Black gold. The genome of the black truffle has now been sequenced.

GENETICS

Rooting Around the Truffle Genome

A favorite of gourmets, truffles are revealing their delicious secrets to the biologists studying the mysterious fungi

ALBA, ITALY—Upon first glance, it's hard to believe that this is one of the most prized and expensive foods in the world. The muddy clods laid out in the display case bear a striking resemblance to animal droppings. But their true identity is revealed the moment the seller, Stelvio Casetta, lifts the glass lid. The aroma—a potent, earthy cocktail of sulfurous chemicals—is unmistakable. Then you see the price tags, ranging from $100 to $400 for a lump of fungus smaller than Brussels sprouts. These are *tartufi* or, as the many English-speaking tourists here call them, truffles.

Casetta plucks out a $300 creamy, golden nugget with the same enormous hand with which he unearthed it just days ago at his secret location in a nearby forest. (You can join *Science* on a truffle hunt and an unusual taste test at www.gonzoscientist.org.) "You can't know a truffle just using your eyes," he tells potential buyers before handing it to a nearby woman, who takes it to her nose, inhales deeply, and smiles at the recognizable odor.

Paola Bonfante, a microbiologist at the nearby University of Turin, knows her truffles, in some ways better than Casetta does. She is part of a Franco-Italian team exploring in intimate detail the prized fungi that make up the truffle genus. At a meeting in November,* the group gave a preview of the first full genome sequence of the black truffle (*Tuber melanosporum*), just the second symbiotic soil fungus to be so deciphered, and the genome sequence of the white truffle (*T. magnatum*) is expected by summer. Already, the European investigators have dug up several surprises among the fungal DNA sequence, including one that may help stem the truffle black market and another that rewrites the sex life of these subterranean organisms.

The truffle is the latest in a series of gourmet genome projects pursued by European researchers. French and Italian scientists clinked glasses last year after sequencing the genome of the grape used for wine production (*Science*, 25 April 2008, p. 475). French scientists are now sequencing the genomes of the microbes responsible for Camembert and Roquefort cheeses. "It is natural that these should be French and Italian projects, … food is so important to our culture" says Francis Martin, a plant and fungal physiologist at the French National Institute for Agricultural Research (INRA) in Nancy who led the black truffle genome project.

*3rd International Truffle Congress, Spoleto, Italy, 24–28 November 2008

Precious fungus. White truffles have resisted domestication and command the highest prices.

Truffle trouble

For commodities such as gold and silver, the market value is the same regardless of where they are mined. But the price of a truffle is strongly determined by its geographic origin. The most highly regarded black truffle is *la truffe noire du Périgord*, harvested for centuries beneath oak trees in southwestern France. French researchers discovered how to reliably cultivate black truffles in orchards in the early 19th century and continue to do so to this day. But the white truffle has resisted domestication and thus commands far more money. The fungi can be found—with the help of pigs or trained dogs—only in a narrow swath of forests between the Istrian peninsula of Croatia and central Italy. And here at the Alba market, *tartufo bianco d'Alba*—the locally grown white truffle—is the undisputed king, routinely selling for $4000 per kilogram.

But a major problem for truffle buyers is "counterfeiting." Black truffles bearing the prestige and price of the name Périgord sometimes originate from less famous regions. White truffles harvested in Croatia are brought to Italy and sold with "d'Alba" labels. Sometimes similar looking species, such as the plentiful but less aromatic Chinese truffles (*T. indicum*), which resemble black truffles, "are sold with a small amount of black truffle included to provide the right smell," says Francesco Paolocci, a fungus researcher at the Institute of Plant Genetics (IGV) in Perugia, Italy.

So the truffle industry is turning to molecular biology for help. Researchers have "assumed for decades that truffles are almost clonal," says Martin, 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. Sampling the genomes of black truffles from around Europe has turned this view on its head, however.

The newly completed black truffle genome revealed areas with highly variable amounts of repetitive DNA. A team of Italian and French geneticists led by Paolocci, Andrea Rubini, and Sergio Arcioni at IGV and Claude Murat at INRA used these markers to take DNA fingerprints of more than 200 black truffles from 13 regions across southern Europe. Far from being a monoculture, black truffles form local varieties that are genetically distinct, the researchers reported at the November meeting. Paolocci's group has also fingerprinted more than 300 white truffles from 26 different areas across

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its range, and early results indicate that it may also form distinct regional populations. "Now that we have the global picture," says Martin, "you see that the Tuber genome is like a mosaic," with "islands" of stable genes separated by "an ocean of repeating DNA" that change rapidly over the centuries.

DNA analysis has already caught imposter species, such as the Chinese truffle, says Bonfante. The next step will be to refine the genetic fingerprinting to determine if truffles of the same species but different regions can be distinguished. "This will become important," she says, if governments adopt the wine industry's "controlled geographical origin" system. The European Union will then require authentication of a truffle's birthplace.

