National Academy of Sciences (*PNAS*), they reported that this method, combined with other techniques, increases the amount of antibody by up to 100-fold, reducing the size of the crop needed and making it feasible to grow plants commercially indoors. Compared with making a transgenic plant, which takes a year or two to develop, this "magnifection" can go from gene to grams of protein in a couple of weeks. "It's incredibly promising technology," says Ma, who, like other academic researchers, is trying out magnifection.

With help from the drug giant Bayer, which bought the company in 2006, Icon Genetics will open a clinical-grade manufacturing plant in June. It expects to begin trials with a cancer vaccine tailored to individual patients in 2009, says CEO Yuri Gleba.

Bayer's move is a healthy sign of regrowth for the pharming field, Ma and others say. And other new sources of support are helping too. Last month, Pharma-Planta, a €12 million, 5-year, European Union–funded project co-coordinated by Ma, described in *PNAS* an anti-HIV microbicide grown in corn or tobacco that could be ready for testing next year. The Defense Department and other U.S. government agencies have provided the Fraunhofer USA Center for Molecular Biotechnology in Newark, Delaware, nearly $14 million to use a technique like magnifection to make vaccines. It has tested anthrax and plague vaccines in nonhuman primates and a pandemic flu vaccine in ferrets. "[We] can do things much faster than any other technology," says Executive Director Vidadi Yusibov, slashing in half the 6 months it now takes to make flu vaccine the traditional way, in chicken eggs. The organization also has $8 million from the Gates Foundation for plant-based vaccines for malaria, sleeping sickness, and flu.

As visions of endless fields of pharma crops have faded, so have unrealistic expectations for pharming. Scientists say they now realize that they need to be smarter about the marketability of the drugs they develop in plants. They think the best bets—Protalix aside—may be high-volume biologics, such as microbicides, monoclonal antibodies, and vaccines, particularly for use in developing countries. Getting these first low-hanging fruits through clinical trials and FDA approval should allay concerns about safety and environmental risks. Says Palmer, now at the University of Louisville in Kentucky, "Once two or three products [win approval], the field should really take off." **–JOCELYN KAISER**

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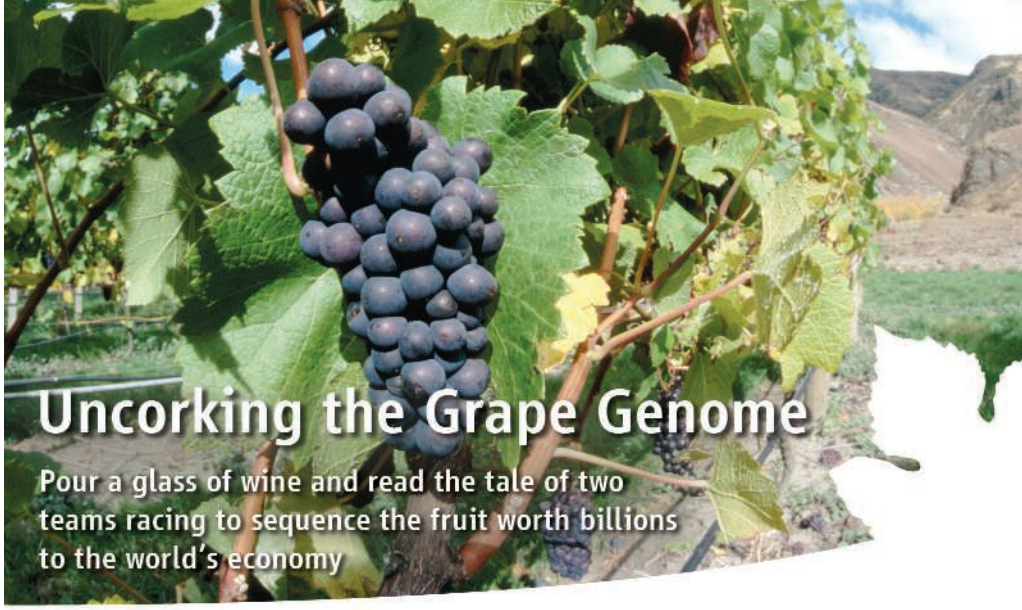

Uncorking the Grape Genome

Pour a glass of wine and read the tale of two teams racing to sequence the fruit worth billions to the world's economy

AMONG WINE CONNOISSEURS, OPINIONS differ about whether 2007 will prove a good year for Pinot Noir. But among plant geneticists, it's the finest vintage ever: Last year, two European teams published high-quality drafts of two Pinot Noir–derived genomes.

