Can we use Molecular Biology methods to help naming *Meconopses*? Part 3
by Prof. Adam Curtis

(Written up by P. Anderson)

The objectives of Professor Curtis' work are:

1. To see if he can measure how alike DNA from two species or cultivars of *Meconopsis* DNA are and
2. If they are slightly different, is this reason to assign different names to each cultivar?

Professor Curtis used an analogy of doing a jigsaw to illustrate the principle of how he analyses how alike two DNAs are. Imagine you are doing a jigsaw that is two pieces wide, but very, very long. It should be fairly simple to assemble the jigsaw along its whole length . However, if some pieces from another similar, but not identical jigsaw are added they can be incorporated into the jigsaw. The completed jigsaw will be imperfect and will easily break into pieces because the match between the two strands of jigsaw is imperfect.

[Section inserted by P Anderson to explain rationale of experiments. The theory of the experimental methods used in these analyses were described in Professor Curtis' talk at the Meconopsis Group Meeting of 8/3/08. Briefly:

Structure and properties of DNA

- In a living organism all the DNA is present as a double strand and each strand is a complementary copy of the other strand.
- The strands are bound together by base pairing. An A in one strand always pairs with a T in the other strand and a G in one strand with a C in the other (where A=adenine, T = thymine, G = guanine and C=cytosine). The more accurate the base pairing the stronger the binding of one strand by the other. However if there is mispairing (e.g. an A in one strand opposite a G or C in the other) the DNA strand will not be base-paired at that position. The more mismatches in DNA, the less strong the binding of one strand to the other.

Behaviour of DNA on heating

- The two strands of DNA from an organism can be separated by heating to 80-90^{o}C, (this is called melting) to give what is termed single-strand DNA. These two strands will recombine and the bases will match up perfectly when the temperature is reduced slowly again.
- But if one DNA strand comes from one organism and the other from a closely-related, but not identical organism, the recombination is not perfect. There will be mismatching of bases and some single-strand DNA will remain.
- If a molecule of DNA that has mismatches is heated, it will melt at a lower temperature than a molecule that is completely double-stranded. So monitoring the temperature at which recombined DNA melts can tell us how well-matched the strands of the two cultivars were.

Behaviour of DNA on hydroxyapatite columns

- Double-stranded DNA binds to hydroxyapatite (a form of calcium phosphate), but single-stranded DNA does not. So, if a mixture of single- and double-stranded DNA is applied to a hydroxyapatite column, the single-stranded DNA will pass through the column (it will be eluted) whereas the double-stranded DNA will be bound.
- If the temperature of the column is raised, double-stranded DNA with many mismatches will become single stranded and will be eluted. Then as the temperature is raised further, double-stranded DNA with a few mismatches will become single-stranded DNA and will be eluted. Finally, as the temperature is raised even further, perfectly-matched DNA will become single-stranded and will be eluted.

Rationale of experiments

- If double-stranded DNAs from two Meconopsis cultivars (A and B) are heated, single strands will form as described above. If the A and B single strands are mixed and the temperature is reduced slowly, double-stranded DNA will form. Some molecules will be composed only of A strands, some only of B strands and some will have one strand of A and one strand of B. These latter are called hybrid DNA molecules.
- Some of the bases along the hybrid molecule will be 'perfectly-matched' and some will not. Mispairing of bases has the effect that when the hybrid molecules are heated, they come apart (melt) at lower temperatures than if they are perfectly matched. The better the DNA strands are matched, the higher is the melting

temperature. And the less well the DNA strands are matched, the lower is the melting temperature So the thermostability of hybrid molecules is a measure of how closely related the cultivars/ species are.

- Experimentally, the behaviour on hydroxyapatite of recombined DNA of cultivar A alone, of B alone and a mixture of A and B together is analysed. If A and B are closely related, the mixture of recombined (A+B) DNA will be eluted at a fairly high temperature, but if they are not closely related, the recombined (A+B) DNA will be eluted at a lower temperature by virtue of the many base pair mismatches. DNAs

Experimental

Professor Curtis' group isolated DNA from the pollen of 20 different named *Meconopsis* strains and species from Scottish gardens and the Himalayas. The yield was only a few million millionths of a gram of DNA per grain of pollen. About 100,000 grains were used per sample.

This is not enough DNA to work with, so the DNA was amplified making many exact copies from each sample. This gave about a 500-fold amplification.

The next stage was to proceed to the DNA-DNA hybridisation.

They heated each sample to make it entirely single-stranded. Some of the samples were kept as they were. Other new samples were made by mixing the DNA from two strains 50:50. Then the samples were cooled to below 60^{o}C at which temperature they should (if they could) have reformed double-stranded DNA. Then they were loaded on to hydroxy apatite columns and heated in 2 or 3^{o}C steps. At each temperature the eluate was tested to see if any single-stranded DNA had formed.

Results

Professor Curtis has pollen from 20 different named *Meconopsis* for analysing DNA .

He showed results from representative experiments:

When the pairs *M. simplicifolia* and *M. Lingholm* were analysed, the hybrid DNA eluted at a much lower temperature than either of the species alone suggesting they are not the same species at all.

Similarly, *M. 'Lingholm'* and *M. 'Barney's Blue'* are very different.

In summary, he finds that: *M.* 'Slieve Donard' and *M.* 'Cecily Crewdson' are closely related

M. 'Brian Conway' and *M.* 'Crarae' are fairly closely related

M. 'Bobby Masterton', *M.* 'Maggie Sharp' and *M.* 'Crarae' are not closely related

M. simplicifolia and *M. primulina* are not closely related

M. 'Lingholm' from Tromso and *M.* .'Barney's Blue' are not closely related

M. grandis and *M.* 'Lingholm' are not closely related

Professor Curtis wants to repeat the work on larger samples of pollen. He will use pollen from 10-12 flowers. He will build up results for more varieties especially from the Fertile Blue Group.

Damp pollen is a problem because fungus can grow on it and ruin analyses. A better system of drying pollen is required. He said he was willing to distribute silica gel or take it round to the gardens of those contributing pollen so that the pollen could be dried thoroughly.

He has confidence in the methods used and sees possible extensions of the work by other techniques to give parallel and interlocking results. He thanked collectors of pollen Evelyn Stevens, Margaret and David Thorne) and Carol-Anne Smith the technician in his lab who has done most of the experimental work. He also thanked the Centre for Cell Engineering, Glasgow University for provision of laboratory facilities.

Questions

Q. Presumably it is small regions of DNA that are being amplified.

A. Yes

Q. Are you using different primers?

A. Only one. Its sequence is unknown because the suppliers of the kits won't disclose the sequence. The primer is an 18-mer (i.e. it is 18 bases in length).