

Hosam Osama Mohamed Nageeb Elansary: Organellar DNA diversity in some ornamental plants related to reproduction system and life strategy

Main topic of the thesis is the diversity of organellar (first of all mitochondrial) DNA in three vascular plant species with different (and contrasting) breeding systems as well as morphological and physiological features, namely in *Silene vulgaris*, *Silene latifolia* and *Aldrovanda vesiculosa*. Gynodioecious *Silene vulgaris* became in the last decades a model species in studies aimed at DNA polymorphism in mitochondrial genes, relationships between cytological male sterility (CMS) and mitochondrial genome, processes leading to rapid changes in mtDNA structure and genome copy number (substoichiometric shift, lineage sorting) and the inheritance of mtDNA (though the inheritance is maternal as a rule, even in *S. vulgaris* occasional paternal inheritance has been discovered). Dioecious *Silene latifolia* is a model species in studies aimed at e.g. sex chromosome evolution. *Aldrovanda vesiculosa*, a water carnivorous plant, is an example of species with very low genetic variation.

Principal aims of the present study are as follows:

(i) to analyse the inheritance of mitochondrial and chloroplast genome in natural population of *Silene vulgaris* by means of PCR-RFLP in coding and Southern-RFLP in non-coding regions as well as sequencing of *atp1* and *cox1* genes, (ii) to elucidate the processes causing the mtDNA polymorphism in *S. vulgaris*, (iii) to analyze the paternal inheritance of mitochondrial genome in the offspring of controlled crosses in *S. vulgaris*, (iv) to evaluate the organellar DNA polymorphism in *Silene latifolia* with respect to the breeding system (comparative study with *S. vulgaris*), and (v) to assess the genetic variation in *Aldrovanda vesiculosa*.

Many previously published generalized statements regarding the mitochondrial genome in higher plants shall be most likely corrected in the light of recent findings. Thus, this thesis is with no doubt an important contribution to this relatively little known and sometimes controversial field. From my point of view, the most important is the section devoted to *Silene vulgaris*, which falls in a long-term research program managed by the supervisor (H. Štorchová), who is a leading expert in this field and co-author of several crucial publications on *S. vulgaris*. Results of the present thesis could be thus a step forwards in this very timely topic. *Silene latifolia* was also a good choice – although it is closely related to *S. vulgaris*, it differs substantially in the breeding system (pure dioecy). *Aldrovanda vesiculosa* possesses completely different position in the Angiosperm phylogeny and any comparison with the *Silene* species must be done very carefully.

Objectives of the study are well defined; experimental design, data collection and statistical analysis are adequate. Discussion is rather short, some topics should be discussed in more detail.

Comments and questions:

1) The author discovered that 20 plants of different Kováry families possessed both hermaphroditic and female flowers on the same plant (i.e. gynomonocy). However, he did not find any differences in mtDNA banding pattern between branches with hermaphroditic and female flowers, respectively. This is not congruent with the statement mentioned in the

thesis that this fact could be caused by different mtDNA populations in the respective branches. I am aware that only one plant was analysed... Secondly, the fact that gynodioecy can occur in this species is very interesting for me and shows that the breeding system in this species seems to be more complicated than e.g. in the *Thymus* species (family Lamiaceae). I miss this fact in Conclusions, where *S. vulgaris* is mentioned as a „gynodioecious species“.

2) The author did not prove the paternal mtDNA transmission in controlled crosses between *Silene vulgaris* from the locality „Kováry“ (mother plants) and from two geographically distant localities (U.S.A., Russia; pollen donors bearing easily detectable mtDNA markers). In the Discussion section he argues that this can be caused (besides others) by presumably large genetic distances between the nuclear genomes of the crossed plants. How this relate to the possibility of paternal transmission of mtDNA? Did you observe e.g. lower seed set which can show restricted compatibility? On the other hand, sequencing of *atp1* and *cox1* genes of plants from „Kováry“ revealed three haplotypes in the coding region of *atp1* gene and two haplotypes in *cox1* gene, which perfectly match the the sequences housed in GenBank and originating from plants from both Europe and North America. This might show that there can be only little geographic separation (of course, another markers can bring another results). By the way, was *Silene vulgaris* a subject of phylogeographic study? Is something known about main migration routes and origin of the recent geographic area?

3) Can be the frequency of paternal transmission influences by e.g. environmental stress (water and nutrient availability, etc.)? It has been repeatedly documented that the frequency of male and female flowers/plants (or hermaphroditic and female) in higher plants is strongly influenced by such factors.

4) *Aldrovanda vesiculosa*, genetic uniformity (p. 120, Discussion): the author offers several explanations of well documented genetic uniformity. However, some of them seem to be hardly probable – (i) it has been shown that *Aldrovanda* is not a newly formed genus (it is most likely older than the genus *Drosera*), (ii) severe bottleneck can be hardly expected in a species with such large geographic area. Water plants are not generally variable in chloroplast genome as mentioned in the Discussion (p. 120).

5) What is known about the frequency of hermaphroditic and female plants in natural populations? Can they differ in e.g. stress tolerance or ecological demands (and thus microhabitat preferences)?

6) I find for extremely interesting that families/plants of *Silene vulgaris* from Kováry bearing the KovC mtDNA haplotype (*atp1* coding region) have significantly lower frequency of hermaphrodites. However, I miss a more detailed discussion of this fact (some of them see p. 116). Is CMS driven by one gene (as given on p. 116) or by a set of chimeric genes?

Minor comments

There are several inconsistencies, e.g.:

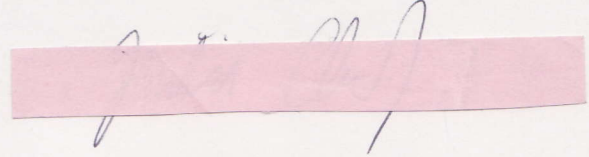
p. 90, subtitle 5.2. Controlled crosses of *S. vulgaris* between females from Kovary and hermaphrodites from **the same** as well as from distant populations
As I realized from the thesis, only pollen from distant populations was used.

Several publications cited in the text are not in the list of references, e.g. Adamec 1996b (p. 121), Koga et al. 2008 (p. 120).

Unfortunately, there are numerous typing errors throughout the text.

Conclusion

The thesis fulfills the criteria necessary for obtaining the Ph.D. degree at the Charles University. I consider it suitable for defense.

A redacted signature consisting of a horizontal pink bar with handwritten ink strokes visible above and below it.