THE IMPORTANCE OF POLYPLOIDY IN MECONOPSIS WITH PARTICULAR REGARD TO THE BIG PERENNIAL BLUE POPPIES

This essay is based on a talk presented at the Meconopsis Group AGM on March 2nd 2013 by Geoff Hill on behalf of the author Ian McNaughton. (Text slightly amended from talk)

Introduction

Polyploidy is the doubling of chromosomes within the cells of a plant. The very first polyploid plant was discovered in 1907, its chromosomes were counted using a microscope technique newly developed at that time. Polyploids are known to occur more frequently in the Angiosperms, the flowering plants, than in other groups such as the Gymnosperms. Not all genera contain polyploid forms, in some they have never been detected, in others they are few. In some genera polyploids are frequent and may be a special feature of that particular genus, as with *Meconopsis*. The *Meconopsis* subgenus, loosely called the Big Blue Poppies, is found to have a range of particularly high chromosome numbers forming a short polyploid series. Only these, along with prospective additions, will be considered in detail.

Taxonomy and collecting expeditions

David Prain and George Taylor, successive directors of Kew Gardens around the turn of the 19th century, were primarily taxonomists and each attempted a formal classification of *Meconopsis*, a complex genus. On leaving Kew, Taylor published the first detailed taxonomic account of the genus *Meconopsis*. This was based almost entirely on herbarium specimens lent to him from various botanic gardens throughout Europe and Asia; he seldom referred to living specimens. This scholarly classification became widely accepted. At that time neither Taylor nor Prain could have had any knowledge of chromosome numbers let alone polyploids. Prain was adventurous and travelled into the Himalayan region, especially Tibet and with regard to the Big Blue Poppies he classified them under the Section Grandes which was comprised of three species, *Meconopsis baileyi*, *M.grandis* and the monocarpic *M.simplicifolia*. Some alterations to Taylor's classification were made by James Cobb in his book 'Meconopsis'. More recently Christopher Grey Wilson wrote his monograph on *Meconopsis* entitled 'Poppies'. He has also written an updated account of the genus which has just been published by Kew, as described by him at the November meeting of the Meconopsis Group.

Other explorers in the early 1900s visited China with its huge and varied flora. Rhododendrons were avidly collected and introduced by sponsored expeditions. Some plant hunters had a special interest in collecting the spectacularly beautiful *Meconopsis* that occurred commonly throughout the whole Himalayan region. Collectors with a particular interest in *Meconopsis* were Frank Ludlow, George Sherriff, Frank Kingdon-Ward, George Forrest and Ernest Wilson. The Scottish born David Prain was not the first person to discover *M. grandis* that was the French monk Pere Delavayi. The type specimen is kept in the Paris herbarium.

George Sherriff, another Scot, introduced many plants, and is noted for his introduction of *M.grandis* forms from E. Bhutan in 1934. His personal collector's code number was GLSG600 for Ludlow and Sherriff. This became misconstrued as GS600 and a few plants and a large quantity of seeds, thought to be collected from a particular population of *M. grandis* was distributed far and wide under this erroneous code. It was common in gardens causing much confusion amongst both amateur growers and professional nurserymen. The Meconopsis Group, was founded in 1998, its main remit being to resolve this problem.

In more recent times several other expeditions have visited China and brought back living specimens and seed of what are considered to be new forms of *M.grandis*. These are currently being assessed for their garden merit.

In 1989 the Kew, Edinburgh Kachenjunga Expedition, (K.E.K.E) collected good new forms and a fine deep blue plant of *M.grandis* was featured in a report by Peter Cox of the 2004 expedition to Arunachal Pradesh. This is now available commercially and is one to be sourced. The numerous expeditions to these regions are meticulously detailed by Christopher Grey-Wilson in an article published in Sibbaldia last year.

The Chinese botanist Fu-Sheng Yang describes graphically the mountains and valleys of S.W. Tibet on an expedition to collect the yellow flowered *M.integrifolia*. The objectives were partly to study evolution in these natural situations but also to determine forms of the greatest medicinal value. The Chinese team collected more than 30 samples from plants growing in various habitats which were taken for molecular analysis. Yang described these mountainous areas, as 'a complex of tectonic events and climatic oscillations' with thin soils, very cold temperatures and high winds. These extreme conditions tend to engender the inevitable struggle for existence amongst both plant and animal inhabitants and ultimately the survival of the fittest by natural selection. They could be expected to favour reproduction from seed and so act against the survival of sterile hybrids. This recent 2012 venture is the first of its kind, during it fossil specimens of *M.integrifolia* and *M.baileyi* were discovered. Most importantly molecular analysis showed quite close relationships this is interesting in view of their ability to form hybrids in the present era see p4 for details.