Beyond fighting counterfeit fungi, the genetic data is filling out the truffle's evolutionary story. Using the new map of black and white truffle diversity, Martin and Murat recently modeled the spread of truffles going back 12,000 years to 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. Black truffles then spread over the Alps and across Europe, but white truffles never did. What held the white truffles back is a mystery. 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," says Martin, but the white truffle, blocked by the Alps, could become extinct.

Sex in the soil

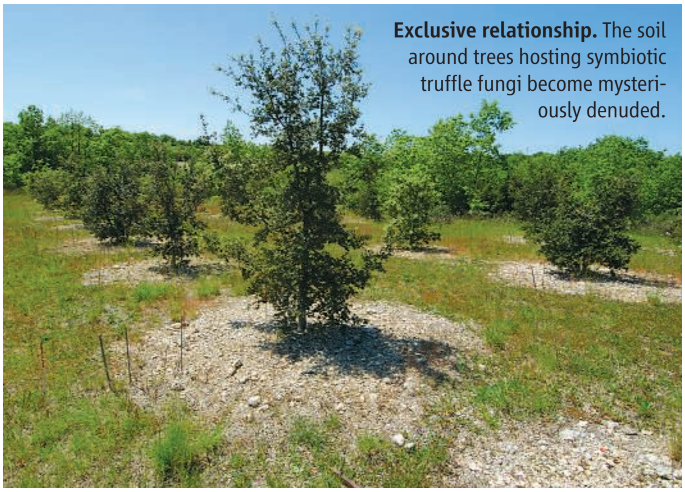
Everyone in Alba seems willing to testify that truffles are an aphrodisiac. The origin of this legend may be that, among the hundreds of volatile compounds that truffles emit, there is indeed a close mimic of androstenol, a mating pheromone secreted in boar saliva. This may explain the frenzied enthusiasm of sows when they locate truffles beneath leaf litter, says Bonfante. Evidence of any such behavioral effect on humans is lacking, but the question of the truffle's own sexual behavior has now been answered, thanks to the new genome data.

The only part of the fungus that ends up on dinner tables is the fruiting body, a temporary reproductive organ created at specific times of the year—between November and December for white truffles, December and February for black. The rest of the organism exists year-round as a fine web of hairlike cells, the hyphae, that sheath the roots of a tree, providing minerals in exchange for food. The spores packed into the fruiting body each contain two copies of the truffle's chromosome complement, whereas the hyphae cells 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.

With the highly variable markers from the fully-sequenced truffle genome in hand, the IGV group has now tested whether the fungi are really so chaste. The investigators compared the DNA of gleba cells and spores in dissected black and white truffles. Like catching an adulterer in the act, they found different genetic fingerprints in the two cell types of both truffles. The gleba cells contained one set of DNA markers, and the spores carried those same markers plus a foreign set, the team reported at the meeting. In one stroke, says Paolocci, 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. Like a mother's womb, all of the fruiting body's gleba cells have just the "maternal" genotype, whereas the spores carry both, like a fertilized egg.

Confirming this, the researchers inoculated the roots of potted tree seedlings in the laboratory with black truffle spores. In each case, the hyphae cells that grew contained a Mendelian assortment of genotypes, exactly as predicted for sexual reproduction.



Exclusive relationship. The soil around trees hosting symbiotic truffle fungi become mysteriously denuded.

CREDIT: ANTONIETTA MELLO

The discovery has "major implications" for the truffle industry, says Charles Lefevre, a mycologist based in Eugene, Oregon, who is considered one of the world's experts on the delicacy. Companies that sell tree seedlings colonized by black truffle fungi "have benefited from low or nonexistent seedling quality standards," he says. Now sellers may have to prove that their trees are colonized by multiple truffle mating types that can produce the edible fruiting bodies.

What's that smell?

The sex life of truffles is only one of their secrets, says Martin. "We really don't understand truffle ecology." For one thing, the nature of the chemical crosstalk between the hyphae and roots is still "a black box," he says. And then there are the countless interactions with all the other residents of the soil. One clue to this ecosystem may literally be under our noses. "All those volatile chemicals that make truffles smell delicious to us may serve other purposes," says Bonfante. 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. Bonfante's group has shown that the microbial community structure in brulé soils is dramatically different when compared with soil around neighboring 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."

Martin and his Italian colleagues plan to take a crack at the problem by creating a "volatile map" of truffle aroma in the field. "The idea is to capture the gases released by truffles at various stages of maturation and freeze samples of the tissue immediately," Martin explains. 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.

Of course, learning the delicious secrets of truffle biochemistry could make it possible to genetically engineer truffle aroma into more easily cultivated organisms, such as mushrooms. But would Europeans accept genetically modified portabellos with the whiff of *tartufo bianco d'Alba*? "Absolutely not!" says Martin.

–JOHN BOHANNON

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