

# THE ROLE OF GENOME DUPLICATION AND VARIATION IN NUCLEAR GENOME SIZE IN THE EVOLUTION OF THE CAPE FLORA

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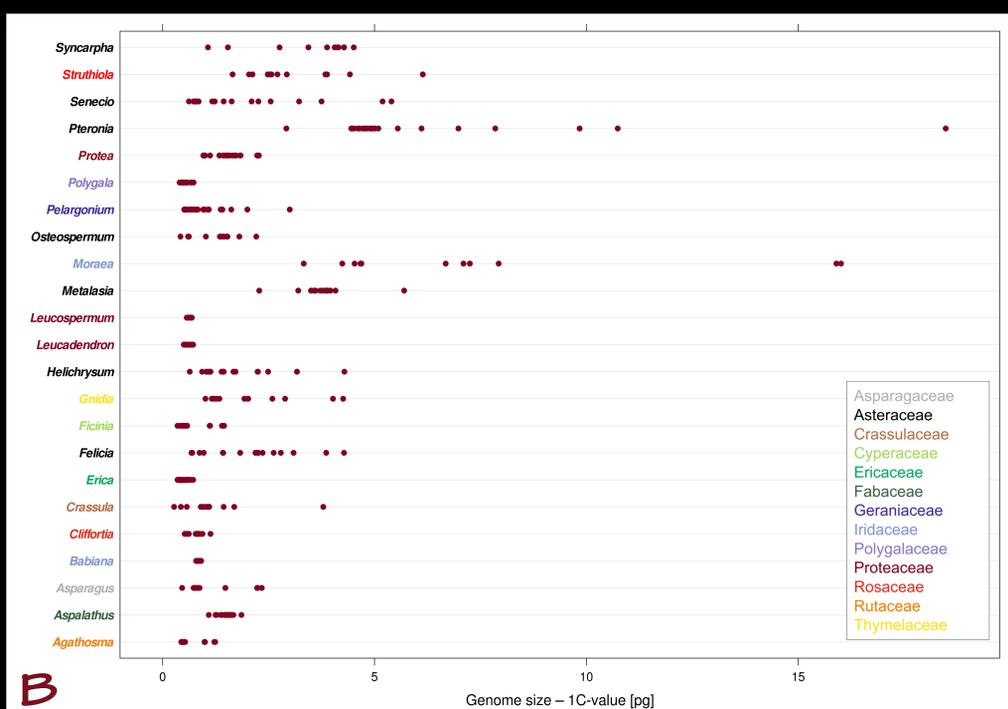
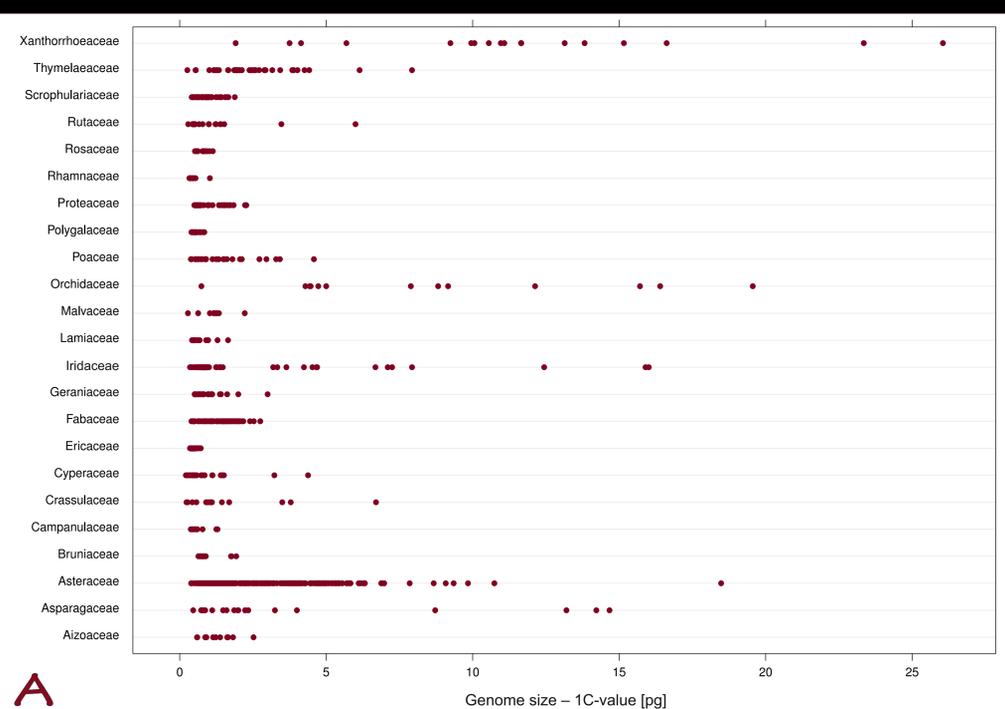
## INTRODUCTION