On a recent trip to Tibet John Mitchell found *M.baileyi* forms occurring at the base of the foothills. Different forms could be distinguished, each confined to a different valley or even to different sides of the same valley. The varied forms appear to be products of geographical isolation. The geographical separation of plants by mountain ranges, acts as an effective barrier to inter-crossing and gene flow between populations or incipient species and results in genotypes which are reproductively isolated from their progenitors. Darwinian evolution is an extremely slow process taking many millennia for a new species to evolve. In stark contrast, the evolution of new species through polyploidy can be virtually instant or certainly takes a very short time.

Features of polyploids

Polyploids in flowering plants have important morphological and physiological differences, distinct from normal diploids. Often these are visual enhancements, referred to as gigas factors and are manifest in a more robust growth habit. The mature plants can be taller with thicker stems and broader, thicker leaves. They have larger flowers with petals of good substance, all parts especially pollen grains and seeds are bigger. *M. grandis* seeds are about twice the size of *M.baileyi* and the seeds of Lingholm much larger than *M.grandis*; an ascending series of seed size. The fact that these morphological features are often measurably and even statistically different means that the likelihood of a plant being a polyploid can be narrowed down without resorting to cytological or molecular techniques, which may follow later.

Table1. Chromosome numbers of Meconopsis species

Species	Chr.Number	Remarks
M. cambrica	2n = 2x = 28	deleted from genus
M. napaulensis	2n = 2x = 56	plus other species
M. horridula	2n = 2x = 56	plus other species
M. integrifolia	2n = 2x = 76	unique chromosome number
M. baileyi	2n = 2x = 82	plus *M.betonicifolia* and *M.simplicifolia*
M. grandis	2n = 4x = 164	three sub-species now described

Table 1 shows the chromosome number of some *Meconopsis* species. Now that *M. cambrica* has been deleted the rest are all polyploids.

The paramount subject of chromosome numbers was not addressed for the genus until 1968 when James (Jimmy) Ratter published details of his studies at the RBGE. Most of these were later verified by cytologists, in particular Hugh McAllister in 1998. This study also included the important hexaploid count of Lingholm.

The two groups with 56 chromosomes have been the subject of several lectures and many discussions, amongst Meconopsis Group members. It is very obvious that there are clear morphological differences and each is classified as a subgenus. Having the same chromosome number does not mean that the two are inter-crossable. The many genetic and chromosome differences separating these sub genera would most likely result in sterile hybrids. The *M. baileyi* and *M. grandis* group form a third subgenus. *M. integrifolia* stands alone with an unusual chromosome number 2n= 76 and is clearly distinct from other species. In spite of this discrepancy, hybrids have been reported between *M. integrifolia* and other species. Several have been described such as *M.sarsonsii,* an artificial cross which is said to be fertile and *M.beamishii* which is found in the wild. I would expect it not to be fertile and therefore not a species.

M.grandis has precisely double the chromosome number of *M. baileyi*. This suggests that *M.grandis* has probably been formed from *M.baileyi*, or close allies, by chromosome doubling. *M.grandis,* as a polyploid, is likely to have arisen spontaneously and at random and thus could occur anywhere at any time. This could occur in the wild and even in cultivation.

Table2. Chromosome numbers of The Big Blue Poppies – A polyploid series

Species or Hybrid	Chromosome Number	Ploidy Level	Seed Fertility
Single genome or chromosome set	n = x = 41	haploid	N/A
M. baileyi	2n = 2x = 82	diploid	fertile
M.x sheldonii M. x Slieve Donard M.x Crewdson Hybrid	2n = 3x = 123	triploid	sterile
M. grandis Many cultivars. Some new introductions	2n = 4x = 164	tetraploid	should be fertile. sterile forms reported
Not yet ascertained	2n = 5x = 205*	pentaploid	predictably infertile or only partly fertile
Species name not yet accorded	2n = 6x = 246	hexaploid	one fertile cultivar Lingholm

* not yet determined, predicted number shown.

Circumstances in which polyploids are formed

There are three situations in which polyploid species have evolved or polyploid forms within a species have been created.

