



Review of the Master's thesis

Bc. Eliška Krtilová: Genome duplication in *Stellaria* genus – the more, the merrier? Link between ploidy levels and sexual polymorphism

The thesis of Eliška Krtilová investigates various evolutionary and reproductive aspects contributing to the complexity of the highly polyploid plant species *Stellaria palustris* in comparison with related congeners showing lower ploidy levels. Special attention is paid to its sexual polymorphism, manifested by the occurrence of hermaphrodite, female and intermediate individuals. The work aims to test correlations between sexual polymorphism, ploidy, phylogeny, morphology and geography.

I consider the topic of the thesis to be a bold one (since high polyploids are not very popular among evolutionary biologists for many reasons) but definitely very interesting and topical. Although nowhere in the thesis (except in the abstract) are its aims explicitly mentioned, the overall scope of the work is very ambitious. The introduction briefly but concisely (and without unnecessary “padding”) summarizes the issues of polyploidy, sexual polymorphism and their mutual relationships. Perhaps it would be worthwhile to pay more attention to the evolutionary and genetic aspects associated with high polyploids; however, there are not many relevant studies on this topic in general. The sampling is large and representative for answering most of the questions, but it has some limitations: (i) it would be better to include a larger number of populations and individuals to investigate the frequency of individual sexual morphs, (ii) to better assess the correlation between latitude and sexual polymorphism, it would be necessary to include some populations from Poland and northern Germany. Nevertheless, I have to appreciate the sampling (at least by the standards of master theses) as undoubtedly respectable, covering a large geographical area. The methods are mostly described adequately, but often not in a clear manner. The text contains many typos and it is difficult to navigate, some passages are not clearly arranged; some data would be better arranged in a table rather than in the text itself (matrices in the morphometric part). Below are some shortcomings concerning the methodological part:

(i) The introductory part of the part concerning the NeighborNet analysis is completely missing (the text starts “After conducting the initial network analysis...”).

(ii) The thesis claims that ploidy was detected in all taxa by direct chromosome counting in combination with flow cytometry. However, this is not the case for *S. longifolia*, for which it is stated “diploid *S. longifolia* was analyzed by Šmarda et al. (2019)”; actually, Šmarda et al. did not count chromosomes in this species, hence direct evidence that the samples of *S. longifolia* from this study are diploid is lacking (although this is very likely).

(iii) It is stated that the DPI character has been removed from morphometric analyses, but it is not described what this abbreviation actually means.

I appreciate the wide range of methods used and the large number of analyses performed. Although the results show complex patterns that are often difficult to assess, many of them are significant and interesting, including:

(i) Revealing of a previously unknown tetraploid “Scandinavian entity”.

(ii) Detecting of extensive karyological and cytogenetic variation in highly polyploid *S. palustris*, including aneuploid chromosome counts.

(iii) *Stellaria palustris* is highly variable in the expression of sexual polymorphism, even at the individual level, but shows consistent flower size polymorphism.



Nevertheless, the Results section is formally the weakest part of the thesis. First of all, it is not logically structured: although individual taxa were obviously defined mainly on the basis of genome size, the chapter Genome size estimation is for unknown reasons included after the genetic and karyological analyses. A large part of the figures are not graphically clear: many of them show illogically (randomly) ordered values on the x-axis, several have incorrectly described axes, Fig. 6 is inverted in such a way that most of the text in it is upside down. The figure captions contain a lot of redundant information that makes it difficult to find what data they actually represent. It is very difficult to read any information from the messy Fig. 3 (ITS tree), here it would be appropriate to discard identical sequences. There is also a discrepancy in the number of karyologically analysed samples (12 individuals in the text, 13 in Fig. 10). Fortunately, the results are adequately and clearly interpreted in the discussion, which finally puts many uncertainties to rest.

Questions:

- 1) It is not described in Methods how polymorphic sites in ITS were handled in Bayesian analysis. However, it is mentioned in the discussion that *S. palustris* as well as other species contain many such positions that are probably the cause of the numerous polytomies and branch collapses in the ITS tree. Have you tried running an analysis that contains only samples without polymorphic sites? Do some samples show a pattern of polymorphic sites that appears to be additive between two directly unrelated taxa?
- 2) How is the “Scandinavian entity” actually defined? The relatively large group of individuals assigned to this entity shows a significantly larger genome size (four such individuals are acknowledged in the text, but in fact there are more than ten, see Fig. 14a), their chromosome numbers are probably unknown (unfortunately, there is not indicated in the thesis which specific samples of the Scandinavian entity were subjected to chromosome counting). In some samples of the Scandinavian entity, genetic analyses suggest a possible hybrid origin. Would it not be more appropriate to classify such individuals separately from the “Scandinavian entity”? The presence of such taxonomically ambiguous individuals within a formally classified group can negatively affect, for example, the results of CDA analyses.
- 3) According to the recent paper of Kučera et al. (2021), the presence of sex chromosomes is suggested in *S. graminea* (both 2x and 4x), based on the difference in genome size between hermaphrodites and female plants. However, this possibility is not mentioned or tested in the thesis. Can you rule out that the possible presence of sex chromosomes cannot affect the data you have presented? What is generally known about the occurrence of sex chromosomes in *Stellaria* and *Caryophyllaceae*?
- 4) Vegetative organs were not included in the morphometric analysis, which is commented as follows: “Stems and leaf-associated traits were considered irrelevant for morphometric assessment due to their susceptibility to ecologically driven phenotypic variability (cf. Kučera et al., 2021).” In fact, this statement is valid only for the distinct cytotypes within *S. graminea*. In fact, individual species of *Stellaria* included in this study are often distinguished from each other on the basis of vegetative characters, including the taxonomically uncertain *S. fennica*, which could perhaps be identified with the “Scandinavian entity”. Do you intend to analyse vegetative traits in the future? It would be useful for a clear morphological definition of the Scandinavian entity.



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Overall assessment: Eliška Krtilová's thesis involves a huge amount of work on an extraordinarily challenging plant group. Unfortunately, this impressive work is also not very good from a formal point of view, especially with regard to the Results section. Despite this, it can be assessed as a very thorough multi-disciplinary study that deserves a rating "výborně" if the defense goes well.

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Jan Prančl, Ph.D.