



CLAUDIA SCHAEFER PHOTOGRAPHY

Researchers at the University of British Columbia hope that by analysing cannabis diversity, they can determine whether it is one species or several.

## BOTANY

# The cultivation of weed

*Researchers are getting closer to answering the centuries-old question of how to label cannabis varieties — a necessary step to bring the plant into mainstream agriculture.*

BY LUCAS LAURSEN

Packets of cannabis seeds line the shelves of legal grow shops in Madrid. Many carry labels reporting the percentage of sativa and indica, two types of cannabis. Breeders often label plants that produce a more exciting high as sativa and plants that provide a more mellow feeling as indica, suggesting that cross-breeding tailors that buzz. The conceit is widespread. Botanist Jonathan Page at the University of British Columbia in Vancouver, Canada, says he sees the same at local grow shops.

For reasons that go beyond assessing the quality of the user experience, botanists such as Page are investigating the evolution and present-day diversity of cannabis. To do this, they must confront centuries-old taxonomic questions, including whether cannabis is one species, *Cannabis sativa*, with several subspecies or varieties, or if it is several distinct species, such as *C. sativa*, *Cannabis indica* and *Cannabis ruderalis*. “It’s complicated taxonomically because of its intimate relationship with humans for long periods of time,” Page says. People have long bred cannabis as a source of fibre, food and oil — as well as for its mind-altering effects

(see page S10). As governments relax cannabis laws, commercial growers want more clarity about the chemical properties and capabilities of the herb’s many varieties. In parallel, regulatory bodies trying to establish a legal framework want to be able to classify whether a given type of plant is for fibre (hemp) or recreational or medical use (marijuana).

Demand for such information is pressing. Last year, the United States granted permission for farmers to grow hemp for research purposes. Several states, including Colorado, have legalized the possession and use of small amounts of marijuana, and are beginning to integrate the plant into the legal economy. Elsewhere, Uruguay has legalized cannabis and other governments are relaxing restrictions on its possession and use. As academic and commercial interest grows, governments and the research community will encounter a rising demand for taxonomic information to help resolve disputes, establish registered cultivars, and create reliable centralized databases of cannabis information. Botanist Ernest Small of the government agency Agriculture and Agri-Food Canada, says that talking about cannabis taxonomy “is

really talking about the ability of countries to rationally regulate important drugs and products.”

## BLURRED LINEAGES

Cannabis diverged around 27.8 million years ago from *Humulus*, the hop plant used to give beer its bitter and floral flavours, according to genetic analysis presented at the International Cannabis Research Society’s 2010 meeting by botanist John McPartland and Geoffrey Guy of London-based GW Pharmaceuticals. Human influence on its diversity is more recent, but still stretches back millennia. The earliest archaeological evidence for human use of the plant comes from hemp ropes found in 10,000-year-old tombs in Taiwan. Cannabis now grows throughout much of the world, and humans have almost certainly had a role in shaping its many forms.

The plant is promiscuous, which confuses the species issue. Most known lineages seem to be capable of producing viable offspring from crosses with each other. Lines domesticated by humans may also have mixed with wild plants, blurring the taxonomic boundaries further.

The first modern taxonomist, the Swede Carl Linnaeus, used geographic origin and

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sex organs to identify five variants of a single species, *C. sativa*. Later, the French naturalist Jean-Baptiste Lamarck used morphology and chemistry to distinguish *C. sativa* from a shorter, less fibrous and more psychoactive species, *C. indica*.

Debate continued throughout the twentieth century. The US botanist Richard Evans Schultes favoured a third species — *C. afghanica*. Small, however, disputed this, maintaining that the genus *Cannabis* had only one species, with several variants that had been selected for by humans. Small's expertise even took him into the courtroom to dispute lawyers' claims that the plant their clients had been caught with was a different — and hence unregulated — species from the *C. sativa* banned by law. Although many botany guidebooks and researchers now agree with Small's view, there is still debate — stoked whenever another scientist revisits or champions the arguments for multiple species — within the grower and user communities. “The issue is exaggerated and tends to mislead people,” says Small. “I almost feel that it's better not to talk about it anymore.” Yet, he and other cannabis researchers continue to encounter public demand for clarity.

Small continues to do research and has even provided authorities with a means of distinguishing between drug-type and non-drug-type cannabis — a chemical threshold. Thanks to Small's work examining the natural range of tetrahydrocannabinol (THC) concentrations, some governments are able to sidestep the taxonomy question by counting plants with less than 0.3% THC as hemp and those with more as marijuana. This has allowed the Canadian agriculture industry to cultivate groups of plants with stable characteristics and register these as formal cultivars of hemp without fear of running afoul of drug laws.

