Genetic Variation in British Campanula rotundifolia L.

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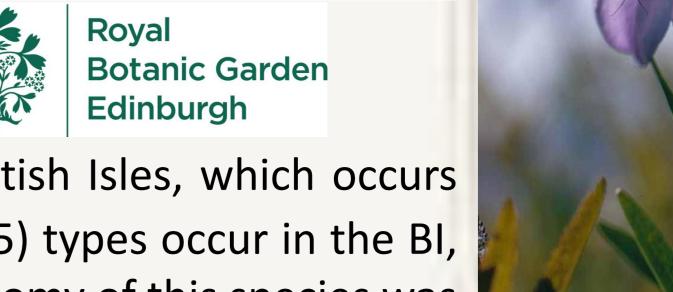
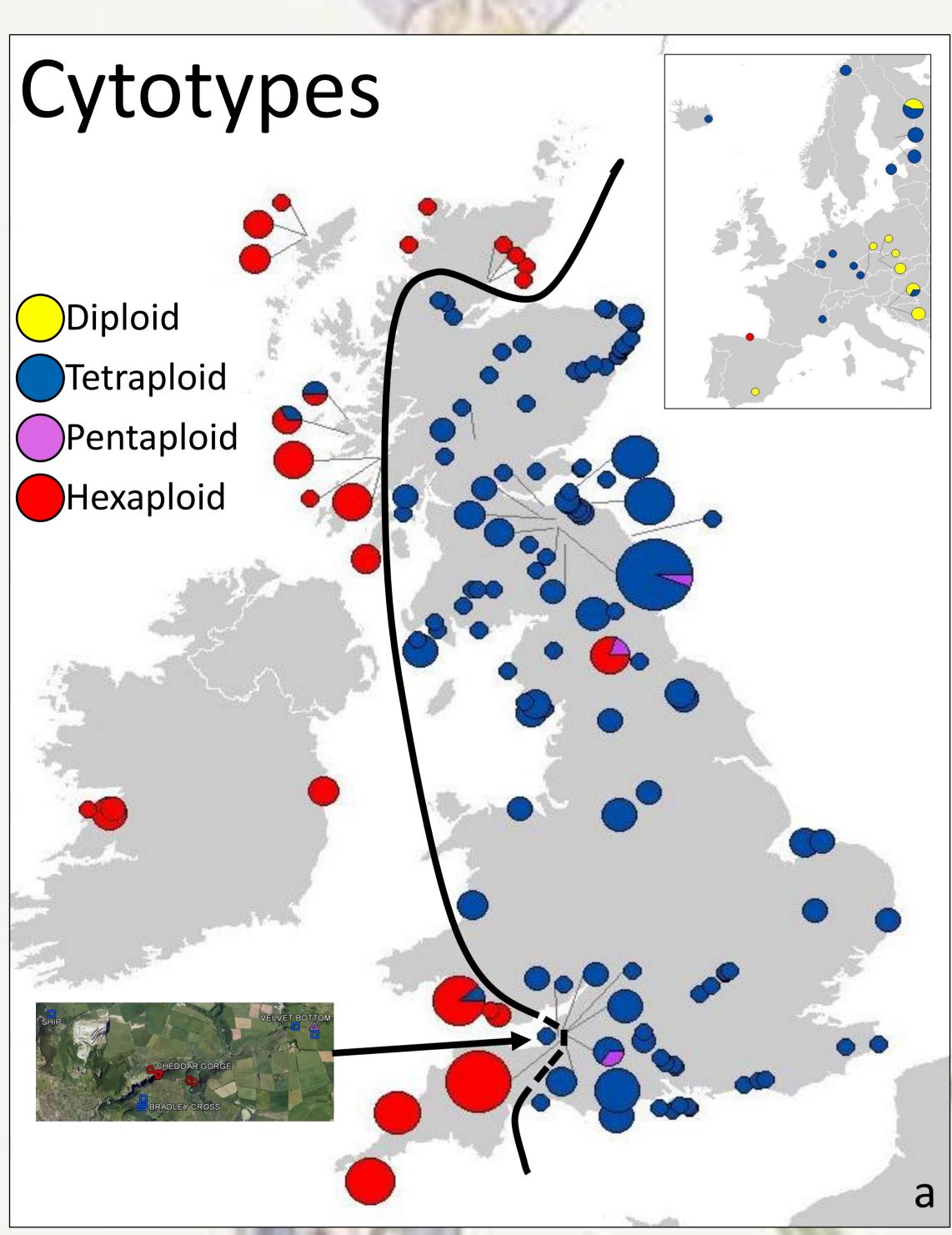