1. In the wild through natural pollination
2. In cultivation also by natural cross pollination
3. Artificially through controlled hand-crossing/pollination and the use of chemicals or irradiation.

Triploids

Since triploids are highly sterile they obviously cannot be species. A species by definition should be fertile. Triploids have their own genetic identity and this is reflected in their characteristic traits. Triploids are important components of the polyploid series in this subgenus of Meconopsis. They are formed by hybridization between the tetraploid species *M.grandis,* and the diploid *M. baileyi* and so are technically allotriploids.

In theory triploids could occur in the wild provided that the parental species are closely sympatric to allow cross pollination. However, triploids have never been located in the wild and are unlikely to survive the rigours of an alpine habitat.

All triploids verified or putative have been formed in cultivation, either arising naturally and spontaneously in gardens or produced artificially by controlled hand-pollination as with *M.x sheldonii* and *M.*x Slieve Donard. Several presumed triploids have been regarded as distinct and have been given cultivar names and have proved to be good, reliable garden plants. The integrity of each cultivar has been maintained by simple division, their only means of propagation. *M. x sheldonii*, *M.*x Slieve Donard and *M.*x Crewdson Hybrid have been verified cytologically as triploid. Since they are also hybrids they are correctly described as allotriploids. Other sterile hybrids, such as Maggie Sharp, are referred to as putative triploid hybrids.

All triploids are open to spontaneous mutation. It must be emphasised that this phenomenon is an extremely rare event, mutation rate in general is estimated to be one in a million and it is an extremely rare occurrence. This explains why the evolution of Lingholm is a very rare event and seems so far to be unique, at least so far.

The creation of M.Lingholm as an example of a hexaploid species

The story of Lingholm began in 1934 when W.G.Sheldon of Oxted in Surrey hand pollinated a Sikkim form of *M.grandis* with *M.baileyi*. A plant raised from the seed produced turned out to be entirely sterile and has remained so ever since. It was officially named *M.x sheldonii* after its raiser. Because of its sterility *M. x sheldonii* had to be propagated clonally mainly by simple division. Later a chromosome count was obtained for *M.x sheldonii* confirming it to be a triploid (2n=3x=123). Plants were circulated far and wide by various nurseries. Some found their way to Cumbria and were grown by Dr.L. Nelson in his large garden in Brampton in Cumbia. This garden may be the site of *Meconopsis* Lingholm's evolution as it was here that fertile plants were first found. Seed was distributed and the plants eventually found their way to Lingholm Garden near Keswick which was a garden open to the general public. They became publicised and many years later this resulted in the plants being given the cultivar name M. 'Lingholm'.

All publications and accounts of this important event state that *M. x sheldonii* is the progenitor of Lingholm. Some believe that it was actually *M. x* 'Slieve Donard', the only other cultivar ascertained to be a triploid and which was also derived through hand- crossing.

Fig 1. Mechanisms and pathwayleading to the evolution of M Lingholm 'Lingholm'Stage 1 - Hand - crossing

M. grandis **x**	***M. baileyi***	**>**	***M. x sheldonii***
tetraploid	**diploid**		**triploid**
(2n=4x=146)	**(2n=2x=82)**		**(2n=3x=123)**

Time gap of 44 years

Stage 2 - Somatic chromosome doubling

M. x sheldonii	**>**	***M.* 'Lingholm'**
triploid		**hexaploid**
(2n=3x=123)		**(2n=6x=246)**

Mechanisms and pathways.

Mechanisms and pathways are words commonly used in modern genetics to describe evolutionary processes. A mechanism may be defined as a process that enables something to happen; chromosome doubling is a good example. A pathway is explained as leading to the emanation of a new form or even a new species, a good example of these processes is provided by a new hexaploid species, exemplified in Lingholm.

There are two ways in which a polyploid can be formed. The first is due to a failure of meiosis leading to the formation of unreduced gametes which pair to give a zygote with double the chromosome number. The second is an asexual vegetative process in which mitosis is inhibited leading to the formation of a plant with double the chromosome number; a process known as somatic chromosome doubling.

Lingholm cannot have evolved by sexual means i.e. reproductive chromosome duplication since its progenitor is a sterile triploid and does not produce gametes. Under these circumstances the only mechanism possible is an asexual one of which somatic chromosome doubling is the logical probability.