Plant biologists are toasting the genomic double-header. This is the first fleshy fruit and just the fourth flowering plant to have its genome decoded. And in economic terms, grapes top the world's fruit crops: We consume them fresh or dried, crush them into juice, and use them to make wine that can sell for many thousands of dollars a bottle. "The contributions of these sequencing efforts are enormous and historical," says grape researcher Steven Lund of the University of British Columbia in Vancouver, Canada.

Wine woes. Powdery mildew (*above*) and other fungal diseases can devastate vineyards.

The story behind the grape genome is one in which a worldwide scientific community came together, then partially splintered into rival camps; money to support sequencing was hard to come by; and success has brought both new insights and delicious questions. The rivalry provided the drama of the story. For a while, a French-Italian grape genome alliance called Vigna/Vigne looked like it was going to be beaten by a disgruntled researcher who started his own genome effort. "Undoubtedly, competition was a driver here, perhaps in a microcosm of the human genome sequence drama of years past," says Lund, referring to the bitter contest between public and private programs to decipher our genetic code. Recently, however, at a workshop* in Udine, Italy, the two grape genome groups began to put aside their rivalry. "I'm hopeful there will be more collaboration now," says Vigna/Vigne member David Horner of the University of Milan in Italy. "It's cool there are two cultivars done. It allows more comparative work."

A key motivation for deciphering the grape genome is to prevent a repeat of the economic devastation that struck the European wine industry in the late 1800s. At that time, phylloxera, sap-sucking insects from North America, ravaged European grapevines. Today, winemakers and grape researchers are struggling to combat new threats, particularly downy and powdery mildew, diseases that have made their way to Europe from the United States over the past century. These fungi are an environmental as well as an economic nightmare: Although only about 5% of Europe's farmland is dedicated to wine vineyards, they account for about 70% of the region's fungicide use.

The new genome information should speed the creation of hardier vines, which has been slow going. "The target now is clearly resistance genes," says Vigna/Vigne member Michele Morgante from the Institute of Genomic Applications (IGA) in Udine. New insights into the locations of these genes can assist breeders as they try to develop better varieties, for example. And identifying genes in the few grapes that are resistant to drought

*Tuning the Taste of Wine, 7 March 2008, Udine, Italy.

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or pests may pave the way for genetically modifying common wine grapes to have the same attributes.

But as viticulturists enter the genomic age, many wonder whether the wine industry, particularly the conservative European sector, would dare bypass conventional breeding for genetically modified (GM) grapevines. Scientists have for years been experimenting with GM grapes—usually putting nongrape genes into the fruit's genome—but most winemakers have shied away from public association with such efforts. "For a lot of consumers of wine, especially high-end ones, history and tradition is a very important part of their experience. If you produce wine from a genetically engineered grape, you strike at the heart of that," says Carole Meredith, a former grape geneticist who now runs a vineyard (see sidebar, below).

Taking root

Although there's a dazzling variety of wines produced around the world, the great majority flow from the juices of a single species, *Vitis vinifera*, commonly called vinifera. Indeed, in Europe, this grape is the only source of fine wines—other grapes are limited to fruit, juice, or so-called table wines. Thanks to centuries of breeding, 7000 cultivated varieties, or cultivars, of vinifera now provide an incredible diversity of flavors, from hearty reds to light whites.

A vinifera genome project began to take root in the mid-1990s, largely through the instigation of Meredith, who was then conducting grape research at the University of California, Davis. Meredith and others wanted to use genetics to identify various grape cultivars—Chardonnay, Cabernet Sauvignon, and so on—that can be tricky to distinguish in other ways. In a business in which wines made from different cultivars can vary enormously in price, correct identification is critical.

The researchers proposed to "fingerprint" grapes based on variable DNA sequences called microsatellite markers. Back then, however, identifying such markers "was expensive and laborious, and no one lab was ever going to develop enough to make their efforts worthwhile," Meredith recalls. "So I approached all the people I knew and suggested working together."

From that suggestion arose an international consortium that amassed hundreds of such markers within several years. The researchers were thirsty for more. In 2001, many of them, including Meredith, formed the International Grape Genome Program, arguing for a full-fledged sequencing of a vinifera cultivar.

But who would pay for it? It was well-known that grapes weren't on the U.S. genomics menu. In 2001, Enrico Pè of the Sant'Anna School of Advanced Studies in Pisa proposed a grape genome program in Italy, sponsored by the private sector. But bank foundations and winemakers declined.

Finally, in 2005, the French research agencies INRA and Genoscope joined with various Italian groups, including IGA and universities in Verona and Udine, to form Vigna/Vigne, which means vine in French and vineyard in Italian, respectively. This time, researchers did not ask winemakers for money, only political support, says Pè, who now leads the Italian side of the collaboration. France ultimately contributed about €8.5 million, Italy, about €12 million.