Photo Courtesy of Olympic National Park
Fig. 1. Campanula rotundifolia

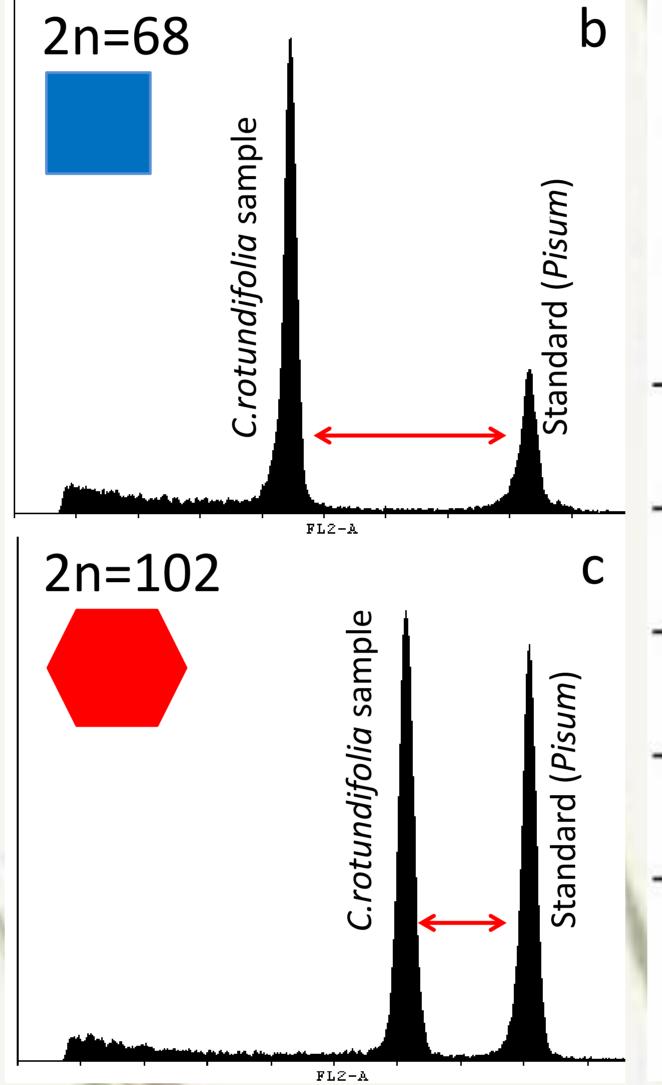
4 CV1

Background: The harebell (*Campanula rotundifolia*, Fig. 1) is a short-lived perennial native to the British Isles, which occurs widely in the northern hemisphere. Tetraploid (2n = 68), hexaploid (2n = 102) and (rarely) pentaploid (2n = 85) types occur in the BI, but not diploids, which occur elsewhere in Europe. The cytotypes display strong spatial structuring¹. The taxonomy of this species was revised in 2010^2 on the basis of combined cytotype and morphological characters, with two subspecies now recognised (ssp. rotundifolia 2n = 68; ssp. montana (Syme) P.D. Sell, 2n = 102). There is evidence that *C. rotundifolia* is declining in Britain¹.

Aims: To determine whether the geographic structure revealed in cytotype distribution is reflected in the morphology and genotypes of *Campanula rotundifolia* individuals across Britain and Europe

