How to Make a Meconopsis by Professor David Rankin

Matthew Heasman, committee member, introduced David Rankin.

The Meconopsis Group was set up 20 years ago because it was realised that we didn't really know a lot about the blue *Meconopsis* in cultivation, what to call them, what GS 600 was and where all the hybrids came from. Over the years we have made a great amount of progress. The naming of the plants has advanced enormously. There isn't yet a definitive key but we know their characteristics. There is also a lot we don't know about them – for instance, how did *Meconopsis* 'Lingholm' originate?

An opportunity arose when a letter came from Australia from a researcher who worked for a company involved in DNA analysis and who wanted to investigate Meconopsis. We realised that this might be very useful and might answer questions about how the plants relate to one another. It was mutually agreed that we would send him lots of leaf samples for investigation. Samples have been collected over the past year, to be sent to Australia. There are no results as yet but The Group will be regularly informed on the progress of this project.

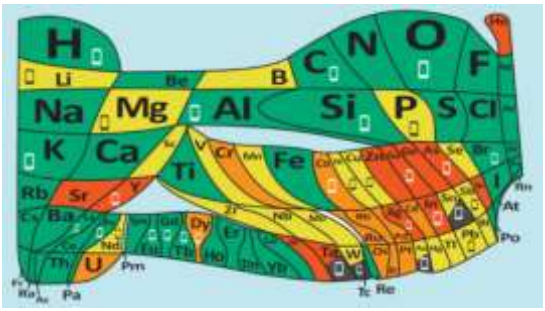
David then outlined his plan for the rest of the talk, which would be an introduction to DNA and how it relates to *Meconopsis*. If we understand the differences in the DNA perhaps we can begin to understand the differences between different *Meconopsis*.

How do you make a Meconopsis?

a) We need all the components plus the instructions. The instructions are all in the DNA, which is made from just six chemicals.

b) These are phosphate, a sugar and four organic molecules. These organic molecules, known as bases, are adenine, thymine, guanine and cytosine, usually abbreviated as A, T, G and C.

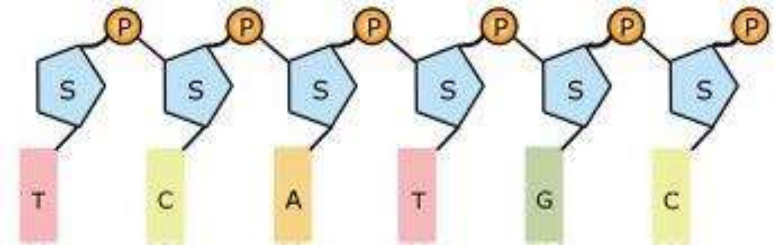
c) There are just five elements found in the compounds listed above which are needed to make the instructions: carbon C, hydrogen H, oxygen O, nitrogen N and phosphorus P.



d) As 2019 is the International Year of the Periodic Table, David showed the version of the table shown here, which indicates their abundance in the earth's crust. Each area is on a logarithmic scale.

Of the 90 naturally occurring elements, plants contain 16 – the five in DNA, plus potassium K, calcium Ca, magnesium Mg, zinc Zn, sulphur S, chlorine Cl, boron B, iron Fe, copper Cu, manganese Mn and molybdenum Mo. In contrast, a typical mobile phone contains 30, many of which are very scarce. The importance of recycling was emphasised..

e) The four base compounds can be joined together in a chain using the sugar and the phosphate as links to form long DNA chains.



f) So far it seems straightforward, but **those bases (letters A, T, G, C) must be in the right order** – and there are a lot of them – to make the correct instructions for a particular plant.

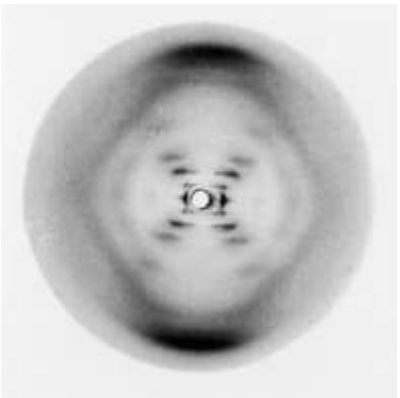
g) The smallest number of letters in a plant is found in bladderwort – 80 million, *Paris japonica* has 149 billion.

