Meconopsis: Seed viability testing, seed coat characteristics and phylogenetic relationships by Alan Elliott

aelliott@rbge.ac.uk

(Written up by P Anderson)

(Terms with an * are defined at the end of the article)

Background Alan's interest in *Meconopsis* stems from his time at the National Trust for Scotland garden at Branklyn three years ago. Before this he had never heard of *Meconopsis*. The year after he started the HND Horticulture at RBGE and for his Specialist Project he chose to do: Germination of *Meconopsis* from the seed bank at the Royal Botanic Garden, Edinburgh. Last year he did seed testing of *Meconopsis* for Evelyn Stevens and Jim Jermyn on behalf of the *Meconopsis* Group seed exchange.
He is now doing a three-stranded project for his Undergraduate Dissertation: Testing seed viability and germination: Looking at the morphology of external seed coats with Prof Rankin and studying phylogenetic* relationships.

Phylogenetics*
Alan's aim is to construct a cladogram• to use the different relationships within the genus as a visual aid for interpreting the results from seed germination and seed morphology studies.

Until recently there have been limited genetic studies on *Meconopsis* as a genus, except as an outgroup of Papaver. Carolan and colleagues in Ireland (2006) published a study into members of Papaver. A cladogram form this work shows *Meconopsis* nested within Papaver which is a bit tenuous as *Meconopsis* is a genus in its own right. However, the cladogram shows some anomalies and the phylogeny and current taxonomy do not agree. For example *M. cambrica* was farther away from other *Meconopsis* than many other Papaver members. So how do you resolve papaver taxonomy? Lump *Meconopsis* with Papaver or do you split Papaver to make *Meconopsis* a distinct group?

A cladogram from a Chinese student's PhD research looking at *Meconopsis* relationships showed that many *Meconopsis* species are closely related. Also the genus Cathcartia which used to have members of *Meconopsis* is an ancestral to both Papaver and Meconopsis.

Alan decided to work out his own relationships from genetic data. He didn't do any genetic testing himself, but all the information came from freely-available genetic information stored on GenBank* to create his own phylogeny. He carried out Bayesian analysis which is based on likelihoods and is a more realistic interpretation of evolution. The output shows relative differentiation on the branches so *M. lyrata* is distinct and has a lot of changes genetically. For *M. grandis* group all the branches are quite short suggesting that they are quite a recent development in the genus. The vertical bars down the right hand side of the phylogram showed Taylor's series. Alan's output based on gene two sequences agreed well with Taylor's monograph that had been made purely on morphology.

There were, however a few oddities. For example, in Alan's analysis, *M. sinuata* comes out in series Primulinae whereas Taylor had it in ser. Aculeatae. Only Series Delavayanae is not represented in the analysis.

Seed Morphology When Alan started this project he was unaware of Professor Rankin's dataset of seed images. In his background reading he found a paper on seed morphology by Sulaiman (1995) This study looked at the seed coats of 5 species of *Meconopsis* under a scanning electron microscope: *M. paniculata: M. simplicifolia: M. horridula: M. sinuata: M. villosa.* He found that differences within species were minimal whereas differences between species were marked.
He looked at seed dimensions, seed coat patterns and the differences between individual cells of seed coat. One of the main findings was that *Meconopsis* doesn't fit the agreed seed coat structure for Papaveraceae which is said to

have a reticulate pattern*. *Meconopsis* has a more reticulate*/rugose* seed coat pattern. *Meconopsis* (Cathcartia) villosa which featured in the study demonstrates the 'classic' papaver seed coat – unlike the others in the study.

So, using Prof Rankin's dataset, Alan and Prof. Rankin selected a set of characters to use for his analyses. These included angularity, colour, curvature of seeds and scanning electron microscope pictures for the seed coat. For measurements he used software that had been developed for analysing blood cells and had been used for primula studies.

So far, his results show that some seeds are very distinctive seed in terms of shape, surface texture and cell appearance. These include *M. punicea*, *M. delavayi* and *M. superba*. *M. delavayi* has a very high ratio of length to breadth and is sickle-shaped. *M. superba* is unique in the angularity of the seed and *M. punicea* because of the external seed coat cells looking like blood cells which you don't find in any other *Meconopsis* seed.

The bulk of the recording of characters has now been done and entered into the database. Additional characters will be added.