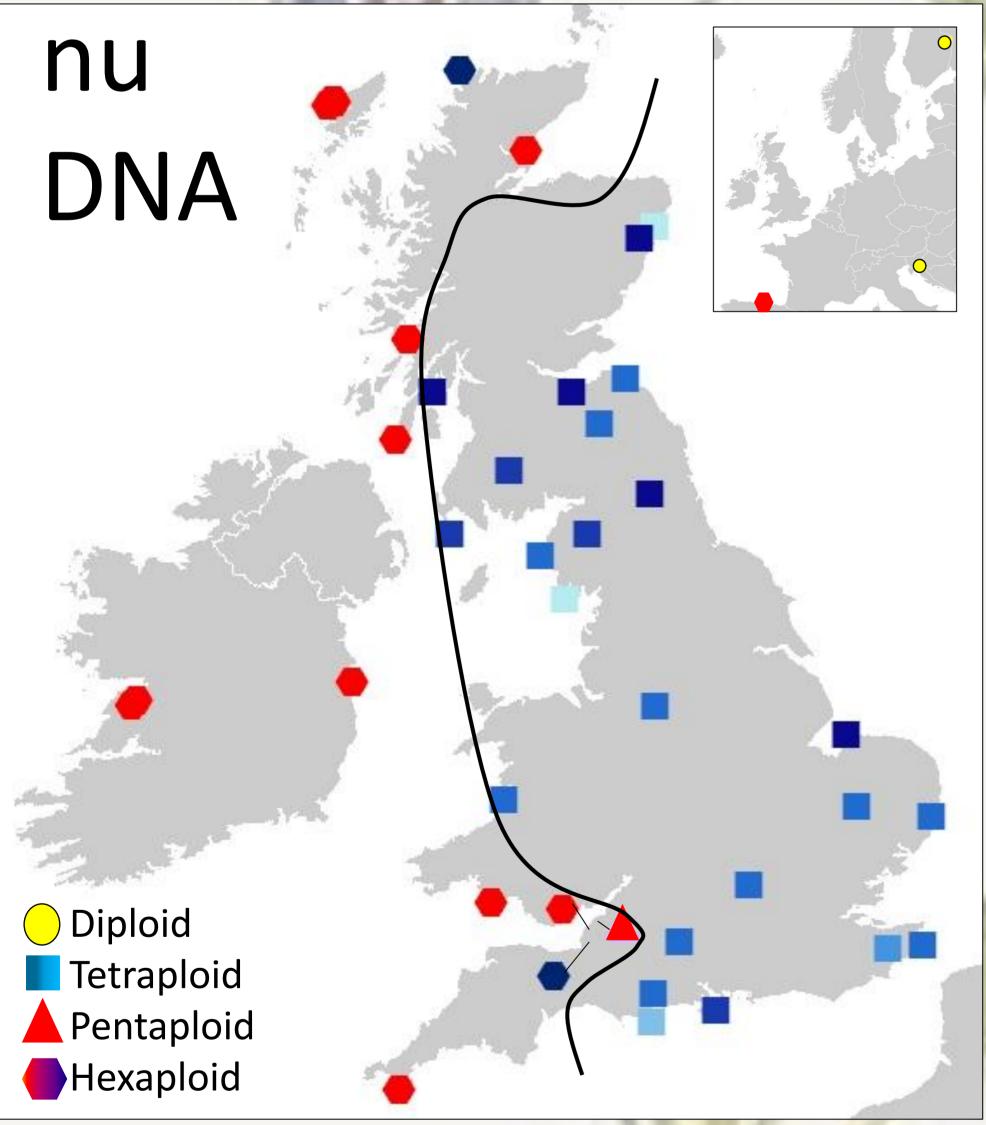


Campanula rotundifolia cytotypes; a. Distribution of cytotypes sampled; b, c. Calculation of DNA amount and ploidy using a FACSCalibur flow cytometer and a standard (*Pisum sativum* L. 'Ctirad') of known DNA content (9.09 pg)³