The formation of a hexaploid through somatic doubling involves two stages. In the first stage, chromosome doubling, takes place within the apical meristem of the sterile triploid plant. In the case of Lingholm this is thought to have occurred in the shoot meristem. A random mutation occurs, which interferes with the process of normal <u>mitotic</u> cell division, resulting in a single polyploid cell. This cell multiplies to form a group of hexaploid cells. Later the doubled cells proliferate and then differentiate to form floral organs which eventually produce pollen and egg cells.

The second phase is reproductive, at anthesis diploid pollen is released, very probably resulting in self-pollination. Male and female balanced euploid gametes, with n=x=123 chromosomes unite to form a zygote with exactly double the number of chromosomes 2n=6x=246. Such cells then proliferate mitotically to form an embryo, seed, seedling and eventually a mature flowering plant, a hexaploid in the case of Lingholm.

In the case of Lingholm this must have happened since only a single capsule on the sterile triploid plant produced seed. It seems therefore that Lingholm is self-compatible. This is confirmed by the copious seed obtained from the original clone of Lingholm when grown in isolation.

It seems that today Lingholm stands alone as the only example so far of a hexaploid *Meconopis* species. An application should be made for a new hexaploid species and not for Lingholm *per se.* It should consist of a clear, concise and correct genetical account of the origin and status of Lingholm together with historical morphological and cultural details with good photos. Such information is well provided by the Meconopsis Group website.

<u>The possibility of pentaploids</u>

Other cultivars previously grouped along with Lingholm seem not to be hexaploids, due to their partial or in some cases complete sterility, but belong to some other group yet to be defined. Two of these, Mophead and Louise are in fact not fully fertile, yet they are stated to have been selected from Lingholm which is consistently highly fertile. I consider that by their sterility and general robust appearance with large flowers, that they are higher polyploids but not hexaploids. I therefore suggest that they could well be pentaploids, with five sets of chromosomes and a predicted chromosome number of 2n=5x=205, a ploidy level not so far encountered or even considered in this group of plants. If this were proved to be so it would complete the one remaining gap in the proposed extended polyploid series. Chromosome studies of Mophead, Louise and other large flowered, sterile plants could prove revealing.

In order for a pentaploid to be produced, a hexaploid and a tetraploid must inter-cross. In this example, Lingholm and *M. grandis* could inter-cross. This could only occur in gardens where they are planted together. There is some circumstantial evidence that Lingholm and *M.grandis* have co-habited in gardens for some time and certainly may do so today. In general, such crosses are more successful when the plant with higher ploidy level is the seed parent so pentaploid hybrids are more likely to be found amongst seedlings obtained from Lingholm than from *M.grandis.*

Three of the taller cultivars are currently listed as selections derived of Lingholm, on the grounds of general similarity to that cultivar. This may be so but perhaps it is not. Seedlings obtained from Lingholm could in fact be pentaploid hybrids, with three of their five genomes derived directly from Lingholm, a basic resemblance is to be expected. As Louise is only partially fertile, as observed by Geoff Hill over a ten year period, it is more likely to be a pentaploid than a selection made at the hexaploid level, a point to ponder.

The well known, sterile *M*. Jimmy Bayne, discovered by the gardener from Kilbryde Castle may also be a pentaploid. The strikingly tall, elegant Dalemain, named after the original garden in Cumbria, where it forms a large floriferous colony, may be another. The same applies to P.C. Albilgaard, found in Denmark with large pale blue, pendent flowers. All of these are maintained vegetatively as clones. There are numerous others described by the Meconopsis Group under the ‘Sterile Blue Group’, not a proper botanical classification, but a useful temporary grouping. A proper classification of these fine plants cannot be made on morphological and physiological traits alone without some form of chromosome analysis. They may or may not be pentaploids, if not, what are they? Only chromosome analysis can solve the problem, just one of many in this intriguing genus.

APPENDIX

Examples of Angiosperm polyploids in Agriculture and Horticulture

Polyploids have been enormously important in both agriculture and horticulture. There are many examples. Triploid forms of snowdrops have proved to be of special value in the garden being long lived and floriferous. The sterility of triploids is put to use in the improvement of fruit shown by the development of seedless watermelons a very valuable crop in tropical countries. Most apples are triploid as are bananas.

About 75% of all daffodils are tetraploid, the remainder are mostly triploid. These higher polyploids have been built up from the wild type diploid species over many decades using colchicine. All potatoes for culinary use are tetraploids produced from wild diploid species. The genetical breeding work is conducted at the diploid level in view of its simpler genetics.