Seed from one species was chosen as a random sample. Its characteristics were measured and the characteristics of other species or hybrids were measured and were entered into a computer. The computer then compares these with all the data in its database, and calculates a 'goodness of fit' for each of them. These were then shown in order. Results showed good matches for some and poor for others. Using *M. grandis* as a random sample, the 5 best matches were all in ser. Grandes. These were *M. x harleyana* lilac form which is a cross between *M. simplicifolia* and *M. integrifolia* both in series Grandes, *M. grandis* ex ES208, *M. grandis* ex NAPE178, M. Fertile Blue Group ex Nelson. Using *M. betonicifolia* (in ser. Grandes) as a random sample, matches were poorer and the best included 2 in ser. Robustae (*M. napaulensis* and *M. staintonii*), 2 in ser. Aculeatae (*M. forrestii* and *M. aculeata* CC4643) and 1 in ser. Grandes (*M. betonicifolia* blue ex wild). *M. betonicifolia* has a reticulate coat whereas *M. staintonii* has a rugose/reticulate pattern. The seed form of *M. forrestii* is distinct as it is kidney-shaped.

Question Did the seed of *M. napaulensis* come from herbarium specimens and would thus be true CC ssw or is it *M. napaulensis* (of hort.)
Answer Yes they are "of hort"
The database needs more characters to get a +ve match.

Alan has started to analyse seed from herbarium specimens. Light microscope and in the future scanning electron microscope measurements will be carried out.

Seed Germination Study
The aim of this study is to collect seed, germinate it, monitor the germination and assess the viability of the seed. It is important to distinguish between viability and germination. Viability takes into account seeds that remain fresh at the end of the experiment and assumes that they will eventually germinate.

Seeds were sown in batches of 3x10 seeds on filter paper in Petri dishes. A weak solution of Gibberellic acid (250ppm) | was present.
Meconopsis seed germination is very sensitive to drying out, so to conserve moisture, plates were sealed in polythene bags. The bags were placed in a growing chamber in which daylight and temperature could be controlled. There was a regime of 12 hours daylight and 12 hours darkness. The temperature was 20^{o}C +/- 5, but the recorded temperature never rose above 21^{o}C.

If contamination was seen, the seeds were transferred to fresh filter paper and infected seeds were recorded and classified as dead.

At the end of 28 days germination was classified according to 4 criteria: Normal seed germination: abnormal seed germination e.g. yellowing in the chlorophyll of the seed leaves or the primary root wasn't properly formed: fresh seeds and seeds that had died.

Selected results.
All species/cultivars tested germinated to a point with the exception of *M. punicea* and *M. quintuplinervia* which failed to germinate.

- Is short term viability affected by storage conditions of the seed? For each storage time sampled, the germination of 3 x 10 seeds was assessed as above. Results were confusing. It would be expected that the viability would decrease with time of storage, but results showed the opposite. The initial reduction in viability was due to higher levels of fungal contamination at the start of the testing. However, the viability of the seed actually holds quite well over the short term.

- Does the temperature of storage affect viability? Seeds were tested after different times at ambient or chilled temperatures. Results showed that the temperature of storage made little difference to the germination.

- Does the presence of Gibberellic acid aid germination? Selected results from *M. napaulensis* seeds showed that at 91 days there was a difference, but this tailed off with increasing storage time.

What's next?
For the seed database Alan aims to add more characters and to analyse additional samples from the herbarium sheets including collections from Forrest, Rock and Ludlow and Sherriff.

Further investigation into the lack of germination of *M. punicea* and *M. quintuplinervia* which generally do not germinate well. Alan did not get any germination. He agrees with James Cobb that the reason may be dormancy because the seeds looked viable at the end of the tests.
Phylogenetics There is a PhD study in Texas which will hopefully analyse the missing series. New species will be added as they are genetically recorded.
Alan thanked Evelyn Stevens and James Cobb for seed: John Mitchell for help: David Rankin use of his data set for seeds and the SRGC for financial support

Definitions
Clade comes from the Greek 'klados' meaning branch. A clade is a lineage branch the results from splitting in an earlier lineage
A **cladogram** is a branching diagram like a family tree that shows how different species are related to each other and when they diverged from each other.
GenBank is a database that contains all the genetic sequences that have been published
Germination The beginning of growth of a seed or spore.
Phylogenetics The study of evolutionary relationships based on genetic data. It looks at relationships within and between taxonomic levels particularly the patterns of lines of descent, often branching, from one organism to another.
Rugose having many ridges, wrinkled
Reticulate marked with a network pattern

References
Carolan J.C., Hook I.L.I, Chase M.W., Kadereit J.W., Hodkinson T.R., (2006), Phylogenetics of Papaver and related Genera based on DNA Sequences from ITS Nuclear Robsomal DNA and Plastid trnL Intron and trnL-F Intergenic Spacers, *Annals of Botany,* **doi:10.1093/aob/mc10789**, freely available online at www.aob.oxfordjournals.org

Sulaiman I.M., (1995), Scanning Electron Microscopic Studies on Seed Coat Patterns of Five Endangered Himalayan Species of *Meconopsis* (Papaveraceae), *Annals of Botany*, **76:**323-326