

Gourmet Genomics – Part II

Truffle Hunting

In our last issue, Jeremy Garwood reported on the recent results of high throughput analyses aimed at unravelling the molecular secrets behind the aromas of wine and cheese. Now he focuses on the genomic efforts towards understanding a delicacy so expensive that many people have never even tried it: truffles.

The white truffle, *Tuber magnatum*, still resists cultivation.



In 2004, when the Coffee Genome Project was announced, there were critics who objected that coffee was a “luxury” and that research should be directed at food that is essential for human nutrition. Many of us might quibble about the luxury status of coffee – it certainly feels essential – but how do you feel about €4,000-a-kilo truffles?

€4,000 a kilo

Genome sequencing has since become a lot cheaper and there is significant commercial interest in the higher prices that consumers are prepared to pay for luxury foods. Biotechnology research is being directed towards improving the quantity and quality of many foods, but the image of genetically modified organisms is at odds with the elite status of gourmet food. The traditional image of gourmet food is one that is closer to the organic food movement than that of the laboratory – it is a celebration of the aromas, textures and flavours that exist “naturally” in food without the addition of chemical flavour enhancers and preservatives. When you pay more for a gourmet food item (ideally purchased from a market stall or specialised boutique, or eaten in a reputed restaurant), you want a real gourmet experience with more taste and aromas and less of the afterthought that it may have been industrially sprayed and processed.

Truffles are the luxury of luxuries in European cuisine. Many readers of *Lab Times* may never have sampled this prized fungi, but given their price who can blame them? There are 60 species of truffle worldwide, 20 of which are found in Europe, but haute gastronomy recognises the unique and delicate “organoleptic properties” (i.e. taste and perfume) of just two of them – *Tuber melanosporum*, the black truffle found principally in France’s Perigord region, and *Tuber magnatum*, the white truffle found in a narrow swathe of forests in central Italy and Croatia, but especially around Alba. Despite extensive attempts at cultivating them – *T. magnatum* still resists cultivation – annual yields for both of these truffles have consistently fallen while their price has soared to upwards of 4,000 euros a kilo! Even a smallish truffle will easily set you back 100 euros.

Symbiotic ecology

This is a cult food, an icon of European gastronomy and culture which despite, or perhaps because of, its elitist status, has been the subject of a Franco-Italian collaboration: The French-Italian Tuber Genome Consortium.

Truffles are the fruiting body of an entire fungal colony that results from the ectomycorrhizienne symbiosis between an Ascomycete fungus of the genus *Tuber* and the roots of a large number of trees and bushes.

Antibiotic weapons

Tuber melanosporum (Perigord Black truffle) is endemic to chalky soils in southern Europe and induces a beneficial symbiosis on hazelnut and oak trees. Unable to produce their own food, the long, branching fungal filaments (hyphae) ramify between cells of the host root’s outer layers, forming a sheath around the root that radiates outwards into the surrounding soil to take moisture and minerals from the earth and infuse the soil with antibiotics, protecting the host tree from disease-causing organisms. In return, the fungus feeds on sugar photosynthesized in the tree canopy.

In late summer, these extramatricial hyphae aggregate to form fruit body initials. The latter develop into the fruit body during autumn and early winter. For *T. melanosporum*, the period of maturation extends from December to March. The spores released from the mature truffles germinate in the following spring, producing a vegetative mycelium, which results in the colonisation of tree root tips and further development of the symbiosis completing the truffle life cycle.

Production in decline

The principal problems encountered by truffle growers are the irregularity and decline in production from year to year. It has fallen from more than 1,000 tonnes at the end of the 19th century to less than 100 tonnes today (although some areas of truffle production tend to be shrouded in mystery). The droughts and excessive heat of recent years have been particularly unfavourable. However, other factors may also be at play, such as soil quality and competition between mycorrhizal fungi.

Since antiquity, truffles have been hunted in forests. By the 18th century, the acorns of truffle trees were being sown in an attempt to produce more truffles. But it was only during the 1970s that French researchers developed techniques to directly inoculate trees with truffle spores. Young trees are planted out in containers containing the spores which are then filled with a fertilised artificial substrate compatible with mycorrhization and tree growth. Today, 80% of the Périgord black truffles produced result from such cultivation, but there’s still a

place for peasants hunting in secret forest locations, guided by the sensitive noses of their truffle-trained pigs and dogs.

Some 300,000 truffle trees, covering between 1,000 and 1,200 hectares, are planted each year but truffle production remains irregular. French researchers are trying to improve upon the sowing of unselected acorns by breeding homogeneous truffle-bearing hazelnut and oak trees through vegetative propagation of the best yielding trees – an approach that has yet to bear truffles for comparison.

Mysterious crosstalk

Despite these efforts, truffle ecology isn’t well understood. For example, the nature of the chemical crosstalk between the hyphae and roots is still a mystery, let alone the countless interactions with all the other residents of the soil. Perhaps those volatile chemicals that make truffles smell so delicious serve other purposes in their ecosystem? When truffle fungi colonize a tree, a denuded zone, or *brulé*, that looks as though the ground has been scorched often develops around the trunk. Paola Bonfante’s group at the University of Turin has shown that the microbial community structure in *brulé* soils is dramatically different to that in the soil around neighbouring trees. “Truffles seem to trigger these changes with chemical signals,” possibly with the help of the tree, says Bonfante, “but we don’t know how it works.” Similarly, we do not know which genes are necessary for recognition between partners and the establishment of symbiosis.

