Microbial Terroirism

BY MATTHEW GODDARD

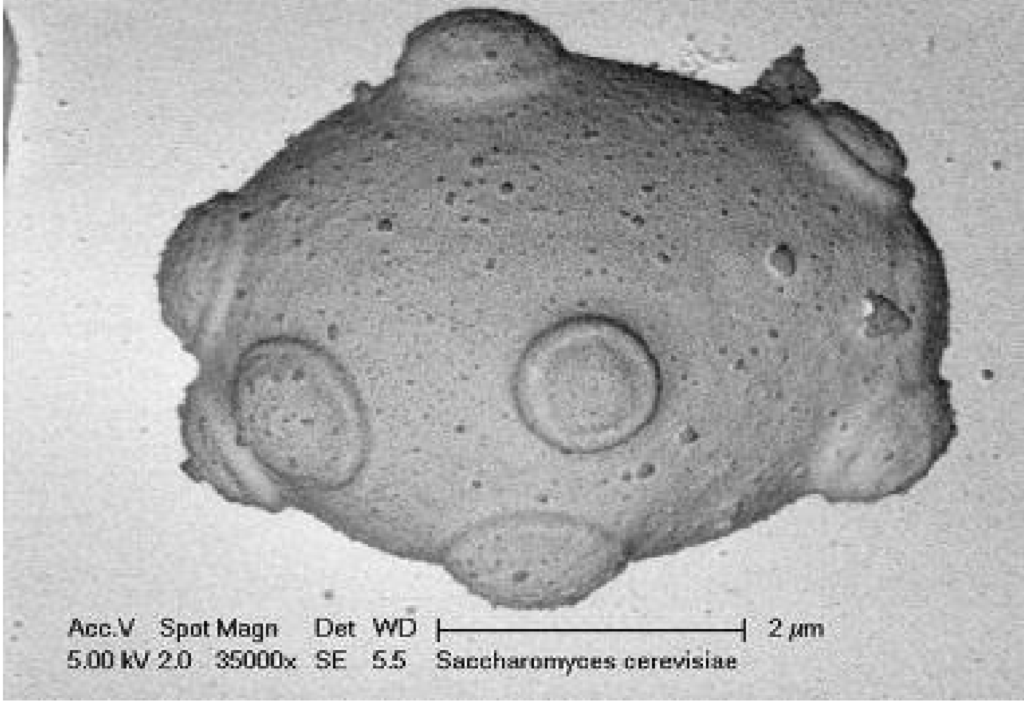
Genetic analysis of yeast has revealed that local species contribute enormously to the regional characteristics of wine.

Saccharomyces cerevisiae is a seemingly humble single-celled fungus. However, humans owe an awful lot to this species of yeast. This debt originated before the dawn of civilisation, well over 7000 years ago, when humans first unknowingly employed yeasts to make wine, beer and bread. Today these yeast products are cornerstones for our modern, sophisticated and enjoyable social lives, and help drive economies – especially in Australia and New Zealand through the fermentation of wine.

Today *S. cerevisiae* is also used in cutting-edge molecular genetics and cell biology research, which includes Nobel Prize-winning advances in understanding the fundamentals of cell division related to cancer.

While we know a lot about how yeast cells work, surprisingly we know very little about the ecology of yeast. My laboratory is attempting to make in-roads into understanding why *Saccharomyces* yeasts convert fruit juices into wine and whether populations of these yeasts vary from area to area. Our recent research has shown that wine is the by-product of *S. cerevisiae*'s sabotage of competing microbes in fruits, and that New Zealand has a distinct population of *S. cerevisiae*.

If different regions harbour different populations of wine yeasts, then the use of these region-specific yeast in winemaking means that the resulting wine more faithfully reflects the sense of place – or *terroir* – of the wine. In short there may be a microbial component to terroir.



The story really begins, and only makes sense, once we consider the ecological and evolutionary forces that have shaped *S. cerevisiae*, and thus our interaction with this species.

The consumption of wine has some health benefits: as well as aiding digestion and being the perfect accompaniment to a good meal, wine contains antioxidants and has positive cardiovascular benefits when enjoyed in moderation. However, excessive alcohol consumption leads to many physiological and social problems – after all, ethanol is a toxin.

Why do humans actively consume a poison? Most higher primates eat a considerable amount of fruit: this makes sense as fruits constitute a valuable store of energy and vitamins. Individuals more adept at finding fruits, particularly easily digestible ripe fruits, have a selective advantage over those who are not.

One way in which ripe fruits are located is by following ethanol plumes they emit as they begin to ferment. Those who are better able to detect and follow the ethanol, which signals the presence of ripe fruits, and tolerate the ethanol when the fruit is consumed, will likely outcompete those who can't.

Quite simply, these individuals obtain more food and are thus more likely to survive and reproduce. They will consequently pass their ethanol-liking genes down through generations. It seems, then, that an appetite for ethanol is ingrained deep in the higher primates' family history.

