# Understanding the Polyploidisation Process of Hexaploid Chrysanthemum

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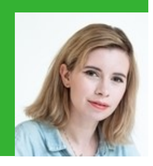

## Background

Chrysanthemum is one of the most important ornamental crops, economically and culturally. It is a member of the Asteraceae family and sits within the Anthemideae tribe. The cultivated varieties are hexaploids, thought to be derived from the hybridisation of several diploid wild species. One of these possible ancestors is the diploid species *Chrysanthemum japonicum*.

## Objective

- Develop an *ab initio* annotated genome assembly of *Chrysanthemum japonicum*
- Study the diversity within the cultivated hexaploid chrysanthemum genomes in relation to their origin

## Results

### *C. japonicum* genome assembly

*C. japonicum* is a wild diploid plant found in Japan. Like all chrysanthemums it has a large and highly heterozygous genome (1C-value ~ 3 pg). We produced Nanopore reads and paired-end Illumina reads using four different insert size libraries. The genome was assembled with Smartdenovo, flattened with Purge Haplotigs and polished with multiple rounds of Racon and ntEdit. Key statistics and a comparison to closely related species is shown in Table 1.

**Table 1.** A comparison of key statistics between our assembly and the closest, published, related assemblies.

| | C. japonicum | C. seticuspe | C. nankingense | Helianthus annuus (Sunflower) | Lactuca sativa (Lettuce) |
|---|---|---|---|---|---|
| **Total Length (Gb)** | 3.135 | 2.722 | 2.527 | 3.642 | 2.38 |
| **No. of Contigs/ Scaffolds** | 14 226 | 354 212 | 24 051 | 17 (pseudomolecules) | 11 474 |
| **GC content (%)** | 36.0 | 36.0 | 35.6 | 37.6 | n/d |
| **N50 (bp)** | 257 742 | 44 741 | 130 678 | n/a | 1 769 000 |
| **Busco Complete (%)** | 89.9 | 88.8 | 92.7 | 92.0 | n/d |
| **Busco Duplicate (%)** | 14.3 | 8.7 | 6.9 | n/d | n/d |
| **Cegma Complete (%)** | n/d | n/d | n/d | n/d | 97.58 |
| **Percent Repetitive (%)** | n/d | 72.5 | 69.6 | n/d | 77.5 |
| **No. of Genes** | 32 205** | 15 796 | 56 870 | 97 436 | 38 919 |

**Consensus transcripts based on nanopore cDNA using the pinfish workflow

**C. seticuspe:** doi.org/10.1093/dnares/dsy048, **C. nankingense:** doi.org/10.1016/j.molp.2018.10.003, **H. annuus:** doi.org/10.1038/nature22380, **L. sativa:** doi.org/10.1038/ncomms14953

### *Diversity and origin of cultivated hexaploids*

The ancestry of the cultivated hexaploid chrysanthemums remains unclear. To gain a better insight, we resequenced a selection of wild diploids, tetraploids and hexaploids as well as a collection of cultivated hexaploids. As the first step in the analysis, we used the resequencing data to assemble the chloroplast genomes to look at the maternal lineage.

**Figure 1.** All of the re-sequenced cultivated hexaploid varieties

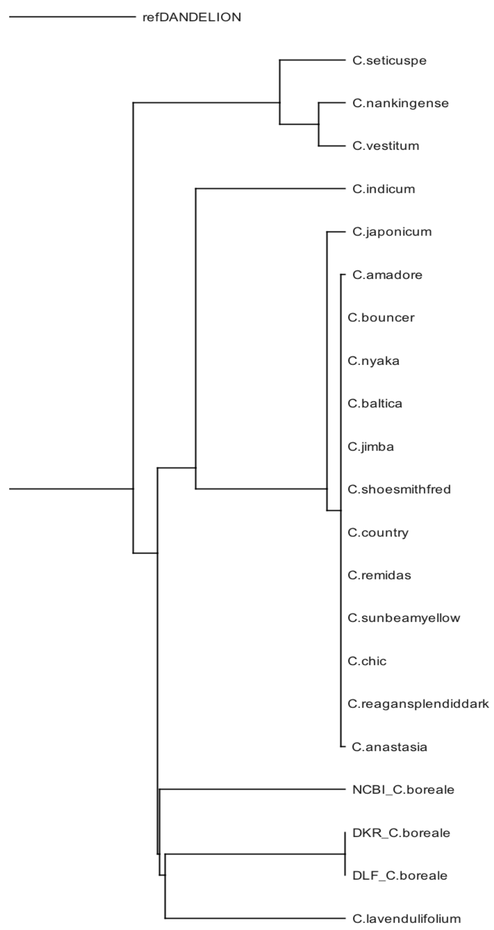


**Figure 2.** The second iteration tree produced by MUSCLE using the SSC chloroplast region, visualized in Archaeopteryx (colours inverted).

Using MUSCLE on the Short Single Copy (SSC) region of the chloroplast it became clear that all the cultivated hexaploids cluster closely together, with the closest wild relatives being *C. japonicum* and *C. indicum*. Our results confirm the finding of Nakano et al. (2019) that *C. seticuspe* and *C. nankingense* are closely related, while *C. lavendulifolium* is not in the same cluster.

## Future Work

- Improve the *C. japonicum* genome assembly
- Establish a k-mer signature library for all accessions
- Perform a comparative study of *C. japonicum* within Asteraceae
- Assess the diversity and find the origin of the hexaploid genomes using the whole genome re-sequencing dataset

## Acknowledgements

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