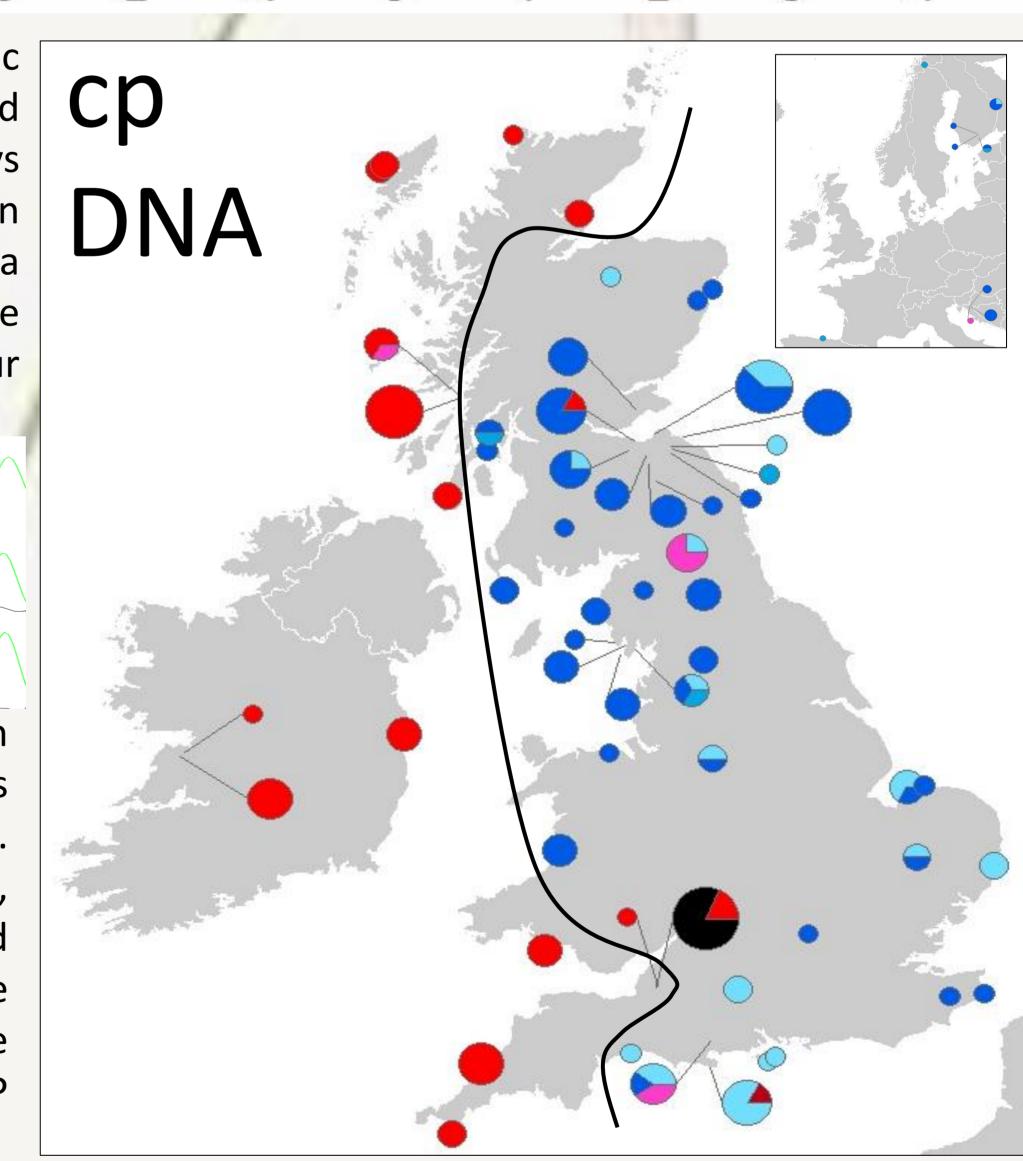
Morphology

Morphometric analysis; a. Campanula rotundifolia flower size and number are used as taxonomic characters for the newly classified subspecies, although these vary with environmental conditions; b. CVA analysis using 17 floral and vegetative characters, with ploidy as a grouping factor. CV1 and CV2 account for 86.5% and 13.5% of the variation respectively. Most of the variation in CV1 is represented by width of the open corolla, supporting the use of flower size as a taxonomic character



Nuclear ITS haplotype distribution: Three polymorphic sites were found over 528 bp of the internal transcribed spacer (ITS) region. Haplotypes were almost always restricted to one cytotype, with only one shared between hexaploids and tetraploids, while European diploids had a unique haplotype. Heterozygous point mutations (see arrow below) were found in 88% of tetraploids (four haplotypes) but only 13% of hexaploids (one haplotype).

Chloroplast haplotype distribution and frequency: An RFLP marker (trnH-trnK) and a microsatellite locus (ccmp2) were combined, resulting in seven haplotypes. Although both cytotypes have similar levels of variation, the haplotypes found in each are nearly always restricted to that cytotype. Individuals from the Cheddar Gorge population (SW England – black) have a haplotype unique to that location due to a mutation in the RFLP locus.



Conclusions:

Molecular data indicate genetic differentiation of cytotypes, which maintain largely distinct chloroplast and nuclear genotypes. These data are reflected in morphometric analyses, especially flower form. Pentaploids seem to have arisen through hybridisation between hexa- and tetraploids, although this appears to be a rare event. Although taxa are highly variable morphologically, genetic data support the recent taxonomic subdivision of *C. rotundifolia*, but suggest species status may be merited.

Future work:

What factors maintain the geographic separation of these cytotypes? Are cytotypes locally adapted to the environment (e.g. maritime vs continental), is separation due to different competitive abilities or is geographic structure due to colonisation history? Do these populations remain separate due to minority cytotype exclusion at their contact zones?

These questions are being tested in a common garden study of both cytotypes in a tetraploid zone in Eastern Scotland and by wider analysis of continental populations.

References: ^{1.} Biological Flora of the British Isles: *Campanula rotundifolia* L. (In prep) Stevens, C. J., Wilson, J., McAllister, H. A.; ^{2.} Stace. C. (2010) New Flora of the British Isles. Third edition; ^{3.} Dolezel, J. *et al.* (2007). Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols 2(9): 2233-2244.