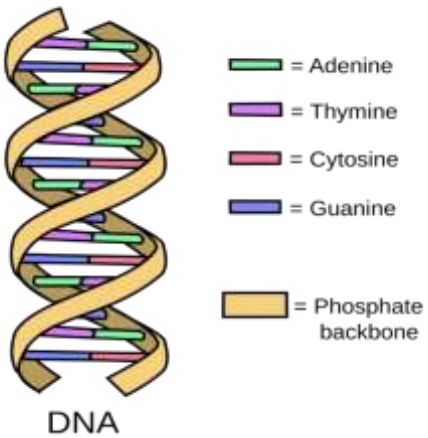
There are two billion **2000000000** bases in *Meconopsis* DNA, which are present in every cell – but the total weight is less than 1 billionth of a gram.

h) Once the letters are in the correct order it is possible to copy them. So to make a *Meconopsis* we need to start with the DNA from an existing *Meconopsis*.
A (alanine) and T (thymine) have shapes that are complementary, so one of them will link to the other one. Similarly, C (cytosine) usually links with G (guanine). So if we start with one strand of DNA we can make the complementary strand.

ATTCGTGAACGCTAGACTTAA
TAAGCACTTGCGATCTGAATT

Repeating this process with the complementary strand results in a copy of the original strand being made. Each time this procedure is repeated the number of copies is doubled. So from one tiny amount of a particular DNA, you can make a lot of it.





DNA

This happens naturally when cells divide. The process could be understood once the structure of DNA was known. Rosalind Franklin worked on the X-ray crystallography of DNA fibres, and obtained the images that allowed to structure to be deduced. Crick and Watson published the famous model of DNA, with the phosphates were on the outside of a double helix.

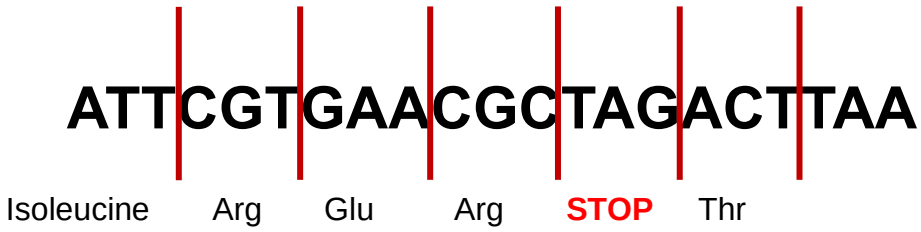
It has been said that this is the most important photograph ever taken. And possibly the most important sentence in scientific literature comes from Crick and Watson. In their paper they said that "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material."

i). **What does DNA do?**

The sequence of the four letters is a code which determines the structure of proteins. The code determines the characteristics of all living things.
In humans there are 75,000 enzymes, 20,000 genes and 23 pairs of chromosomes. There are big differences between species, but also smaller variations between individuals of each species.

Enzyme proteins catalyse the production of everything in the plant, which account for all of its charateristics.
Some enzymes determine the ability to photosynthesise – a characteristic of all green-leaved plants.
Other characteristics, such as the structure of the seed capsule, determine whether a plant is a member of the genus *Meconopsis*.
Within the genus *Meconopsis* there are variable characteristics – not the same for all *Meconopsis* – including flower colour, whether blue, red, yellow or a mixture of colours, the shape of the leaf, smell, hardiness etc.

j) All the different protein enzymes in virtually all living things are produced from the same four letters found in the DNA – **A, T, G** and **C**.
Proteins are made of 20 different amino acids.
Groups of 3 letters code for a particular amino acid.
The order of the amino acids determines the properties of the protein.



(Arg – Arginine, Glu – Glutamine, Thr – Threonine)

second base

first base	T	C	A	G	third base
T	TTT Phe	TCT Ser	TAT Tyr	TGT Cys	T
	TTC Phe	TCC Ser	TAC Tyr	TGC Cys	C
	TTA Leu	TCA Ser	TAA stop	TGA stop	A
	TTG Leu	TCG Ser	TAG stop	TGG Trp	G
C	CTT Leu	CCT Pro	CAT His	CGT Arg	T
	CTC Leu	CCC Pro	CAC His	CGC Arg	C
	CTA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CTG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	ATT Ile	ACT Thr	AAT Asn	AGT Ser	T
	ATC Ile	ACC Thr	AAC Asn	AGC Ser	C
	ATA Ile	ACA Thr	AAA Lys	AGA Arg	A
	ATG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GTT Val	GCT Ala	GAT Asp	GGT Gly	T
	GTC Val	GCC Ala	GAC Asp	GGC Gly	C
	GTA Val	GCA Ala	GAA Glu	GGA Gly	A
	GTG Val	GCG Ala	GAG Glu	GGG Gly	G

There is a three-base code for **STOP** to limit the length of a protein and start the initiation of a new protein.
The copying of the code is not perfect. Mistakes occur, leading to slight differences in individual individuals of the same species.
Every time a cell divides around 100,000 mistakes are made. A letter may be missed out, duplicated or changed. But there is a mechanism that corrects incorrect copies, and this mechanism is almost – but not quite – perfect. There is an interesting question about how this self-correcting mechanism came about, because it is itself dependent on enzymes, which have to be copied more or less perfectly to function properly!

If there are too many mistakes the result would be chaos. An example was given of a single base mutation which led to a child who couldn't walk or talk.