The southernmost tip of Africa is famous for its extraordinary rich and unique flora, forming worldwide diversity hotspot and separate floristic kingdom – the Greater Cape Floristic Region (GCFR). The evolution of such diversity is

ascribed to stable climate, variability in soil types, altitudinal gradient, etc. On the other hand, broadly accepted concept of a weak contribution of polyploidization events in evolution of tremendous diversity in the GCFR is suggested<sup>1</sup>. Our aim

is to reveal if the hypothesis is based on realistic assumptions or on simple underestimation of local flora in terms of ploidy level and genome size variation.

## RESULTS



**Figure 1.** Overview of genome size variation in picograms for families (A) and genera (B). Only taxa (families and genera) with at least more than 10 estimated specimens are included.

All together 1265 species out of 331 genera and 80 families were already analysed. It covers 29.6% of recognized genera of the GCFR<sup>2</sup> and 44.9% of recognized families.<sup>3</sup> Because of the huge species richness of the GCFR (more than 11 420 recognized species), our dataset represents only fraction of local plant diversity (~11.1%).

Intra-generic variation possibly ascribable to ploidy level variation (i.e. exceeding 50% difference) was recognized for 87 genera out of 151 genera with more than two analysed species. The highest intra-generic variation was found in the genus *Tetraria* from Cyperaceae family, reaching almost 20-fold difference between species *Tetraria fimbriolata* (1C = 0.21 pg) and *T. brachyphylla* (1C = 4.38 pg).

Surprisingly, the highest intra-family variation was found in Santalaceae, because of including of the genus *Thesium*, bearing very small genome sizes (*Thesium lineatum*, 1C = 0.30 pg) and *Viscum*, which is characterized by huge genomes (*Viscum continuum*, 1C = 87.16 pg). It represents almost 290-fold intra-familial difference, and exceed so far record holder Melanthiaceae (230-fold difference<sup>4</sup>).

### Method - Flow cytometry (FCM)

Holoploid genome size<sup>5</sup> was estimated by means of propidium iodide FCM. For each plant, piece of intact tissue (usually leaf or stem or ovary) was chopped along with an appropriate amount of an internal reference standard using a new razor blade in a Petri dish containing 0.5 ml of ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20)<sup>6,7</sup>. The resulting suspension was filtered through a 42-µm nylon mesh and incubated at room temperature for at least 5 min. After incubation, the suspension was stained by using 1 ml of Otto II buffer (0.4 M Na<sub>2</sub>HPO<sub>4</sub> · 12 H<sub>2</sub>O) supplemented with the intercalating fluorescent dye propidium iodide, RNAase IIA (both at the final concentrations of 50 µg/ml) and β-mercaptoethanol (2 µl/ml). The samples were stained for 5 min at room temperature and analysed using a Partec CyFlow cytometer equipped with a 532 nm diode-pumped solid-state laser Cobolt Samba as the source of excitation light. Fluorescence intensity of 5 000 particles was recorded, and the data were analysed using Partec FloMax Software version 2.4d. *Pisum sativum* 'Citrad' (2C = 8.76 pg)<sup>7</sup>; served as the primary reference standard.

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## CONCLUSIONS & FUTURE PROSPECTS

- more than half representatively covered genera are variable enough to suspect them for ploidy variation
- demonstrable ploidy variation was so far found in 64 genera out of 32 plant families
- prominent heterogeneity in ploidy is shown by numerous genera of Asteraceae family
- Santalaceae in the GCFR uncovers extraordinary intra-family variation in genome size and sets a new record in plants
- Starting work on Genome size database of the Greater Cape flora: <https://botany.natur.cuni.cz/gfdb/>



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