Human tolerance of ethanol was probably consolidated once fruits were gathered and made into wine for one simple reason: waterborne diseases. Drinking of water before the advent of modern water purification technologies was a potentially lethal pursuit due to the host of parasites and pathogens contained in the water. Those individuals who drank more water than wine would be less likely to survive. Since ethanol sterilises drinks, wine drinkers were more likely to survive and pass on their wine-drinking genes to the next generation.

Thus, disregarding psychological reasons, certain individuals probably have a liking for alcohol as they descend from a long line of ancestors who also did.

But why did humans bother gathering grapes and making them into wine? They clearly had no knowledge of the intricacies of modern microbiology and thus no understanding that water harboured diseases but could be made safe by the addition of ethanol.

The answer is likely a more practical one: fruit cannot be stored but wine can. Since fruits are seasonal, one can easily imagine that early attempts to store fruits for the lean winter months would have involved filling large vessels and putting these in a safe place. Of course, without the advantage of modern freezers the fruit would have rotted. Fruit rotted by yeast is wine.

It would not have necessarily taken the boldest of individuals to have given the putrefied contents of this jar a taste: the rest is history. So, apart from tasting nice, wine and its host of vitamins and beneficial compounds can be stored for much longer than fresh fruits.

We do exactly the same thing today: gather fruits once they are ready, put them in big containers and let the fruit rot by yeasts. Rotted grapes are now one of the most sought after and prized beverages on the face of the planet.

Modern winemaking has evolved to become a highly sophisticated process. Humans have taken wine grapes and bred them to create a number of different varieties, each with different properties. Many of us are not only able to easily differentiate between wines made from different types of grapes, but from grapes grown in different regions and possibly in different years. Indeed, this is part of the enjoyment of wine: it is not a consistent product but influenced by the type of grape and the region in which it is grown.

This is the core of the long-standing French concept of terroir, which roughly translates into "the sense of place" of a wine. The particular soil type and microclimate in a specific location, coupled with a particular variety of vine, potentially produces a unique type of wine. For example, a Barossa shiraz or Marlborough sauvignon blanc is notably different from a French syrah or sancerre, even though the grape varieties are the same.

Vines planted in different regions clearly produce different wines, so the concept of terroir make sense. However, wine is not simply grape juice where the sugars have been converted to ethanol: approximately half of the volatile compounds that contribute to the smell and taste of a wine are derived from yeast during the fermentation process.

Surprisingly we know relatively little of the natural process of fermentation. This is probably due to the fact that fermentation is conducted by microbes that we have only been able to study properly relatively recently with the advent of modern scientific instruments and techniques. For example, humans have been propagating and experimenting with vines for more than 5000 years but yeasts were only shown to be the agent that conducts fermentation by Pasteur in the last 100 years or so. Before this it was believed to be a magical process!

Modern molecular genetic techniques mean that yeasts can be identified relatively easily to the species level. These techniques have been used to get an idea of the microbes associated with grape juice.

Many yeast species are associated with grape juice derived from commercial vineyards. However, the number and types of species may vary dramatically between samples. We have no clear idea of the nature of the distribution of these wine microbes, nor a good idea how or why these species vary in space and time as they do.

Thus we have very little understanding of the ecology of microbes associated with wine. Understanding this ecology offers not only a window into fundamental ecological processes generally, but also a window into the applied and commercially relevant science of winemaking.

Although we know little of the yeast diversity associated with grape juice, one strikingly consistent observation is that *S. cerevisiae* is extremely rare in grape juice brought in from the vineyard. The vast majority of yeasts initially present in juice are known as non-*Saccharomyces* yeast species. These are yeasts that can grow in grape juice but can't ferment well. They need oxygen to grow and don't make much ethanol. Put simply, these yeasts cannot convert grape juice into wine. So what changes during fermentation?

If one crushes grapes and leaves these in a large container, initially the non-*Saccharomyces* species begin to grow. However, *S. cerevisiae* is also present in this microbial community at very low frequencies. Even though it starts out as only a small component of the community, *S. cerevisiae* increases and eventually displaces the other microbial species, turning grape juice into wine as it does so.

All things considered it's a remarkable observation: that one member of a community, from such relatively small numbers, eventually displaces the others. How is *S. cerevisiae* able to invade the fruit niche? To understand this we need to know a little about how organisms deal with sugar.

Yeast and humans, and pretty much everything in between, use carbohydrates as a source of energy. The first step in this energy-harvesting pathway is glycolysis (sugar-splitting), through which glucose is split into two three-carbon chains called pyruvate. The breaking of these bonds releases two molecules of ATP, and a lot of energy is wasted into the environment as heat. ATP molecules may be thought of as biological batteries that store and transfer energy for the cell to drive other reactions, such as building biomass.

While there is still a lot of energy stored in pyruvate, pyruvate cannot be broken down any further in the absence of oxygen. In order to prevent the process backing up, the cell must remove excess pyruvate. In humans this is converted to lactic acid, but *Saccharomyces* yeasts first strip out a molecule of CO_2 (this is where the bubbles in sparkling wine and beer come from and why bread rises). This leaves ethanol, which diffuses out of the cell.