One *Meconopsis* plant produces many thousands of seeds, the majority of which are viable. But on average only one of these viable seeds grows to maturity to pass on genes to its offspring. Seedlings may differ from their parents due to changes in their DNA. Natural selection tends to favour beneficial changes, so that new generations are able to survive better than their parents. This will, over many generations, lead to a change in the DNA of the species.

Species of living organisms

Over time populations in different places may evolve different DNA because they are isolated from one another. So *Meconopsis* on one mountain may be different to those on another mountain, but will still have much genetic information in common. How different their DNA is depends on how long they have been separated – thousands or millions of years.

Closely related species share more genetic information with each other than more distantly related species. For example, humans share 99% of their DNA with chimpanzees, but only 45% with cabbages!

Populations may be isolated by distance. For example, the three subspecies of *Meconopsis wilsonii*.are in different areas of the Sino-himalaya, separated by hundreds of kilometres. *Meconopsis wilsonii* subsp. *wilsonii* is found in SW Sichuan, *Meconopsis wilsonii* subsp. *orientalis* is found in NE Yunnan and *Meconopsis wilsonii* subsp. *australis* is found near the Myanmar border in NW Yunnan. They are genetically distinct.

Populations may also be isolated by altitude, flowering time, habitat and pollinators.

The project

By comparing DNA for different *Meconopsis* we can find out how they are related to each other. The bigger the difference, the longer ago they separated from one another.
Only a tiny amount is needed, as the copying procedure increases it indefinitely.
The DNA is taken from leaf samples.

i) Collect samples (10 sq cm) of leaves
ii) Dry with silica gel. This removes the moisture from the leaf. The silica gel changes colour as it dries the leaf.



iii) Take photographs of each specimen plant e.g both sides of the leaf, the flower, the fruit capsule, and the whole plant. Ideally a herbarium specimen should also be taken.

All of these procedures ensure that the specimen plant can be exactly identified.

iv) Label the sample and keep records of where and when it was collected and who collected it.

v) Send samples to Australia.
The samples are sent to Jason Carling in Canberra who is analysing them using Diversity Array Technology sequencing (DArTseq).
This method targets the most significant part of genome (about 1.5 %) avoiding repetitive DNA fragments. The method targets the Exosome, which is the protein-coding portion of the genome.
'Next Generation Sequencing' gives the letter (base) sequence.
Once the sequences of letters are is known each individual *Meconopsis* studied can be compared with others.

The cost of analysing DNA has decreased dramatically. In 2001 it cost thousands of pounds to analyse a million letters, in 2017 it cost pennies for the same analysis.
It is being done for The Meconopsis Group for nothing.

David also mentioned The Tree of Life Project, which will use published DNA data to relate all living organisms Its aim is to study the whole genome of all plant and fungi species. It is expected to take ten years to complete,

What do we hope to discover?

So far five people have collected 130 samples from 65 plants. This represents 10 sections out of 18 in the genus, and 18 taxa out of a possible total of 81.

Questions we hope to answer

- How different is the DNA between subgenera, sections and series?
- Is the DNA of species within each category consistent?
- How different is the DNA of different species?




For example, we hope to see how *M. integrifolia* is related to the three other similar species, *M. lijiangensis*, *M. sulphurea* and *M. pseudointegrifolia.*

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Then we may be able to decide whether this plant is one of these species, or something else.

Similarly, we will be able to look at the differences between the sub species of *Meconopsis wilsonii*, referred to earlier.

Hybrids

- Can we work out the parentage of the hybrids that are grown?

So far four hybrids have been sampled.
The genetics should confirm that the parents of *M* x *cookei* are *M. punicea* and *M. quintuplinervia*. Then we may be able to answer other questions.

- What is 'Sichuan Silk'? Is it a perennial form of *M. punicea* or is it *M* x *cookei* x *M punicea*?
- What is *M.* 'Lingholm'?
  Is it *M. grandis* x *baileyi* with chromosome doubling?
  If so, should be it a new species?
- What is the parentage of *M*.'Inverewe'?
  Is it *M. bailey?*
  If so, why is it infertile?

Named clones

- Can we distinguish varieties by their genetics?
  Can we work out their parents?
  Are they hybrids?
- What exactly is GS 600?
- Is *M*. 'Dalemain' the same as *M*. 'Huntfield'?

So far four named clones have been sampled.

Some supposedly infertile clones do set a few seeds, for example *Meconopsis* 'Jimmy Bayne'. One plant arising from seed is on display at Branklyn. Is that identical to its parent, or a hybrid?

What next?

So far just a few people have been involved
The Group hopes to include more people, especially

- if you grow unusual species
- if you can help practically with samples
- if you can enter data.

There will be regular updates at Meconopsis Group meetings.

Matt thanked David for the talk.