Sequencing the truffle genome

In 2006, a strain of *T. melanosporum* from the INRA collection in Clermont-Ferrand was chosen for sequencing at the Génoscope (French National Sequencing Centre). A consortium of 10 French and Italian laboratories was formed to annotate and analyze the genome, including the INRA-Nancy, the Università degli studi in Parma, the Istituto per la Protezione delle Piante del CNR in Turin, the Istituto di Genetica Vegetale in Perugia and the Università degli Studi in Urbino. At a meeting in November 2008, the group presented a preview of the genomic sequence of the black Perigord truffle, the second symbiotic soil fungus to be sequenced, and said the genome sequence of the white truffle (*T. magnatum*) should be ready this summer.

The newly completed black truffle genome revealed areas with highly variable amounts of repetitive DNA. Paolocci in Pe-

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rugia's Institutue for Vegetative Genetics and Murat from INRA have been using these regions as markers for DNA fingerprinting of more than 200 black truffles from 13 regions across southern Europe.

Strong variations in the organoleptic properties of a single species like *T. melanosporum* have been observed. Previously it was assumed that truffles are almost clonal with hardly any genetic differences between the fungi growing in different regions. Any distinct flavors, aromas, or appearances are chalked up to variations in the environment but sampling the genomes of black truffles from around Europe revealed considerable heterogeneity. However, far from being a monoculture, black truffles form local varieties that are genetically distinct. Likewise, Paolocci's group has fingerprinted more than 300 white *T. magnatum* truffles from 26 different areas across its range, and early results indicate that it may also form distinct regional populations.

Naturally, one of the great questions researchers hope to answer is: What are the genetics of truffle odour? Researchers from the genome consortium plan to create a "volatile map" of truffle aroma in the field, capturing the gases released by truffles at various stages of maturation and immediately freezing samples of the tissue. Then, back in the lab, gene-expression patterns in the fruiting body and other tissues will be correlated with changes in the cocktail of chemicals released. The ultimate aim is to find the genes responsible for fruiting body development and for the symbiosis between tree and fungus. However, fear not. Given its status, it seems unlikely that such a prized gourmet item as the truffle could ever be subjected to genetic manipulation.

The sex life of truffles

The only part of the fungus that ends up on the dinner table is the fruiting body. The spores packed into the fruiting body each contain two copies of the truffle's chromosome complement – they're diploid. But the rest of the organism exists year-round as a fine web of hairlike hyphae cells that are haploid and only contain a single set. The elusive question has been what sexual acts hyphae get up to when producing spores. According to the prevailing model, truffles do not have sex with strangers. Instead, they self-mate, with two hyphae of a single fungus fusing. The resulting cell,

which would have two identical sets of the genome, then divides rapidly to form the spores of the fruiting body, surrounded by a matrix of cells, called the gleba, each containing single-copy genomes.

A tasteless invader

Now, using the highly variable markers, the investigators have compared the DNA of gleba cells surrounding the spores and the spores themselves in dissected black and white truffles. They found different genetic fingerprints in the two cell types of both truffles. The gleba cells contained one set of DNA markers, but in addition to these same markers, the spores carried a foreign set. This work showed not only that the fruiting bodies were the result of a sexual encounter between two different fungi but also that gleba and spores have different cellular origins. All of the fruiting body's gleba cells have just the "maternal" genotype, whereas the spores carry both.



The 'black brother', *Tuber melanosporum*, in situ

There is an interloper in the oak groves of Perigord, a highly competitive interloper that looks even to experts just like *T. melanosporum* but tastes, appallingly, of nothing much. Enter *T. brumale*, the so-called "Chinese" truffle, that has shaken the black truffle world to its very foundations. *T. brumale* is the dreaded enemy of *T. melanosporum*, an invader species up to 10,000 times harder and superior in its ability to compete. The *brumale* is almost identical in outer appearance to *melanosporum*, but it is crisscrossed with thick white veins inside, tends to smell of alcohol and, horror of horrors, it is flavourless! Unfortunately for truffle lovers, the bad news is that *T. brumale* grows better in the dry summers that are becoming the norm in Perigord. And unscrupulous retailers and restaurants are now passing off the *brumale* as *melanosporum* in the hope that fewer members of each new generation of truffle-eaters will know the difference.

To identify a truffle species, the morphology of spores used to be observed under the microscope. Now a molecular test has been developed, based on analysing the DNA sequence motifs from the internal transcribed spacer of the nuclear rRNA. This DNA analysis has already caught imposter species, such as the Chinese truffle. The next step will be to refine the genetic fingerprinting to determine if truffles of the same species, originating from different regions, can be distinguished. This could be important if governments adopt the wine industry's "controlled geographical origin" system because the European Union will require authentication of a truffle's birthplace.

Post-glacial migration

The genetic data is also filling out the truffle's evolutionary story. Using DNA footprinting, INRA researchers at Clermont Ferrand have established a genetic map for the Perigord truffle going back 12,000 years to



Digging for results – a motto for truffle hunting ... and genomics

the last Ice Age. By the time that Europe was thawing, only two small populations of black truffles existed, in southern Italy and Spain, and white truffles were restricted to central Italy. They found that there are at least 10 genotypes, deriving from two ancestors that followed separate post-glaciation migratory routes over the Alps into

France, in association with their favourite host, the oak tree. Genotypes I and II are abundant (60 and 28% respectively), but type I occurs mostly in Western France and type II in the East. Other genotypes are more or less localised. For example type X is exclusive to the Hérault. A post-glacial explanation for this distribution would be that populations of black truffles recolonised France from the north of Italy: genotype II colonised the limestone regions of the Rhone valley as far north as Lorraine, while type I diffused through the Roussillon and Languedoc regions as far as Périgord and the Atlantic coast.

But, for some reason, white truffles never succeeded in leaving Italy. What held the white truffles back is a mystery but, considering changing climates, the question is of more than academic interest. It is expected that the black truffle will be able to adapt to global warming by moving northward, but that the white truffle, blocked by the Alps, could become extinct.