In the presence of oxygen, however, pyruvate may be completely respired to yield about 38 molecules of ATP. Fermentation harvests only about 2% of the energy locked in glucose and is around 18 times less efficient than respiration.

In an environment with high sugar and oxygen, the sensible strategy is to respire the glucose to yield as much energy as possible. While most organisms do this (including humans), yeast don't. Why do *Saccharomyces* yeasts preferentially ferment sugars, even in the presence of oxygen?

Even though it is the energetically wasteful strategy, fermentation produces ethanol, heat and CO_2 and generates a hot, suffocating alcoholic environment that serves to poison the other microbes in the fruit niche. Until recently this attractive hypothesis had not been proven, but recent work in our lab was the first to experimentally demonstrate that, by modifying the environment and sabotaging their competitors, *Saccharomyces* yeasts ensure that they secure the valuable resources in the fruit. Thus, *Saccharomyces* yeasts make wine from grapes as a by-product of killing their competitors. Humans have learned to harness this process.

Fruits are found, and wine is made, in many areas of the globe. In the past 40 years or so, specific strains of *S. cerevisiae* have been isolated and are sold as commercial wine starter cultures that may be deliberately added at high numbers in an effort to increase the consistency and reliability of ferments. However, for more than 7000 years humans relied on *S. cerevisiae* strains that were naturally present to do their work and make wine, and many still do so today.

Yet we actually know very little of the diversity and distribution of *S. cerevisiae*. To date we have had very little idea of where the microbes contributing to spontaneous ferments come from.

One school of thought suggests that *S. cerevisiae* associated with wine is rarely found in nature but instead inhabits wineries. Another school of thought suggests the opposite – that these yeasts come from the local environment.

Our group was interested in this question, and chose to scrutinise a spontaneous ferment of chardonnay grapes from Mate's vineyard at Kumeu River in West Auckland, New Zealand. We employed the same technique used in the forensic

DNA profiling of crime scene samples to identify between and ascertain the relatedness of different strains of *S. cerevisiae*.

The first striking finding was that this one ferment harboured approximately 100 different strains of *S. cerevisiae*, and relatedness analyses suggest that they cluster into six different but connected groups. Since many wineries employ commercial wine strains to conduct the ferment, the first thought was that these strains simply represent commercial strains escaped from neighbouring wineries. So we compared these profiles to a comprehensive database of commercial strain profiles we have generated and found that none of them matched even closely.

This supplies evidence that a natural population of *S. cerevisiae* resides in New Zealand. Since we failed to find any yeasts in Kumeu River's winery between vintages, we hypothesised that these strains derived from the local environment.

We went to a local vineyard only 6 km away and sampled the soil, flowers and vine bark. We found and identified roughly 20 strains of *S. cerevisiae*. When we included these in the relatedness analyses we found that these local soil/flower/bark isolates clustered strongly with some of the groups from the wine ferment. This supplies reasonable evidence that some of the strains in the Kumeu River ferment derive from the local environment. How, then, might strains travel this distance and find themselves in the ferment?

It has long been suggested that yeasts may be dispersed by insects, but until now there has been no direct evidence to support this. We sampled an apiary situated close to the vineyard we sampled and Kumeu River. We managed to isolate *S. cerevisiae* and found that the DNA profiles were very closely related to strains isolated from the soil/bark/flowers. Our inference, therefore, is that at least bees may move *S. cerevisiae* on a scale of a few kilometres.

This was the first study worldwide to show that yeasts in spontaneous ferments derive from local populations, and may be brought in by insects. We then asked how the population of *S. cerevisiae* inhabiting New Zealand is related to other *S. cerevisiae* from around the globe. To do this we compared the New Zealand isolates to a global collection of geographically and genetically diverse strains of *S. cerevisiae*.

The prominent finding was that none of the New Zealand strains were even remotely related to any of the international strains – the New Zealand population stood out by itself. This was the first study to show that an area contains unique microbes associated with winemaking and provided strong evidence that New Zealand harbours a genetically distinct population of *S. cerevisiae*.

This brings us back to the concept of terroir. While it is widely accepted that the particular climate and soil conditions within an area serve to make distinctive wines, there is also a microbial component to terroir. More than half of the volatile compounds that contribute to a wine's aroma and flavour come from yeasts during fermentation, and there is strong evidence to show that different *S. cerevisiae* strains have significantly different effects on a wine's aroma and flavour.

If different areas harbour different microbial communities, as we have shown, then it seems reasonable that, given the contribution of yeasts to a wine's aroma and flavour, these also potentially contribute to the distinctiveness of wines from different areas.

The use of local yeasts to make wine is precisely in keeping with the terroir concept. These wines more faithfully reflect their sense of place as they are produced by the particular microbial communities inhabiting that area.

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