The race is on

Except when discussing European football—their national teams are bitter rivals—the French and Italian groups meshed smoothly. Still, agreeing on what to sequence wasn't easy. Compared with many plants, vinifera grapevines are extremely heterozygous: The female and male versions of the plant's chromosomes differ significantly. This complicates the sequencing and assembly of an accurate genome. The Italian scientists first considered a Sangiovese grape, then a Pinot Noir. Their French counterparts lobbied for a Cabernet Sauvignon grape, but after studying its DNA further, the team decided it was too challenging as well.

Finally, an almost-forgotten grapevine growing in a French greenhouse provided a solution. In the 1980s, a French viticulturist hoping to develop a better vine for winemakers began inbreeding a Pinot Noir. Several generations of selfings produced a few lines with simplified genomes but also stunted their growth and made them unappealing for wine production. Vigna/Vigne decided one of those, PN40024, would offer the best potential for sequencing, even if it was, as Pè jokes, "pathetic, hardly a grape."

Just months after Vigna/Vigne began its PN40024 sequencing effort, Pè and his colleagues were shocked to learn of a rival effort. In March 2006, Riccardo Velasco and his colleagues at the Istituto Agrario San Michele

CREDIT: STEPHEN LAGIER

A Life With Grapes

CAROLE MEREDITH SPENT 3 DECADES STUDYing grape genetics, and she helped start the grape genome efforts that bore fruit last year. But in 2003, she traded her lab bench for a life of winemaking in California's Napa Valley.

Before that career shift, Meredith had earned fame for using genetic fingerprinting to resolve the origins of some of the world's great wines, including Chardonnay and Cabernet Sauvignon (*Science*, 3 September 1999, p. 1562). She and her colleagues garnered the most attention by tracing the roots of the iconic American wine grape Zinfandel to an ancient Croatian grape called Crljenak kastelanski. Such research, Meredith says, "was very attractive to the popular press and wine-geek consumers."

It didn't pay the bills, however. Her grapevine sleuthing was never directly funded, and Meredith argues that there is a bias in the United States against supporting grape research despite the fruit's economic importance. "I think that, in large part, it's due to our history of Prohibition, and there's still the feeling in Washington that research related to the alcoholic beverages is not the best use of taxpayer money."

To Meredith, it's fitting that Europeans sequenced the grape genome. "In Europe, grapes for wine are a fundamental part of their agricultural heritage and modern economy," she says.

Burned out from the bureaucracy of research and the constant search for grants, Meredith retired and joined her husband, Stephen Lagier, who had been working for the Robert Mondavi Winery, to make their own wine using land they purchased in 1986. Doing almost everything themselves—vineyard work, winemaking, bottling, sales—the

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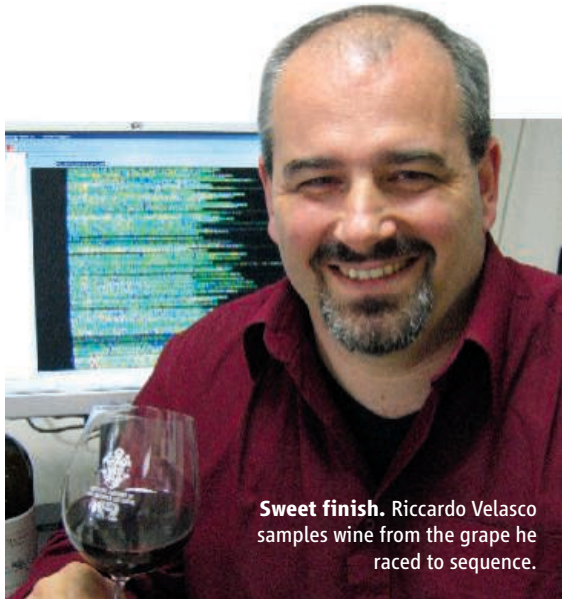
all'Adige (IASMA) in Trentino, Italy, announced that they were almost done sequencing the genome of a Pinot Noir grape used in many countries to make red and sparkling wines. Velasco had been involved in some of the original genome planning efforts, including Pè's Italian proposal, but he disagreed with the initial decision to switch from sequencing an outbred Pinot Noir to a Cabernet Sauvignon. His institute then lobbied the Trentino regional government for about €10 million to pursue its own grape genome. IASMA initially kept quiet about the true scope of the project, says Velasco, because "the possibility of failure was high." Unlike Vigna/Vigne, which kept sequencing in-house, IASMA contracted with Myriad Genetics in Salt Lake City, Utah, to sequence and help assemble most of the genome.

After the IASMA bombshell, Anne-Francoise Adam-Blondon of INRA says that the French-Italian effort ground to a halt for several months as members discussed whether to spend their governments' money on something that had apparently already been accomplished. But IASMA hadn't published its genome, so Vigna/Vigne decided to press on.

