanism against this insect.

Scott stresses that this is just one example of farmers exploring new varieties to make better tea, along with theanine-rich, catechin-poor albino mutants and purple-leaved varieties. Zhang agrees, saying that tea production in India is focused on "big plantations, industrialized processing and more central quality control", whereas in East Asia, tea is grown mostly on small farms and with more diversification. "Tea is constantly moving," he says.

Because the selective force of people never stays still, the genetics will always be changing, says Orians. "Domestication never ends." ■

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