## MOLECULAR AND GENETIC TECHNIQUES

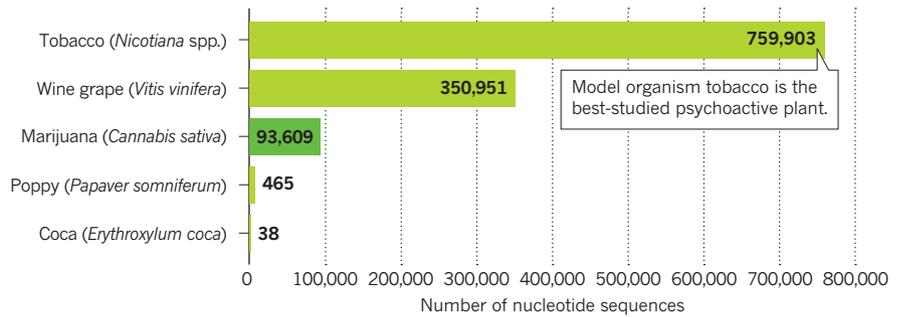
A chemical threshold is useful, but an official taxonomy would provide a clear and common language for researchers, regulators, growers and users to share information about the plants. In the past 20 years, researchers have turned to a variety of molecular and genetic techniques to tackle some of the questions that previous generations sought to resolve through morphology.

In 2004, biologists Paul Mahlberg and Karl Hillig, both then at Indiana University in Bloomington, analysed the enzyme-encoding genotypes of 157 sample varieties. Based on proportions of CBD and THC levels, they suggested that there are two species, *C. sativa* and *C. indica*, that contain six subspecies<sup>1</sup>. Hillig later published a broader study identifying three species — adding *C. ruderalis* to the mix<sup>2</sup>.

Genetic analysis, however, may not offer an immediate resolution to taxonomic debates: a 2003 study<sup>3</sup> examining THC/CBD ratios identified five different lineages of cannabis, but all within one species. And in 2013, in perhaps the most comprehensive book on the subject,

## GENOTYPING OUR HIGHS

Fewer published nucleotides exist for cannabis than for tobacco and grapes. Recent interest may narrow the gap.



botanist Mark Merlin of the University of Hawaii at Manoa and cannabis researcher Robert Clarke of the International Hemp Association in Amsterdam argued for three species of cannabis (*C. sativa*, *C. indica* and *C. ruderalis*), divided into a total of seven subspecies<sup>4</sup>.

Researchers continue to bring ever more sophisticated genetic tools to bear. In 2011, Page and his colleagues published a draft set of the DNA and RNA of a marijuana plant (*C. sativa*) and compared the RNA with that of a hemp cultivar. They found tantalizing differences in the expression of cannabinoid-controlling genes<sup>5</sup>. Botanist Nolan Kane of the University of Colorado Boulder is working with colleagues on a ‘genetic map’ that will involve complete DNA sequencing of some plants. They are also using a faster, cheaper method called genotyping by sequencing to study about 500 plants. By the end of 2015, they aim to have placed around 60,000 genes — about double the number reported by Page's group — onto the plant's 10 pairs of chromosomes. In addition, Kane's team has been working on determining the complete DNA sequence of 66 individual plants, with plans to extend to several hundred more. This work could be used to provide information about breeding new plants, and could also afford an “unprecedented” insight into the relationships between many of the major lineages of *Cannabis*, says Kane.

## INDICA BY ANY OTHER NAME

Names and well-defined lineages matter because they help researchers to know what they are working with. “Many taxonomic studies and genetic studies work with *Cannabis* hybrids, and generate inconclusive results,” McPartland says. Establishing groups of plants with stable features, each with some known characteristics such as certain THC and CBD levels or ideal growing conditions, could help pharmaceutical firms and others to exploit the plant (see page S6). What is more, without a clear taxonomy, existing lines with unique and useful traits may be neglected and even go extinct, he warns. McPartland's own research

suggests that some northern European strains have already disappeared.

This type of scientific omission might seem odd for such an apparently valuable plant. *Vitis vinifera*, a grape species used for making wine, has been subject to several genome-wide studies so far, and its cultivars are a matter of economic interest and national pride. And, tobacco (*Nicotiana tabacum*), although heavily regulated, is a model organism in basic biological research and has a well-documented pedigree (see ‘Genotyping our highs’). But given cannabis's regulatory history and its stigma in many cultures, perhaps it is not surprising that there has been some reticence about its study. “This is a sensitive subject,” Small says.

However, with an ever-growing number of jurisdictions permitting research and creeping towards cannabis commercialization, the need for a solid taxonomy is clear. Grow shops, with their labelled wares, are providing researchers with a bounty of specimens against which to test such ‘folk taxonomies’. This year a study<sup>6</sup> of 81 commercial marijuana samples demonstrated that the advertised percentages of *sativa* and *indica* show little correlation with the genetic reality. Unlike hemp, with its genetically stable registered cultivars, “in the marijuana world we don't have varieties or registered cultivars — we have things called strains”, says Page. Strains are informally named by breeders and are not associated with a genotype in the same way that formal varieties or cultivars are. “You need to put a name to something to [research] it accurately,” Page says.

“What is a species is a somewhat subjective concept,” says Small. Whether a group of plants is a cultivar, a subspecies or a species may matter less than that everyone agrees on their evolutionary relationships. ■

Lucas Laursen is a freelance science writer based in Madrid.

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