The race ended with Vigna/Vigne winning by a nose. It published the PN40024 genome online 27 August in *Nature* (sciencenow.sciencemag.org/cgi/content/full/2007/827/1). Velasco's Pinot Noir genome appeared online 19 December in *PLoS One*. Some Vigna/Vigne members felt that Velasco initially downplayed their feat, by, for example, reminding the media that PN40024 wasn't a real Pinot Noir or used for wine. But Velasco attended last month's workshop, which focused on Vigna/Vigne's results, and he was greeted warmly. "The two projects are fully complementary," he says.



CREDIT: IASMA COLLECTION

Sweet finish. Riccardo Velasco samples wine from the grape he raced to sequence.

Decanting the genome

It's already clear that the two genomes vary significantly. Although both efforts predict that the vinifera genome contains about 30,000 genes, the Pinot Noir sequenced by Velasco's team has about 270 members of a gene family associated with disease resistance, whereas the inbred PN40024 strain has almost 360, Adam-Blondon reported at the workshop. The two grapes also differ in the number of the many hundreds of genes related to the production of polyphenols, flavenoids, and resveratrols—all of which contribute to a wine's color, aroma, and taste.

Several of the talks in Udine centered on how genomic data could aid the breeding of disease-resistant grapevines. Gabriele Di Gaspero of IGA, for example, is trying to harness the powers of a Central Asian grape that is used for raisins. It is the first vinifera shown to stand up to powdery mildew, a discovery by a Hungarian group that electrified grape biologists a few years ago. Di Gaspero and colleagues have been crossbreeding the grape with a mildew-vulnerable vinifera and have used DNA markers identified through the genome projects to pinpoint the chromosomal location of a disease-resistance gene.

In theory, researchers can now—even without identifying the exact gene—breed resistance into popular wine grapes such as Chardonnay and Pinot Noir. After crossing the Central Asian grape with their favorite wine grape, they can select and continue to breed just the seedlings whose DNA contains the markers bracketing the mystery resistance gene. Ultimately, such marker-assisted selection could result in a disease-resistant grape that retains most of the qualities needed to make a good wine, instead of raisins. Still, bringing a new grape to market can take decades—and scientists have to be sure the transferred resistance gene is stable in its new genetic surroundings. "When you plant a grape field, it's for 30 years, so you really need durable resistance," notes Adam-Blondon.

Grape researchers are also using the new genome data to probe the interplay of genes, environment, and wine flavors in a variety of cultivars. In Udine, Mario Pezzotti of the University of Verona detailed genetic studies of the unusual process that produces the Italian red wine Amarone. The grape involved typically produces a sweet wine, but decades ago winemakers realized that if those same grapes dry for several months after harvest, the withered fruit make a more intense and bitter wine that has since become highly valued. Although some of his colleagues predicted that genetics had little to do with the withering process, Pezzotti revealed that large numbers of genes are active during this period, including many that influence a wine's taste and aroma. Amarone makers are now following the research with intense interest, he says.

The Vigna/Vigne and the IASMA teams are now on more cordial terms, but they do have some scientific disagreements. Both have found evidence of whole-genome duplications in vinifera's past—a common feature of plant evolution in which new species arose after an ancestral plant accidentally duplicated its genome or hybridized with another to expand its gene set (see p. 481). Velasco and his colleagues argue that such an event happened relatively recently, after grapes had split off from the branches on which *Arabidopsis* and poplar belong. Vigna/Vigne, on the other hand, has concluded that the grape genome did not undergo any recent expansion. It instead suggests that vinifera derives from an ancient hybrid that once had six sets of chromosomes. Because each team used different strategies for discerning and dating duplications, says Velasco, "who knows who's right?"

Indeed, Pè ended the Udine workshop with the reminder that having a grape genome—or two—in hand merely provides a foundation for future work. "Most of the data still have to be digested," he notes. A glass or two of vinifera's valuable juices, perhaps a nicely aged Cognac, should speed the process. **–JOHN TRAVIS**

couple now produce a well-regarded Syrah.

Although she may have helped pave the way for genetically modified (GM) vines, Meredith says she wouldn't plant them herself, for practical reasons. She has no concerns about the safety of GM grapes and believes vines engineered to resist disease could be useful. But Meredith predicts "tremendous consumer resistance" to GM wines. "I'm a realist," she says. "The only thing that would convince me to switch to a genetically engineered grape … is if my alternative was a dead vineyard."

Meredith loved her research career, particularly the historical studies of wine grapes. But today, sitting in her house on a Napa mountainside watching birds fly over her vineyards, she's content making delicious wine with grapes from her own land. "It's a lovely existence," she says. **–J.T.**

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